



HAL
open science

Exploring the plasticity of chromosomal domains upon overexpression of silencing factors in *Saccharomyces cerevisiae*

Antoine Hocher

► **To cite this version:**

Antoine Hocher. Exploring the plasticity of chromosomal domains upon overexpression of silencing factors in *Saccharomyces cerevisiae*. Genomics [q-bio.GN]. Sorbonne Université, 2017. English. NNT : . tel-04067988

HAL Id: tel-04067988

<https://hal.science/tel-04067988>

Submitted on 13 Apr 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Thèse de doctorat de l'université Paris VI-Pierre et Marie Curie

Ecole doctorale Complexité du vivant - ED 515; Spécialité: Génétique

Présentée par :
Antoine Hocher

**Exploring the plasticity of chromosomal domains upon overexpression
of silencing factors in *Saccharomyces cerevisiae***

Thèse dirigée par Dr. Angela Taddei

UMR3664 CNRS /Institut Curie / UPMC

Equipe Compartimentation et Dynamique des Fonctions Nucléaires

Soutenue publiquement le 29 septembre 2017

Rapporteuse :	Prof. Dr. Ann Ehrenhofer-Murray
Rapporteur :	Dr. Cédric Vaillant
Examinatrice :	Dr. Sandra Duhaucourt
Examineur :	Dr. Gianni Liti
Examinatrice et présidente du jury :	Dr. Teresa Teixeira
Directrice de Thèse :	Dr. Angela Taddei

A la mémoire de mon grand père, André Peynaud.

Remerciements

Je tiens d'abord à exprimer ma gratitude aux membres du jury qui ont accepté d'examiner ce travail. Il faudra c'est certain plus qu'un paragraphe et quelques années pour bien remercier tout le monde de vive voix, mais en guise de programme voilà l'inventaire à la Prévert des personnes que je remercie, le plus sincèrement. J'ai eu le privilège de travailler avec Angela Taddei qui a bien voulu m'accueillir dans son laboratoire. J'ai eu la chance de bénéficier de ses conseils, de son expertise et de son attention; je ne pourrais pas compter exactement les napolitains et les cafés que nous avons pu partager mais ils ont assurément pavé un chemin sûr et motivant le long de ma thèse. Merci Myriam, Isabelle Micol, Ivaylo, Marie, Marie, Han, David, Camille, Arnaud, Aurore, Yann, Tanguy, Dominique et Caroline, c'est grâce à vous que venir au labo a toujours été, au fond, un vrai plaisir, grâce à vous que j'ai tant ri pendant ma thèse mais aussi avec vous que j'ai pu travailler consciencieusement. Je remercie aussi Guillaume et Layla que j'ai eu beaucoup de plaisir à encadrer durant leur stages.

Mes amis, je pense avoir réussi à presque tous vous inviter pour le café pendant ma thèse, comme mes frères et ma soeur je ne saurais dire à quel point vous comptez pour moi, à quel point notre amitié a pu m'aider à traverser cette thèse. Merci Rémi, merci Benoit, merci Raph, si je pouvais je graverai vos noms au canif sur les arbres de Curie, mais je suis presque sûr que ça n'est pas ok. Merci encore à tous mes amis, Hatch, Nilda, Thibaut, Nil, Will, Julie. J'ai hâte de vous rejoindre à Londres Nil et Will.

J'ai l'impression d'écrire une carte postale, un journal de bord; je veux remercier enfin mes frères François et Octave, ma soeur Fanie, ma mère, Emma, Clara et Alfred parce qu'ils sont simplement la matière qui me tient et qui me porte au jour le jour. Sans que je n'ai vraiment ni à l'écrire ni à le dire, mes pensées les plus douces vont à Giulia.

Table of content

Introduction

I Organization and properties of eukaryotic chromosomes	7
A. Telomeric structures	7
1- Telomeres in bacteria virus and mitochondria.....	9
2- Eukaryotic telomeres	13
4- Eukaryotes without telomerase.....	21
5- General considerations on subtelomeres	22
B Conformation and organization of chromosomes: central role of chromatin	27
1- Histone variants.....	30
2- Histone variants and associated chaperones.....	31
3- Chromatin as an interface between DNA and nuclear machinery	33
4- Chromatin and transcription	34
C. Spatial organization of chromosomes and nuclear compartments	38
1- Nuclear organization is dynamic and shows cell type specificities.....	38
2- Nuclear bodies.....	41
II Chromatin based repression of chromosomal domains	44
A. Diversity, properties and origin of heterochromatic structures	44
1- Position effect variegation.....	44
2- Constitutive and facultative heterochromatin	46
B Molecular description of heterochromatic silencing	48
1- X chromosome inactivation in mouse	48
2- HP1 family	48
3- SIR based silencing	54
C. Functional impact of heterochromatin on nuclear processes	71
1- Telomere clustering	71
2- Chromosome segregation.....	72
3- Heterochromatin and genome stability:	73

III Mechanisms of silent domains delineation	75
A Heterochromatin barriers set boundaries	75
1- Barriers.....	75
2- Desilencing.....	77
3- Directional silencing	78
4- Borderline RNA	78
B Chromatin antagonism at budding yeast subtelomeres	79
1- Local environment.....	79
2- Chromatin acetylation.....	80
3- The intriguing role of Rpd3 at subtelomeres	81
4- Methylation.....	83
Aim of the thesis	85
Results	86
Results, part I: Dose-dependency of heterochromatin domains reveals subtelomeric structuration in budding yeast.....	87
Results, part II: Subtelomeric properties and Sir3 recruitment at internal loci	128
A. Causality and subtelomeres	128
B Nucleation of the SIR complex at non-canonical sites.....	136
Results part III: Screening viability upon overexpression of <i>SIR3</i>	143
General discussion	153
Bibliography	160
List of abbreviations:	188
<i>Annex I : Guidi et al. 2015</i>	
<i>Annex II: Batté et al. 2017</i>	

Introduction figures:

Figure 1 McClintock's diagram of the Breakage-Fusion-Bridge cycle..... 8

Figure 2 Telomeric structures from prokaryotes, mitochondria, and viruses10

Figure 3 Different ways to solve the end replication problem12

Figure 4 Simplified view of canonical telomeres exhibiting TTAGG-like sequences in mammal, fission yeast and budding yeast.....14

Figure 5 Rif-Rap1 binding in trans on telomeric repeats.....18

Figure 6 Two models for regulating elongation of telomeres by telomerase.....19

Figure 7 DNA supercoiling in prokaryotes and eukaryotes.....28

Figure 8 Nucleosome structure and electron microscopy images of chromatin.....30

Figure 9 Asf1 is an example of a histone chaperone within a network of binding partners.....32

Figure 10 Some of the known sites of histone modification and corresponding modifying enzymes in S.cerevisiae.37

Figure 11 Electron microscopy image of a nuclei and schematic organization of chromatin in the nucleus40

Figure 12 Examples of nuclear bodies42

Figure 13 Schematic representations of important characteristics of ideal liquids and ideal solids.....43

Figure 14 Sex chromatin.....47

Figure 15 Phylogenetic tree of phylum Ascomycota.....49

Figure 16 Schematic representation of a HP1 dimer50

Figure 17 The nascent transcript model, and a self-reinforcing epigenetic loop mediating silencing in S.pombe ..52

Figure 18 SIR proteins: domain structure and interactions56

Figure 19 DNA binding sites organization of silencer and protosilencers.....62

Figure 20 Model of the nucleation spreading and maturation of silent chromatin65

Figure 21 Heterochromatin and telomere clustering.....72

Figure 22 Schematic illustration of the nucleosome excluding barrier mechanism.77

Figure 23 Domain organization by directional initiation of heterochromatin.....78

Figure 24 Schematic representation of the equilibrium regulating SIR spreading at subtelomeres81

I Organization and properties of eukaryotic chromosomes

A. Telomeric structures

Well before the discovery of deoxyribonucleic acid (DNA) as the support of genetic information, the advance of x-rays as mutagenic agents shed light on a major defect associated to linear chromosomes: distinguish their ends from a break. In the late 1930's, the cytogeneticist Herman Muller studied X-ray induced chromosomal rearrangements. However he never obtained chromosomes carrying terminal deletions (H. Muller 1938). This led him to conclude that "the terminal gene must have a special function, that of sealing the end of the chromosome, so to speak". He noted, "for some reason, a chromosome cannot persist indefinitely without having its ends thus sealed". Muller named this portion of chromosome the telomere (from the greek telos, end and meros, part). Simultaneously, the work on dicentric chromosomes initiated by Barbara McClintock in the early 30's led her to discover the fusion bridge breakage cycle (**Figure 1**). Consequently, Barbara McClintock deduced that the natural ends of chromosomes are protected from fusion. She later -1983- wrote to Elisabeth Blackburn that she had isolated a mutant unable to heal chromosome ends, suggesting that an enzymatic activity might be required for this process (Blackburn 2010).

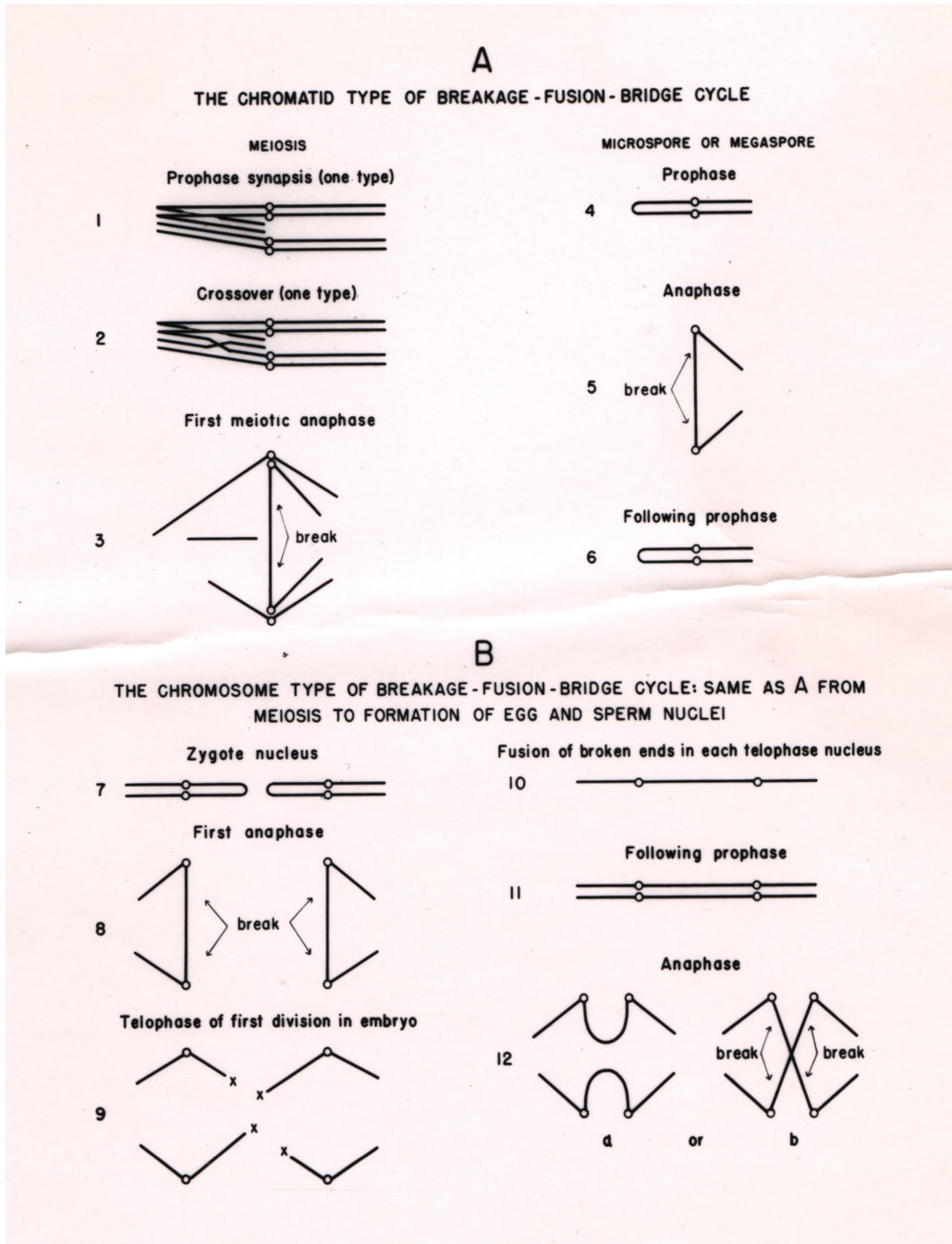


Figure 1 McClintock's diagram of the Breakage-Fusion-Bridge cycle.

source : American Philosophical Society, Library, Barbara McClintock Papers

During the fall of 1966, Olovnikov, who had just heard a lecture on the Hayflick limit (Hayflick 1965) -human cells divide a finite number of times and then enter senescence-, understood the second fundamental property of telomeres: they can't be replicated entirely with the known polymerase based replication. This idea is generally referred to as 'the end replication problem'. In a short essay (Alexey M Olovnikov 1996), Olovnikov tells how Moscow subway inspired him. He draws a telling analogy between the first door of a subway car and the catalytic site of a DNA polymerase which both can't reach the end of the trail. Few years later he linked the end replication problem as a causal agent of aging explaining Hayflick limit. He postulated that in some cases, such as in germ line cells or during cancer the cell expresses a specific polymerase. His theory of marginotopy was published in russian in 1971 and in english in 1973 (A.M. Olovnikov 1973). A year later, J.Watson independently proposed the end-replication problem (Watson 1972). Olovnikov's theory would prove amazingly precise with the decades of results obtained on telomeres.

The outline of this first part is as follow: in an effort to show that all organisms with linear chromosome face the same issues, we will first describe how bacteria viruses and mitochondria possessing linear chromosomes protect their chromosome ends and solve the end replication problem. Then we will describe the structure of eukaryotic telomeres maintained by the telomerase. We will see how different complexes protect the telomeres from being recognized as double strand breaks and describe the sophisticated mechanism of telomere length regulation. Lastly, we will elaborate considerations on the end of chromosomes and see how the genome specificities associated with chromosome ends extends within domains called subtelomeres.

1- Telomeres in bacteria virus and mitochondria

While linear chromosomes are ubiquitous in eukaryotic genomes, they are not exclusive to eukaryotes. Linear forms of DNA have been found in some bacteria species but also in viruses or in a subset of eukaryotic organelles such as mitochondria or plastids. As we will see through this part, even if the large majority of eukaryotic telomeres are maintained by a conserved retro-transcription based mechanism, a large variety of other systems exists.

a. Hairpins close the deal

The bacterium responsible for the Lyme disease has a peculiar genome. It is composed of one linear chromosome (900 kb) and over 20 linear and circular plasmids. All linear dsDNA of these bacteria end with covalently closed hairpins (**Figure 2A**); in a sense, the whole chromosome is single stranded. As no DNA extremities exist the capping problem is absent. Once the replication is complete, the new telomeres are processed into terminal hairpins. This step is mediated by the telomere resolvase ResT, which cuts and re-ligates the chromosome termini and forms new terminal hairpins (Kobryn, Briffotiaux, and Karpov 2009). Similar telomeric structures are found in *A. tumefaciens* c58, the agent of the crown-disease and an important tool for plants genetic transformation, as well as in phages (N15, PhiKO2) and in some candida species such as *C.frijolesensis* (for review, Fulcher et al. 2014).

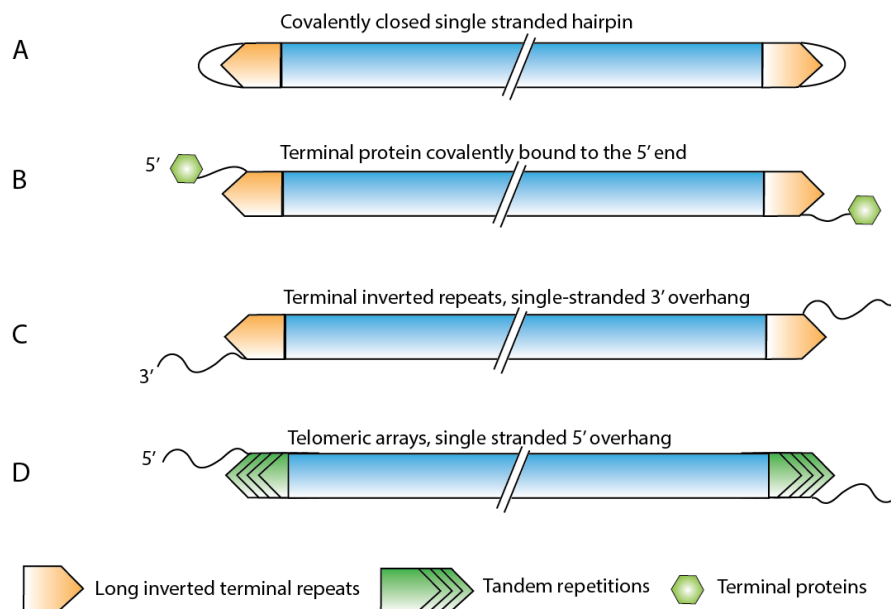


Figure 2 Telomeric structures from prokaryotes, mitochondria, and viruses. Covalently closed hairpin structures at terminal regions has been observed within *B. burgdorferi*, *A. tumefaciens*, and the *Vaccinia virus* (VACV) (A). 5' _overhangs of the human Ad2/5 adenovirus are bound by a terminal protein (TP) (B). Mitochondrial telomeres from the green algae *C. reinhardtii* comprise long inverted terminal repeat regions with a 3' _overhang (C) whereas *C. parapsilosis* exhibits tandem repeats with 5' _overhangs (D) *Figure adapted from the review by Fulcher et al. (2014)*

b. Covalently associated terminal proteins

Some bacteria of the genus *Streptomyces* as well as bacteriophages or some adenoviruses possess linear chromosomes at the extremities of which terminal proteins are covalently bound (**Figure 2B**). The 36 kilobases of the Ad5/2 human adenovirus ends with terminal inverted repeats homing the replication origin. 5' single-stranded ends of this double stranded DNA (dsDNA) are covalently bound by a single terminal protein (TP) (Rekosh et al. 1977). TP of Ad2/5 protects the viral genome from the host exonuclease and also primes replication. Replication is initiated by the coupling of the first deoxynucleotide (dNTP) to the TP by the viral DNA polymerase. This protein priming mechanism ensures the complete duplication of the viral genome (De Jong and Van Der Vliet 1999).

c. Telomeric arrays

The yeast pathogen *Candida parapsilosis* possesses linear mitochondrial DNA. The ends of *C. parapsilosis* mtDNA consist of arrays of tandemly repeated sequences flanked by 5' 110 nucleotides single stranded partial repeats at the extremity (**Figure 2D**). Protection from nucleases and DNA repair machinery is ensured by mitochondrial telomere binding proteins (mtTBP) which share homology with bacterial single strand binding proteins (SSB). In addition, mitochondrial DNA ends in *C.parapsilosis* form T-loop (Tomaska et al. 2002). Those peculiar structures are generated by invasion of ssDNA into the duplex telomeric region (See figure 5, T-loops at mammalian telomeres). First observed in human, those structures have a protective role in eukaryotes. Together with mtTBP, T-loop might contribute to the protection of mtDNA termini from nucleases (for review, Fulcher et al. 2014). Recent experiments analysing recombination intermediates by 2D gels suggest that those mitochondrial DNA might be replicated by a recombination driven mechanism (**Figure 3B**) (Gerhold et al. 2014).

d. Other telomeric structures

The mitochondrial genome of the green algae *C.reinhardtii* also ends with terminal inverted repeats (TIRs) (**Figure 2C**). In contrast to Ad5/2 virus, those TIR ends with non-complementary 3'

overhangs preventing circularization. In this case the mechanism of capping and replication remains to be discovered (Vahrenholz et al. 1993).

Interestingly, researchers could linearize *E.coli* genome using the protelomeraseN enzyme encoded by the lambda phage. In this study, cells with linearized chromosomes and cells with circular chromosomes had similar growth rate, nucleoid shape and global gene expression (Cui et al. 2007). Given our partial knowledge of virus and prokaryote diversity, it is safe to assume that many more structures ensuring telomere functions remain to be discovered.

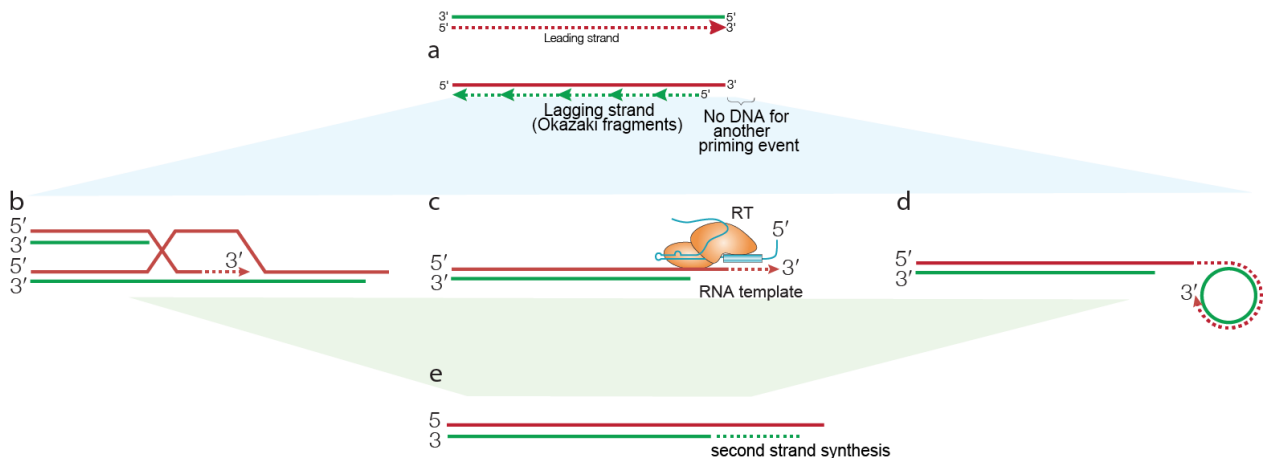


Figure 3 Different ways to solve the end replication problem. Due to the replication mechanism a portion of the lagging strand will not be replicated (a), which can ultimately lead to chromosome shortening. The leading strand can be elongated by break induced Repair (BIR) or by recombination, using another telomere as a template (b). Alternatively, retro-transcription of an RNA template -be it the one associated to the telomerase or a retrotransposon- can be used to elongate the leading strand (c). Lastly, rolling circle replication using extra-chromosomal DNA as template is another possible way of extending the leading strand (d). Irrespective of the leading strand elongation fashion, second strand synthesis completes the elongation of the telomere (e). *Figure inspired by the opinion letter by Titia de Lange (2004)*

In the vast majority of eukaryotic genomes studied so far the end replication problem is solved by a conserved reverse transcriptase, the telomerase. In contrast, the conservation of protein complexes dedicated to capping is not as strict, and there are variations depending on the specie of interest.

2- Eukaryotic telomeres

a. Telomeric DNA

Due to the mechanism leading to their formation, telomeres formed by a telomerase have distinctive features. The telomerase uses a genomically encoded structured RNA as template to add repetition of a short, generally G rich, DNA motif to the end of chromosomes (**Figure3B**). The sequence of telomeres synthesized by the telomerase was first determined by Elizabeth Blackburn and Joseph Gall in *Tetrahymena thermophila* (Blackburn and Gall 1978). This motif is identical within all chromosomes of an organism and its specie. However, variation of the telomeric repeat sequences exists albeit identical sequences are shared by very diverse species. Vertebrate's telomeres for examples consist in thousands of tandemly repeated TTAGGG sequences. The same TTAGGG sequence constitutes telomeric ends of very diverse organisms such as *Neurospora crassa*, jellyfish or common tobacco. Yeast telomeric sequence is somehow degenerate; in *S.cerevisiae* it consists in repeats of a [TG]₁₋₃. The prevalence of G-rich sequences with strong strand bias is thought to have important contribution to telomere properties (Blackburn 2010). While telomeric sequences are relatively well conserved, the number of repeats forming the telomere is dynamic within an organism and shows dramatic variations in between species. A non-exhaustive list: average of 300bp in budding yeast, 2-15 kb in humans, 150kb in tobacco (for review, Fulcher et al. 2014).

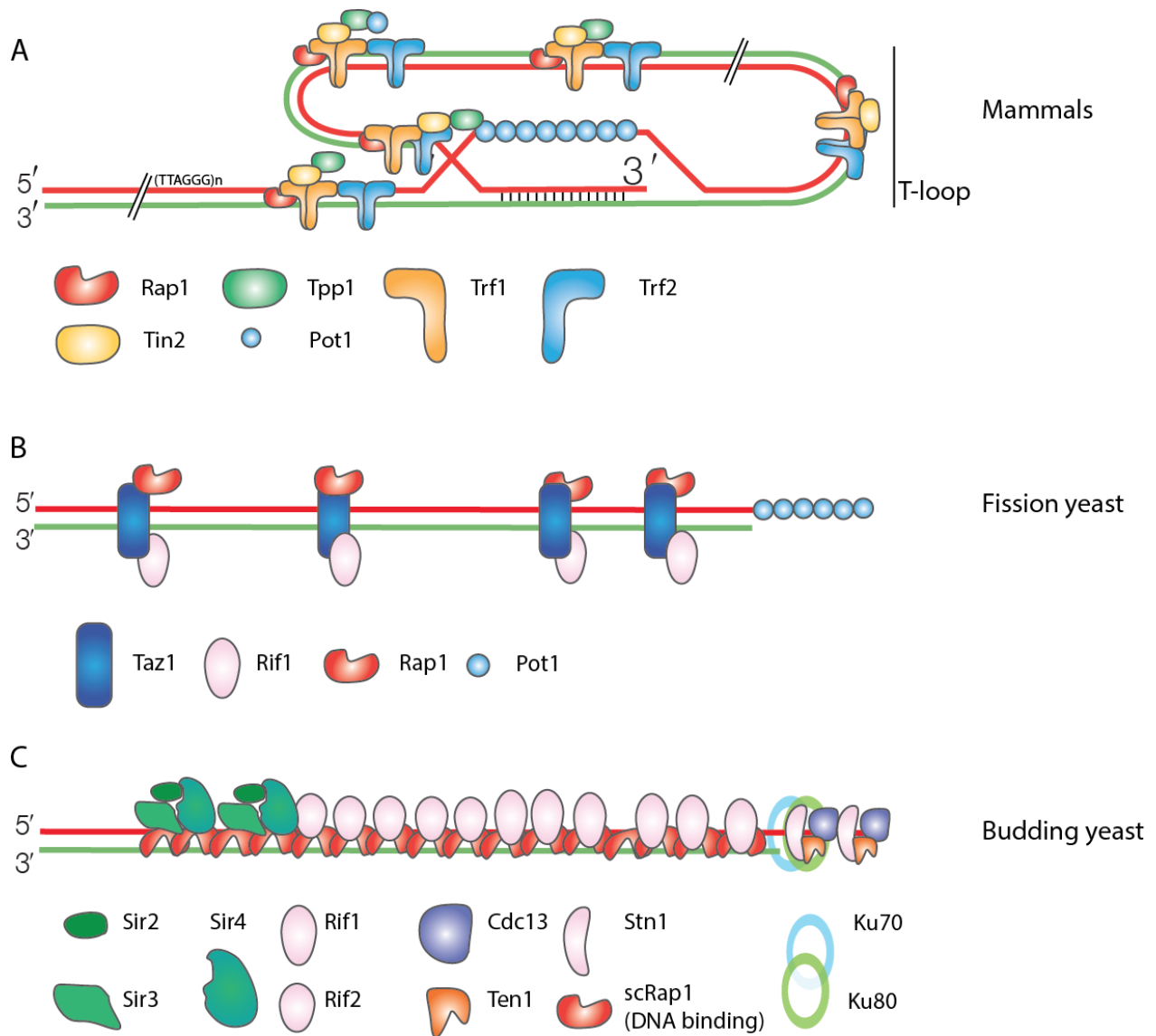


Figure 4 Simplified view of canonical telomeres exhibiting TTAGG-like sequences in mammal, fission yeast and budding yeast. In mammals the shelterin complex sequesters the G-overhang into duplex double-stranded telomeric DNA(A), while in fission yeast, the single strand DNA binding protein Pot1 protects telomeres and prevent checkpoint activation(B). The CST (Cdc13, Stn1, Ten1) complex ensures the efficient capping of budding yeast telomeres(C). See the core text for references.

b. Capping by OB (oligo nucleotide Binding) fold protein binding to G-overhang

Budding and fission yeast telomeres are apparently devoid of T-loop structures although technical difficulties prevent any strong conclusion on this subject. The ordinary procedure used to detect T-loops cannot be employed in yeasts for two reasons. First, psoralen crosslinking requires TA sequences, which are absent from telomeres in both species; second, isolation of

telomeric sequences by size selection of digested genomic DNA is not efficient due the relatively small size of yeast telomeres compared to mammal telomere (de Lange 2004). Despite those technical difficulties, the degenerated telomeric sequences found in most yeast might impede the homology driven sequestration of the G overhang within the duplex telomeric sequence.

In those organisms, telomere capping is mediated by the binding of OB-fold containing proteins to the single stranded DNA overhang. In fission yeast the OB-fold containing Protection of telomere (Pot1) protein binds ssDNA (**Figure 4B**). Pot1 is evolutionary conserved between yeast and human as a part of the sheltering complex; its inactivation in fission yeast has dramatic consequences as it leads to chromosome fusions and acute DNA damage signaling (Baumann and Cech 2001). In budding yeast, protection of the telomere also involves the binding of an OB-fold containing protein, Cdc13, to the 3' ssDNA overhang. However, Cdc13 is unrelated to Pot1 but might rather represent a RPA-like particle. Cdc13 binds telomeric ssDNA in complex with Stn1 and Ten1, forming the so-called CST complex (**Figure 4C**). Of note, it has been suggested that fission yeast and human possess a second capping complex independent of Pot1 and involving Stn1 and Ten1 homologs but devoid of Cdc13 (Moser and Nakamura 2009; Lloyd et al. 2016).

c. Capping by T-loop

The relatively long mammalian telomeres adopt a T-loop structure. T-loop structures have been observed using electron microscopy in various organisms including human, mouse and chicken (for review, de Lange 2004). The sheltering complex mediates the formation and the stabilization of this structure (**Figure 4A**). Within this complex, Trf1 and Trf2 possess DNA-sequence specific affinity for dsDNA telomeric repeats; Pot1 selectively binds to the single stranded overhang (see paragraph below). Tin2 interacts both with Trf1/2 and Tpp1. Tpp1 can interact with Pot1, so that Tin2 is bridging the interaction between Trf1/2/Tpp1 and Pot1. Lastly, the mammalian homologue of yeast Rap1 interacts directly with Trf2. Current models for T-loop formation suggest that Trf2, which has DNA bending properties in vitro, mediates the formation of a primary loop, which is later stabilized. Stabilization is mediated by invasion of the single-stranded overhang into duplex telomeric DNA. This last step requires the DNA repair proteins

Rad51-52. Importantly, T-loop structures are dynamic as they are disassembled during DNA replication, in a process that requires *RTEL1* (de Lange 2004; Fulcher et al. 2014).

Naively, because capping structures protect telomeres from being recognized as double strand breaks, one could think that capping might also block their replication and their elongation by the telomerase. In essence, the whole telomeric structure has to be flexible enough to allow both capping and telomerase recruitment when required. At the core of this system, the cell somehow measures telomere length. Mechanisms favouring or inhibiting telomerase recruitment responds to this measure. Last, capping has to be dynamic to allow replication and elongation of telomeres when needed. Focusing on budding yeast we will see how the whole telomeric structure contributes to the maintenance of telomere length.

d. Budding yeast telomeres

In budding yeast, Repressor/Activator site binding Protein (Rap1) is the major protein binding double stranded telomeric DNA (Mark S. Longtine et al. 1989). The name of this protein stems from its dual functions in the nucleus (Shore and Nasmyth 1987). At telomeres Rap1p-bound arrays constitute the foundation of a proteic structure dedicated to gene silencing, telomere length regulation and telomere capping whereas in the genome, Rap1 is a prominent regulator of the transcription of genes involved in ribosomal biogenesis and glycolysis. Perhaps surprisingly, various proteins present at telomeres assume different roles within the genome, very much like Rap1; as we will see throughout the introduction, this is a recurrent theme in telomere biology, which relates to fascinating questions.

According a popular model, telomere length is monitored through the number of Rap1 molecules bound to a given telomere (**Figure 6A**) (Marcand, Gilson, and Shore 1997). Rap1 is a 92 kDa protein organized in at least three domains; unstructured regions located between domains are predicted to account for 40% of the protein. The N-terminal third of the protein is a BRCA1 C-terminus (BRCT) domain. The central DNA binding domain is composed of two Myb-like domains joined by a linker sequence (König et al. 1996). A transactivation domain is located within the unstructured linker located between the DBD and the C-terminal domains (Hardy,

Balderes, and Shore 1992). Lastly, Rap1 regulatory C-terminal domain (RCT) is required for interactions with the Sir3, Sir4, Rif1 and Rif2 (Gartenberg and Smith 2016).

Rap1 binds telomeric DNA via its two MYB domains. Each domain recognizes the GGTGT motif and their arrangement in tandem allows the whole protein to recognize the 5'-GGTGTGTGGTGT motif (Konig and Rhodes 1997). In vitro studies probing the binding of purified Rap1 to long telomeric DNA molecules indicated continuous binding of Rap1 along the telomeric track, with an average periodicity of 18bp (Gilson et al. 1993). The presence of two structurally similar Myb domains within the same protein has been proposed to be advantageous for highly specific binding to a short DNA motif compared to the use of single proteins which would likely require cooperativity. Indeed the coupling of the two domains is supposed to allow a flexibility that might be necessary due to the irregular spacing of Rap1 binding sites within telomeric repeats (König et al. 1996). The function of the BRCT domain of Rap1 has yet to be found but it is not required for Rap1's known functions: no phenotype could be observed upon deletion of Rap1's first 279 aa (P Moretti et al. 1994), which include the BRCT domain.

Telomeric tracks being ~300bp long on average, an estimated 16 Rap1 molecules would be present at a typical telomere. This Rap1 array is key in the articulation of telomeric processes. On the centromere proximal region, some Rap1 are required for the recruitment of the Silent information regulator (SIR) complex, which represses the transcription of neighbouring genes. The remaining Rap1 proteins are interacting with Rif1 and Rif2. Interestingly, binding of Rap1 to DNA induces conformational changes leading to localization of N-terminal and C-terminal domains on opposite sides of the DNA. This conformational change has been proposed to favour RCT interactions with Rif and Sir proteins (Matot et al. 2012).

At budding yeast telomeres, Rif1 and Rif2 have been shown to regulate the formation of a 'velcro like' structure by interactions of Rif proteins with Rap1 (**Figure 5**). This structure potentially brings robustness to the array of Rap1 bound proteins (Shi et al. 2013). In addition, recruitment of Rif proteins to telomere is important for telomere length regulation as telomere length is roughly doubled in rif mutants (Hardy 1996). However the Rif-Rap1 interaction is not

itself sufficient to maintain telomere length since mutation in the conserved RVxF/SILK domain involved in PP1 phosphatase binding (Glc7 in budding yeast) causes telomere lengthening (Unpublished results from, Mattarocci et al. 2014). The mechanism by which Rif proteins influence telomere length in the framework of the counting model remains yet to be described.

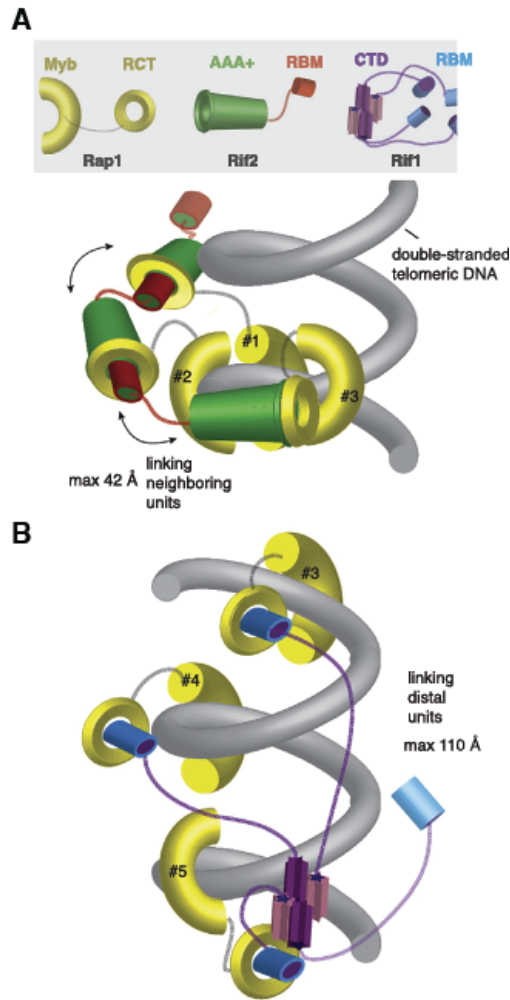


Figure 5 Rif-Rap1 binding in trans on telomeric repeats. (A) Three closely positioned Rap1 molecules (1 to 3, 19 bp spacing between each Rap1- binding site) are interlinked by Rif2-AAA+ and Rif2-RBM domains in trans. A cartoon color legend is shown on the top. Rap1 is depicted with Rap1-RCT and Rap1-myb, Rif1 with Rif1-CTD and Rif1-RBM only. The Rap1-BRCT and Rif1 N-terminal domains are omitted for clarity.(B) Rif1-Rap1 binding in trans on telomeric repeats employing the tetramerization domain. The long flexible linker bridging up to 110 Å between Rif1-CTD and Rif1-RBM allows Rif1 to interconnect distal Rap1 molecules (Rap1- binding sites 3 and 5 are spaced by 30 bp). *Original figure and legend from Shi et al.2013*

Although originally identified for their role at yeast telomeres, Rif proteins are conserved and play various roles in the genome. Rif1 has been shown to regulate the global timing of DNA replication. Rif1 inhibits origin firing through its interaction with PP1 phosphatase (Glc7 in *S.cerevisiae*) (Mattarocci et al. 2014). Additionally Rif1 inhibits resection at double strand breaks in mammals and in yeast (Martina et al. 2014). Even the telomeric function of Rif proteins has been mostly studied in yeasts, a putative role of Rif1 at mammalian telomeres can't be excluded. Rif1 is highly expressed in mouse testis and ESC. Depletion of Rif1 in ESC leads to increased telomere length, presumably as an indirect consequence of subtelomeric heterochromatin destabilization (Dan et al. 2014).

An alternative model (Replication fork model) postulating that the telomerase travels with the replication forks proposes that Rif1 influences telomere length by inhibiting neighbouring origin firing. Inhibition of telomere proximal origins of replication would diminish the probability of the telomerase of reaching the end of the chromosome and thus inhibit lengthening (**Figure 6B**)(Greider 2016).

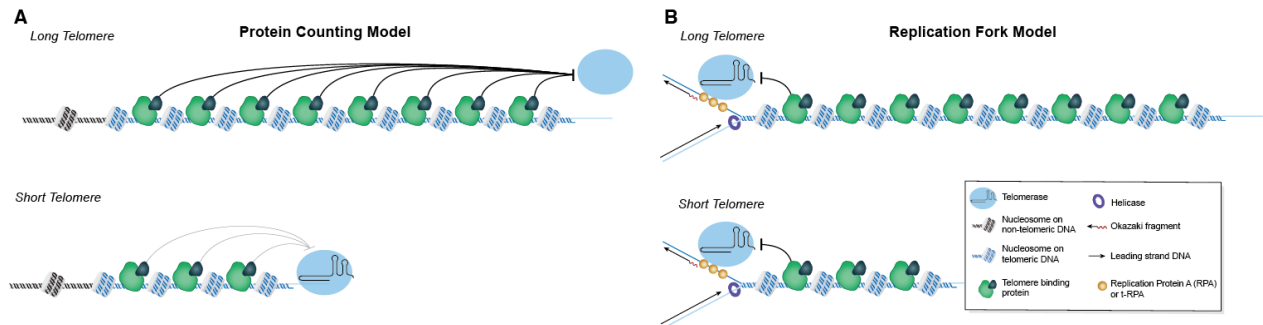


Figure 6 Two models for regulating elongation of telomeres by telomerase. (A) Protein-counting model: the proteins bound to the telomere (Rap1 in budding yeast) inhibit telomerase recruitment. As the amount of telomere bound is proportional to the length of the telomere, short telomere exert less inhibition and are thus preferentially elongated (B) Replication fork model: In this model, the telomerase must remain bound to the fork until it reaches the extreme terminus for the telomere to be extended. The telosome might inhibit neighbouring origins of replication or replication fork progression. *Figure from Greider et al. (2016)*

e. Telomeres and telomerase recruitment

In budding yeast protein Est2 and the TLC1 RNA form the catalytic core of the telomerase, and are sufficient for in-vitro telomerase activity. *In vivo*, proper regulation of telomerase activity requires at least three additional proteins: Est1, Est3 and Cdc13. Deletion of any of those genes leads to the progressive shortening of telomere, a phenotype termed ever shorter telomere

(est) (Lundblad and Szostak 1989). While elongation of a short telomere by the telomerase is restricted to late S/G2(Marcand et al. 2000), Est2 is also bound to telomere during G1 phase and early S phase. Association of the telomerase to telomeres during G1/early S depends on the Ku complex. While the Ku complex is generally involved in non-homologous end joining (NHEJ), an activity that should paradoxically be avoided at telomeres, it also interacts with TLC1. Cdc13-Est1 direct association mediates telomerase recruitment during late S/ G2 (Taggart, Teng, and Zakian 2002). Lastly, the ATM-like kinase Tel is required for proper recruitment of Est1 and Est2 to telomeres (Goudsouzian, Tuzon, and Zakian 2006).

As introduced earlier, telomerase activity is also regulated by telomere length, this regulation ultimately leading to telomere length homeostasis. *In vivo*, elongation by the telomerase only takes place at a subset of telomeres at a time. While the extension length per event is independent of telomere length, telomerase preferentially targets short telomeres (Teixeira et al. 2004). In this context, it has been shown that both Est1 and Est2 are preferentially recruited to short telomeres in a Tel1 dependent manner(Sabourin, Tuzon, and Zakian 2007). In contrast, the binding of Ku70, Rif1 and Cdc13 appears independent of telomere length(Sabourin, Tuzon, and Zakian 2007).

There is a gap of knowledge concerning the competition between the Sirs and the Rif proteins for Rap1 association and their organization along the telomere track. Furthermore we still lack convincing mechanism explaining the influence of the Rif proteins in telomere length regulation. While the counting model does not offer clear mechanistic, the replication fork model fails to explain why short telomeres are preferentially elongated even if they have similar amount of Rif1 bound. However, the observation that binding of Rif1 and 2 is independent of telomere length(Sabourin, Tuzon, and Zakian 2007) might be biased. In this study, Chromatin immunoprecipitation was estimated at sequences neighbouring the telomere track so that it might have been blind to more distal sequences. Exciting results might arise from the study of the influence of Rif mediated structure on Rap1 binding and turnover.

4- Eukaryotes without telomerase

In some cases, the cell uses telomeres to limit the number of times that it will divide. In dividing human somatic cells for example, telomerase activity is too low to counteract losses due to the end-replication problem. Thus telomeres will shorten at each replication round. Eventually this leads to a state in which capping is not functional anymore and telomeres are perceived as a source of stress. Consequently, the cell cycle will be stopped and the cell will enter senescence (for review, Shay and Wright 2000).

In theory, deletion of telomerase in a clonal population of yeast should thus lead to senescence of the whole population after a certain amount of divisions. While this prediction holds for the vast majority of the population, a subset of mutants surviving senescence emerges at low frequency (Lundblad and Blackburn 1993). Those mutants still have linear chromosomes, indicating that they developed another way of elongating telomeres. In budding yeast survivors elongate their telomeres by two types of Rad52 dependent recombination. Type I survivors maintain telomeres by amplification of Y' elements and require Rad51, Rad54 and Rad57. Type II survivors are characterized by the amplification of [TG]₁₋₃ repeats. Telomere amplification in Type II survivor is Rad51 independent but requires the MRX complex (Mre11, Rad50 and Xrs2), Rad59 and Sgs1 (Q. Chen, Ijima, and Greider 2001). Furthermore, the normally essential capping function of Cdc13 is dispensable in both types of survivors, suggesting that capping can also take alternative routes (Larrivée and Wellinger 2006).

Interestingly, telomerase is constitutively absent in some eukaryotic species such as *Drosophila melanogaster* (Levis et al. 1993), the mosquito *Anopheles gambiae* (Roth et al. 1997) as well as in some onion related plants (Pich and Schubert 1998). *Drosophila* telomeres stand out as they apparently break the general principle of telomere maintenance in eukaryotes. Telomere maintenance in *Drosophila* is ensured by the addition of transposable elements to the end of chromosomes. The adjunction of two classes of retrotransposons, HeT-A and TART (telomere associated retrotransposon), maintains chromosome length in this organism (**Figure 3C**). In addition, truncated ends devoid of HeT-A and TART sequences can be recovered and maintained, despite the progressive shortening of chromosome ends. While those ends will

eventually be healed by addition of HeT-A or TART cDNA, the ability to propagate a truncated end without inducing cell cycle arrest indicates that the end-protection function is independent of DNA sequence. Part of the explanation lies in the use of a particular protection system implicating the Heterochromatin Protein 1 (HP1). Thus, in analogy with centromeres, telomere function in *Drosophila* might be epigenetically regulated by their chromatin state (E. J. Louis 2002).

Throughout this part we have briefly described the structures that have evolved to face the challenges associated to linear chromosome ends. Despite the variety of telomeres, we have seen that some common themes emerge from the study of chromosome ends. First it is striking to see how multiple proteins acting at other chromosomal locations join at telomere to play a specific role. Cells thus managed to evolve a complex system that uses repair (Ku, MRX, ATM) and replication (Rif proteins) machinery for telomere surveillance.

5- General considerations on subtelomeres

Telomeres are associated to the end of linear chromosomes. But defining the end might be more difficult than it seems. First, one can naively appreciate that defining the end requires an agreement on the way we see the whole chromosome. Conceptually, if one thinks of a chromosome as a discrete entity, then 'the end' can be a discrete element. One has to agree on a scale at which the 'end' term is coined. From the genome point of view, the end would be the last genes, however from the DNA fiber point of view, it would be the last base pairs or the single stranded DNA ends. In this regard, the end of linear chromosomes is generally not restricted to the telomeres. The domains located between the core of the chromosome and the telomere generally have peculiar properties compared to the rest of the genome. Those domains are generally referred to as subtelomeres or telomere associated sequences. Defining subtelomeres is not an easy task (E. Louis and Becker 2014). While those domains often have specie specific properties compared to the chromosome core, no property enables a univocal definition of subtelomeres. Gene density, chromatin structure or recombination rate generally differs in the subtelomeres in comparison with the core genome. However there are no

generality in the way that those properties diverge from the core genome. If in many species gene density is lower in subtelomeres, in others it is higher. The same is true for the aforementioned properties: some organisms have a decreased recombination rate in the subtelomeres while some don't. At last, subtelomere length varies substantially among chromosomes arms and between species (E. Louis and Becker 2014). The lack of univocal criterion to define what a subtelomere exactly is makes it hard to delineate unambiguously those domains. While subtelomeres are often designated as regions next to the telomere that show low gene density or that are devoid of essential genes (C. A. Brown, Murray, and Verstrepen 2010; Pryde and Louis 1997; Leonid Teytelman, Eisen, and Rine 2008), none of those property is a fully satisfying definition. For example the essentiality of a gene can be context dependent or background specific. For those reasons, it has been proved relevant to define subtelomeres by exclusion. For example by setting the limits of the chromosome core, within which gene order (synteny) is conserved within specie and its close relative. Whole genome sequencing of several closely related yeast species using long read technology recently enabled a rigorous definition of budding yeast subtelomeres based on synteny (Yue et al. 2017). Major subtelomeric properties in several eukaryotic species are listed in Table 1.

Organism	Long homology blocks	Extracellular genes	VNTRs	Helicase	TEs	rDNA	TRs
Saccharomyces	Yes	Yes	Yes	Yes	Yes	No	Yes
Other yeasts	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Human	Yes	Yes	Yes	No	Yes	Yes	Yes
Pneumocystis	Yes	Yes	Yes	?	?	No	Yes
Fungi	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Drosophila	No	?	Yes	No	Yes	No	No
Bombyx	No	?	No	No	Yes	No	Yes
Chironomus	No	?	Yes	?	?	?	No
Anopheles	No	?	Yes	?	?	No	No
Giardia	Yes	Yes	Yes	Yes	No	Yes	Yes
Plasmodium	Yes	Yes	Yes	No	Yes	No	Yes
Trypanosoma	Yes	Yes	Yes	No	Yes	No	Yes
Alliaceae	Yes	?	Yes	?	?	No	No
Plants	Yes	?	Yes	?	Yes	No	Yes

Table 1 The generic structure of eukaryotic chromosomes with subtelomeric details of several organisms, adapted from the book Subtelomere, E.J. Louis. The core genome, which is generally stable over time can be opposed to the chromosome ends. Chromosome ends are composed of the subtelomeres and the telomeric repeats (TRs), when presents. Subtelomeres are generally composed of long homology blocks which often contains genes dedicated to the interaction with the environment. Most subtelomeres contain variable number of tandem repeats which sometimes function as telomeres. Most subtelomeres also contain transposable elements such as the Ty5 in *S.cerevisiae*. Several yeast subtelomeres contain genes encoding for helicases - see text for example-. Lastly ribosomal DNA arrays are subtelomeric in many species, including *Saccharomyces pombe*.

The interest for repeated sequences located near telomeres stems from the early 1980's. Yeast geneticist revealed the high degree of polymorphism of telomere associated sequences along with the presence of gene families such as the SUCrose genes (Horowitz, Thorburn, and Haber 1984; Carlson, Celenza, and Eng 1985). Following work in insects, parasites, humans confirmed the polymorphic nature of subtelomeres and extended the repertoire of their specificities. Among them is the fascinating observation of the location of ribosomal DNA arrays at subtelomeres in various species such as humans, the diarrhea agent *giarda lamblia* or *schizosaccharomyces pombe* (for review, E. Louis and Becker 2014). Lastly, many species host mobile elements that preferentially transpose within subtelomeres, examples include the Ty5 family in *S. cerevisiae* (S. Zou, Wright, and Voytas 1995).

Another property often associated with subtelomeres is the relative abundance of gene families dedicated to interaction with the environment. Hereafter we will develop two well-studied examples.

a. Olfactory receptors genes in mammals

The first step leading to our capacity to smell is the interaction of odorant molecules with G-protein transmembrane receptors. The variety of receptors expressed in sensory neurons contributes to our ability to discriminate complex smell. Interestingly olfactory receptor (OR) genes form the largest mammalian gene family known as it contributes to 1/30 to 1/50 of the genome in humans. OR genes are often clustered, and among the 42 loci detectable by FISH, 55% are subtelomeric or pericentromeric (Young and Trask 2002). It has been suggested that the subtelomeric position of OR genes contributes to the diversity of this gene family (Mefford and Trask 2002). Selectivity olfaction is achieved by the monoallelic expression of one OR of the multiple OR genes available. In mouse this selectivity is achieved by epigenetic silencing of the other OR genes (Magklara et al. 2011). Olfactory sensory neurons that express the same OR gene will converge to the same location within the olfactory bulb (Axel 2005). To which extend the subtelomeric position of human OR genes contribute to their transcriptional regulation and evolution remains to be explored.

b. Antigenic variation in parasites

Many pathogens have the ability to escape the host immune system by systematically altering the epitope accessible to antibodies. Here we will briefly describe how the malaria agent *Plasmodium falciparum* uses a subtelomeric gene family for successful infection. *P. falciparum* infects red blood cells (RBC). The primary antigen characterizing infected RBC is a highly variable transmembrane protein named *Plasmodium falciparum* erythrocyte membrane protein 1 (pfEMP1). This protein is potentially encoded by the 60 var genes within pfEMP1's genome. However, a single var gene is expressed at a time while the remaining var genes are epigenetically silenced. By exposing a single variant of pfEMP1 at a time and by switching to a new variant when the host has developed an efficient antibody, the parasite maximizes the chances of infection. In contrast to the core genome, which is largely conserved within the same species, the var genes exhibit virtually unlimited sequence diversity. This sequence diversity, which is driven by gene conversion events, combined with the shuffling of the expressed var gene efficiently prevents the building of immune memory and thus further strengthens pathogenicity. Var genes have been classified into three classes, A, B and C based among other things on their chromosomal location. Class A and B var genes are present in the vicinity of most telomeres. The subtelomeric location of class A and B var genes, and the association of telomere with nuclear periphery favor the preferential recombination of class A and class B with each other and has been proposed to favor the epigenetic silencing of var genes. Thus, in this organism, the subtelomeric location of var genes contributes to the variety of the parasite defence strategy (Kirkman and Deitsch 2012).

c. Subtelomeres in S.cerevisiae

Budding yeast telomeres are flanked by middle repeat elements of two types, the X element and the Y' element (Pryde and Louis 1997). The X element is present at all telomeres, sometimes separated from the telomeric repeats by a Y' element. All X elements have in common the presence of a core sequence (XCS) of 473 nt which contains a potential replication origin. Small elements designated STR-A, B, C or D make up the rest of X elements, and are present in different combinations depending on the chromosome end (E. J. Louis et al. 1994). The conserved Y element is found at some chromosome ends in up to four copies depending on the

chromosome end. This element contains putative ORF, one of which codes for a putative helicase of unknown function with similarities with viral helicases (M. Yamada et al. 1998), that is expressed during meiosis and in telomerase mutants. It has been suggested that the Y' is a relic of transposable element (E. J. Louis and Haber 1992). Structurally, most budding yeast subtelomeres have a low gene density and contain long blocks of homology. Subtelomeric gene families can be split into three categories:

- Genes involved in carbohydrate metabolisms

- Genes involved in cell wall composition

- Uncharacterized genes

Gene families involved in sugar metabolism include the MAL genes, which confer the ability to use maltose as carbon source, the SUC genes which gene product allows the conversion of sucrose into glucose and fructose. There is extensive variation in the number of *SUC* genes present in a given yeast isolate (Yamashita et al. 1985), which is poorly represented by the laboratory backgrounds which only contain one non-subtelomeric gene of this family (*SUC2*). The STA genes are only present in some strain and allow the use of starch as carbon source (Yamashita, Takano, and Fukui 1985). Genes from the FLO family contribute to the capacity of *S.cerevisiae* to form cell aggregate. Interestingly the expression of the FLO genes contributes to the directed evolution toward multicellularity. Other gene families such as the PAU or the COS genes have been suggested to influence cell wall properties but their actual function remains unknown.

The idea that emerges from the study of subtelomeres in yeast is that the genic composition of subtelomeres somehow reflects the organism lifestyle and capabilities. In association with the rapid turnover and the variable copy number of subtelomeric genes, this part of the genome is reminiscent of the use of plasmids by bacteria.

In the following part we will adopt a bottom-up approach to describe the principles driving the spatial and functional organization of the eukaryotic genome. This very active field of research, sometimes denominated as functional architecture of the nucleus, remains full of surprises.

B Conformation and organization of chromosomes: central role of chromatin

« Quoi de plus manifeste et de plus clair, quand nous avons porté nos regards vers le ciel et contemplé les corps célestes que l'existence d'une divinité d'intelligence absolument supérieure qui règle leurs mouvements ? [...] non seulement la demeure céleste et divine a un habitant, mais celui qui l'habite exerce sur le monde une action directrice, il est en quelque sorte l'architecte d'un si grand ouvrage et veille à son entretien [...] » Cicéron

In the three kingdoms of life genome organization occurs in such a way that compact packaging of DNA into the nucleus (in eukaryotes) or into the cell (in bacteria and archaea) is compatible with replication, repair and transcription. Four major forces are driving the organization of chromosomes: the coiling of the dsDNA molecule, the self-organization resulting from the high molecular crowding, the wrapping of DNA along proteins and the interaction of chromatin with nuclear landmarks (for review, Luijsterburg et al. 2008).

The concentration of macromolecule in a living organism ranges from 100-400 mg.mL⁻¹. At this range of concentration, entropy driven organization is expected to occur (Marenduzzo, Finan, and Cook 2006). Importantly entropic effects are largely independent of the actual structuration of DNA by architectural molecules. Moreover molecular crowding can influence chemical equilibrium; thus proteins binding to DNA or protein-protein self-association are likely favored in a crowded environment. Lastly, molecular crowding can favor ribo-nucleic phase separation, as we will discuss later on. Because it is not the subject of this work we will not expand much the discussion on molecular crowding. However, this aspect of biochemistry surely has a major influence on cellular organization (Luijsterburg et al. 2008).

Prokaryotes lacking histones induce active DNA compaction by the use of DNA gyrase. Gyrases are ATP-dependent enzymes that introduce negative supercoiling. Introduction of supercoils leads to the formation of plectoneme structures, reducing the volume explored by the DNA molecule. Potential coiling energy is constrained by the binding nucleoid associated proteins

(NAP) to DNA (**Figure 7**). In organisms lacking gyrases (eukaryotes and some archaea encoding histone proteins), negative supercoils are introduced through the interaction of DNA with nucleosomes. These supercoils are constrained by Van der Waals interactions with the surface of histones. The positive supercoils induced to compensate nucleosome formation are then relaxed by the action of Topoisomerases.

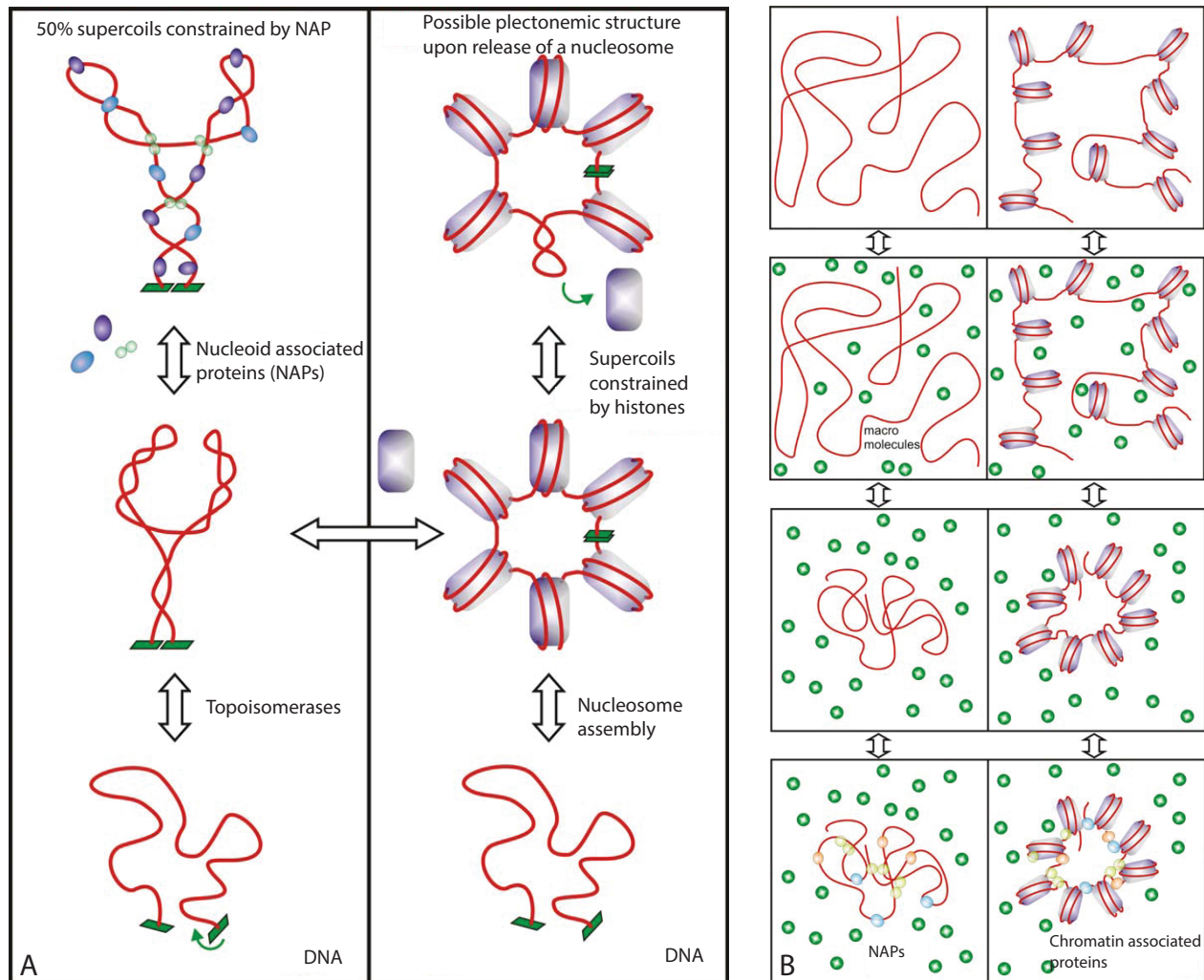


Figure 7 DNA supercoiling in prokaryotes and eukaryotes. Bacteria and some archaea have enzymes allowing the introduction of supercoils using ATP. In bacteria DNA gyrase introduce negative supercoils while reverse gyrase leads to the formation of positive supercoils (A). In eukaryotes and in some archaea expressing histones, supercoils are introduced by wrapping of DNA around the nucleosome. These supercoils are constrained on the surface of histone proteins. Disassembly or remodeling of a nucleosome can release supercoils, which results in a topology similar to that in bacteria (i.e plectonemic). *Figure adapted from the review by Martijn S. Luijsterburg (2008).*

Histone are small proteins made of 3 hydrophobic α -helices separated by two small loops. This conserved motif, the histone fold, allows histones to dimerize. Dimer of histones can assemble in tetramers or octamer to form the functional nucleosome structure, around which DNA will wrap. Histone proteins can be grouped into 5 major families: H2A,H2B,H3,H4 and H1. Archaeal nucleosomes studied so far consist of tetramers of H3-H4 histones wrapping one helical turn of DNA. In eukaryotes, the assembly of two H2A-H2B dimers and one H3-H4 tetramer form a nucleosome. This octameric structure wraps 1.6 helical turns of DNA (Kornberg 1974; Oudet, Gross-Bellard, and Chambon 1975). Resolving the crystal structure of recombinant histones forming nucleosomes with human alpha satellites sequences showed that the basic charges of histones neutralize the negative charges of DNA backbone (Luger et al. 1997). This charge neutralization likely favours the formation of higher scale structures.

H1 histone bridges DNA from both sides of the nucleosome (McGhee and Felsenfeld 1980). H1 is different from other histones in the sense that its function is not necessary for the formation of a nucleosome. In addition, homologs of H1 are present in many bacterial genomes. The Hc1 and Hc2 proteins from *Chlamidia tachomatis* are homologous to H1 (Hackstadt, Baehr, and Ying 1991); their heterologous expression in *E.coli* induces nucleoid compaction (Barry, Hayes, and Hackstadt 1992). Contrary to other core histones, which are believed to originate in archaea, H1 family potentially has a bacterial origin (Kasinsky et al. 2001; Sandman and Reeve 2006). A major distinction between archaeal and eukaryotic histones is that eukaryotic histones possess extruding terminal tails, which experience important/numerous post-translational modification. As we will see at the end of this part, these modifications are key for the regulation of numerous genomic processes.

In metazoan, a typical nucleosome binds and wraps ~146 bp of DNA, and the addition of histone H1 bridges an additional 20bp. The resulting structure is called a chromatosome and wraps 2 helical turns of DNA. In vivo, nucleosomes wrap entire chromosomes and are regularly spaced by a DNA linker which length varies between species (~20bp). This structure, when observed by electronic microscopy, recalls beads on a string (Olins and Olins 1974; Oudet, Gross-Bellard, and Chambon 1975). Further evidence for the regular spacing of nucleosomes along the

chromosomes came from enzymatic digestion of DNA by the micrococcal nuclease. In presence of chromatin this enzyme, which specifically cuts within the linker DNA, produces a ladder-like structure that suggest that nucleosomes are regularly spaced on chromatin (Kornberg 1974). For those reasons, the association of DNA with nucleosomes is considered as the fundamental unit of chromatin.

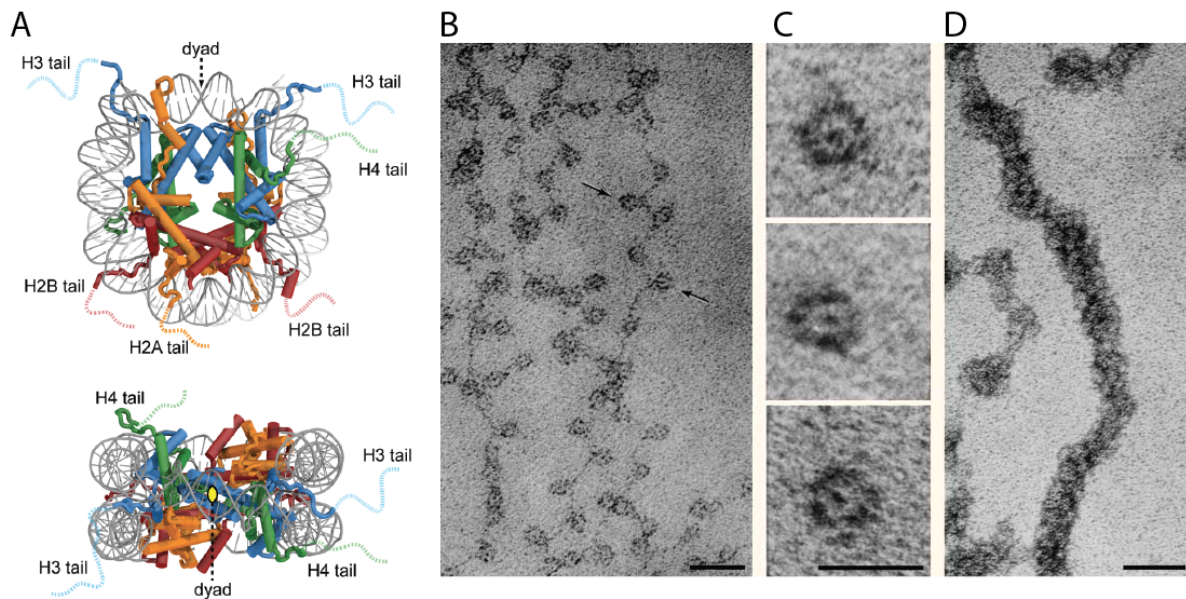


Figure 8: Nucleosome structure and electron microscopy images of chromatin. (A) Representation based on the crystal structure of a nucleosome, Davey et al. (2002). The double stranded DNA helix is in grey and dashed segments represent histone tails. (Top) : face view, Bottom : top view) Adapted from the review by Bowman and Poirier (B) low ionic-strength chromatin spread, the "beads on a string" structure. (C) Isolated nucleosomes obtained nuclease digestion of chromatin (C) moderate ionic-strength chromatin spread, the so-called 30 nm fiber. Images from the review by Olins and Olins (2003).

1- Histone variants

In eukaryotes, all histones but H4 exist as protein variants. Each variant uniquely contributes to the properties of the whole nucleosome and can affect local chromatin properties, along with the post-translational modification of histone tails. *In vitro* experiments clearly showed that the different variants give rise to nucleosome with different stability or wrapping DNA on different lengths (Jin and Felsenfeld 2007; Mariño-Ramírez et al. 2005). Histone variants fall into three classes:

- The replicative or canonical variants, which are transcribed synchronously during S phase. Their incorporation is actively coupled to DNA synthesis.

-The replacement variants that are constantly transcribed and potentially incorporated during the whole cell cycle.

-Tissue specific variants, which expression is developmentally regulated.

Budding yeast encodes two histone variants, CenH3 that is required for centromere assembly, and H2Az, which generally replaces H2A at the +1 nucleosome of transcribed genes. Furthermore, the main H3 variant encoded by budding yeast is similar to mammalian histone H3.3, which happens to be a replacement variant (Kusch and Workman 2007; C. M. Weber and Henikoff 2014).

2- Histone variants and associated chaperones

The dynamics of nuclear processes requires frequent eviction or incorporation of histones to chromatin. When they are not associated to DNA, histones are generally associated with proteins called chaperones. This class of proteins not only prevents aspecific interactions of histones but catalyses a variety of reactions:

-Histone transfer onto DNA (deposition)

-Histone transfer from DNA (eviction)

-Histone transfer to enzymes using histone as a substrate

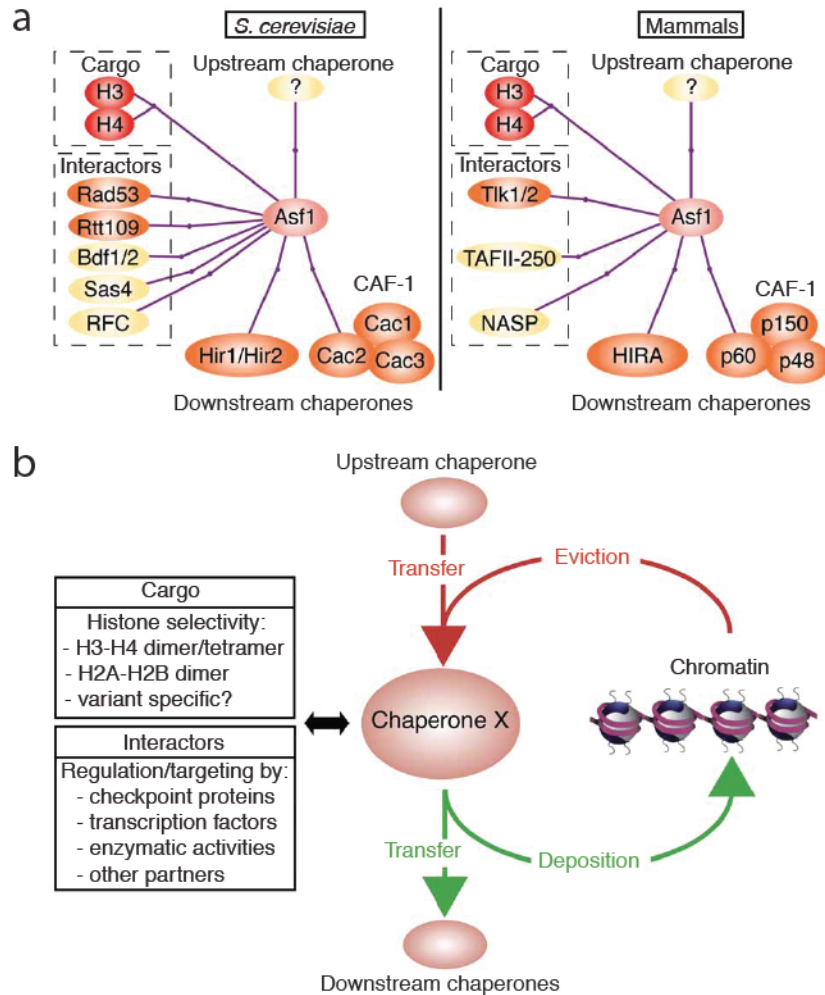


Figure 9 Asf1 is an example of a histone chaperone within a network of binding partners. (a) Asf1 binding partners in *S.cerevisiae* and in mammals. This cartoon illustrates how chaperone interacts with multiple partners including other chaperones (CAF-I and HIRA) or enzymes (Rtt109). The color code (red to yellow represents the amount of evidence supporting each interaction, red being well documented). (b) Schemating representation of the integration of an histone chaperone within its network of binding partners. A given chaperone will have various degree of selectivity for histone dimers or tetramers, which it will either accept from or/ and transfer to to chromatin or to another chaperone. *Figure adapted from the review by De Koning et al. (2007)*

Additionally, chaperones can pass histone to other chaperones, as exemplified for the H3-H4 chaperone Anti-silencing factor1 (Asf1) which passes histone dimers to the HIR complex for replication independent assembly of histones, or to the CAF-1 complex for replication associated assembly (**Figure 9**). These downstream chaperones can handle specific variants or dimer of variants. Thus in eukaryotes, histone dynamics are closely linked to a complex network of partners that influences histone fate (for review, De Koning et al. 2007).

3- Chromatin as an interface between DNA and nuclear machinery

In eukaryotes, nucleosomes are not merely structural components of chromatin. Such as every other protein, histones can be modified post translation. In vivo histones tails are the subject of numerous different modifications, the most frequent being acetylation, methylation, phosphorylation or ubiquitination. The abundance of modifications and their interplay have significant information content, which is key for the articulation of nuclear processes. Post-translational modification of histones (PTM) can either disrupt nucleosome structure and affect its dynamics or modulate the relative affinity of nucleosome binding proteins (Kouzarides 2007). Much like transcription factors recognize DNA sequences, many proteins selectively recognize chromatin, on the basis of nucleosome composition and histone modifications. The analogy between chromatin modifications and a code is often used to describe how the nucleo-proteic structure has a role of interface between DNA and nuclear machinery. While controversial because identical modifications can have different outputs in vivo, this notion of code contributed to the general interest in the field of chromatin signalling.

Within this framework, enzymes modifying histones tails are named writers. Some histone modifications are reversible; the enzyme responsible for the reversion reaction are named eraser. Lastly, Proteins that selectively recognize modified histones are referred to as readers. In a given organism, the post-translational modifications (PTM) of histone tails coupled with the different histone variants offer virtually unlimited complexity. However, only a subset of the possible configurations is observed in vivo, as many combinations of modifications occur together, while some others are mutually exclusive. Such cross-talk between histone modifications has been described both in *cis*, *i.e* along the same histone tails, and in *trans*, *i.e* between marks located on different histone tails (Nakanishi et al. 2009; J. Kim et al. 2013).

Here we will describe the dynamics of histones marks and variants associated with transcription. When required we will specifically focus on results obtained in *Saccharomyces cerevisiae*.

4- Chromatin and transcription

In eukaryotic organisms, transcription of a gene is the consequence of a complex process. The integration of transcription factor binding, local chromatin state and transcriptional machinery availability eventually leads to transcription. Chromatin can affect transcription during its earliest steps: the actual binding of a transcription factor can be influenced by the local chromatin state of the promoter. This is the case of scRap1, which is associated with a limited number of promoters albeit its binding motif is localized throughout the genome, including gene bodies (Lieb et al. 2001).

In vitro biochemical experiment demonstrated that the presence of reconstituted nucleosomes inhibits the initiation while histone tetramers can block the elongation of transcription by PolII (Lorch, LaPointe, and Kornberg 1987; C. H. Chang and Luse 1997). However, in vitro transcription of chromatin isolated from the repressed *S.cerevisiae* Pho5 suggests that native chromatin favors transcription (Nagai et al. 2017). This suggests that histone variants, histone marks and chromatin-associated proteins can collectively favor transcription.

Nucleosomes in the bodies of active genes generally have acetylated H3 and H4 tails, methylated H3K4 and H3K79 and are ubiquitinated at H2BK120 (H2BK123 in *S. cerevisiae*). In contrast, phosphorylation of H2AS129 is prevalent on poorly transcribed genes. Recent work on budding yeast demonstrated a central role of the PolII associated Paf1 complex in the coupling of transcription with (J. Kim, Hake, and Roeder 2005)modification. The histone mark domain of Rft1 directly interacts with the ubiquitin conjugase Rad6. The evolutionary conserved Rad6-Bre1 duo conjugates ubiquitin to H2BK120 (H2BK123 in *S. cerevisiae*). This mark is required for the di and tri methylation of H3K4 and H3K79 residues respectively by Set1 and Dot1 (Z.-W. Sun and Allis 2002),(Ng et al. 2002). Both histone lysine methyl transferases (KMT) also physically interact with elongating PolII through the Paf1 complex (Krogan et al. 2003). This crosstalk does not extend to the deposition of H3K36 methylation, which is also associated with transcribed genes. While the cross-talk between H3K4 methylation and H2BK120 ubiquitylation is

conserved in humans, the relationship between hDot1l and H2BK120 is likely more complex, as hDot1l activity also depends on the phosphorylation state of PolII (S. K. Kim et al. 2012).

With the exception of H3K79 methylation for which no demethylase has been found yet, those marks can be actively removed independently from histone turnover. Ubp8 and Ubp10 catalyse the removal of H2Bub, Jhd2 the removal of H3K4me3 and Rph1/Jhd1 the demethylation of H3K36. Cross-talk regulating histone mark removal has also been identified. In *S. cerevisiae*, H3K14 acetylation and H2BK123Ub prevent H3K4me3 demethylation (Maltby et al. 2012)(Huang et al. 2015). The need for H3K14 deacetylation illuminates the loss of H3K4 methylation concomitant with gene repression, for which HDAC activity is a prerequisite.

The methylation of H3K4, H3K36 and H3K79 residues is essentially universal, occurring throughout gene coding regions, and is highly regulated. Paradoxically, the deletion of *SET1*, *SET2*, or *DOT1* only affects the expression of few genes. *set2* mutants are viable and grow as wild type; however because H3K36 methylation is required to recruit the histone deacetylase complex Rpd3S, acetylation is higher over coding regions in *set2* mutants. This high level of acetylation leads to the initiation of transcription at normally unused promoters located within coding regions. The effect of *set2* is a canonical example of a mechanism explaining the localized effect of the absence of a ubiquitous histone mark (Carrozza et al. 2005);(Joshi and Struhl 2005)(Keogh et al. 2005) (Pokholok et al. 2005)(Govind et al. 2010). In this example, loss of H3K36 methylation will only affect genes in which a cryptic promoter is present. The role of H3K4 and H3K79 methylation is less clear. Both marks are antagonistic to the silencing of reporter genes in budding yeast (Van Leeuwen and Gottschling 2002) but have little effect on silencing of native genes. It seems unlikely that this would be their only role on transcription (Y. Takahashi et al. 2012)(Fingerman et al. 2005). It remains that deletion of *SET1* or *DOT1* has very little effect on the genome wide transcription, leaving the role of those marks open.

a. Cumulative and specific properties of histone tail acetylation

The acetylation of histone tails differs from their methylation in at least two manners. First, the methylation is neutral whereas the acetylation neutralizes the positive charge of lysines. Charge neutralization by acetylation could impact the compaction of chromatin structure (Hong, Bradbury 1993; Luger 1997 Nature). Second, the enzymes catalysing the acetylation generally have a broader substrate than the KMT. Histone H2B has two acetylated lysines (K11/K16), H3 five (K9/K14/K18/K23/K27) and H4 four (K5/K8/K12/K16). Within the SAGA complex, Gcn5 catalyses the modification of H2B and H3 lysines. Newly synthesized H4 is acetylated at K5 and K12 in multiple organisms (Sobel et al. 1995). Esa1, which is essential for yeast viability, mediates the acetylation of K5/K8/K12/16 from nucleosomal H4. Within the SAS-I complex (Sas2,Sas4,Sas5) (Meijsing and Ehrenhofer-Murray 2001)the catalytic subunit Sas2 is responsible for 60% of cellular H4K16ac (Heise et al. 2012)(Osada et al. 2001). The absence of Sas2 causes a global loss of acetylation with pronounced effect within lowly expressed genes. In contrast to Esa1 or Gcn5, the SAS-I complex is not recruited via specific interaction with transcription factors. The SAS-I complex acetylates bulk histones upon passage through S-phase (Reiter et al. 2015). The rate of this process, but not the steady state level of H4K16ac, is influenced by the histone chaperones Asf1 and CAF-I, which directly interact with SAS-I (Meijsing and Ehrenhofer-Murray 2001). Intriguingly, the incorporation of acetylated histone H4 into chromatin is not synchronized with its modification (Reiter et al. 2015). Consistent with the pronounced loss of H4K16ac at lowly expressed genes in sas2 mutants, Spt6-dependent incorporation of acH4K16 into chromatin is observed upon gene repression. This observation has been proposed to reflect the decreased activity of the histone chaperone Spt6, which preferentially incorporates unacetylated H4 histones during transcription. Of note, H4K16 acetylation impacts chromatin structure by preventing the interaction of the H4 tail with the H2A/H2B acidic patch of the neighboring nucleosome (Shogren-Knaak et al. 2006).

Because yeast encodes two copies of each canonical histone genes, it is experimentally compatible with the use of mutants. Systematic mutation of lysine residues from H3 histones demonstrated that individual acetylation has little effect on transcription, while cumulative

effects are observed upon mutation of 4 and more H3 lysines (A. M. Martin et al. 2004). Combinations of point mutation of H4 lysines demonstrated that H4K16 has a specific role on transcription, while the effects of K5/K8 and K12 acetylation on transcription are cumulative and independent of the mutated residue (Dion et al. 2005).

b. Globular domain modifications

Increasing number of modification of histone residues located in the globular domain has been discovered. The acetylation of H3K56 is probably the most studied example of such residues. This mark is present in newly synthesized histones and is deposited throughout the genome during DNA replication in *S.cerevisiae*. In this yeast, Rtt109 acetylates H3K56 in cooperation with the histone chaperone Asf1 (Recht et al. 2006; Schneider et al. 2006), which let CAF-I and Rtt106 incorporate the modified histone to chromatin during replication (Q. Li et al. 2008). H3K56ac is deacetylated after S phase by the sirtuins Hst3 and Hst4 (Celic et al. 2006). The acetylation of H3K56 affects genome stability (Celic et al. 2006) and peripheral positioning of telomeres (S. Hiraga, Botsios, and Donaldson 2008).

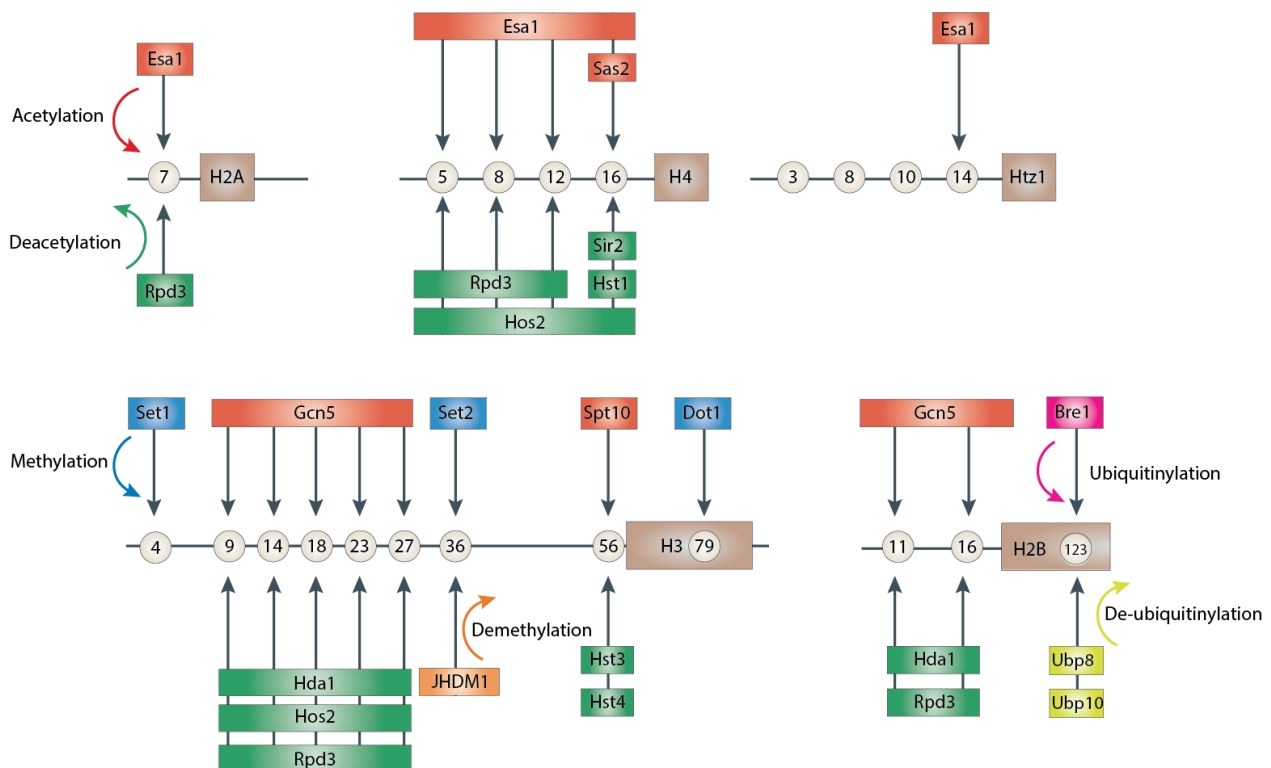


Figure 10 Some of the known sites of histone modification and corresponding modifying enzymes in *S.cerevisiae*. Histone tails are depicted by black lines and globular domains by brown boxes. Selected modification sites are drawn as circles, each labelled with the residue number. Enzymes are shown as

coloured rectangles, with histone acetyltransferases in red, deacetylases in green, methyltransferases in blue, demethylases in orange, ubiquitin ligase in pink and ubiquitin proteases in yellow. When an enzyme has multiple targets, target specificity is not shown for clarity purposes. Sir2 has been reported to deacetylate H3K56ac in heterochromatin (Xu et al. 2007). *Figure adapted from the review by Millar & Grunstein 2006, Bre1, Ubp8 and Ubp10 modification of H2BK123 have been added to the original figure.*

C. Spatial organization of chromosomes and nuclear compartments

1- Nuclear organization is dynamic and shows cell type specificities

a. Heterochromatin / euchromatin

Early cytogenetic studies extensively characterized the inhomogeneous distribution of chromatin within the nuclei along with the inhomogeneous compaction of chromosomes. The discrimination of euchromatin and heterochromatin by Emil Heitz has undoubtedly stemmed this field to fame (Heitz 1928). Those were differentiated on the basis of density when observed by light microscopy. Contrary to euchromatin, which experiences compaction/decompaction during the cell cycle, heterochromatin remains compacted through the cell cycle (**Figure 11**). This sub-organization of the genetic material is dynamic, as heterochromatic structures can evolve during cell differentiation and consequently often displays cell type specificities. A molecular description of heterochromatin will be drawn in the second chapter of this introduction.

b. Chromosome territories, hierarchical organization of chromatin

The organization of chromosomes within the nuclear space is a very active field of research. As often in biology, major advances were tightly coupled with technological progress. The advance of fluorescent in situ hybridization, and its derivative chromosome painting, enabled the description of the conformation of entire chromosomes within the nucleus. Work from the Cremer laboratory described how each chromosome generally occupies a separate space (Cremer and Cremer 2001) (**Figure 11**). Development of chromosome conformation capture (3C) and its coupling with high throughput sequencing enabled the description of yet another level of organization of chromosomes (Dekker et al. 2002). Within chromosome territories, chromosomes are partitioned compartments, containing either the active and open (A compartments) or inactive and closed chromatin (B compartments). The spatial partitioning of chromosomes into compartments correlates with functional features such as chromatin

composition, gene expression and replication timing (Dixon Nature 2012). Compartments are composed of different self-interacting subdomains, named Topologically Associated Domains (TADs) (**Figure 11**). TADs are prevalent in many organisms. The confrontation of TADs with lower scale properties such as chromatin marks revealed the co-segregation of both properties (Ramani, Shendure, and Duan 2016).

The loci lying between two TADs are termed, boundaries. In mammals, boundaries are generally associated with CTCF binding (~75%), and are enriched for housekeeping genes and repeats. Degradation of CTCF destabilizes TAD insulation blurring the overall TAD segmentation (Nora et al. 2017). The conformation of chromosomes appears to possess multiples intrinsic scales, as sub-domains can also be defined within TADs (Wijchers et al. 2016). Chromatin loops likely a lower scale of chromatin organization. Two main types of loops have been described: loops associated with enhancer activity, which mediate long-range trans contact, and gene loops. Primarily identified in *S. cerevisiae*, gene loops connect the promoter of a gene to its terminator, which has been proposed to provide robustness to transcription orientation (O'Sullivan et al. 2004)(Tan-Wong et al. 2012).

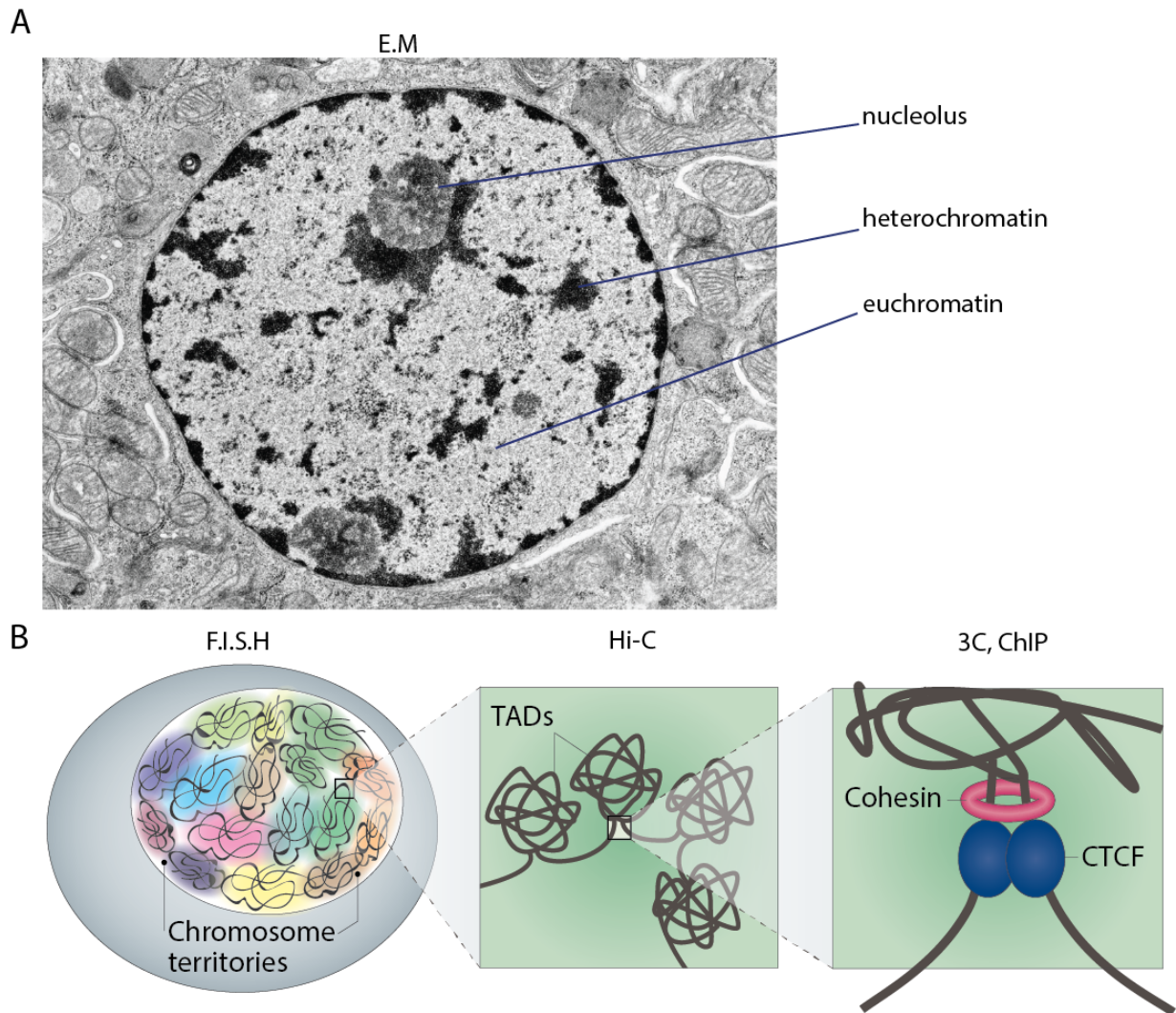


Figure 11 Electron microscopy image of a nuclei and schematic organization of chromatin in the nucleus.(A) Electron Microscopy (E.M) image from <http://medcell.med.yale.edu/>. (B) Within the nucleus, chromosomes generally occupy territories that can be observed by chromosome painting, a technique based on Fluorescent In Situ Hybridization (F.I.S.H). Although they occupy defined territories, chromosomes can contact each other's, as observed by Hi-C, a genome wide variation of the chromosome conformation capture (3C) technique. Chromosomes form multiple intrachromosomal interactions leading to the formation of topologically associated domains (TADs) with an average size of 1 Mb. The TAD boundaries are often enriched for binding sites of the architectural proteins CTCF and Cohesin. Binding of those proteins can be probed by chromatin immunoprecipitation, and induced contacts by 3C. *Panel B is adapted from the review by Jégu et al. (2017)*

The spatial organization of *S. cerevisiae* cannot be described adequately with the TAD nomenclature. The yeast genome adopts a Rabl-like configuration. As described earlier, telomere clusters are anchored to the nuclear membrane during exponential growth. In addition centromeres are clustered all together at the spindle pole body. Overall, this configuration is well described by a polymer model that includes trans contact at centromeres and at telomeres (Taddei and Gasser 2012).

2- Nuclear bodies

DNA and more generally chromatin display high level of organization within the nucleus. As discussed earlier, this organization is believed to be driven both by the physical properties of the chromatin and the nucleoplasm as well as by architectural proteins. But obviously, the nucleus cannot be restricted to DNA or chromatin. Among the various machineries necessary for DNA usage, maintenance and replication or nuclear import/export, some important actors of nuclear biology also display non-random organization. Indeed, a prevalent feature of the nucleus is the presence of discrete sub-nuclear structures, referred to as nuclear bodies (Zhu and Brangwynne 2015). In contrast to organelles, lipid membranes do not delimit those sub-compartments. At least ten different types of nuclear bodies have been described in the mammalian nuclei. They are dedicated to various functions, often associated to RNA processing, and largely differ in their composition or size. Nuclear bodies are fundamentally different from nuclear aggregates such as amyloid plaques. Indeed, components of nuclear bodies are frequently exchanged with the neighbouring nucleoplasm. This fundamental difference is at the core of nuclear bodies biology.

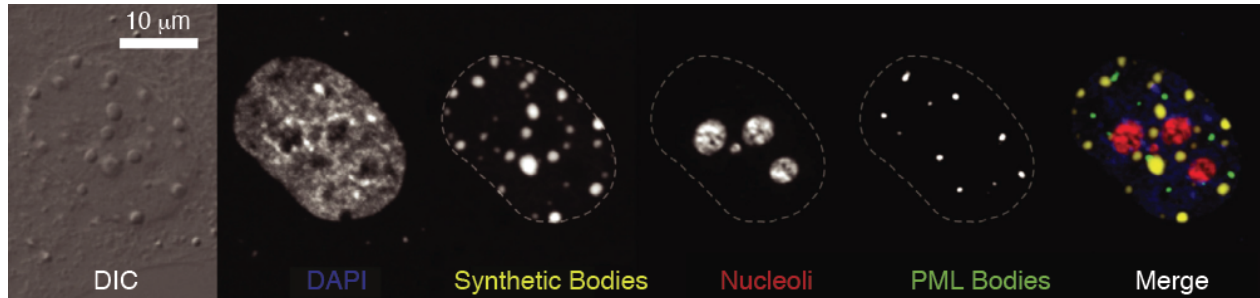


Figure 12 Examples of nuclear bodies Examples of nucleoli, PML bodies, and synthetic Ddx4YFP bodies assembled in HeLa cells. DNA is stained with DAPI. Images from the review by Zhu & Brangwynne (2015)

a. Formation of nuclear bodies

The mechanism leading to the formation of nuclear bodies is a current subject of investigation. Current studies argue for a prevalent role of RNA in the seeding of some of those structures. Artificial tethering of ncRNAs from diverse nuclear bodies is sufficient to nucleate the formation of histone locus bodies, nuclear speckles, paraspeckles and nuclear stress bodies (Shevtsov and Dundr 2010). RNA based seeding has been demonstrated in the case of paraspeckles (NEAT1 lncRNA), nucleolus (rRNA) (Falahati et al. 2016) and stress bodies (centromeric RNA) (Biamonti and Vourc'h 2010).

b. Liquid-liquid phases:

While the bodies, granule or speckle nomenclature is somehow vague, studies focusing on the prevalence of liquid-liquid phase separation in biology offer a larger framework to consider those fascinating objects (Hyman, Weber, and Jülicher 2014). Liquid-liquid phase transitions might represent a physical state, which properties contribute to many processes including heterochromatin formation in mammals. In this context, we will briefly recapitulate the principal properties of simple liquids. A liquid state of matter is characterized by the ability of its components to rearrange easily. Due to the free movements of liquid components, a liquid can be mixed and change shape but remains a liquid. In the absence of a container, a liquid can be deformed and even flows.

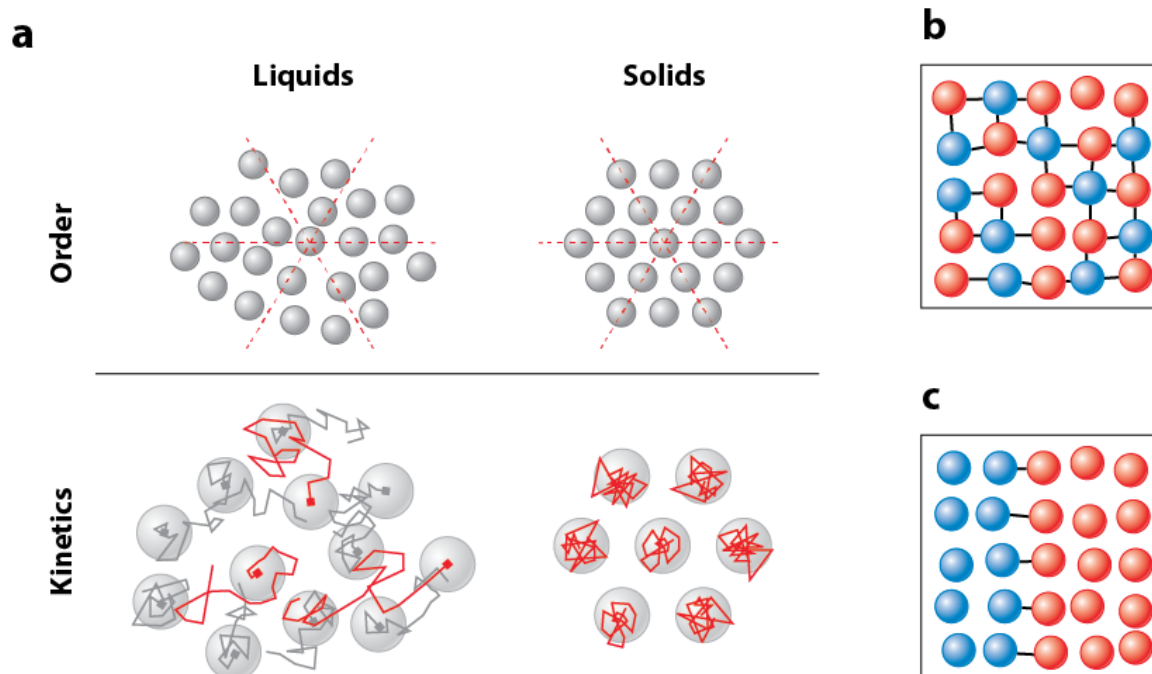


Figure 13 schematic representations of important characteristics of ideal liquids and ideal solids and of liquid demixing. (a) For a crystalline solid, positional order exist over long distances, so that one can draw straight lines along which particles are separated by approximately equal distances. In an ideal liquid this is not the case and spatial order only exists at short distances. Kinetics: in liquids the particles can rearrange and diffuse, while in solids they are constrained by the neighboring particles. (b,c) In a liquid composed of two types of particles, interactions that favor like neighbors and disfavor unlike neighbors, a mixed state has a larger energy cost than a demixed state as illustrated by the number of disfavored bounds (in black). *Figures adapted from the review by Hyman et al. (2014)*

The resistance of a fluid to shearing flows defines its dynamic viscosity that becomes the kinematic viscosity when normalized by the fluid density. In some situations different liquids will not mix, such as oil and vinegar in vinaigrette or water and anethol in pastis (Grillo 2003). Somehow annoyingly when one makes a vinaigrette or pastis and forget those emulsions for a while, it will have demixed into two different phases. The demixing of two non-miscible liquids A and B is driven by the differential energy involved in A-A, B-B and A-B bonds. If A-B bonds have a higher free energy than A-A or B-B, liquids will eventually demix (**Figure 13 b,c**). While entropic considerations would in principle favour the mixing of the two liquids together, the equilibrium reached by demixing at room temperature represents a situation of lower free energy and is thus favoured.

P granules found in the *C.elegans* embryo are likely the first convincing example of cytoplasmic liquid drop. Those granules can fuse, dynamically exchange components with the cytoplasm and

are deformed by surrounding flow. Within this framework, the apparent viscosity of P granules is comparable to the one of runny honey (C. P. Brangwynne et al. 2009), whereas *X. laevis* nucleolus is at least ten times more viscous (Clifford P Brangwynne, Mitchison, and Hyman 2011).

II Chromatin based repression of chromosomal domains

A. Diversity, properties and origin of heterochromatic structures

1- Position effect variegation

"In a pedigree culture of *Drosophila*, which had been running for a considerable number of generations, a male appeared with white eyes. The normal flies have brilliant red eyes." In his seminal 1910 article, T.H. Morgan unveils his discovery on the inheritance of the *white* phenotype. Morgan's work will soon after participate to the transformation of Mendel's hereditary "factors" into Johanssen's definition of "genes". During the course of its X-ray-induced mutagenesis experiments, Muller noticed the occurrence of a "group of distinctly peculiar variants" with white and red compound eyes (H. J. Muller 1930). As he describes: "To the great surprise of the writer, the Notch winged offspring of this cross had neither white nor normal red eyes nor even eyes of any uniform intermediate colour. They had mottled eyes, and exhibited various grades and sizes of lighter and darker areas". The co-existence of both phenotypes suggested that the *white* gene had remained functional, but was repressed to various extent in a fraction of ommatidia. Patterns of variegation vary seemingly continuously in the size of pigmented patches and the concentration of pigment per cell. Examination of the polytene chromosomes revealed that those variegating phenotypes were associated with chromosomal rearrangement leading the re-localization of the *white* gene in peri-centric heterochromatin (H. J. Muller 1930). Thereafter, *white* flies could be obtained following X-ray treatment of the white-mottled-5 mutant. Cytological observation of polytene chromosomes showed that this phenotype reversion corresponded to relocation of the *white* gene within euchromatin (Griffen A 1940). Collectively, these experiments demonstrated that heterochromatic re-localization of the *white* gene affected its expression but not its physical

integrity. Because those phenotypes are caused by the re-localization of a gene along a chromosome, this phenomenon is referred to as Position Effect Variegation (PEV).

While position effect variegation in flies might be the first palpable evidence of the influence of chromatin state on the expression of a gene, major advances in recognizing the dynamic nature of heterochromatin came from studies of transposable elements in plants. Again, Barbara McClintock's contribution was a milestone in this field. Soon after her arrival in Cold Spring Laboratories, she focused on the self-fertilized progeny of the crops that experienced fusion-bridge-fusion cycle involving chromosome 9. Within those plants, 40 different mutable loci were identified, some of them affecting aleurone color. In 1950 she brilliantly unfolded her discovery of the transposition of the Dissociation (Ds) element that she thought has some link with the heterochromatin knob. In the conclusion of her report she asked, "Is this transposition of heterochromatin? Is it a reflection of a process that normally occurs in nuclei? Is it responsible for controlling the rates and types of exchange that occur between nucleus and cytoplasm? Is it usually an orderly mechanism, which is related to the control of the processes of differentiation?" and postulated that "changes in quantity, quality or structural organization of heterochromatic elements may well alter the kind and/or degree of particular exchanges that occur, and in this way control the chromosome organization and the kind and the relative effectiveness of genic action". At last, she believed that " In many cases, there can be little question about the similarities in expression of variegation in *Drosophila* and maize" (McClintock 1950). Causal relationship between position effect and heterochromatin emerged with the observation that the region containing the variegating gene was packaged within heterochromatin only in the larvae of flies with repressed expression (Zhimulev et al. 2003). In *drosophila*, virtually all gene tested experienced variegation when re-localized within pericentric heterochromatin, and this position effect was independent of the pericentromere considered (Girton and Johansen 2008). Position effect variegation has subsequently been observed in many species including Yeast and mammals. Analogous phenomenon has been observed at the vicinity of telomeres. Positioning of reporter gene within subtelomeres led to variegating phenotypes in a variety of organism ranging from yeast (Gottschling et al. 1990;

Nimmo, Cranston, and Allshire 1994), to mouse (Pedram et al. 2006) and humans (Baur et al. 2001; Koering et al. 2002).

The observation of PEV has major consequences on our understanding of gene expression, as it shows that the transcriptional output of a gene can be influenced by its position along the chromosome or by the elements present at its vicinity. In this sense the chromosome as a support of genetic material is not a neutral element.

2- Constitutive and facultative heterochromatin

In the late 1960 Jacob and Monod, our national heroes, established the model of gene regulation by transcription factors on the basis of their work on the operon lactose (Jacob and Monod 1961). With time it became evident that this system of regulation of genetic information takes a central place in genome function and during development. But PEV cannot be accounted by this model. The progress of staining techniques and electronic microscopy, enabled to discover that the quantity of heterochromatin varies between organisms but more importantly between cell types. The distinction between constitutive heterochromatin and facultative heterochromatin was first restricted to the heterochromatinization of one allele of a homozygous pair (S. W. Brown 1966). Pioneer observations arose from the study of the unusual mealy bug males chromosomes in 1921 (Schrader) (**Figure 14 A, B**). At the early stages of the development of a male mealy bug, the set of paternally-inherited chromosomes is heterochromatinized. Whereas those chromosomes are maintained in the body of the insect, they are discarded at the onset of sperm formation and are thus not transmitted. Such sex-specific chromatin was later observed in the cells of the larvae of the spruce bedworm, allowing sex determination prior to the development of gonads. In those organisms female nuclei are characterized by the presence of a dark staining body absent from male nuclei (Smith 1944). Even if this extreme case of imprinting is fascinating, most of the interest for facultative heterochromatin arose from an unexpected result obtained during the course of neurocytology

experiments. While studying the nerve cells of cat, Barr and Bertram noticed the appearance of a small body specific to female nuclei upon Nissl staining (Barr and Bertram 1949). The use of different staining methods -Feulgen and methyl green staining- demonstrated that this new body had a composition similar to chromatin. This sex-specific chromatin is frequently called the “Barr body” and corresponds in mammals to one of the two X chromosomes (for review Mittwoch 1964). An updated definition of facultative heterochromatin would correspond molecularly to condensed and repressed chromatin that will eventually open and experience transcription either in the course of differentiation *i.e* during development, or in response to environmental cues (For definition, (Trojer and Reinberg 2007)).

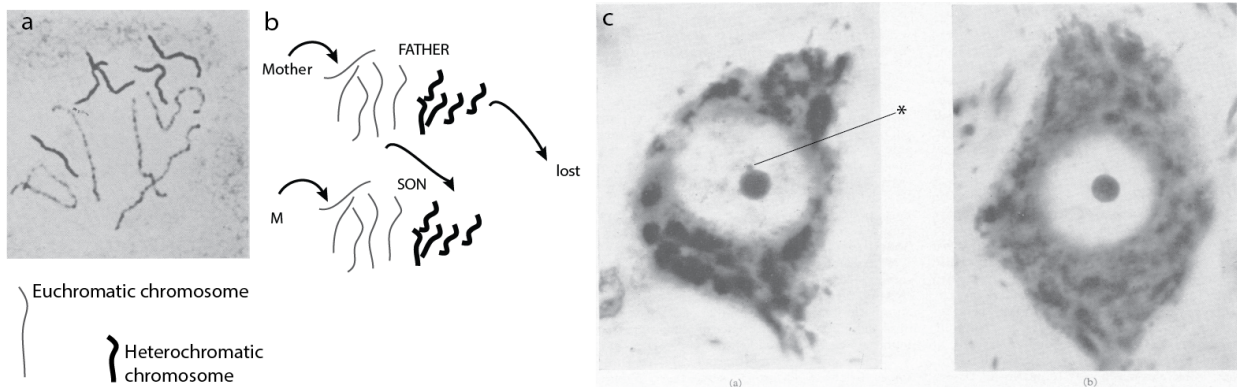


Figure 14 Sex chromatin. (a) Chromosome spread of a male mealy bug. Darkly stained chromosomes are heterochromatic and paternally inherited (b). Paternally inherited chromosomes are not transmitted to the male progeny. (c) Sex differences in the neurone of cat, on the left 'nucleolar satellite' nowadays called 'Barr body' adjacent to the nucleolus in females, no nuclear satellite in the male (right). (a) Picture from the review by Spencer Brown (1966), (c) pictures from the review by Ursula Mittwoch, courtesy of Pr. M. L. Barr

Intensive cytogenetic and genetic studies demonstrated the commonality and the variety of heterochromatic structures in a plethora of organisms and cell types. Since then, considerable progress have been made in the description of the machineries and the mechanisms involved in heterochromatin formation. Conserved families of genes dedicated to heterochromatin have been identified, but also more exotic and less conserved ways of implementing heterochromatin have been shown to coexist.

The outline of the following part is as follow: first, we will briefly describe the mechanism leading to X chromosome inactivation in mouse to exemplify a RNA-based process of heterochromatin formation. Then we will elaborate on the contribution of the heterochromatin

protein 1 (HP1) family to heterochromatin formation. In the last mechanistic part, we will describe in more details the molecular details of gene silencing in budding yeast. Despite the major focus on gene silencing of this part, we will see how heterochromatic structures potentially contribute to various nuclear processes ranging from genome stability to protein storage.

B Molecular description of heterochromatic silencing

1- X chromosome inactivation in mouse

In mammals the process of X-chromosome inactivation insures the correct dosage of genes located on X chromosomes by silencing randomly one of the two X-chromosomes in females cells. In mouse, silencing is initiated early during development by the programmed transcription of the X-inactive specific-transcript (Xist) long non coding RNA (lncRNA). Xist lncRNA is produced from a single locus, located on the X chromosome that will be silenced. Once produced the Xist lncRNA physically associates with loci spatially close to the Xist locus. Additional Xist transcripts progressively associate with the vast majority of the X-chromosome. Xist coating leads to a cascade of changes in chromatin state, which correlates with the propagation of silencing. While the exact mechanism by which Xist binding leads to gene silencing remains elusive, this chromosome wide mechanism of silencing exemplifies the potentialities of lncRNAs in genome function. Interestingly, targeting a XIST transgene to an autosomal chromosome triggers its coating and silencing by the Xist RNA (Gendrel and Heard 2014). This finding has promising therapeutic implications, as successful silencing of one of the three chromosome 21 of a population of Down's syndrome pluripotent stem cells by a Xist transgene could improve cell growth and differentiation (Jiang et al. 2013).

2- HP1 family

HP1 was initially discovered in drosophila in a screen for proteins predominantly localized at polytene chromosomes chromosomocenter. HP1 gene was later found necessary for gene repression in the process of position effect variegation. HP1 is a highly conserved protein present in one or multiples isoforms or variants depending on the specie of interest. HP1 homologs include Swi6 and Chp2 in *S.pombe*, HP1 α , β , γ in mammals and the plants specific

Like-HP1 protein. Budding yeast has lost Hp1 silencing and associated proteins (**Figure 15**) (Hanson and Wolfe 2017).

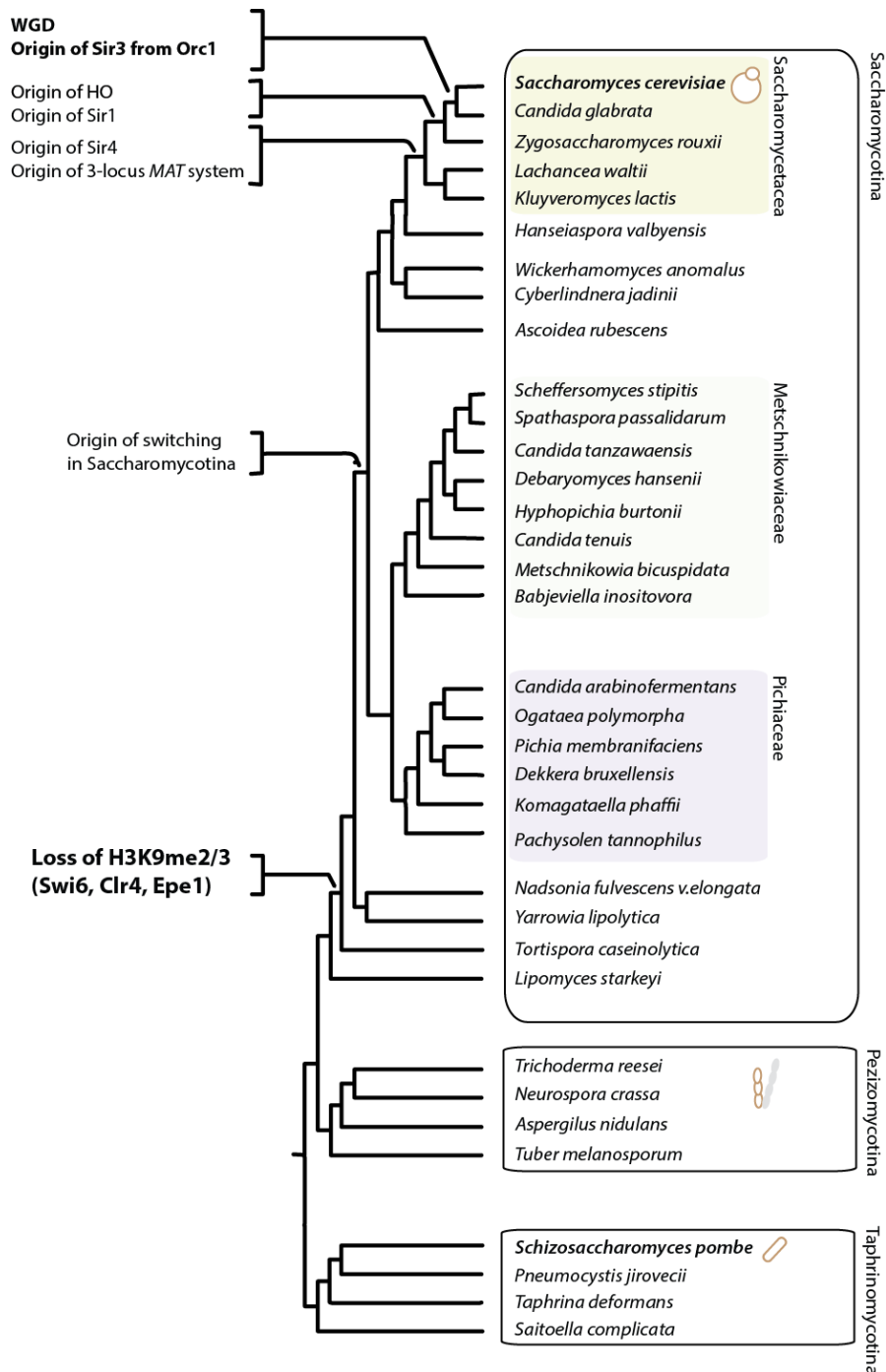


Figure 15 Phylogenetic tree of phylum Ascomycota. The tree shows major clades along with gain or loss of heterochromatin related proteins. *Tree adapted from the review by Hanson et al. (2017)*

There is approximately 50% conservation at the amino-acid level between drosophila and human HP1 (Y. Li, Kirschmann, and Wallrath 2002). Isoforms are transcribed from different genes, known as the chromobox (CBX) genes. HP1 proteins belong to the superfamily of proteins containing chromatin organization modifier (chromo)domains. They form a subclass characterized by the presence of another domain located in the C-terminal half of the protein, the chromoshadow domain. There is no question on the functional importance of HP1 proteins in evolution. Ectopic expression of mouse HP1 β rescues swi6 *S.pombe* mutants (G. Wang et al. 2000), while expression of human HP1 α can rescue su(var)2-5 mutants (Norwood et al. 2004; T. Yamada et al. 1999).

From the amino-acid point of view, HP1 proteins are organized into three domains (Figure 16). The chromodomain mediates interaction with chromatin with specificity for nucleosome methylated at H3 lysine 9, the chromoshadow domain is involved in homo/heterodimerization. An unstructured linker, the hinge domain, separates both domains. The hinge domain is amenable to post-translational modification, notably phosphorylation and sumoylation (Romeo et al. 2015).

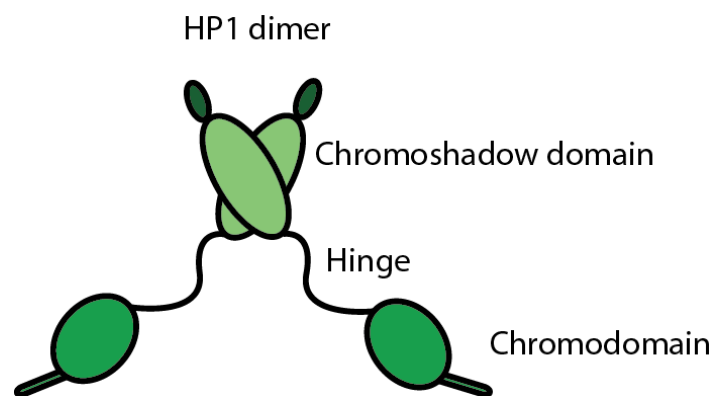


Figure 16 Schematic representation of a HP1 dimer. This scheme is not to scale and depicts the major domains of HP1 proteins. *Image from Larson et al. (2017)*

While their name suggest a unique function in heterochromatin, some HP1 isoforms also localize to euchromatin sites. In mouse, while HP1 α is specific to peri-centromeric heterochromatin, HP1 β and HP1 γ also have euchromatic roles associated with transcription. For example, HP1 γ has been implicated in alternative splicing (Saint-André et al. 2011) and transcription regulation during infection (Harouz et al. 2014). The localization of HP1 γ

correlates with post-translational modification of Ser83 (Lomber et al. 2006), suggesting that an active determination of the proportion of euchromatic HP1 might exist. However for the purpose of this work we will restrict the discussion to heterochromatic functions of HP1. The main focus is on *S.pombe* homologs Swi6 and Chp2 to illustrate the mechanisms that can lead to heterochromatin formation.

a. Heterochromatin establishment in S.pombe

Centromeres, mating type locus and subtelomeres are heterochromatic in *S.pombe* (Cam et al. 2005). They have in common the presence of dg and dh repeats, which transcription potentially leads to the production of small interfering RNAs that can contribute to heterochromatin assembly, propagation and maintenance. The involvement of sRNA in heterochromatin is particular at peri-centromeres compared to mating type loci or subtelomeres.

Nucleation of heterochromatin at fission yeast centromeres largely depends on a RNA based mechanism. The repetitive nature of centromeres rather than specific DNA sequences seems to be what triggers silencing establishment (**Figure 17**). Current models propose that dh and dg centromeric repeats are transcribed by RNA polymerase II during S phase of the cell cycle. Those transcripts are processed into double stranded RNA by the RNA-dependent RNA polymerase complex. Processing of dsRNA by Dicer leads to the production of small RNA that are loaded onto Ago1. Base pairing of Ago1 bound siRNA to nascent centromeric transcript allows the association of the RNA-induced initiation of transcriptional silencing (RITS) complex. Small RNA processing occurs through the ARC complex, which chaperone siRNA generated by dicer to the RITS. The RITS complex associates with centromere chromatin through its subunit Chp1. Chp1 chromodomain association to chromatin is sensitive to histone modification, and requires nucleosomes di-methylated at H3K9. The RITS complex recruits the Clr4 methyltransferase complex (CLRC), within which Clr4, the homolog of human SUV39H, can methylate H3K9 and Lid2 can demethylate H3K4. The chromodomain of Clr4 specifically recognizes methylated H3K9 and is required for heterochromatin spreading (K. Zhang et al. 2008). Thus in this system, Clr4 is both the reader and the writer of the H3K9 methylation mark. Within the CLRC complex, its enzymatic activity paves the way for Swi6 association to chromatin through interactions with H3K9me2 nucleosomes. Both Swi6 and the other HP1 protein Chp2 contribute to limit PolIII

Several studies demonstrated that the contribution of RNAi is dispensable in certain contexts. First, Swi6 and H3K9 methylation are still detected at low levels at centromeres in RNAi mutants (Sadaie et al. 2004). In the absence of RNAi, loss of the Mst2 H3K14 HAT, of the putative H3K9 demethylase Epe1, of the RNA quality control protein Mlo3 or the shelterin component Poz1, independently rescue centromeric silencing (Trewick et al. 2007; Reddy et al. 2011; Reyes-Turcu et al. 2011; Tadeo et al. 2013). While the dynamic of H3K9 modification tunes the efficiency of heterochromatin establishment as exemplified by *EPE1* deletion, increasing Swi6 dosage also rescues silencing in a dicer mutant (Tadeo et al. 2013). Interestingly, tethering of TetR-Clr4 is sufficient to induce heterochromatin formation and requires the Clr4 'reader' chromodomain. This suggests that self-recruitment of heterochromatin participates in the propagation mechanism. Removal of the nucleating element, TetR-Clr4 in this example, leads to loss of silencing within 10 generations. The absence of Epe1 prevents this loss of silencing, suggesting that active pathways erase otherwise epigenetically transmitted information. In this context, current model considers that maintenance of a transcriptionally silent state is an equilibrium determined by Clr4 reading and writing of H3K9 methylation opposed to the turnover of nucleosomes and to a Epe1 dependent demethylation of H3K9 (Ragunathan, Jih, and Moazed 2014).

b. HUSH silencing, another H3K9 methylation based system in human

As we have just seen, H3K9 methylation is at the core of heterochromatin formation via the HP1 protein family. A recent forward genetic screen for proteins involved transgene silencing in human cells identified the Human silencing Hub complex (HUSH). Interestingly, this complex also involves the recognition of H3K9 methylation by a chromodomain protein (MPP8), which recruits the TASOR protein. Crucial to the function of the HUSH complex is the recruitment of the H3K9 methyltransferase Setdb2. HUSH silencing presents mechanistic similarities with HP1 based silencing, and represents a new chromatin based system of epigenetic control of gene repression (Tchasovnikarova, Iva et al. 2015).

3- SIR based silencing

In budding yeast, position effect was first observed at the mating type loci (HML and HMR) and later at the vicinity of telomeres and within the rDNA array. Mutation disrupting mating type silencing enabled the identification of the Silent Information Regulator (SIR) complex. Silencing of the mating type loci by the SIR complex is crucial for budding yeast to control its sexual identity. Since its discovery, the SIR complex represents a paradigm of chromatin-based repression of transcription. In the following part we will describe each member of this complex separately, and see how the dynamic interplay of those proteins with chromatin leads to gene repression.

a. Sir1

Within the SIR complex Sir1 has a particular role. Contrary to the other SIRs which absence causes a complete loss of mating type silencing, Sir1 is not necessary for silencing. Disruption of *SIR1* yields a mixed population of cells: a fraction of the population silence one or both HM loci while the remaining cells de-repress HMs loci (Pillus and Rine 1989)(Xu, Zawadzki, and Broach 2006). In *sir1* mutants, silencing at the HM is semi-stable, switching approximately every 10 generations (Fox et al. 1997; Enomoto et al. 1997; A L Kirchmaier and Rine 2001). By interacting both with Orc1 and Sir4, Sir1 contributes to the efficiency of silencing initiation and maintenance (Dodson and Rine 2015).

b. Sir2

Sir2p couples the deacetylation of lysine to the hydrolysis of NAD⁺. This reaction stoichiometrically exchanges acetyl group and Nicotinamide (NAM) from NAD⁺, producing 2'-O-Acetyl-ADP-ribose and nicotinamide (Tanner et al. 2000). Sir2 activity is inhibited by addition of nicotinamide to the growth medium. Pnc1, a nuclear enzyme that converts NAM to NA, attenuates this feedback inhibition. Soon after its discovery, Sir2 homologs have been found in variety of organism ranging from bacteria, archaea to humans; those constitute the Class III KDACs, sirtuins, which were named after Sir2. Mammals express seven sirtuins (SIRT1 to 7), one of which (SIRT1) can partially complement scSir2 function in silencing (Gaglio, D'Alfonso, and Camilloni 2013). Budding yeast expresses four other sirtuins, Hst1, Hst2, Hst3, Hst4 (Brachmann

et al. 1995). Hst1 and Sir2 are paralogs of whole genome duplication; their functions diverged, but they retain some redundancy (Kellis, Birren, and Lander 2004; Hickman and Rusche 2010).

Substrate specificity:

In yeast, Sir2 can associate either with Sir4 to form the SIR complex (**Figure 18**) or with Net1 to form the regulator of nucleolar silencing and telophase exit (RENT) complex, which mediates silencing at the rDNA array. It has been proposed that free Sir2 can homo-trimerize and is only able to interact with the RENT complex in this configuration (Tanny et al. 2004; Cubizolles et al. 2006). In vitro studies demonstrated that Sir2 preferentially deacetylates H4K16ac and that its activity is highest when Sir2 is associated to Net1 or to Sir4. Lastly Sir2-Net1, Sir2-Sir4 or Sir2/3/4 complexes were able to deacetylate free histones but failed to modify acetylated nucleosomes (Tanny et al. 2004). Conformational requirements might prevent Sir2 from acting on mono-nucleosomes but might only act on nucleosomal arrays, or additional factors might be required for Sir2 to deacetylate nucleosomes. It is still unknown if free Sir2-Sir4 complex deacetylate histones in-vivo.

Post-translational modifications:

Recent reports of the post-translational modification of Sir2 indicate that the sumoylation of Sir2 regulates its distribution between telomeres and the rDNA, likely by impairing Sir2-Sir4 interactions. Additionally, the phosphorylation of Sir2 catalytic domains -Ser437- affects cellular lifespan by modulating *PMA1* gene expression. Lastly, *in-vitro* studies reported the ability of Sir2 to self-modify by mono-ADP ribosylation (Tanny et al. 1999). However the physiological relevance of this activity remains to be explored.

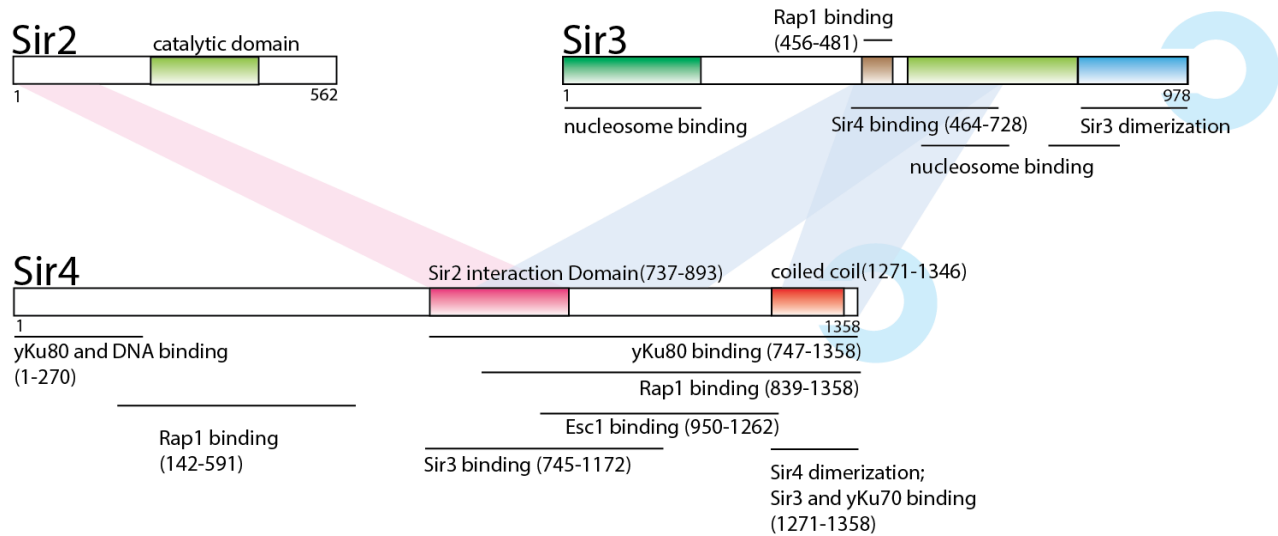


Figure 18 SIR proteins: domain structure and interactions. Domain structures of Sir2, Sir3 and Sir4, shaded shapes indicate interactions between the Sir proteins. Dark lines indicate additional interactions and corresponding residues. Scale is only indicative but not exact. *Figure compiled from the review by Gartenberg (2016).*

c. *Sir3*

SIR3 is the paralog of *ORC1*, the gene encoding the largest subunit of the origin recognition complex (ORC). Both emerged from the whole-genome duplication of the ancestor of budding yeast (100-300 MYA), (Wolfe and Shields 1997; Friedman and Hughes 2001; Kellis, Birren, and Lander 2004). While Sir3 and Orc1 cannot substitute for each other in budding yeast (Bell et al. 1995). N-terminal domain exchange between the two proteins maintains the usual C-terminal function of each protein –probed by viability or mating ability- (Bell et al. 1995).

To understand if the gene that gave rise to *ORC1* and *SIR3* possessed both silencing and replication functions, researchers studied yeast species that diverged from *S.cerevisiae* before the WGD (Fig 15), such as *K.Lactis*. Interestingly, *K.Lactis* Orc1 independently mediates both replication and silencing functions. At last, *Saccharomyces Kluyveri* and *Plasmodium falciparum* *ORC1* genes can complement *SIR3* silencing function when expressed in budding yeast (Van Hoof 2005; Varunan et al. 2013). Mechanistically, *K.Lactis* Orc1 requires Sir2 and Sir4 to implement silencing at *HMR* and at telomeres – HMLalpha is silenced by Hst1/Sum1-. To conclude, silencing is not a new function acquired by *SIR3* but most likely *SIR3* specialized in silencing, taking over HML for example (Hickman and Rusche 2010).

Sir3 protein structure:

Sir3 protein can be subdivided into three structured domains: a N-terminal bromo-adjacent homology (BAH) domain, which can bind histone with great specificity (see below), a central AAA+ ATPase- domain and a C-terminal winged-helix domain. As C-terminal domains of Orc1 and Sir3 are not interchangeable (Bell et al. 1995), it is safe to assume that this domain of Sir3 might have retained and evolved functions promoting silencing and lost replication related function.

Current knowledge of Sir3 BAH domain has been built over intensive genetic assays and crystal structure studies. The requirement of histone H4 tail to silence the HM loci was the first indication that the SIR complex requires histones to implement silencing. Following studies established that the N-terminal domain of Sir3 is responsible for histone binding. Determination of the crystal structure of Sir3 BAH domain and the nucleosome core was a major step forward (Armache et al. 2011). In detail, the hypermorphic D205N allele was chosen instead of WT BAH, which did not form stable complex in vitro when expressed from bacteria. This is likely due to the absence of N-terminal acetylation that was shown to be essential for SIR mediated silencing and spreading of the SIR complex in vivo (X. Wang et al. 2004) and to stabilize the interaction of Sir3 with the nucleosome in vitro (Arnaudo et al. 2013). The D205N allele of Sir3, which is considered as an Orc1-like mutation, rescues silencing at the mating type in H4K16Q mutants and at telomeres in a rap1-17 mutant that is normally unable to implement silencing (Norris, Bianchet, and Boeke 2008). Consistent with genetic studies, crystal structure of the Sir3 BAH domain on nucleosome indicates that H4K16 acetylation and H3K79 methylation are expected to disrupt Sir3 association with nucleosome. In the context of this thesis, it is important to note that the authors predict that H3K79 can potentially form three hydrogen bonds with BAHsir3. Mono-methylation of H3K79me is predicted to weaken this interaction while tri-methylation of H3K79 would lead to its disruption (Armache et al. 2011).

AAA+ ATPase- domain:

The AAA+ proteins are ubiquitous proteins that are characterized by a highly conserved ATPase module of ~200 amino acids, structured by a alpha-beta-alpha core domain. Those proteins use ATP hydrolysis energy to remodel macromolecules. AAA+ proteins are involved in a variety of process ranging from protein unfolding, peroxisome biogenesis to DNA replication (Orc1) and repair. AAA+ proteins have been found in all kingdoms of life, *i.e.*, viruses, bacteria, archaea and eukaryotes (for review, Snider, Thibault, and Houry 2008).

The central portion of Sir3 (aa530-845) is analogous to an AAA+ ATPase domain, but possesses non-canonical residues that likely affect the function of its nucleotide-binding pocket. Although it has been suggested that Sir3 AAA+ domain was able to bind Sir2 deacetylation by-product OAADPR (Martino et al. 2009; Ehrentraut et al. 2010) crystal structure revealed that this domain of Sir3 largely diverged and do not have a nucleotide pocket (Ehrentraut et al. 2011). The central portion of Sir3 mediates interactions with Sir4 (aa657-660), Rap1 (aa456-481) (P Moretti and Shore 2001; Y. Chen et al. 2011) and histones (Ehrentraut et al. 2011). Interestingly, the specificity of this domain for histones is similar to that of the BAH domain. Indeed, methylation of H3K79 and acetylation of H4K16 reduce the affinity of this domain for histones (Ehrentraut et al. 2011). Those protein-protein interactions mediated by the central domain of Sir3 are required for silencing (**Figure 18**). Thus, in comparison to Orc1, Sir3 likely lost its ability to use nucleotide hydrolysis energy and acquired interactions with Rap1 and Sir4.

The winged-helix domain:

The third structurally identified domain of Sir3 forms the C terminal part of the protein (aa 843-978). Seminal experiments showed that this domain self-interacts in two-hybrid assay, and that the tethering of this domain in SIR+ cells can recruit the SIR complex. Analysis of the crystal structure of this domain revealed that it formed a winged-helix (wH), which is unable to bind DNA and confers strong self-interactions properties (Liaw and Lustig 2006). Deletion of this domain abolishes silencing (Oppikofer et al. 2013).

An elegant experiment replacing Sir3 wH domain by the self-interacting HlyU transcription factor from *Vibrio vulnificus* demonstrated that conferring dimerization to Sir3 Δ wH is

sufficient to completely restore silencing. It is thus considered that this domain is dedicated to Sir3-Sir3 interactions (Oppikofer et al. 2013).

Post-translational modifications of Sir3:

Post-translational modifications of Sir3 are essential for its function. In particular, acetylation of alanine 2 by NatA (Geissenhoner, Weise, and Ehrenhofer-Murray 2004), increases the affinity of Sir3 for nucleosomes (Connelly et al. 2006; Onishi et al. 2007; van Welsem et al. 2008; Sampath et al. 2009) and is required for silencing (Whiteway et al. 1987; Ruault et al. 2011a). Crystal structure of unacetylated BAH domain indicates that this modification is essential to stabilize the surface of the BAH/nucleosome interface (Arnaudo et al. 2013; D. Yang et al. 2013). Accordingly, Sir3-A2Q point mutant is totally defective for telomeric silencing and partially defective for silencing at the HM (F. Wang et al. 2013; Ruault et al. 2011a). A patch of 5 serines is phosphorylated by the kinase Slt2 in response to environmental stress. It has been suggested that this phosphorylation causes partial release of the SIRs from the telomeres (Stone and Pillus 1998; Ai et al. 2002; Ray et al. 2003). Current work carried in our laboratory investigates the occurrence of other phosphorylation events associated to quiescence. According to large scale study, Sir3 is also modified by the small ubiquitin-related modifier (SUMO) (Denison, Kirkpatrick, and Gygi 2005), but the role of this modification has not been studied extensively. Work from Wang Chia-Lin PhD suggests that Siz1 is responsible for sumoylation of Sir3 at K247, and that Uls1 regulates the amount of sumoylated Sir3.

d. Sir4

Sir4 is the largest protein of the SIR complex in which it plays a major structural role. Sir4 interacts with each of the other Sir proteins and is considered as a scaffold that maintains the complex assembled (Hecht, Strahl-Bolsinger, and Grunstein 1996; Triolo and Sternglanz 1996; Moazed et al. 1997; Strahl-Bolsinger et al. 1997; Rudner et al. 2005; Cubizolles et al. 2006).

Interaction with Sir proteins

Sir4 can self-interact through its C terminal coil-coil domain (J. F. Chang et al. 2003; Murphy et al. 2003), its only known structured domain (**Figure 18**). This domain also mediates contact with Sir3 (P Moretti et al. 1994; Moazed et al. 1997; Park, Hanish, and Lustig 1998), and both interactions are required for silencing (J. F. Chang et al. 2003; Rudner et al. 2005). Sir3-Sir4 contacts can also be mediated by a module located within aa 745-1172 (Liou et al. 2005). Sir4 contacts Sir2 through a central domain spanning amino acids 737-89 (Moazed et al. 1997; Cockell, Perrod, and Gasser 2000; Ghidelli et al. 2001; Hoppe et al. 2002; Hsu et al. 2013). This interaction is required for silencing at the HM and at telomeres. Allosteric regulation of Sir2 is mediated by its interaction with Sir4, which has been proposed to limit ectopic activity of Sir2 (Hsu et al. 2013).

Proteic interactors of Sir4

Sir4 contributes to the anchoring of telomeres to the nuclear membrane through its interaction with Esc1 (Ansari and Gartenberg 1997; Andrulis et al. 2002; Taddei and Gasser 2004), Mps3 (Bupp et al. 2007), Nup170 (Van De Vosse et al. 2013) and the ku70/Ku80 heterodimer (Tsukamoto, Kato, and Ikeda 1997). Interestingly, those anchoring pathways are largely independent from one another and pathway choice is telomere dependent (Hediger et al. 2006). The Ku and the Esc1 pathway anchor telomeres during the G1 and S phases of the cell cycle respectively (Taddei and Gasser 2004). While Mps3-Sir4 interaction also predominates during S phase, it is independent from Esc1, Ku70 as well as the other Sirs (Bupp et al. 2007). Lastly, Nup170 dependent anchoring predominates during M and G1 phases of the cell cycle, and is mediated through interactions with Sir4 and Rap1 (Van De Vosse et al. 2013). The exact residues mediating Nup170-Sir4 or Nup170-Rap1 are unknown. Last but not least, Sir4 interaction with Sir1 and Rap1 are key for the establishment of silencing (Luo, Vega-Palas, and Grunstein 2002).

Regulation of Sir4 levels

Ubiquitinylation of Sir4 by Dia2, a component of the E3 ubiquitin ligase complex SCFDia2, occurs in vitro and in vivo. In the absence of Dia2, Sir3 and Sir4 are mis-localized in a fraction of the population. It should be noted however that dia2 mutant cells are sick and appear to have an altered nuclear morphology; this might affect indirectly the clustering of the Sirs. Consistent with a role of ubiquitination in regulating Sir4 function, silencing is partially lost in dia2 mutants (Burgess et al. 2012). In accordance with a potential role of Sir4 level modulation in silencing regulation, Sir4 levels drop with 2 hours in a-factor arrested cells (Larin et al. 2015).

e. Silencing mechanism

Nucleation

Native silencers

The initial step of silencing establishment is the nucleation of the SIR complex at specific loci, known as silencers (Loo, Rine 1995). Natural silencers have been extensively characterized. Two silencers flank each Hidden MAT (HM) loci and the telomeres themselves are silencers. At both HM, the 'essential' E and the 'important' I silencers collectively stem the recruitment of the SIR complex (**Figure 19**). While the E elements are necessary and sufficient for silencing of both HM, I element deletion only weakens silencing. Silencers of both HM differ in sequences and are not exactly similar, for example the HML I silencer is sufficient for silencing whereas HMR I is not (Mahoney 1989). Elements functionally analogous to HMR I silencers, that is to say elements that cannot stem silencing on their own but reinforce the potential of a nearby silencer, are known as proto-silencers (Fourel, Lebrun, and Gilson 2002).

The E silencer is a compact and unique (<150bp) combination of binding sites for Rap1, Sum1, and an ARS, binding site for Abf1 and the origin recognition complex (ORC). The Sir4-Rap1, Sir3-Rap1 and Sir1-Orc1 interactions collectively allow the recruitment of the SIR complex. The contribution of Abf1 to silencer strength is more difficult to apprehend, as no direct interaction between Abf1 and any of the Sirs has been reported so far. Interestingly, collective binding of Abf1 and Orc1 impact nucleosome positioning in the vicinity of the HMR-E silencer, which

confers apparent directionality to this element (Y. Zou, Yu, and Bi 2006). Studies dissecting the respective contribution of each binding sites of the E silencer in the absence of the I silencer showed that Abf1, Orc1 and Rap1p-binding sites contributions to silencing are not equivalent. In this context, Abf1-BS is dispensable while Rap1-BS is necessary (X. Zhang et al. 2012). At HMR, binding of the transcriptional repressor Sum1 to the D2 element contributes to the E silencers efficiency, albeit its contribution is only appreciable in a HMR- Δ I context (Irlbacher et al. 2005).

In contrast, only Orc1 and Abf1 binding participate in proto-silencing at I elements (X. Zhang et al. 2012). In the absence of the SIR complex, neither Orc1 nor Abf1 binding affect nucleosome positioning or occupancy, arguing that their role in protosilencing might not be mediated by changes in local chromatin structure (X. Zhang et al. 2012). Nevertheless it has been shown that inverting HML-I orientation increases silencing outside of HML. Thus, at both HM, loci nucleating the Sir complex also impose directionality to silencing (Bi et al. 1999).

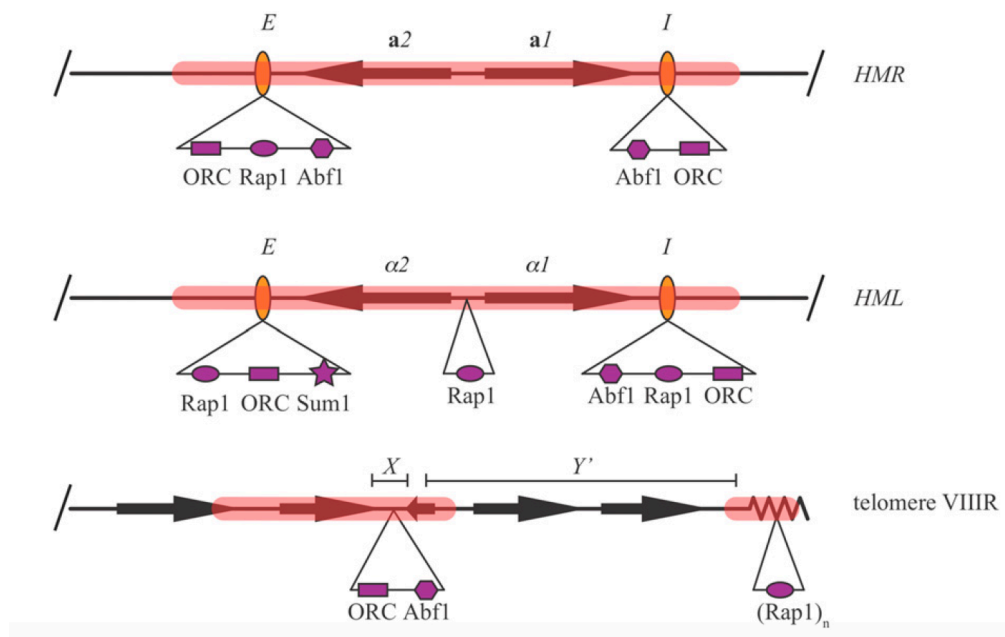


Figure 19 DNA binding sites organization of silencer and protosilencers. Domains silenced by the SIR complex are highlighted in red. X and Y' telomeric associated repeats are shown. *Figure from the review by Gartenberg (2016)*

At telomeres, Sir3-Rap1 and Sir4-Rap1 interactions mediate silencing nucleation (P Moretti and Shore 2001). Interactions of Sir4 with the Ku heterodimer and the telomerase strengthen silencing, but are not required for nucleation. Among the estimated ~ 16 Rap1 molecules binding

a typical ~300 bp telomeric track, only a fraction is thought to recruit the SIR complex. Rif1 and Rif2 occupy the remaining Rap1 sites. It has been proposed that the number of Sir free Rap1 is kept constant (Marcand, Gilson, and Shore 1997), which might contribute to the observation that telomere length proportionally strengthen silencing (Kyrion et al. 1993). In parallel, destabilization of Rap1-Rif1 interactions increases silencing, largely independently of telomere length (Shi et al. 2013).

Proto-silencers at telomeres:

Similarly to the I elements at the HM loci, proto-silencers located within telomere associated sequences contribute to telomeric silencing. The protosilencing activity of the core X element is attributed to the ACS and the Abf1p-binding site (Fourel et al. 1999). Lastly, it has been suggested that the Ume6 binding site associated to PAU genes also acts as a proto-silencer element (Radman-Livaja et al. 2011).

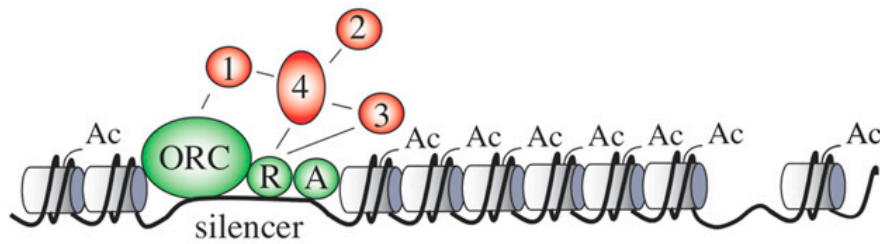
Synthetic and minimal silencers:

Recruitment of the Sir complex can be achieved through various synthetic ways giving further mechanistic insight on Sir complex recruitment. First, artificial tethering of Sir3 or Sir4 to chromatin using a Gal4 binding domain (GBD) is sufficient to induce silencing (Marcand et al. 1996). This observation illustrate how once recruited to chromatin, the SIRs exert their self-recruitment potential. Search for the minimal requirements for silencer activity led to the conclusion that Rap1p and ORC binding sites combined with the D2 element, a Sum1 binding site- are sufficient to establish silencing at HML (Weber and Ehrenhofer-Murray, 2010). Interestingly, *in-vivo* evolution of a tethered RNA successfully demonstrated that structured RNAs could nucleate silencing in combination with the ORC complex. While the involvement of RNA in silencing has not been reported in *S.cerevisiae*, this works suggest that RNAi-independent silencers can exist (Kehayova and Liu 2007).

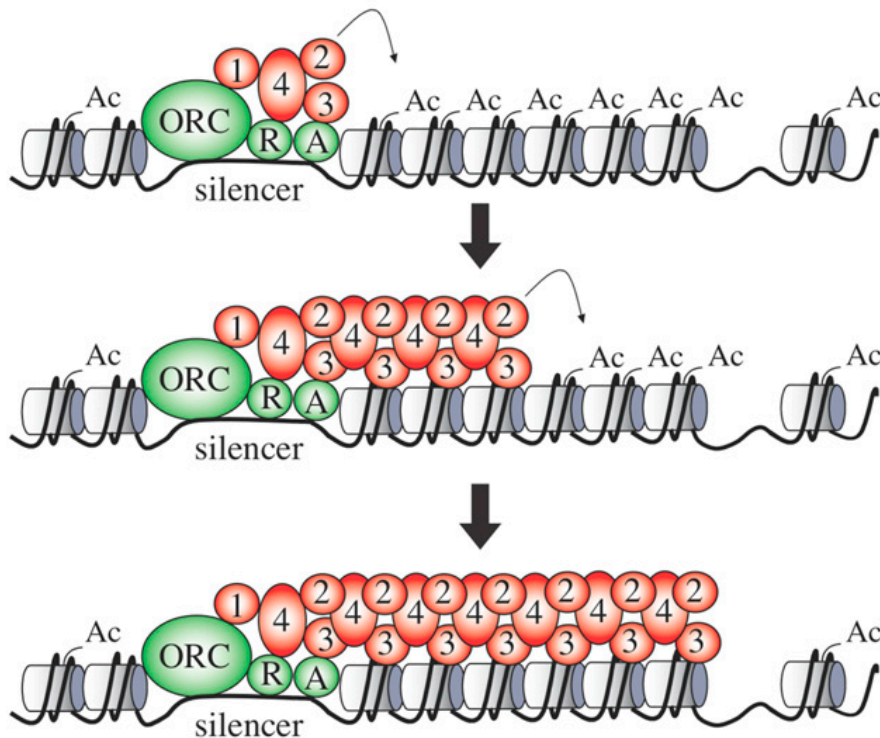
From nucleation to propagation

The prevalent model for SIR complex nucleation is recapitulated by the different interactions listed below. As shown on **(figure 20)** Sir3 and Sir2-Sir4 interact with Rap1 while Sir4 interacts with Orc1-bound Sir1 at the HM or Ku70/80 at telomeres. In wild type cells, Sir2 activity is required for the complex to propagate on chromatin. Accordingly, in strain expressing a catalytically dead mutant of Sir2, the Sir complex is restricted to silencers (Hoppe et al. 2002; L N Rusche, Kirchmaier, and Rine 2002; Ellahi, Thurtle, and Rine 2015). In the classical model of silencing, Sir2 deacetylates H3 and H4 histone tails, allowing the Sir complex to bind neighbouring nucleosomes. Alternatively, it has been proposed that the Sir complex first binds acetylated histones and later modifies them (Oppikofer et al. 2011), which is reminiscent of Clr4 properties. Either way, iterative rounds of deacetylation and binding lead to the linear spreading of the whole complex along chromatin (Hoppe et al. 2002; L N Rusche, Kirchmaier, and Rine 2002).

A Nucleation



B Spreading



C Maturation

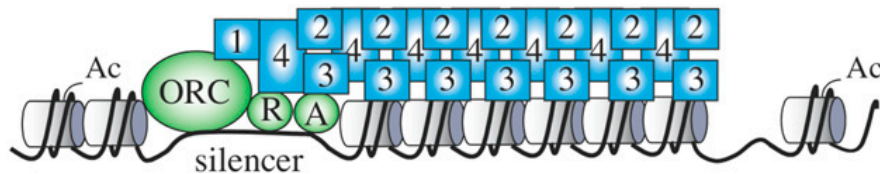


Figure 20 Model of the nucleation spreading and maturation of silent chromatin. (A) minimal representation of the nucleation process at the HM loci. (B) Spreading : deacetylation of neighboring histone by Sir2 creates favorable binding sites for Sir3 and leads to the propagation of the whole complex, until a barrier is eventually reached or until the chemical equilibrium to create a new binding site for Sir3 is not favorable anymore. (C) Maturation : numerous experiments have shown that a maturation step is required for

the SIR complex to implement silencing. This step potentially involves modification of the underlying chromatin. *Figure from the review by Gartenberg & Smith (2016)*

While this model explains properly the propagation of the Sir complex in some cases, several experiments demonstrated that the actual picture is more complex. First the requirement for Sir2 catalytic activity might not be limited to H4K16 de-acetylation. This is supported by the absence of silencing in strains lacking both Sir2 activity and Sir2 main substrate, H4K16ac (B. Yang and Kirchmaier 2006). Further studies demonstrated that Rpd3 activity is antagonistic to silencing in those mutants. This antagonism is independent of known Rpd3 histone substrates - *see part III*- as well as of H3K56 acetylation (Thurtle-Schmidt, Dodson, and Rine 2016). Those observations along with the deacetylation of Ifh1 and Pkc1 by Sir2 (Lin et al. 2009; Downey et al. 2013), give credence to the existence of non-histone substrate of Sir2 required for silencing, as proposed in (Thurtle-Schmidt, Dodson, and Rine 2016). Despite this grey area, the spreading model would predict the assembly of continuous structures, which is apparently incompatible with the occurrence of discontinuous silent domains at telomeres (Fourel et al. 1999; Pryde and Louis 1999). Given the ability of Sir3 and Sir4 to self-interact, it is possible that unsilenced elements such as telomeric Y' repeats, are looped out in such a way that they are not accessible as a substrate in contrast to the centromere proximal nucleation site.

Attempts to elucidate the mechanism of Sir spreading by in vivo experiments can be classified in two categories. Population levels studies have addressed the kinetics of Sir complex binding and associated chromatin mark changes (Katan-Khaykovich and Struhl 2005; Lynch and Rusche 2009; Radman-Livaja et al. 2011) and single cell studies focused on silencing establishment through the use of fluorescent reporter proteins. Collectively those studies indicate that silencing is nucleated stochastically (Xu, Zawadzki, and Broach 2006), at equivalent rate at telomere and HM (L N Rusche and Lynch 2009). Propagation of the SIR occurs away from the nucleation sites and increases with time. This propagation occurs at different rates depending on the genomic location, the highest rate being observed between E and I HMR silencers (Katan-Khaykovich and Struhl 2005; Lynch and Rusche 2009; Radman-Livaja et al. 2011). Two studies reported the simultaneous binding of Sir2 and of Sir3 throughout HMR, at a 5-minute time scale in the most precise study (Lynch and Rusche 2009). Furthermore, addition of an ectopic copy of

the E silencer at a telomere accelerates Sir binding (Katan-Khaykovich and Struhl 2005). At last, the co-expression of a catalytically dead version of Sir2, which should block propagation if one assumes a linear polymerization model, had no effect on HMR silencing but significantly perturbed silencing at two telomeres (L N Rusche and Lynch 2009). Those observations led to the suggestion that two modes of propagation might exist, a locally non-linear one at the HMR and a linear one at telomeres. The different kinetics observed at subtelomere and at the HM loci were attributed to silencers and not to chromatin substrate (Lynch and Rusche 2009).

Despite the great details brought by those studies, the unsynchronicity of ChIP approaches prevent any firm conclusion on Sir spreading mechanism, leaving the door open. Additionally, the differences observed between HM and telomere could be due to differential access of Sir2 to its substrates before the induction of Sir3: In the absence of Sir3, which is the starting point of those experiments, Sir2 is recruited to nucleation sites through its interaction with Sir4. It is possible that the quantity of chromatin accessible to Sir2 differs in both cases. Accordingly it has been shown that a histone acetyltransferase tethered to chromatin by a LexA binding domain can modify neighboring chromatin in a 2 kb long zone (Chiu et al. 2003). Independently of the exact mechanism of propagation, de-novo silencing following Sir3 induction occurs within at least 100min and near complete silencing (>95 %) at the population level occurs within 3 to 5 cell cycles.

f. What does silencing stand for?

As discussed bellow, binding of the Sir complex to a gene can abolish its transcription albeit all the machinery required for its transcription is available in the nucleus. However many steps of potential regulation exist from the binding of a transcription factor to the termination of transcription. It is therefore of prime importance to understand which steps of transcription are impacted by the Sir complex. Sir silencing has been associated with virtually all steps of transcription. The steric occlusion of transcription factor binding, the impairment of pre-initiation complex (PIC) formation and the inhibition of transcription elongation have all been proposed to be regulated by the Sir complex. Precursor study established that Ppr1, a positive

regulator of *URA3* expression can only promote transcription just after replication (O M Aparicio and Gottschling 1994), arguing in favor of a steric occlusion model. However, later studies demonstrated that Ppr1 binding also occurs within silenced domains (L Chen and Widom 2005; T Kitada et al. 2012), ruling out steric occlusion in this precise case.

Impairment of pre-initiation complex assembly postulates that steric occlusion of individual members of the PIC collectively leads to the impairment of its assembly (Steakley and Rine 2015). Last, the inhibition of transcription elongation model proposes that the PIC can assemble within silent domains while mRNA capping factors and elongation factors would be specifically absent of silent domains (L. Gao and Gross 2008).

Elegant experiments carried by the Rine laboratory observed that a prokaryotic T7 polymerase also has hard times to transcribe a reporter gene within HMR (Steakley and Rine 2015). Moreover, this study excludes transcription elongation impairment by probing stalled RNA PolII, and argues that other studies using ChIP-exo-seq (Rhee and Pugh 2012) or nascentRNA-seq (Churchman and Weissman 2011) also lack of evidence toward this model.

While the latest study (Steakley and Rine 2015) postulates that steric hindrance plays a major role in Sir silencing, the use of this rather broad terminology might hide something more complicated. Particularly, the accessibility of dam methylase and several restriction enzymes have been reconsidered quantitatively (L Chen and Widom 2005). It appears that most of those enzymes, along with LexA can access silent domains and that deletion of *SIR2* has at most a 2-fold impact on accessibility. This is interesting because it shows a small protein such as TFIIB (~38kDa) is excluded from silent domains while proteins from similar or even larger size (Dam is 32 kDa and Ppr1 is 102 kDa) can access silent chromatin. This observation led Chen & Widom to postulate that there is more than steric hindrance, it echoes the above considerations on liquid-liquid phases and the recent findings of the ability of HP1 to form liquid-like structures (Larson et al. 2017).

A setup enabling the isolation of OFF and ON cells from a population expressing a variegating telomeric gene demonstrated Sir3 binding -assessed by chromatin immuno-precipitation- was comparable in both populations. However, while Ppr1 exhibited comparable binding in both

populations, the general transcription factor TFIIB was only bound at the promoter of ON cells (T Kitada et al. 2012). This last observation, as we will discuss hereafter suggests that Sir binding might not always be sufficient to preclude PIC assembly and lead to silencing.

g. From Sir binding to silencing

In exponentially growing wild type cells, Sir binding is associated to the repression of telomeric genes and HM. However, there are conditions during which Sir interaction with chromatin -as probed by chromatin immunoprecipitation- is not sufficient for transcriptional repression. Experiment that aimed at understanding the heritability of silencing led to the observation that despite similar binding of the Sirs, cells blocked before their first G2/M passage after Sir induction were deficient for silencing. In contrast cells that have passed the G2/M phase are silencing efficiently (Lau, Blitzblau, and Bell 2002; A L Kirchmaier and Rine 2001). Replication *per se* does not affect this process as excised HM rings, which never replicate, successfully implement silencing (A L Kirchmaier and Rine 2001). Consistent with an antagonistic implication of sister chromatid cohesion in silencing, passage through M phase is not required in the absence of the cohesin subunit Scc1 and silencing is not established in presence of a non-cleavable Scc1 allele (Lau, Blitzblau, and Bell 2002). However, caution is required: it has been suggested that the temperature sensitive Sir3-8 allele used in (Lau, Blitzblau, and Bell 2002) associates slowly to chromatin and might artificially affect the involvement of Scc1 (Ann L Kirchmaier and Rine 2006). In the same line, binding of the Sirs has no effect on transcription in *hst3 hst4* double mutants. In this case, H3K56 remains acetylated even when the Sir complex is bound to chromatin (B. Yang and Kirchmaier 2006).

Similarly, interesting observations attribute a role to H3 tail in promoting silencing. In H3 Δ 4-30 mutants, the Sir complex binds to chromatin but silencing is not fully functional (Thompson, Ling, and Grunstein 1994). Additionally, Sir bound chromatin in those mutants displayed increased dam-methylase activity, suggesting that H3 tail promotes structural changes upon binding of the Sir complex that are required for efficient silencing (Sperling and Grunstein 2009).

Lastly, the on and off states of variegating telomere gene were shown to differ by the extent of histone methylation within the gene body. It was reported that despite similar binding of the Sir, the on state was comparatively enriched for H3K4me3, H3K36me3 and H3K79me1/2 but not H3K79me3, H4K16ac or H3K56ac. Thus Sir binding is necessary but not sufficient for silencing in a wild type context (T Kitada et al. 2012). In vitro, transcription assays led to the conclusion that only H3K79me2 - H3K79me1 has not been tested- allows transcription to occur while the Sir complex is bound to chromatin (T Kitada et al. 2012). The discrepancy between this study and others (Martino 2009) that demonstrated that H3K79 methylation abrogates Sir binding has yet to be solved. The model put forward by this study proposes that histone gene methylation acts as a feedback loop that contributes to the maintenance of ON states. Somehow this conclusion is at odds with all observations regarding silencing in dot1 mutants. Indeed, in this mutant H3K79 methylation is lost, so that one expects OFF state to predominate. However in dot1 mutants, silencing of reporter genes is lost while silencing of natural telomere appears unaffected (Y. H. Takahashi et al. 2011).

Irrespective of the role of H3K79 methylation on silencing variegation, the reason why TFIIB can access ON telomeres but not OFF telomeres remains unknown. Post-translational modifications of the SIR complex might influence its access to chromatin.

h. Subtelomeric Silencing is Influenced by Nuclear Architecture

The localization of telomeres at the nuclear periphery strongly influences silencing processes by creating local environment concentrating silencing factors. The 32 telomeres of an exponentially growing haploid yeast cell generally cluster into 3–8 foci, involving Ku proteins to anchor telomeres to the nuclear envelope (Hediger et al. 2002; Taddei and Gasser 2004; Taddei et al. 2009). This clustering in space favours silencing through an increase in the local concentration of Sir proteins. This is the reason why delocalizing a weak silencer element to the nuclear periphery enables to implement silencing. In addition, the clustering of silenced regions has been proposed to prevent Sir proteins from binding promiscuously to other sites in the genome (Taddei et al. 2009).

C. Functional impact of heterochromatin on nuclear processes

*"It is well known that the various knobs and centromeres may coalesce in the resting nuclei."*B.McClintock 1950

1- Telomere clustering

In fission yeast, Swi6 foci generally coalesce in 2 to 5 foci, much like peri-centromeric heterochromatin in mammals. Upon disruption of heterochromatin, in *clr4* mutants for example, Swi6 pattern is diffused throughout the nucleus and the nucleolus (Ekwall et al. 1996). In contrast, the number of Swi6 clusters increases in RNAi mutants, although those foci are smaller and less intense. Colocalization of those Swi6 clusters with Taz1 demonstrated that this change likely corresponds to the loss of telomere clustering (Ira M Hall, Noma, and Grewal 2003)(**figure 21**). In budding yeast, the clustering of telomeres is also sensitive to changes in the environment. Work from our lab described that upon entry into quiescence by glucose exhaustion, nuclear organization experience dramatic changes driven by heterochromatic factors. Following the diauxic shift, the telomeres progressively cluster all-together at the nuclear interior (Guidi et al. 2015) (**figure 21**). The relocalization of telomere from the nuclear periphery to the interior and their grouping is dependent on the silencing factor Sir3. This reversible organization change is thought to imply nucleus-mitochondria cross-talk, and glucose sensing pathway. Consistently, telomere clusters return to their exponential phase state in less than 30 minutes after glucose addition to the growth medium (unpublished). This fast reversal of the system suggests that this change is likely mediated by post-translational modifications and is independent of replication (Guidi et al. 2015). While RNAi is required for fission yeast cells to survive quiescence (Roche, Arcangioli, and Martienssen 2016), its impact on telomere clustering in those conditions remains to be studied.

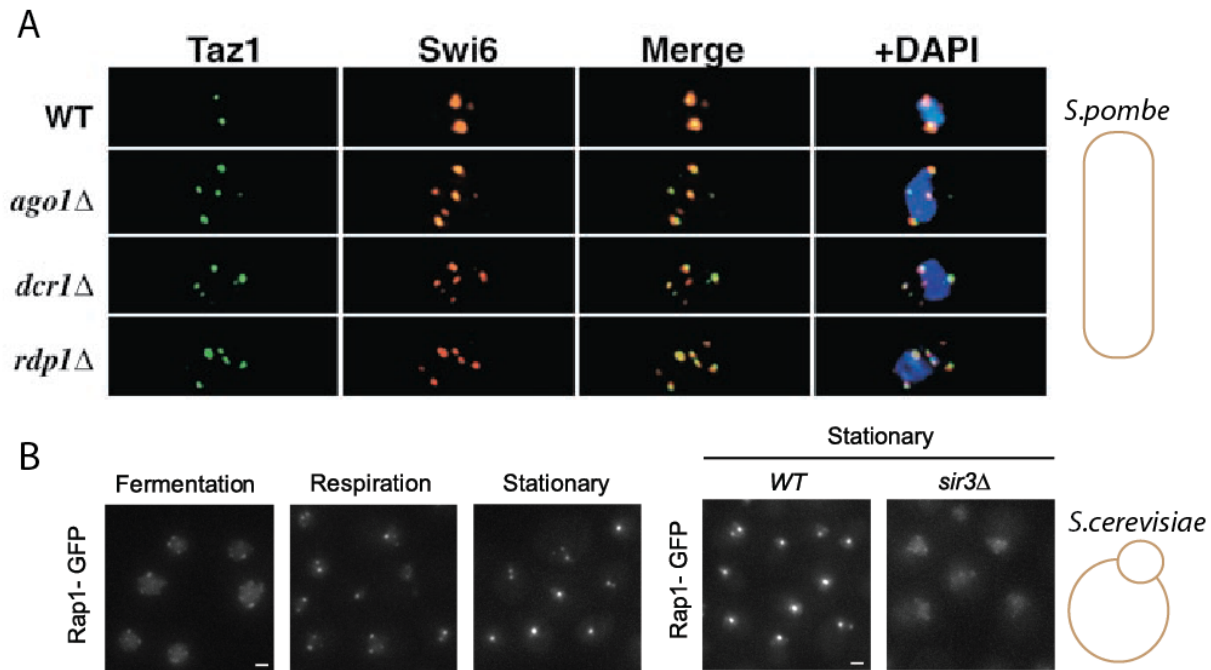


Figure 21 Heterochromatin and telomere clustering. (A) In fission yeast component of the RNAi machinery are required for the clustering of telomeres. Immunofluorescence images of the telomere bound protein Taz1 shows increased number of foci co-localizing with the HP1 homolog Swi6 in mutants of the RNAi pathway. DNA is stained with DAPI. (B) In budding yeast, the SIR complex is responsible for the clustering of telomeres at the nuclear periphery in exponentially growing cells, as illustrated by live cell imaging of the telomere bound Rap1-GFP protein. During the diauxic shift, the nuclear distribution of telomeres changes as they groups into fewer foci. Upon entry into quiescence by exhaustion of available carbon sources the telomeres ultimately cluster at the nuclear interior into one or two foci. This process is requires the presence of Sir3 as shown by the images of Rap1-GFP fluorescence in *sir3* mutants. *Panel A images are from Hall et al. (2003) and panel B images are from Guidi et al. (2015)*

2- Chromosome segregation

Regional centromeres are stereotypically organized around a central domain where kinetochore forms, surrounded by peri-centromeric heterochromatin regions. The presence of peri-centromeric heterochromatin contributes to the faithful segregation of chromosomes during mitosis in several ways. First evidence of this link came from the observation that *S. pombe* *swi6*, *clr4* or *rik1* mutants often experience chromosome loss (Allshire et al. 1994). This transcription independent role of peri-centromeric heterochromatin is believed to emerge from the ability of Swi6 to recruit cohesin (Bernard et al. 2001). Accordingly, forcing cohesin recruitment in the absence of *swi6* rescues mitotic stability (Yamagishi et al. 2008). Additionally, the association of Swi6 with the meiosis specific protein Shugoshin is yet another contribution of heterochromatin

to centromere function (Yamagishi et al. 2008). HP1 is required for appropriate chromosome segregation in drosophila embryos (Kellum and Alberts 1995), and the association of HP1 with Shugoshin is conserved in humans (Yamagishi et al. 2008). Interestingly heterochromatin might not only accompany centromere function but could also favour regional centromere establishment. Accordingly, recent work shows that heterochromatin boundaries are sites of preferential kinetochore formation in drosophila (Olszak et al. 2011).

Heterochromatin and transcriptional regulation

The general propensity of heterochromatin loci to cluster might be evolutionarily conserved to bring in close proximity regulatory elements capable of exerting trans-regulation. Given that heterochromatin content can evolve during cellular differentiation -or de-differentiation in plants- it offers a theoretical mean to control long-range interactions of neighboring loci. While this original mode of transcriptional regulation has been discussed (Yasuhara and Wakimoto 2006), its prevalence awaits further studies.

3- Heterochromatin and genome stability:

Even if the protein actors differ, heterochromatin systems in *S.pombe* and *S.cerevisiae* mating processes are illustrious examples of the role of heterochromatin in silencing and recombination. In *S.pombe* M cells, the binding of Swi6 enables the spreading of the recombination-promoting complex (RPC), which in turns favors one mating type cassette as a donor (Jia, Yamada, and Grewal 2004). In budding yeast the SIR complex not only inhibits the transcriptional process but can also modulate the access of enzymes to DNA. As a matter of fact, this property of the Sir complex is key to switch mating type. Indeed the HO endonuclease, which can potentially target the MAT locus and the two HM, only cuts at the MAT locus (Strathern et al. 1982). While initial experiments attributed the lack of HO activity at the HM as the effect of its exclusion by steric hindrance (Loo and Rine 1994), it appears that HO might not be excluded from silent HM but could be inactive at those loci (Lingyi Chen and Widom 2005).

In addition to the peculiar example of mating type, several studies point toward a role for the SIR complex in the maintenance of genome stability. First, deletion of either *SIR3* or *SIR4* leads to a modest telomere shortening, and increase of both chromosome loss and spontaneous

recombination rate (Palladino et al. 1993). Second, overexpression of *SIR2* and *SIR3* but not *SIR4* was shown to increase chromosome loss (Holmes et al. 1997). Third, sites of “chromatin stress” such as double-strand breaks or chromatin stretching were shown to recruit SIR proteins (S. G. Martin et al. 1999; Mills, Sinclair, and Guarente 1999; Thrower and Bloom 2001) although the significance of this recruitment remains unclear. Fourth, Sir4 together with Rap1 and Rif2 prevent telomere fusion (Marcand et al. 2008). This core telomere function is based on a full cis inhibition of the NHEJ repair pathway at telomere ends. Redundant mechanisms involving Rap1, Rif2 and Sir4 mediate this inhibition (Pobiega and Marcand 2010; Lescasse et al. 2013). Our laboratory has reported that replication stress arising from tight DNA–protein interactions favours heterochromatin formation by recruiting the SIR complex (Dubarry et al. 2011). Lastly, collaborative work between the laboratory of K.Dubrana and our laboratory, demonstrated that binding of the SIR complex can also affect repair pathway choice by inhibiting Exo1-mediated resection of double strand breaks. (Batté et al. 2017)

A potential dual role of heterochromatic small RNAs

The involvement of transcription in the process of centromeric heterochromatin formation in *S.pombe* seems paradoxical, especially as the similar task can be carried without RNAi in the same organism. This potentially means that using RNAi in this context might offer additional benefits. It has been proposed that the small RNA generated by the RITS complex might be exported to the cytoplasm to mediate post-transcriptional silencing of homologous RNA (Grewal and Jia 2007). Given that there are several examples of viruses that preferentially integrate within heterochromatin, restricting their transcription to the production of small RNAs might serve as a genomic vaccination and prevent further infection. Accordingly, it has been shown that ectopic expression of a hairpin RNA can induce Clr4 dependent silencing in trans in *S.pombe*. The efficiency of this process is decreased by Paf1 (Kowalik et al. 2015) and depends on the distance of the target gene from heterochromatic loci and on the dose of Swi6, as increasing Swi6 dosage improves trans-silencing efficiency (Simmer et al. 2010).

Through this part we described some of the fascinating properties of heterochromatic structures. It appears that heterochromatin is a crucial component of the nuclear environment. It impacts the overall organization of DNA within the nucleus, and the transcription of large chromosomal domains. Current knowledge leads us to conclude that what was initially viewed as silent domains are in fact a dynamical and pluri-functional nuclear hub. However, this potentiality comes at a cost, and heterochromatin domains need to be constrained for this reason. In the next paragraph we will focus on the structures that have the ability to block or limit heterochromatin propagation in budding yeast.

III Mechanisms of silent domains delineation

The complexes responsible for heterochromatin formation have in common the ability to self-propagate along chromatin and to mediate trans-interactions. This property comes with a cost, which is the necessary limitation of heterochromatic domains. This poses the question of how heterochromatin domains are limited. Studies in drosophila, chicken, fission and budding yeasts revealed that at least some heterochromatin domains are precisely delimited by punctual loci. In this part we will discuss our current knowledge on heterochromatin spreading limitation.

A Heterochromatin barriers set boundaries

1- Barriers

Specific DNA sequences have the ability to block the propagation of heterochromatin. In general, experimental setups used to study such loci consist in the insertion of a candidate sequence in-between a heterochromatin nucleation site and a reporter gene. Alternatively, insertion of a tethering site can allow the targeting of a given protein to test its impact on silencing propagation. When a DNA locus or a tethered protein located between the nucleation site and the reporter gene leads to the de-repression of the previously silenced reporter, it's a barrier.

First examples of barrier loci came from the study of silencing at the mating type loci in yeast. Silencing at the HMR is confined by a tRNA loci located 3' from the I silencer (David Donze et al.

1999). Removal of this locus, or mutation in cohesin, perturbs the barrier function of the tRNA. This effect is not common to all genes transcribed by PolIII as the 5S ribosomal gene of *S. cerevisiae* has no barrier properties. Binding of TFIIC is believed to be sufficient to block the spread of heterochromatin, as non-tRNA TFIIC binding loci - or extra TFIIC sites (ETC)- mediate boundary activity. Apart from TFIIC, loci with barrier activity generally consist in multiple transcription factor binding sites. This is the case for *TEF2* upstream activating sequence (uas) (Bi and Broach 1999), as well as of the Subtelomeric anti-silencing regions (STARS) (Fourel et al. 1999). Similarly to TFIIC, the proteins responsible for the barrier properties of *TEF2* and STARS loci have other functions. *TEF2* uas is a seemingly extreme case, as binding of Rap1 to 2 contiguous sites is responsible for the barrier activity. Rap1 can thus function as a nucleator and/or as a barrier, depending on the chromosomal context. At STARS elements, the joint binding of Reb1 and Tbf1 insulates Y' elements from their neighboring nucleation sites. Following those pioneer studies, the tethering assay has been very popular in the search for proteins that impairs silencing propagation. Subsequent studies for proteins having a barrier effect when tethered to DNA identified more than 40 factors, including drosophila BEAF (Kojiro Ishii and Laemmli 2003), a known insulator -element preventing the trans association of an enhancer with a promoter-.

It seems that, at least in some cases, the mechanism at the origin of the barrier effect has to do with changes in local chromatin structure. Accordingly, binding of Rap1 to the *TEF2* promoter induces a local relaxation of DNA that theoretically corresponds to the loss of two nucleosomes (Xin Bi, Zou 2004). Similarly, the binding of multiple LexA ($n>3$) has a barrier activity that correlates with nucleosome exclusion. Further support for the "nucleosome excluding structure" model comes from the observation that long polyA-tracks or CCGNN repeats, which strongly impair nucleosome formation, impose a barrier to the spread of the SIR complex (Bi, Yu, Sandmeier, and Zou 2004).

The observation that NFR can block the spread of heterochromatin is somehow surprising as establishment of silencing requires to propagate over genes, and thus over the nucleosome depleted regions corresponding to promoters. Thus, it seems that only certain NFR can block the spreading of the SIR complex. In this context, it is crucial to understand why

heterochromatin structures can replenish some NFR but not others (Garcia et al. 2010). Combinatorial and context dependent effects likely determine the 'strength' of a barrier. Barrier activity often requires the synergistic action of multiple proteins as exemplified by the contribution of Abf1 or Reb1 to Rap1's barrier property (Q. Yu et al. 2003); however more study is required to understand the details of this property. Furthermore, we still lack understanding of why the same elements do not have the same barrier efficiency depending on their location (Q. Yu et al. 2003). One explanation could be that local chromatin conformation affects the function of barrier elements, as one barrier elements can act downstream of the insulated gene on a minichromosome (Chakraborty, Simpson, and Grigoryev 2011).

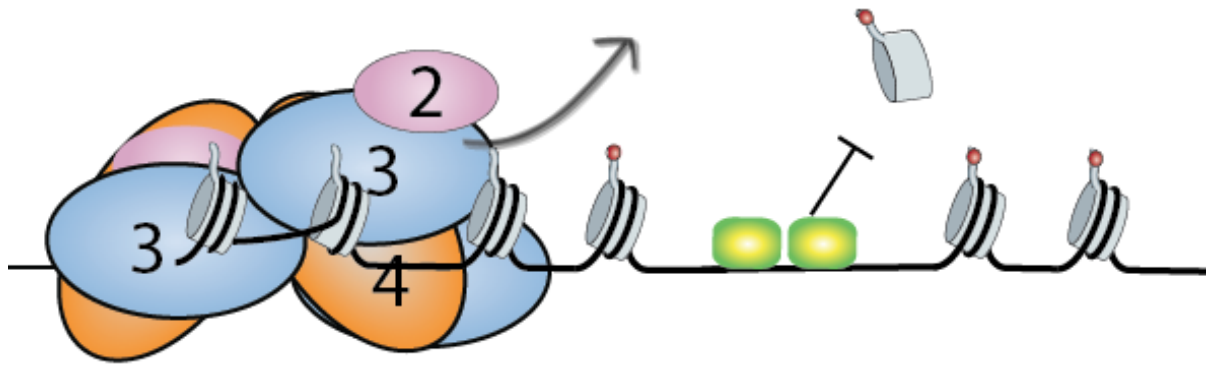


Figure 22 Schematic illustration of the nucleosome excluding barrier mechanism. The SIR complex is drawn as large globular entities. Each Sir protein is labeled with its number (color code : Sir2 pink, Sir3 blue, Sir4 orange). The two green boxes represent a transcription factors that has the ability to disrupt local chromatin structure.

Another mechanism contributing to the barrier property is the localized modification of neighboring nucleosomes. Tethering of Dot1, Gcn5, Esa1 or Sas2 to chromatin also halts the spread of heterochromatin, likely by shifting the chemical equilibrium ruling histone tail modification to a configuration dis-favourable to SIR complex binding. Interestingly, overexpression of Sir3 can counter-act the anti-silencing affect of targeted LexA-Gcn5 or LexA-Esa1 (Chiu et al. 2003).

2- Desilencing

The concept of desilencing differs from the one of a barrier in the sense that a desilencing structure will only insulate a given loci from the spread of silencing but allow propagation past

this locus. Assays used to demonstrate such a property generally consist in the tethering of candidate protein to DNA in a configuration called "boundary trap". In such an assay, the association of chromatin to nuclear pore components such as exportins confers efficient Nup2 dependent desilencing property (K Ishii et al. 2002). Interestingly, mammalian CTCF or drosophila GAGA factors also have desilencing activity when tethered to chromatin (Kojiro Ishii and Laemmli 2003). Despite the very clear results brought forward by those two studies, it is puzzling that nuclear pore connection favours HMR silencing in native conditions (Ruben et al. 2011).

3- Directional silencing

A specificity of silencing at the HML loci is the absence of identifiable barrier sequences, despite the sharp limitation of Sir bound domain. Because reversing the orientation of the I silencer reinforces silencing outside of HML, it has been proposed that the I element acts as a boundary by establishing directional silencing (Bi and Broach 2001).

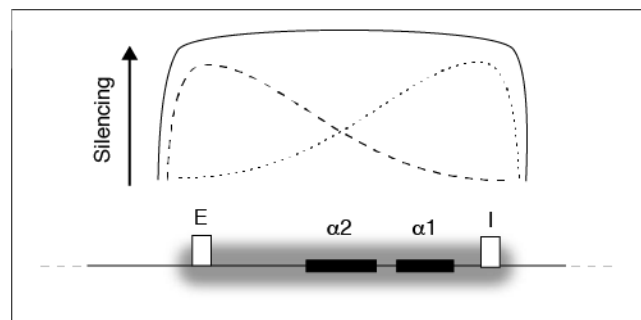


Figure 23 Domain organization by directional initiation of heterochromatin. the HML locus is pictured showing the location of the E and I silencers along with the mating type genes alpha1,2. Silencing initiated from the E silencer is represented by a dashed lines and silencing initiated from the I silencer by a dotted lines. The sum of both effects leads to uniformly high silencing between the E and the I silencer, as depicted by a plain line. Figure from the review by X.Bi & J.R Broach (2001)

4- Borderline RNA

One study reported the ability of a non-coding RNA to block the spread of Swi6 at *S.pombe* centromeres. This so-called 'Borderline' RNA is processed into small RNA by the RITS complex. However the small RNA is not handed over to Ago1, in contrast to the pro-silencing role of small RNAs at centromeres. Instead, this RNA promotes Swi6 eviction from chromatin. Direct Swi6-RNA interaction is suspected as cells expressing a Swi6 mutant unable to interact with RNA

have extended heterochromatin domains (Keller et al. 2012). While non-coding RNA has been implicated in the barrier function of the drosophila Gypsy insulator (Matzat et al. 2012), this is the first evidence of direct HP1-RNA interaction acting as barrier. The prevalence of such mechanism awaits further investigation.

B Chromatin antagonism at budding yeast subtelomeres

With the exception of the STARS, which are telomere-proximal, no barrier element has been described at budding yeast subtelomeres. In the absence of such containment, the extent of Sir binding and silencing results from the balance of pro and anti silencing activities. In the next part we will briefly describe the specificities of budding yeast subtelomeres, and focus on how various chromatin modifiers contain SIR based silencing to the end of chromosomes.

1- Local environment

Specific features characterize subtelomeric chromatin. First, loci bound by the SIR complex are largely devoid of histone modification with the exception of H2AS129ph and H4R3 methylation. However in wild type cells, SIR silenced loci occupy a limited portion of subtelomeric genes. The domains located between euchromatin and the end of SIR silenced domains hold distinctive chromatin structures. Those include Hda1-affected subtelomeric (HAST) domains and Htz1 activated domains (HZADs). HAST domains are characterized by a local increase in H3K18ac in *hda1* mutants, while HZADs have been identified as domains transcriptionally repressed in cells lacking the histone variant H2Az. Of note, while H2Az containing nucleosomes are generally located at the +1 nucleosome of euchromatic genes, H2Az covers the gene body of several subtelomeric genes. Both HAST and HZADs genes are lowly expressed under normal growth conditions but are not silenced by the SIR complex. 22/32 subtelomeres have distinctive HAST domains, lying ~10-25 kb from telomeres and hosting genes that are activated in response to stress. The activity of Hda1 is responsible for the particular chromatin state of HAST genes. Regardless of their transcription frequency, those are relatively less acetylated at H2B and H3 residues. 18 HZADs have been identified at budding yeast subtelomeres, they are characterized by the presence of H2Az all over gene body, and slight hyper-acetylation of H3 over gene body (C B Millar et al. 2006).

Due to the self-propagating properties of the SIR complex and the apparent lack of silencing barrier at subtelomeres, the domains occupied by the SIRs are the result of an unstable equilibrium (**Figure 21**). In the following part we will see how the SIR complex spreads further towards centromeres or retracts towards telomeres in different mutants affecting chromatin.

2- Chromatin acetylation

The first indication of the implication of histone acetylation in restricting subtelomeric SIR complex came from the study of *sas2* mutants and H4K16R point mutants. Sas2 has a histone acetylase activity and is responsible for the majority of genomic H4K16 acetylation (Meijsing and Ehrenhofer-Murray 2001). In those mutants SIR bound domains extends up to 13 additional kilobases towards centromeres than in WT cells. Conversely, overexpression of Sas2 leads to the confinement of the SIR complex towards telomeres (Suka, Luo, and Grunstein 2002; A Kimura, Umehara, and Horikoshi 2002). These results suggest that the equilibrium ruling H4K16 acetylation state is key to restrain subtelomeric silencing. Interestingly, whereas Sir3 and Sir4 promote the deacetylated state implemented by Sir2 activity, binding of Bdf1 and Yta7 to nucleosome unacetylated at H4K16 restrains silent domains expansion (Ladurner et al. 2003; Jambunathan et al. 2005; Tackett et al. 2005). Thus the status of H4K16 not only changes the charge of histone H4 tail but also its interactors. Lastly, deposition of the histone variant H2A.z is deficient in *sas2* mutants independently of the SIR complex (Shia 2006). While the mechanistic are not clear, H2A.z presence is also antagonistic to the spreading of the SIR complex (Meneghini, Wu, and Madhani 2003). Citation of unpublished data from Dion et al. reports that global histone turnover is decreased in *htz1* mutants. This change in nucleosome turnover might be the mechanistic explanation of Htz1 antagonism to silencing.

Thus the increased spreading in *sas2* mutants is likely the result of synergistic changes in chromatin properties. It is important however to bear in mind that the action of Sas2 is not specific at subtelomeres, so that the anti-silencing mechanism that we described is most likely not restricted to subtelomeres (Heise et al. 2012). As the majority of nucleosomes are acetylated at H4K16, this implies that most of the genome is not favourable to SIR binding.

Interestingly, perturbing H4K16 acetylation is not the only way to allow extended spreading of the SIRs. Albeit the mechanistic details are not understood, lowering H3 tail acetylation by serial point mutations of its lysines progressively allows further spreading of the SIR complex. In this case however, silencing is not fully functional (Sperling and Grunstein 2009). In the same direction, co-deletion of the H3 specific HAT elp3 and gcn5 leads to increased spreading of the SIRs at subtelomeres (Arnold Kristjuhan et al. 2002). The reason why SIR binding doesn't lead to full silencing in H3 tail mutants remains obscure. Regardless of the silencing status of SIR bound chromatin, H4K16 is unaffected in gcn5/elp3/sir3 mutants compared to sir3, suggesting that the two pathways are parallel (A Kristjuhan et al. 2003).

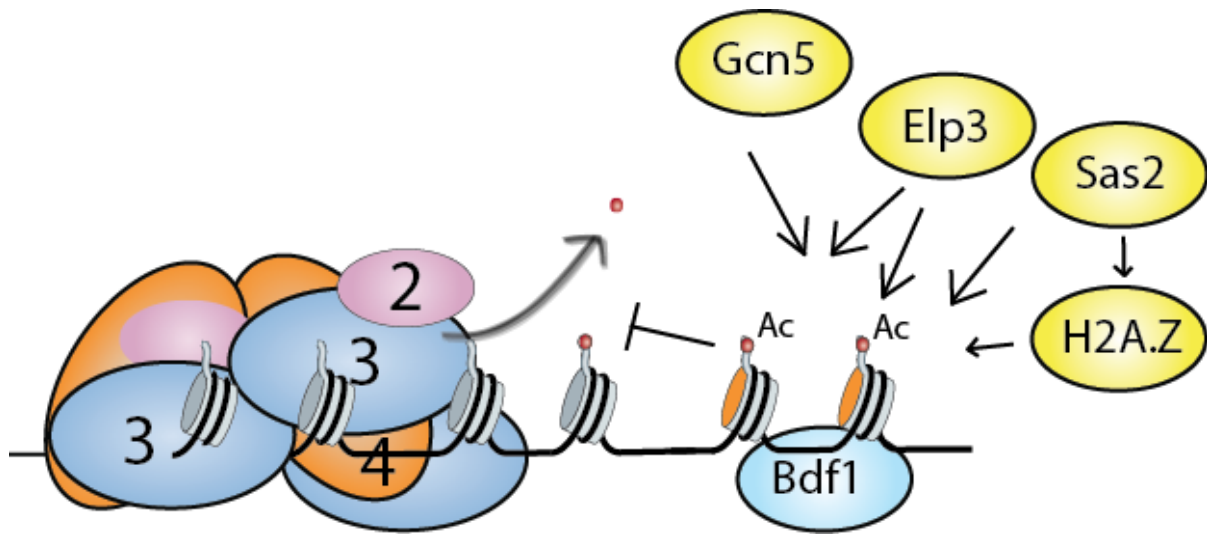


Figure 24 Schematic representation of the equilibrium regulating SIR spreading at subtelomeres. The SIR complex is represented as in figure 22. The collective action of histone modifying enzymes leads to the formation of a chromatin state disfavoring the association of the SIR complex. Red balls represent a histone modification such as the acetylation of H4K16. There might be other factors important for the restriction of the SIR complex within subtelomeres.

3- The intriguing role of Rpd3 at subtelomeres

Rpd3, Sin3 and Ume1 form the core of two complexes, Rpd3L and Rpd3s (See figure). Rpd3L is targeted to specific DNA sequences by the zinc finger repressor ume6. This targeting allows Rpd3L complex to deacetylate histones at promoters and leads to repression of targeted genes. Rpd3s recognize nucleosomes containing methylated H3K36 (modified by Set2) through the chromo-domain of Eaf3. Recognition efficiency of Rpd3s depends on Rco1. Rpd3s recruitment to

gene bodies inhibits intragenic transcription. Thus both Rpd3 complexes act as transcriptional repressors.

As often, first Rpd3 implication in silencing was revealed by genetic screens. Back in 1995, Sussel et al. screened for suppressors of defective silencing of a crippled HM loci (*deltaHMRa*) in a *rap1s* mutant (Sussel, Vannier, and Shore 1995). Among the dozens of effectors, *sds6* was later identified as Rpd3. An independent transposon-based screen identified a mutation leading to an increase of the white phenotype and sporadic disorganization of ommatidia, characteristic of *roughest* mutants. The *roughest* locus being located near white and distal from heterochromatin, authors concluded that Rpd3 mutation allowed silencing to spread past the white locus and eventually silence *roughest*. In the same study, telomeric silencing enhancement of *rpd3* mutants demonstrated a conserved role of Rpd3 in silencing (De Rubertis et al. 1996). Later, it was shown that loss of Rpd3 also increases silencing of PolIII reporter genes at the rDNA array (Z. W. Sun and Hampsey 1999). This effect is not limited to reporter genes, as genome wide profiling of transcription in *rpd3* mutants reports that 40% of endogenous genes located within 20kb of telomeres are silenced upon *RPD3* deletion (Bernstein, Tong, and Schreiber 2000). Genetic analysis established that Rpd3L but no Rpd3s is responsible for the silencing phenotype (Zhou et al. 2009). The involvement of Rpd3 in silencing is thus independent of H3K36 methylation. Intriguingly, deletion of *RXT3*, *ASH1* or *UME6* does not lead to a silencing phenotype. This observation seems inconsistent with the proposed role of Ume6 in Rpd3L targeting. It might suggest that only a sub fraction of Rpd3L is involved in silencing (suggested by (Zhou et al. 2009)) or that Ume6 has independent function that counteracts silencing.

Deletion of Rpd3 enhance bulk acetylation of H4K5,8,12,16 and H3K9,14,18 (Rundlett et al. 1996). The finding of Rpd3 antagonizing silencing is seemingly paradoxical, as histone acetylation is antagonistic to silencing as we have seen. Arguing for a direct and local role of Rpd3 in antagonizing SIR spreading, Rpd3 silencing phenotype is abrogated by H4K5Q and H4K12Q or by mutation decreasing Esa1 HAT activity (Zhou et al. 2009). Furthermore, tethering of Rpd3-GBD or Dep1-GBD to chromatin act as a barrier to the spread of silencing (Ehrentraut et al. 2010). Based on a putative OAADPR binding site located AAA+ ATPase- like domains required

for silencing, it was proposed that deacetylation of histones by Rpd3 diminishes the available substrate of Sir2 which leads to decreased production of OAADPR. In this model, the decrease in local OAADPR concentration prevents proper interaction between Sir2, Sir3 and Sir4 and limit silencing propagation (Ehrentraut et al. 2010). The OAADPR model is appealing and supported by in-vitro evidences (Martino 2009), however it was shown that silencing can occur in the absence of sirtuins. Thus there is no direct evidence for its role in vivo to date. Arguing for an indirect role of Rpd3 on silencing, a genome wide study probing Rpd3, Ume1 and Ume6 distribution observed the relative exclusion of Rpd3 from subtelomeres (Robyr et al. 2002); While this result could be affected by normalization of the array, a later study observed that Rpd3 enrichment by ChIP-qPCR is similar to the negative control along subtelomere VIR (Milliman et al. 2012). Nevertheless, those ChIP studies cannot be taken as firm conclusion of the absence of Rpd3 as many large chromatin modifier complex are known to act genome wide but found at only few loci by ChIP. Finally, it was claimed that the absence of significant overlap between decreased transcription and increased H4K12 acetylation at subtelomere was evidence against a direct role of Rpd3 in silencing (Robyr et al. 2002). While this conclusion might be erroneous as the binding of the SIR subsequent to *RPD3* deletion prevents further acetylation of underlying histones and thus certainly prevent any conclusion. Studying H4K12 acetylation in an *rpd3/sir3* double mutant would shed some light here.

A recent study has shown that silencing in a *sas2* mutant expressing a catalytically dead Sir2 enzyme can be rescued by deletion of Rpd3L or S complexes. While restoration of silencing required the Sirtuin Hst3, it occurred independently of known Rpd3 and Hst3 targets. This last study further complexifies the involvement of Rpd3 in silencing, leaving the OAADPR hypothesis open and at the same time directing our attention to non-histone substrates (Ellahi, Thurtle, and Rine 2015).

4- Methylation

The role of histone methylation on subtelomeric silencing has been extensively debated. There has been much controversy regarding the role of Dot1 in restricting telomeric silencing as discussed in part II. Considering that genome wide binding of Sir3 exhibits relatively little changes in *dot1* mutants (Y. H. Takahashi et al. 2011), we will consider that *dot1*'s contribution

to the restriction of Sir spreading at equilibrium is negligible. It remains that Dot1 slows silencing implementation (Osborne, Hiraoka, and Rine 2011) and impairs silencing when overexpressed (Singer 1998), which is not in contradiction with the previous assertion.

Set1 deletion affects mating and silencing of a reporter gene (Nislow, Ray, and Pillus 1997) and significantly alters the expression of telomere proximal genes. However, Set1 mediated methylation of H3K4 is largely absent from subtelomeres leaving the mechanistic of Set1 effect on transcription rather obscure. One possibility is that the shortened telomeres of set1 mutants (Nislow, Ray, and Pillus 1997) are the cause of reduced telomeric silencing. Alternatively, based on the observation that Sir3 has more affinity for nucleosomes unmodified at H3K4 it was postulated that set1 Δ effect on silencing is due to the emergence of low affinity binding sites genome wide, that reduces the effective amount of Sir3 available for silencing. The latter option has some support from a study showing increased association of Sir3 with euchromatin genes in set1 mutants using Dam-ID (Venkatasubrahmanyam et al. 2007).

Aim of the thesis

The initial aim of this work was to characterize the consequences of *SIR3* overexpression on the genome and to find potential factors involved in the clustering of telomeres. Second, I aimed at further describing the properties of subtelomeres to understand why they would be a good substrate for silencing. In the same line, I aimed at understand what determines if Sir3 will nucleate or not within euchromatin and what could be the consequences of this process. Last I searched for potential new functions of Sir3 by conducting a high throughput genetic screen.

Results

In this section I will first report a manuscript describing the extension of subtelomeric silent domains upon overexpression of the Sirs. This manuscript will first be submitted to genome biology. The second results part details experiments aiming at uncoupling subtelomeric properties from the influence of telomere proximity and report an analysis of Sir3 binding to euchromatic sites that was not included in the manuscript presented in the first part. The last part is dedicated to results obtained during the course of a screen aiming at discovering potential new functions of Sir3.

Annex I and II correspond to publication to which I contributed.

Results, part I: Dose-dependency of heterochromatin domains reveals subtelomeric structuration in budding yeast

Antoine Hocher^{1,2}, Myriam Ruault^{1,2}, Marc Describes^{1,2}, Mickael Garnier^{1,2}, Antonin Morillon^{1,2},
Angela Taddei^{1,2}

Affiliations:

¹Institut Curie, PSL Research University, CNRS, UMR3664, F-75005 Paris, France

²Sorbonne Universités, UPMC Univ Paris 06, CNRS, UMR3664, F-75005 Paris, France

Dose-dependency of heterochromatin domains reveals subtelomeric structuration in budding yeast

Background

A shared property of several eukaryotic genomes is the presence of heterochromatic chromosomal domains experiencing transcriptional variegation. The intensity and the extent of position effect variegation are sensitive to the dosage of silencing effectors in many systems. Mechanistically, the self-propagating properties of heterochromatin machineries come with a cost, which is the requirement for mechanisms preventing ectopic spreading of silencing. This work explores the dose-dependency of telomere position effect, using the budding yeast SIR system as a model for chromatin based heterochromatic silencing.

Results

To assess the dose-dependency of telomere position effect in budding yeast, we built a set of strains in which SIR2 and SIR3 endogenous promoters are changed. We systematically characterized the impact of Sir3 overexpression by quantifying the clustering of telomeres, the genome wide binding of Sir3 and its impact on transcription. Analysis of published data sets enabled to uncover candidates potentially responsible for the limitation of subtelomeric silent domains that were confirmed by genetic assay. Our study reveals that extension of silent domains can reach saturation, associated with the anti-silencing properties of histone marks deposited by the conserved enzyme Dot1. An outcome of this work is the determination of the maximal extends of subtelomeric silent domains, which offer a new point of view on subtelomeric properties.

Conclusion

Our work uncovers the dynamics of the dose dependency of heterochromatin propagation in budding yeast. It uncovers previously uncharacterized discrete chromosomal domains associated with specific chromatin features and demonstrates how telomere position effect is efficiently restricted to subtelomeres by the preexisting chromatin landscape.

Introduction

Heterochromatin classically designates chromosomal domains that remain condensed throughout the cell cycle (Heitz, E 1928). It impacts many aspects of chromosome biology including genomic stability and gene expression (Grewal and Jia 2007). In opposition to gene specific repressors, heterochromatin based regulation of transcription allows the silencing of genes independently of DNA sequence (Talbert and Henikoff 2006). Its prevalence in eukaryotic genomes makes heterochromatin a major system of gene regulation, key to processes ranging from gene dosage to differentiation and speciation (Grewal and Jia 2007). Silencing generally initiates at defined loci and tends to propagate from those sites by self-recruitment mechanisms (Grunstein 1997; Hoppe et al. 2002). The coupling of histone modifying enzymes to the specific association of silencing effector with nucleosomes drives the formation of regional domains of heterochromatin (L N Rusche, Kirchmaier, and Rine 2002). However, this potent mechanism comes at a price and requires the establishment of mechanisms to limit the ectopic spread of heterochromatin (David Donze and Kamakaka 2002). Albeit punctual barrier elements have been shown to block the spread of heterochromatin, the mechanism underlying those process remains elusive.

In budding yeast, a similar system of epigenetic silencing is found at the silent mating type loci (HML and HMR), at subtelomeres and within the ribosomal DNA array. Silencing at the ribosomal DNA array involves the RENT complex, while the silent information regulator (SIR) proteins, Sir2 Sir3 and Sir4, implement stable repression of mating type loci and semi-stable repression of genes at the vicinity of telomeres (Gartenberg and Smith 2016; Grunstein and Gasser 2013; Oscar M. Aparicio, Billington, and Gottschling 1991; Moazed et al. 1997; Rine and Herskowitz 1987; Rudner et al. 2005; Laura N Rusche, Kirchmaier, and Rine 2003).

Sir2/Sir4 heterodimers and Sir3 are recruited at the HM through interaction with Orc1-bound Sir1 and Rap1 (Hoppe et al. 2002; Luo, Vega-Palas, and Grunstein 2002; Paolo Moretti et al. 1994; P Moretti and Shore 2001; L N Rusche, Kirchmaier, and Rine 2002; Triolo and Sternglanz 1996). At telomeres, interactions of Sir3 and Sir2/Sir4 with Rap1p arrays are sufficient to

nucleate silencing (Marcand et al. 1996). Additional interaction of Sir4 with the Ku heterodimers reinforces silencing (Tsukamoto, Kato, and Ikeda 1997; Roy et al. 2004)

Once nucleated, the activity of Sir2, a conserved NAD⁺ dependent histone deacetylase, creates favorable binding sites for Sir3. The bromo-adjacent-homology (BAH) domain of Sir3 drives the selectivity of Sir3 association with nucleosomes. Crystal studies and genetics evidences demonstrated that Sir3 preferentially binds nucleosomes unmodified at H3K79 and at H4K16. Iterative cycles of histone modification and binding allow the self-propagation of the SIR complex on chromatin until a barrier is eventually reached (Grunstein and Gasser 2013; Gartenberg and Smith 2016).

Boundaries restrict silent domains at the mating type loci (David Donze et al. 1999; D. Donze and Kamakaka 2001). A tRNA confines the Sir complex to HMR (David Donze et al. 1999) while directional nucleation restricts HML silencing (Bi et al. 1999). In contrast subtelomeric silencing is rather constrained than restricted. The collective action of chromatin modifying enzymes implements chromatin states that potentially decrease Sir3 affinity for nucleosomes. In addition to the acetylation of H4K16 by the SAS-I complex, acetylation of histone H3 tails by Gcn5 and Elp3, methylation of H3K4 and H3K79 residues and H4K16ac dependent incorporation of the H2A.Z histone variant were all proposed to contribute to the instable equilibrium ruling Sir3 propagation at subtelomeres (Gartenberg and Smith 2016) .

In mutants lacking those enzymes the SIR complex propagates further away from the telomeres. However the respective contribution of each mechanism and what further limits silencing spreading in those mutants remains unknown. In addition numerous factors have been identified on the basis of their barrier properties using boundary assays, which consists in targeting candidate factors fused to a DNA binding domain between a silencer and a reporter gene, but their role *in vivo* remains to be explored (Oki et al 2004). This is the case for nuclear pore complex components or transcription factors.

A key parameter regulating heterochromatin dynamics is the concentration of silencing factors. In *S.pombe* increasing the dosage of the silencing factor Swi6, bypasses the need for the RNA interference machinery at centromeric regions (Tadeo et al. 2013). In *S.pombe* and *S.cerevisiae*,

increasing the level of Swi6 or Sir3 stabilizes variegation toward OFF states (Renauld et al. 1993a, Nakayma et al. 2000). Importantly, silencing factors also influence the spatial distribution of heterochromatin, *S.pombe* RNAi machinery and *S.cerevisiae* Sir complex being required for telomere clustering (Jia, Noma, and Grewal 2004; Guidi et al. 2015; Ruault et al. 2011a; Gotta et al. 1996). In budding yeast, SIR protein concentration is limiting silencing in wild type cells (Maillet et al. 1996, Renaud et al, 1993a). SIR proteins are unevenly distributed in the nucleus (Gotta et al, 1996). In cycling cells, the clustering of the 32 telomeres in discrete foci leads to the sequestration of SIR proteins at the nuclear periphery favoring SIR mediated repression in this subnuclear regions and preventing promiscuous repression elsewhere in the nucleus (Thompson et al. 1994; Maillet et al. 1996; Marcand et al. 1996 ; Andrulis et al, 1997 ; Taddei et al, 2009).

Furthermore, increasing Sir3 dosage in budding yeast leads to further expansion of silent domains toward the chromosome core (Renauld et al. 1993a) and to a concomitant increase of telomere clustering (Ruault et al., 2011). However, the dose-dependency of heterochromatin propagation is largely unknown. In this work we explore the consequences of controlled increases in Sir protein dosage genome wide. We show that saturating the levels of silencing effectors unveils the maximal extend of heterochromatin domains revealing discrete subtelomeric domains. Analysis of published data set in combination with ours revealed that these domains are characterized by the absence of H3 tri-methylation at lysine 4, 36 and 79 and the presence of H2A phosphorylation. Our study demonstrates that histone marks are a major factor restraining the spread of heterochromatin. In particular, our results points to a particular role of the tri-methylation of H3K79 in genome protection against silencing.

Results

Dose-dependency of telomere clustering and Sir3 spreading upon Sir3 overexpression.

To systemically examine the impact of high doses of Sir3 on the genome, we compared strains stably over-expressing *SIR3* at different levels. To minimize the cell-to-cell variability of Sir3

amounts, we replaced *SIR3* endogenous promoter by three different constructs (pADH, pTEF and pGPD) (Janke et al. 2004). We measured Sir3p levels by western blot (FigS1A), and by fluorescence quantification at the single cell level (Fig1A) in live cells expressing Sir3-GFP (FigS1B). Using this system, we measured telomere clustering and genome wide binding of Sir3p in strains WT producing 1x (WT), 9x (pADH-SIR3), 16x (pTEF-SIR3), and 29x (pGPD-SIR3) Sir3p, with minor overlap in Sir3p levels between strains. Potential confounding effects due to differences in the cell cycle were excluded as FACS profiles of WT and pGPD-SIR3 strains are largely similar (SupFigS1D).

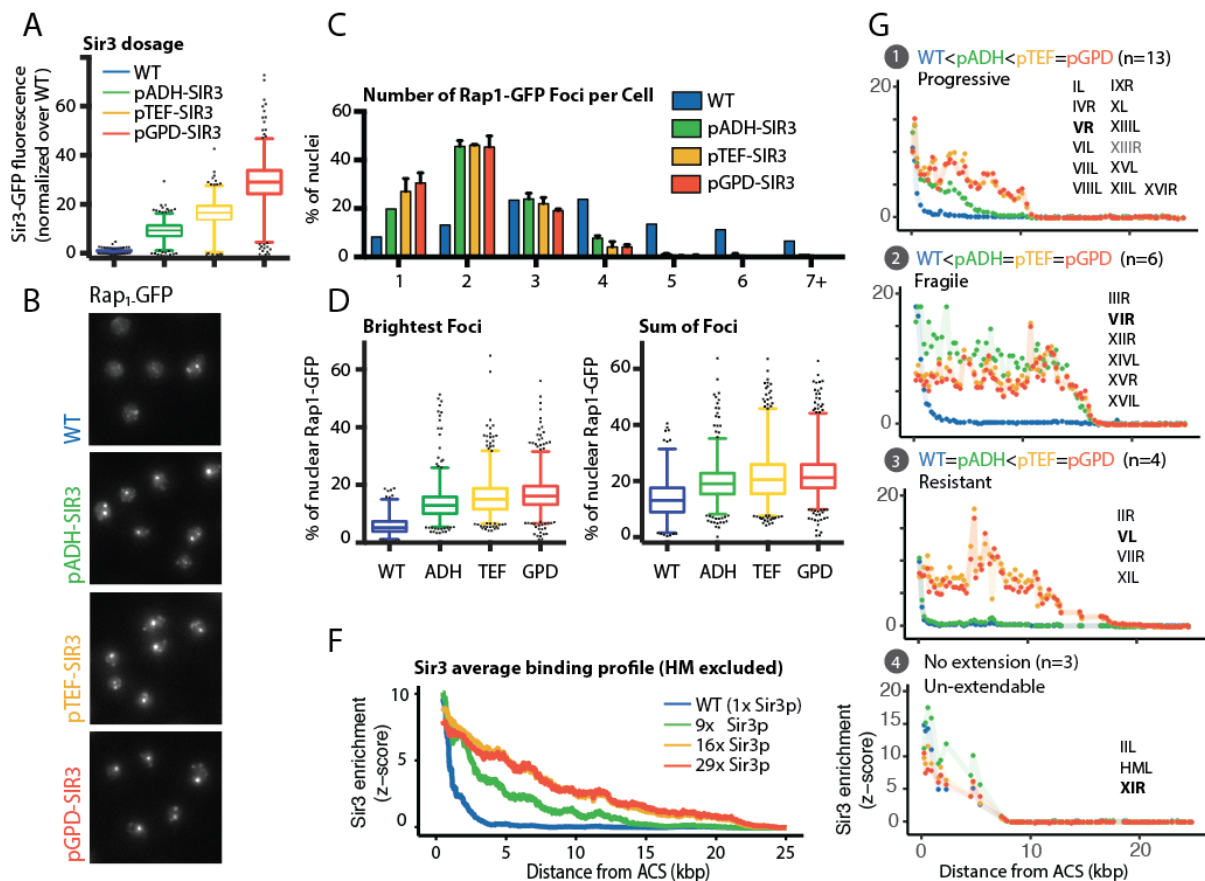


Figure 1 Increasing Sir3 dosage leads to telomere clustering and SIR spreading saturation. (A) Quantification of Sir3 levels by integration of Sir3-GFP signal in strains expressing SIR3-GFP (B) Rap1-GFP foci grouping in strain differing for Sir3 levels. Cells were grown in YPD overnight, diluted to OD_{600nm}= 0.2, and imaged at OD_{600nm}= 1. (C) Quantification of Rap1-GFP foci distribution in images from A. (D) left: Distribution of Rap1-GFP signal attributed to the brightest foci in each nucleus. (E) Distribution of the relative amount of Rap1 measured within foci relative to total nuclear Rap1 signal. (F) ChIP-chip against Sir3 was carried in strains from A. Moving average of Sir3 binding (block = 1000 bp, window = 10) at telomeres (with the exception of TELIIL and TELIIR which contain HM loci) as a function of distance from telomeric X core sequence. Enrichment is measured as the standardized IP over Input (See mat meth). (G) Stereotypical

examples of Sir3 binding in function of Sir3 dosage, numbers correspond to the subtelomeres constituting each group, in bold is the subtelomere plotted.

We monitored telomere foci in function of Sir3 concentration by live microscopy imaging of Rap1-GFP (Fig1B). In the range of concentration probed, we observed that telomere clustering increase non-linearly in function of Sir3 levels.

Consistent with previous studies telomeres clustered into 2 to 6 foci in WT cells. Above 9x, changes in Sir3 concentration only had subtle effect and telomeres clustered within 1 to 3 foci, a configuration compatible with our previous observations (Fig1C). We reasoned that despite changes in the distribution of foci, the total fluorescence corresponding to Rap1-GFP foci would be conserved if all the telomeres were detected in wild type cells. To test this hypothesis, we summed the fluorescence within foci for each nucleus independently of the number of foci (Fig1C). We found that the proportion of nuclear Rap1-GFP increases from 13.6% in WT cells to a maximum of 21.6-22.2 % for Sir3 dosage above 16x. We did not detect significant differences between the proportions of Rap1 in foci in cells overexpressing 16x or 29x Sir3p (Holm-Sidak's multiple comparisons test). Thus at a given time only a fraction of telomeres are present in a telomeric foci visible by fluorescent microscopy. On average, the brightest focus of a wild type cell concentrated 5.6% of nuclear Rap1, significantly (p -value <0.0001 , Holm-Sidak's multiple comparisons test) less than the brightest focus of a 9x, 16x or 29x Sir3 typical nuclei, which respectively accounts for 13.6, 16 and 16.8% of nuclear Rap1. The last differences being detected with low significance (p -value 0.0038).

Increase in foci intensity thus parallels the decrease in number of foci, which is consistent with an increase in telomere grouping in cells overexpressing Sir3. Telomere clustering reaches a maximum for Sir3 levels superior to 9 and inferior to 16 fold over WT.

In parallel, we probed genome-wide Sir3 binding in function of its dosage by chromatin immuno-precipitation analysed on chip (ChIP-Chip). In wild type cells, Sir3 binding was detected until ~5 kb away from the telomeric repeats on average, consistent with previous studies. Upon 9 fold increase of Sir3p, Sir3 bound domains covered on average ~15 kb and up to ~22 kb upon

16 or 29 fold overexpression (Fig1D). Subtelomeric domains covered by Sir3 were undistinguishable (pearson correlation 0.98, p-val < 1.e-16) in 16x[Sir3] and 29x[Sir3] strains (Fig1F). Sir3 binding domains expansion upon increase of dosage thus reaches saturation for Sir3 levels superior to 9x and inferior to 16x. Supporting this interpretation, we observed that the level of nuclear background Sir3-GFP fluorescence is almost doubled in 29x strains compared to 16x strains (Sup FigS1C).

Heterogeneous expansion of subtelomeric domains upon increase of Sir3 expression

Interestingly individual telomeres showed very different behaviours in response to Sir3 dosage elevation. We established four stereotypical categories (Fig1F):

Consistent with the average binding profiles, the largest group (12/26) is composed of subtelomeres at which Sir3 spreading increased progressively with Sir3 dosage, reaching saturation at 16X. Two other groups were distinguished on the basis of their response to the first increase in Sir3 levels. Sir3 binding directly reached its saturation state in a second group (6/26), whereas it only changed upon 16x increase at four subtelomeres (4/26). Lastly, 3 subtelomeres were apparently insensitive to Sir3 levels changes.

Irrespective of the different impact of changes in Sir3 concentration depending on the subtelomere, Sir3 occupancy at saturation covered diverse domain lengths (ranging from 7kb to 25kb -HM excluded-), independently of chromosomal arm length or middle repeats content. Sir3 spreading ends right before essential genes at three subtelomeres (*RPN12* at TELVIR, *ERO1* at TELXIIL and *GAB1* at TELXIIR). Lastly, we found only few examples of euchromatic nucleation sites that were revealed upon Sir3 overexpression (FigS1E).

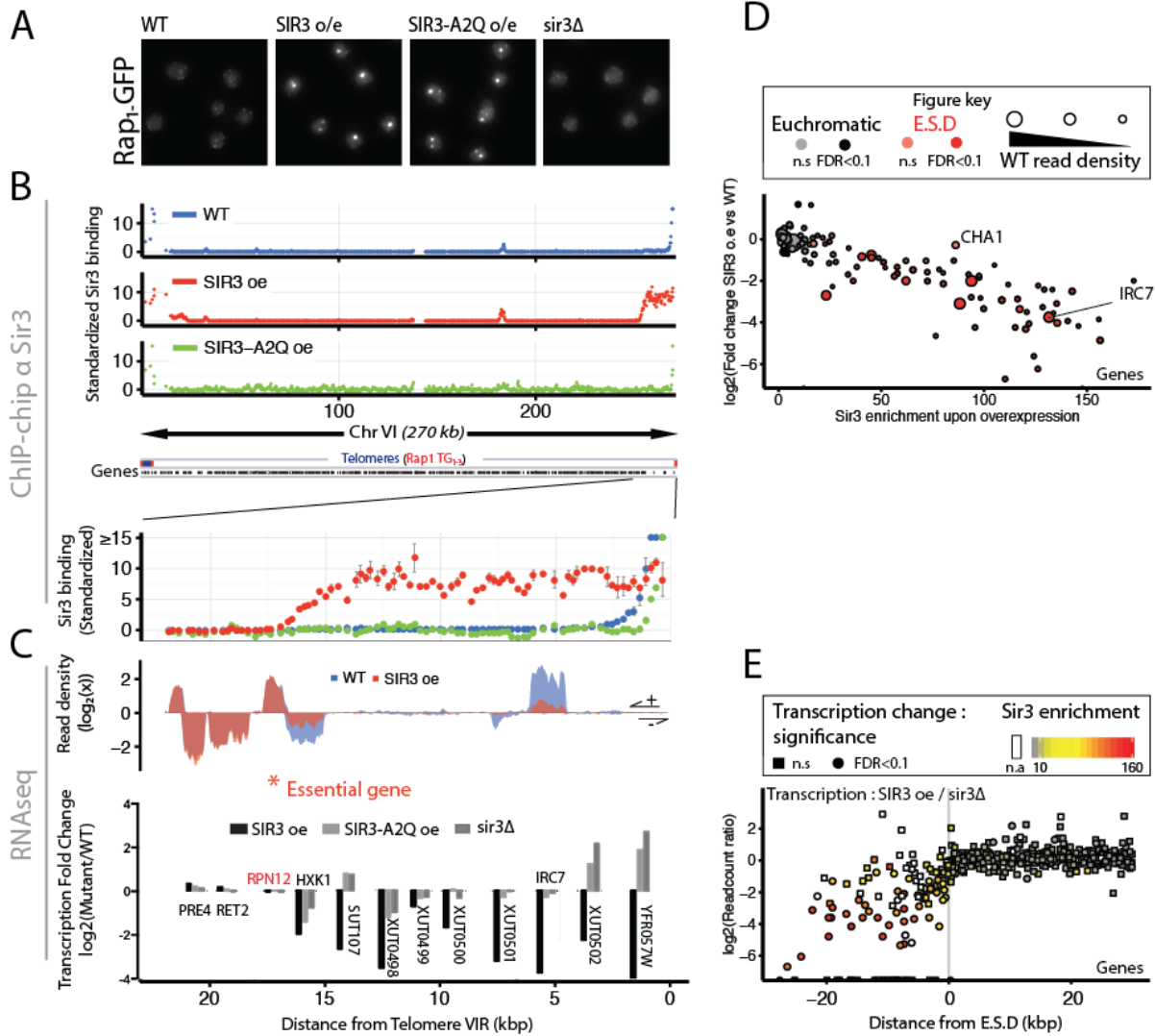


Figure 2 Sir3 extended domains are silenced and restricted to subtelomeres. (A) Representative Rap1-GFP images of exponentially growing strains different for Sir3 amount or expressing the SIR3-A2Q point mutant. (B) Chromosome wide binding of Sir3 in the same strains as in A and blow-up on subtelomere VIR. Enrichment is measured as the standardized IP over Input and scale is thresholded at 15 for visualization purposes. (C) Total RNAseq read density and corresponding transcriptional fold change along subtelomere VIR in indicated exponentially growing ($OD \sim 1$) strains. (D) Sir3 binding and corresponding transcription changes of subtelomeric genes (Distance from chromosome end < 50 kb) upon overexpression of SIR3. Color code indicates if a gene is annotated as within E.S.D (see math et meth) and shade indicate significance ($FDR < 0.1$) of the detected changes. Read density in WT cells is proportional the disk area. (E) Transcription changes in function the distance from the end of silent domains. Symbols indicate significance and color code indicate Sir3 enrichment averaged over gene bodies.

Thus, Sir3 propagation reaches saturation for different amount of Sir3 depending on which subtelomere is probed. Overexpression of *SIR3* creates continuous Sir bound domains of heterogeneous extents at individual telomeres.

Telomere hyperclustering has a minor impact on transcription

It was shown that increasing Sir3 levels extend subtelomeric silent domains by genetic assays (Renault et al. 1993, Strahl-Bolsinger et al. 1997; Ruault et al, 2011) and telomere clustering by microscopy (Ruault et al, 2011). However, the direct impact of telomere clustering on transcription is unknown. To address this question, we took advantage of a strain overexpressing the Sir3-A2Q point mutation. This mutant is impaired for gene silencing yet competent for telomere clustering (Ruault et al 2011, Wang et al, 2004) (figure 2A). We probed Sir3-A2Q binding and confirmed that the binding of this mutant is restricted to nucleation sites (Figure 2B). Next we conducted transcriptome analysis by RNAseq to compare *pGPD-sir3-A2Q* to *pGPD-SIR3* strains, to uncouple the effects mediated by Sir3 silencing function from the potential impact of telomere clustering.

The cells overexpressing Sir3-A2Q exhibited a transcriptional signature consistent with a partial loss of *HM* silencing, the amplitude of the pseudo-diploid transcriptional response being smaller than in *sir3Δ* (FigS2C). This last observation demonstrates that this mutant, when overexpressed can achieve partial silencing at the *HM* even if its spreading capacities are impaired. In agreement with the inability of Sir3-A2Q to spread on chromatin, we observed almost no difference in the transcription of subtelomeric genes when we compared the *sir3Δ* strain to the *GPD-Sir3-A2Q strain* (FigS2E). However, we observed two exceptions, *HXK1* and *PHO89*, that were down-regulated upon Sir3-A2Q overexpression. The down regulation of *HXK1* (Fig2C) might reflect the fact that the perinuclear localization of this gene contributes to its optimal expression (Taddei et al, 2006). Perinuclear localization of *HXK1* is probably lost in a *GPD-Sir3-A2Q* strain since telomeres relocalize in the nuclear interior upon *sir3A2Q* overexpression (Ruault et al.2011). Thus globally neither telomere clustering nor internal localization of telomeres impact the basal transcriptional status of subtelomeric genes in strains overexpressing the *sir3-A2Q* allele.

Extended heterochromatin domains are functional for silencing

In contrast, overexpression of Sir3 strongly repressed subtelomeric transcription. The extension of Sir3-bound domains upon Sir3 overexpression systematically led to the repression of underlying transcripts, independently of their coding status as exemplified for the right subtelomere of chromosome VI (figure 2C) and genome-wide (figure 2D). At the 26 subtelomeres included in our study, extended silent domains (ESD) included 100 genes that were not bound by Sir3 in WT cells. Differential expression analysis indicated that transcripts affected by Sir3 binding were all repressed (*see material and methods*).

We observed that the logarithm of transcriptional repression was linearly proportional to Sir3 binding signal, reflecting the absence of escapers from silencing and the good agreement between RNA-seq and ChIP-chip results. Interestingly, repression was largely independent of initial transcript level (Figure 2D). To gain access to the expression of repeated gene families we carried out a second analysis including reads mapping to multiple loci (*see mat et meth*). It appears that entire gene families characteristic of subtelomeres and Y' elements are repressed upon Sir3 overexpression (FigS2B S2F) suggesting that the portion of subtelomeres devoid of chip probes is collectively silenced.

Transcriptional activity does not account for the extent of Sir3 spreading

We next aimed to identify the mechanisms that could limit the extent of Sir3 spreading. To test directly whether the limit to Sir3 spreading is dependent on the distance covered by the SIRs, we compared Sir3 spreading at the wild type telomere VIII versus a 15 kb truncated version. Strikingly, in both cases Sir3 binding ended within the *HXK2* promoter (figure 3F), with a somewhat sharper decline rate in the truncated strains. This comparison indicated that the determinants of Sir3 bound domain ends are either defined relative to the core of the chromosome or is a local feature.

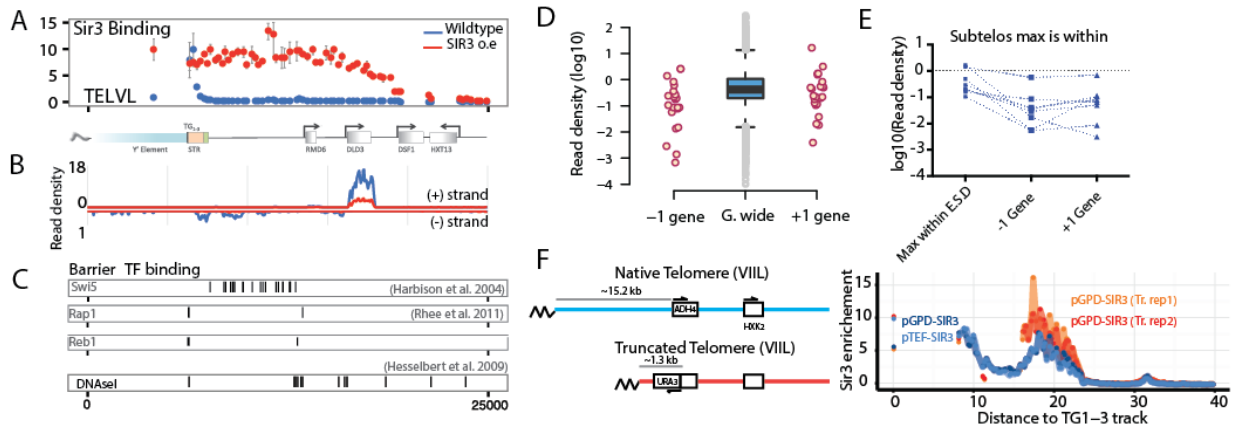


Figure 3 End of extended silent domains is defined locally and independently of transcriptional activity (A) Sir3 binding at TELVL in WT and Sir3 overexpressing (pGPD-SIR3) strains, X-axis coordinate is shared with B and C. (B) Corresponding read density along plus (upper curve) and minus (lower curve) strands. (C) Transcription factor binding and DNaseI hypersensitive sites along TELVL (D) Read density of genes located before and after the end of extended silent domains compared to genome wide distribution (central boxplot) (E) Same as F exemplifying the 7 subteleromeres at which a gene within E.S.D show larger transcript amount than the genes located at the end of the domain. (F) Sir3 binding at native and truncated TELVIII, x coordinates correspond the native telomere VIII

Focusing on silent domains ends, we quantified the slope of Sir3 binding profile in the strains overexpressing SIR3 at each subteleromere whenever it was possible (24/32 subteleromeres). We observed that the slope at the end of a silent domain is not correlated to the distance from the telomere (*i.e* nucleation point) and found no correlation with the groups defined based on the response to Sir3 dosage changes (FigS3A).

We conclude that when the dose of silencing factor is not limiting the spread of heterochromatin, the delineation of the silent domain does not depend on the distance from the nucleation site. Our results rather suggest that extended silent domains are defined relative to the core chromosome.

Most genes covered by Sir3 upon overexpression are lowly transcribed in wild-type cells, which could argue that Sir3 spreading is limited by transcription. However, this not the case as we could find highly expressed genes lying within ESD as exemplified by IRC7 (Fig.2A) and DLD3 (Fig.3A). Both genes belong to the decile of most expressed genes and to the first quartile of most frequently transcribed genes in wild-type cells (Figure 3D) (Pelecano et al 2010). Despite

these high transcription rates, these two genes are repressed upon Sir3 binding, indicating that transcriptional activity per se is not sufficient to stop Sir3 spreading. Accordingly, at 7 subtelomeres at least one gene within the ESD had higher read density than the gene adjacent to the ESD (Figure 3F). While the limitation of Sir3 spreading could be the consequence of the counter selection of cells silencing essential genes, we do not favour this hypothesis. It is noteworthy that no essential genes were found within ESD and that 3 ESDs ends are contiguous to three essential genes. However, we did not detect significant decrease in mRNA levels for these genes upon Sir3 overexpression suggesting that they are protected against Sir3 spreading.

Punctual binding sites of barrier factors are not efficient barriers to silencing

As mentioned above, we observed a 3-fold (2.96) repression of Y' elements upon Sir3 overexpression (FigS2F), which implies that the barrier effect mediated by Tbf1 and Reb1 (Fourrel 1999) does not hold, at least at a fraction of Y' containing telomeres or in a fraction of the population. To explore the possibility that other DNA sequence specific barrier elements are involved in the confinement of Sir3 within subtelomeres, we listed all the transcription factors that have been proposed to have a barrier activity, and concatenated binding data when available, ending up studying the binding of 10 transcription factors (Adr1, Gcn4, Rgt1, Hsf1, Sfp1, Reb1, Abf1, Leu3, Swi5: Harbison et al. 2004, Rap1 : Rhee et al. 2011 ,Tbf1 Preti et al. 2010).

We identified DNA-sequence specific elements, reported to have barrier activity, in the first genes before or after the end of the E.S.D at 12 subtelomeres (FigS3); However, each of these factors were also found at other sites within the E.S.D (Fig3C) indicating that those factors alone are not sufficient to limit the propagation of the SIR complex.

We found known barrier elements flanking Sir3 bound domains at the three subtelomeres that were categorized as insensitive to Sir3 levels (group4). Silent domain invariably remains constrained by the Leucine tRNA at subtelomere IIL. A previously identified barrier sequence homologous to the left barrier of HML (Xin Bi Genetics 2002) lies at the end of subtelomere XIR Sir3 binding domain, while the right end of subtelIIIL silent domains is irresponsive to Sir3 dosage, likely as a consequence of the directional properties of the I silencer (Figure S3B).

Thus, with the exception of these three subtelomeres, we could not identify the factor blocking the extension of silent domains.

Sir2 activity is a minor limitant of SIR spreading

A possible limitation to silent chromatin spreading is the capacity of the SIR complex to deacetylate H4K16 residues. We reasoned that this might be particularly relevant when Sir3 is not limiting the propagation of the SIR complex.

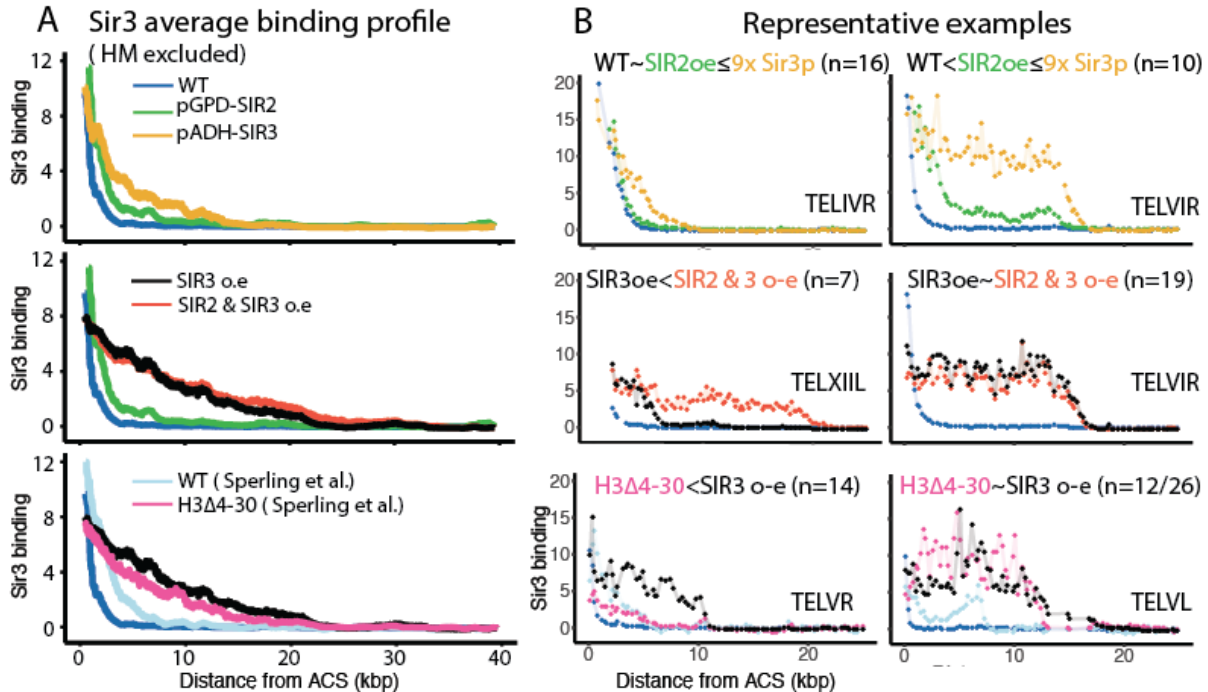


Figure 4 H3 & H4 acetylation is a major buffer of silent domain extension (A) Moving average of Sir3 binding at telomeres (with the exception of TELIIL and TELIIR which contain HM loci) as in fig1F, in the indicated genotypes. (B) Representative examples of Sir3 binding in the indicated genotypes. Qualitative comparison of Sir3 spreading between conditions is indicated as legend with the number of subtelomere attributed to this stereotypical category.

Consequently, we probed the effect of increasing Sir2 dosage on Sir3 spreading. We monitored the genome wide occupancy of Sir3 in strains overexpressing Sir2 and in strains co-overexpressing Sir2 and Sir3. On average Sir3 binding profile progresses toward centromeres

upon Sir2 overexpression, but to lesser extent than upon 9x Sir3 overexpression (Figure 4A). Sir3 bound domains extended at a subset of 12 subtelomeres, 11 of which were also extended upon mild 9x Sir3 overexpression (11/12). However, at these subtelomeres Sir3 enrichments were lower upon Sir2 overexpression than upon Sir3 overexpression (Fig4B). We note that whereas the overexpression of Sir3 had no apparent effect on its propagation at the left border of the rDNA array, Sir3 binding was extended upon overexpression of Sir2. At this locus, a tRNA restricts the binding of the SIR or the RENT complex, and protects the essential acetyl-Coa synthetase gene *ACS2* from deleterious silencing (Biswas et al. 2009).

We next turned our attention to strains co-overexpressing Sir2 and Sir3. To our surprise, in most cases overexpression of Sir2 had no effect (20/26). Only in a minority of cases Sir3 spreading was increased in strain co-overexpressing Sir2 and Sir3 (Figure 4B). In the latter cases E.S.D remained devoid of essential and tRNA genes.

We conclude that at a majority of subtelomeres, Sir2 activity is not limiting the spread of heterochromatin even when sir3 is over-abundant. As Sir3 bound domains were either unchanged or extended upon Sir2 and Sir3 co-overexpression, we deduced that Sir3 and Sir4 were not limiting the extension of silent domains in the strain overexpressing Sir3. Collectively, this lent credence to the notion that we reached a situation in which spreading is likely limited by local chromosomal features, leading to the blocking of the extension phenomenon.

Sir3 overexpression mimics the absence of H3 tail acetylation at a subset of subtelomeres

Albeit the mechanistic details are still obscure, several studies point to a role of H3 tail acetylation in limiting silencing (Thompson et al. 1994) (Kristjuhan et al. 2003) (Sperling et al. 2009). In detail, Sir3 bound domains extend at half of subtelomeres in a mutant expressing a histone with truncated tail, H3 Δ 4-30. Consistent with a prominent role of acetylation in this process, mutation of the 5 acetylatable lysines located on H3 tail (K9, 14, 18, 23 and 27) led to a similar extension of Sir3 bound domains (Sperling and Grunstein 2009). We undertook to compare the effect of Sir2/3 overexpression to the effect of disrupting H3 acetylation on Sir3 binding using

published data(Sperling and Grunstein 2009) and to compare telomere clustering in those mutants.

First we noticed that the subtelomeres that were insensitive to Sir3 dosage were also insensitive to changes in H3 tail acetylation. Globally, we found that when effective, disruption of H3 tail acetylation had a greater effect on Sir3 spreading than Sir2 overexpression (Fig4A). Among the 18 subtelomeres at which Sir3 binding is extended in the H3Δ4-30 mutant, most had Sir3 binding profiles similar and in some cases identical to the one obtained at a given level of Sir3 overexpression: for example, 9x for the IL, 16x for the VL (Fig 4B). However, contrary to strains overexpressing Sir3, telomere clustering was not affected in H3Δ4-30 mutants (FigS4).

Overall, those results demonstrate that Sir2 activity is a minor limitation of SIR complex propagation relative to H3 tail acetylation. Furthermore, comparing H3 tail mutants to strains overexpressing Sir3 led us to conclude that extension of Sir3p binding in subtelomeric regions is not sufficient to promote the clustering of telomeres. Lastly the similarities of Sir3 bound domains in H3 tails mutants and in strains overexpressing *SIR3* suggest that the domains defined by overexpression of Sir3 likely exist independently of Sir3 dosage.

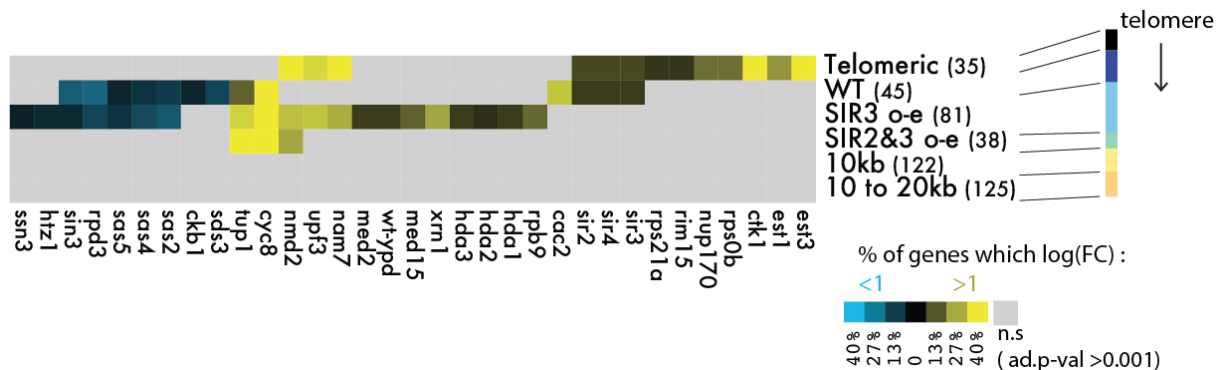


Figure 5 Localized effects of mutations affecting subtelomeric transcription. Grey areas are zone in which no enrichment was detected. Color code indicate the proportion of genes which fold change is > 2. The different subtelomeric subdomains are defined according to Sir3 binding. The number of genes in each domains is into brackets.

Identification of subtelomeric sub-domains

Based on our description of the different Sir3 binding domains unveiled by Sir3 or Sir2 and Sir3 overexpression, we searched for factors having a localized effect within these subtelomeric subdomains. To this end, we analysed a compendium of over 700 transcription profiles (Kemmeren et al. 2014) for mutants having a significant impact on the subtelomeric domains previously defined. We classified subtelomeric genes into four different groups. The genes or pseudo-genes associated to middle repeat elements constitute the first group. The remaining three groups are the genes bound by the Sirs in WT cells, the genes to which Sir3 has access upon saturated overexpression and the genes to which Sir3 binds following co-overexpression of Sir2 and Sir3. To search for potential factors having a localized effect at the domains flanking the one we described, we also consider the group of genes located within 10kb from the end of Sir3 accessible subtelomeric domains (SASD) and located between 10 and 20kb from SASD ends. For each mutant we tested if the proportion of genes up or downregulated ($|\log_2(\text{FC})| > 2$) in a given subtelomeric domain is higher than expected by chance, considering the effect of the mutation on the genome. The main outcome of this analysis is the identification of genes which mutation only affects particular subtelomeric subdomains (Figure5). With this approach, the transcriptional outcome of sir2, sir3 or sir4 deletion were as expected restricted to 'telomeric' and 'WT Sir3 bound domains. Twenty other mutants have a significant impact on the expression of these two domain, including mutants previously known to affect subtelomeric transcription such as telomerase components, the nucleoporin NUP170 (Van De Vosse et al. 2013), the mediator complex tails proteins Med2 and Gal11 (Peng and Zhou 2012; Lenstra et al. 2011), the hda1/2/3 complex, components of the non-sense mediated mRNA decay pathway and the repressors Tup1/Cyc8. While the direct contribution of ribosomal proteins to telomere clustering has been reported, we found that rps0b and rps21 mutants specifically affect transcription of the telomeric domain. More importantly, we observed that gene silencing due to Sir spreading in rpd3 or sas2/4/5 mutants does not extent further than the domains observed upon overexpression of Sir3 implying that those genes are likely not responsible for the restriction of Sir3 upon overexpression. At last, no mutant had a significant localized effect outside of the domains defined by Sir3 overexpression. Even if the dataset chosen does not

cover all gene deletions, this last observation indicate that subtelomeric position effects, in their broad acceptance, are likely absents outside of the domains defined.

A specific chromatin landscape pre-exist in Sir3 accessible domains

To seek for potential chromatin determinants of silent domain propagation and limitation, we analyzed the genome wide distribution of 26 histone marks or variants (Weiner2015). We first computed the correlation between Sir3 binding signal and histone modifications at subtelomeres. Consistent with previous results, we recovered the anti-correlation expected between Sir3 binding and H4K16 acetylation in wild type cells. Interestingly, we observed that upon overexpression, Sir3 binding signal is better correlated with histone H3 methylation and histone H2A phosphorylation (Figure6A &B). This implies that probing Sir3 binding upon overexpression somehow reveals the subtelomeric chromatin landscape of wild type cells.

Next, we probed histone marks distribution within the different ChIP-defined subdomains previously described. Sir bound nucleosomes were depleted of most histone marks, with the exception of H4R3 methylation and H2A phosphorylation, which were enriched within silent domains, as expected. A second category of marks were depleted from silent domains but enriched within the SASD and at background levels past SASD. Within SASD, the average nucleosome is hyper-acetylated at H3K27 and H4K5,8,12 and mono methylated at H3K79. In addition, we observed that the histone variant H2A.Z is enriched with SASD (Figure 6B, C & FigS6).

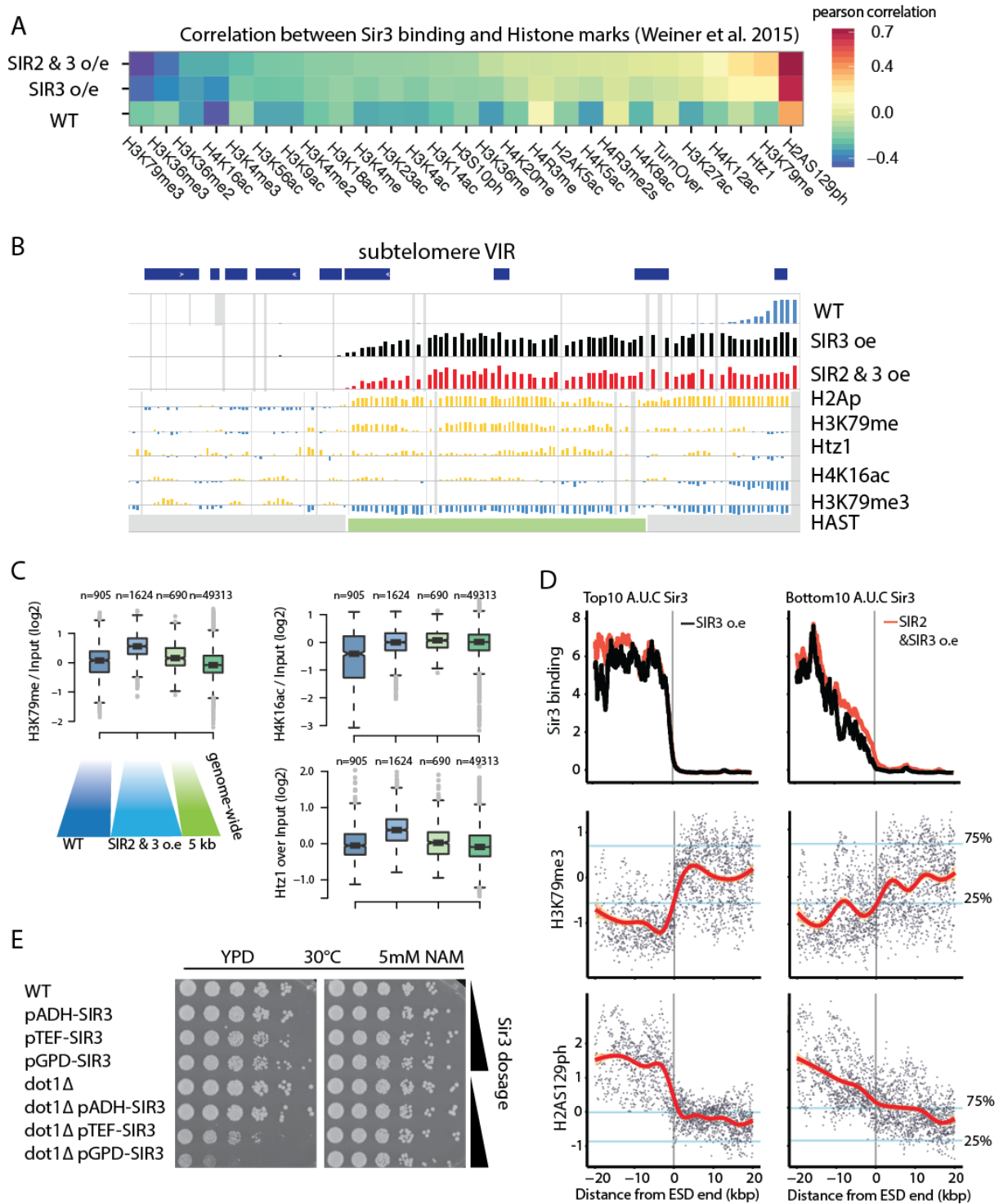


Figure 6 Subtelomeric chromatin landscape structuration correlates with silent domain expandability and shed light on the protective role of H3K79 methylation (A) Pearson correlation matrix between Sir3 binding and histone marks, SIR3 oe corresponds to yAT1254 and SIR2 & 3 to yAT1668. (B) Genome browser visualization of Sir3 binding in WT, pGPD-SIR3 or pGPD-SIR2 pGPD-SIR3 strains and selected histone modification or variants (from Weiner et al. 2015) in WT strains at TELVIR. Border of H.A.S.T domains were

obtained from Robyr et al. 2002. (C) Distribution of selected histone marks relative to H3 (data from weiner et al.2015) along wild type silenced domains and within the contiguous subtelomeric domains accessible to Sir3 upon overexpression. As a control the distribution of those marks within the 5 kb contiguous to the end of extended silent domains as well as the genome wide distribution of those marks is shown. (D) Moving average of Sir3 binding at telomeres. The top and bottom 10 telomere with regards to Sir3 signal in strains overexpressing Sir2 and Sir3 were plot separately. The two histone marks the best associated (either positively or negatively correlated) are shown in the same groups. Genome wide lower and higher quartiles for each mark are indicated by blue line. Red line correspond to the local smoothing of histone modification data (E) Dot assay to probe viability of dot1 mutants upon overexpression of Sir3. Cells were constantly grown in presence of 5mM Nam prior to this assay. Cells were grown overnight, and 0.5 O.D of cells were plated in 5x serial dilution on YPD or YPD 5mM NAM.

In contrast, we observed that the depletion of H3K4, H3K36 and H3K79 tri-methylation extends until SASD ends. A notable exception was H2A phosphorylation which enrichment is still significant within the 5kb flanking SASD ends. We reasoned that the longer intergenes present within subtelomeres might bias our analysis, artificially leading to the depletion of marks associated to gene bodies. To control for this potential artifact source, we conducted a second analysis, separating promoter nucleosomes (-3, -2, -1) from gene body nucleosomes and obtained essentially the same results (not-shown).

In a complementary approach, we focused on Sir3 binding domains ends. We classified each subtelomere according to the area under the curve (AUC) corresponding to the logistic-like fit of Sir3 binding signal upon co-overexpression of Sir2 and Sir3 (See mat & meth). It appears that at the ten telomeres showing the highest AUC, several histone marks display sharp changes. This is especially true for H3K79me3, H3K36me3 and H2AS129P. In contrast, the bottom ten subtelomeres in terms of Sir3 AUC show rather smooth changes (Fig 6D). Thus, upon overexpression Sir3 extends on a pre-existing chromatin landscape associated with specific histone modifications (low levels of H3K79me3 and H3K36me3 and high levels of H2AP).

H3K79 methylation is essential to protect euchromatin from the spread of silencing

Our results demonstrate that when extendable, the end of WT silent domains are generally located within a subtelomeric area devoid of H3K79me3 and enriched for H3K79me1. In

contrast, SASD ends coincide with H3K79 tri-methylation discontinuity zone. As H3K79 methylation has been shown to impair Sir3 binding in vitro (Altaf et al. 2007; Wang 2013), this mark appeared as a good candidate to stop Sir3 spreading when overexpressed.

To test this hypothesis, we overexpressed Sir3 in this absence of Dot1, the only methyltransferase responsible for H3K79 methylation, we found that the *GPD-SIR3 dot1Δ* strains are extremely sick and are generating suppressors upon streaking. To avoid any artifact due to these potential escapers, we designed a transformation experiment in which the *dot1Δ* mutant is transformed with the *GPD-SIR3* construct in the presence of 5mM NAM that efficiently inhibits silencing. After selection of positive clones, we assessed the growth of those mutants on medium without NAM, allowing initiation of silencing in the presence of large excess of Sir3, similarly to what has been done in (Osborne, Dudoit, and Rine 2009). Our results showed that Dot1 is essential to sustain viability when Sir3 is overexpressed (Fig 6E). We used the same method to test the requirement of other histone modifiers in the context of increased Sir3 dosage. In contrast to Dot1, Set1 or Set2, which deposit H3K4 and H3K36 methylation, marks, or the histone de-acetylase Rpd3 were dispensable for viability in presence of high Sir3 dosage (SupFig6). Thus among the chromatin modification best anti-correlated with Sir3 binding, only H3K79 methylation appears essential to restrict the ectopic spread of silencing. Interestingly, the phenotype of *dot1* mutants overexpressing Sir3 was only appreciable at Sir3 amounts above 9x. In those cases, lethality of *dot1Δ SIR3* o/e was fully rescued by 5mM NAM treatment (Fig6E). Overexpression of the *Sir3-A2Q* point mutant in a *dot1Δ* strain was viable and leads to the hyperclustering of telomeres, demonstrating that the lethality of *dot1* mutants overexpressing *SIR3* is not due to the clustering of telomeres (Sup Fig6). In addition, co-overexpression of *DOT1* and *SIR3* leads to loss of silencing, showing that H3K79 methylation prevails on Sir3 binding (Sup fig6C).

Intrigued by the observation that mono and tri-methylation state of H3K79 showed opposite behaviors, we hypothesized that the two methylation state might have different functions regarding silencing. *bre1* mutants are lacking H3K4me3, H3K79me3 methylation and have increased H3K79me1 (Frederiks et al. 2008). As set1 mutants overexpressing Sir3 were viable we reasoned that potential effect of *bre1* deletion would likely come from H3K79 methylation

changes. Interestingly, while *bre1* mutants over-expressing Sir3 29x have subtle growth defect when grown at 30°C, they do not sustain viability at 37°C. As for *dot1*, *bre1* lethality is rescued by 5mM NAM treatment, suggesting that the absence of H3K79me3 allows deleterious transcriptional silencing, with acute effects at 37°C. Our results are consistent with a distinct role of the different methylation of H3K79 in silencing restriction.

Discussion

The Sir complex has been a model for chromatin complex propagation and gene silencing for decades. Pioneer studies demonstrated that increasing the dose of Sir3 extends silenced domains at subtelomeres (Renauld et al. 1993b; Pryde and Louis 1999), a property common to several heterochromatin complexes. However there has been controversy on the generality of this finding at natural telomeres (Pryde and Louis 1999), and the details of this process along with its link with telomere clustering (Ruault et al. 2011b) remain unclear. Here we systematically studied the impact of increasing Sir2 and Sir3 dosage on the propagation of the SIR complex, on the clustering of telomeres and on genome wide transcription.

Gradual overexpression of Sir3 revealed that the increase in telomere clustering and the concomitant spreading of Sir3 over subtelomeres reaches saturation for Sir3 levels between 9 and 16x. Surprisingly the responses to increase in Sir3 levels were not continuous at all subtelomeres as Sir3 spreading was only affected above a certain threshold of concentration at some subtelomeres. At 29x Sir3, extended silent domains covered at least an additional 226 kb, associated with the repression of a hundred of genes. However, while most telomeres are clustered in those conditions, the spreading of Sir3 along subtelomeres varied greatly depending on the subtelomere probed. At few subtelomeres silent domains are already constrained by punctual elements in wild type cells while at others the extension observed varied up to 30 kb this extent being largely independent of middle repeat elements or chromosomal arm length.

Irrespective of the extent of the subtelomeric domain covered by Sir3 we observed that the relationship linking Sir3 binding to transcriptional repression is largely independent of the gene or subtelomere under consideration. This suggests that silencing efficiency is largely dictated by the ability of Sir3 to associate with chromatin. Accordingly, the domains covered by Sir3 upon overexpression shared similar chromatin marks suggesting that the chromatin landscape is the main determinant of maximal Sir3 spreading extension.

Our data indicate that the methylation of H3K79 by Dot1 is required for viability when Sir3 is present in large excess. Our work determines the maximal subtelomeric domains accessible to the Sir complex and uncovers a previously undetermined sub-structuration of subtelomeres. This approach is an original way of probing the extent of subtelomeric chromatin specificities.

Reaching the borders of subtelomeric silent domains

By overexpressing Sir3 at different levels, we studied the dose dependency of heterochromatin spreading at equilibrium. Our data are qualitatively different from previous genome wide studies that described Sir3 binding after 4 hours of induction (Radman-Livaja et al. 2011), out of equilibrium (Katan-Khaykovich and Struhl 2005). Co-overexpression of Sir2 and Sir3 demonstrated that Sir2 activity is not limiting Sir3 spreading at most subtelomeres. In addition, as few silent domains were extended in those conditions, we deduced that Sir4 was not limiting the extension of the subtelomeres unaffected by the additional overexpression of Sir2. As ESD were associated to different middle repeat elements and were of different length, we ruled out a potential effect of nucleation site, and a potential maximal size of silent domains that would be intrinsically regulated. Current model depicting the limitation of heterochromatin spreading oppose negotiable and fixed borders (Akatsuki Kimura and Horikoshi 2004). Interestingly, only fixed borders are expected to be independent of silencing factor concentration. Thus our results collectively suggest that the saturation of silent domain expansion likely correspond to the reaching of fixed borders along subtelomeres.

Different categories of Sir chromatin antagonism

At subtelomeres the extent of spreading of the Sir complex results from the contributions of the nucleation element strength, of chromatin modifying enzymes and Sir concentration (Gartenberg and Smith 2016; Grunstein and Gasser 2013). Ultimately, those parameters influence the affinity of Sir3 for chromatin. While most studies characterized in detail the effect of abrogating one or several chromatin modifying enzymes we chose to tune Sir3 concentration. At high concentrations of silencing factors we observed that Sir3 binding extends within regions that not only contain chromatin marks reported as antagonistic to its spreading but are even enriched for some of them such as the histone variant H2A.Z (Guillemette et al. 2005) and the mono-methylation of H3K79 (Altaf et al. 2007). Although this suggests the existence of fixed borders, our search for punctual border elements only retrieved convincing candidates at the three subtelomeres for which the extension was already limited in wild-type cells. Oppositely, we report that native binding sites occupied by transcription factors that block silencing when tethered to chromatin (Oki et al. 2004) are not efficient barriers to Sir3 spreading. This is perhaps not surprising for two reasons: first, there are precedent showing that multiple binding sites are required for efficient barrier effect (Bi, Yu, Sandmeier, and Zou 2004). Second, the binding strength of those transcription factors is likely different than the one of the GBD used to target candidate barrier factors. Consequently, our work indicates that histone tail acetylation; H2A.Z presence and binding of some transcription factors are likely buffering the spread of the SIR rather than blocking it.

End of extended silent domains: the specific role of Dot1

We observed that the end of extended silent domains coincide with a major histone mark transition zone, characterized by the abrupt enrichment of H3K4me₃, H3K36me₃ and H3K79me₃. While deletion of *SET1* or *SET2*, the genes encoding for the enzymes responsible for the two first marks had no impact on cell growth upon Sir3 overexpression, deletion of *DOT1* that encodes for the H3K79 methyltransferase was lethal in this condition.

Dot1 is a conserved enzyme, which enzymatic activity is distributive and leads to the mono, di or tri methylation of the lysine 79 of histone H3 (Stulemeijer et al. 2015). *In vitro*, binding of Sir3 to H3 peptides is abolished by mono, di and tri methylation of H3K79 (Altaf et al. 2007; Wang 2013). Using reconstituted nucleosomes, mono and di methylation reduces Sir3 affinity for nucleosome by a factor 5 for the tri methylation of H3K79 (Behrouzi et al, 2016; Martino et al, 2009). Studies of the crystal structure of Sir3-BAH domain bound to a nucleosomes predicts that methyl group contributes to decrease Sir3 affinity to nucleosome by decreasing the potential of K79 to form hydrogen bond with the BAH of Sir3 (Armache et al. 2011). In contrast, *in vivo* study suggest that all levels of H3K79me states are functionally equivalent (Frederiks et al. 2008).

Nevertheless they exhibit differences in distribution and localization. Di-methylation is present at gene promoters and over gene bodies and H3K79 tri-methylation is restricted to gene bodies. The two methylation states differ in at least two major ways. First the di methylation is cell cycle dependent whereas the tri methylation is not (Schulze et al. 2009), second, only tri-methylation of H3K79 requires that nucleosomes carry the H2BK123Ub modification (Nakanishi et al. 2009; Schulze et al. 2009). At last, tri-methylation of H3K79 is not correlated to transcription frequency and its removal only occurs through histone turnover (Schulze et al. 2009; Weiner et al. 2015). Upon overexpression, Sir3 spreads over domains enriched for H3K79me implying that *in vivo*, this mark is not an obstacle to Sir3 spreading, which is in agreement with the observation that Sir3 is bound to H3K79me histones at telomeres (T Kitada et al. 2012). H3K79 di-methylation being mutually exclusive with tri methylation (Schulze et al. 2009), we hypothesized that loss of tri-methylation is responsible for allowing deadly spreading of the Sir. We reasoned that if H3K79me3 specifically blocks Sir3 spreading, then a *bre1* mutant would have a similar phenotype as it completely lacks this modification (Nakanishi et al. 2009). However this is not exactly the case as this mutant is slow growing at 30°C yet non viable at 37°C. One interpretation of this observation is that the increased levels of H3K79me in *bre1* mutants (Frederiks et al. 2008) are sufficient to slow down silencing spreading at 30°C, but fail to prevent ectopic spread of silencing at 37°C, a condition known to strengthen silencing (Bi, Yu, Sandmeier, and Elizondo 2004). Our data thus indicate that the tri methylation of H3K79

observed at the boundary of extended silent domains block the spreading of Sir3 and thus protects euchromatin from heterochromatin.

Conversely co-overexpression of *DOT1* and *SIR3* led to loss of silencing. We propose that H3K79me3 mark has a specific function in the restriction of silencing, to which its cell-cycle independent status might contribute (Schulze et al. 2009). At last, we stress the observation that lethality associated to *SIR3* overexpression in *dot1* mutants is dose dependent. While we could not differentiate 16 and 29x Sir3 overexpression strains, they exhibited clear differences in the absence of Dot1 suggesting that the saturation of the dose-dependent increase in silencing is associated to Dot1 activity. We consider that the dose-dependency of this process explains the apparent contradiction of our study with the results presented in (Verzijlbergen et al. 2009) and further exemplify the importance of probing different levels of overexpression.

Subtelomeric specificities

In most organisms, the specificities associated to chromosome ends extend beyond telomeres, within domains generally referred to as subtelomeres (E. Louis and Becker 2014). In budding yeast, several points of view enable to discern such specificities. Recent study comparing the conservation of synteny among closely related yeast species enabled a precise definition of budding yeast subtelomeres (Yue et al. 2017). It turns out that chromatin also exhibit specificities within domains located proximal to chromosome ends.

The first is undoubtedly the presence of heterochromatin, which has a unique signature in terms of histone marks. However specific properties associated to chromosome ends often extend beyond heterochromatic domains (Matsuda et al. 2015; Catherine B Millar and Grunstein 2006). At most *S.cerevisiae* subtelomeres, Hda1 affected subtelomeric (HAST) domains (Robyr et al. 2002) and Htz1 activated (HZAD) domains (Guillemette et al. 2005) lie contiguous to SIR silenced chromatin. In addition, phosphorylation of H2AS129 and mono methylation of H3K79 also extend further away than SIR silenced domains. When we compared the domain accessible to heterochromatin to HAST domains we observed that extended silent domains ends

often coincide with a HAST domain end. Importantly, our study indicates that the subtelomeric domains revealed upon overexpression of Sir3 likely exist independently of Sir complex dosage, as other genetic contexts such as H3 tail mutants or *tup1/ssn6* mutants also revealed similar regional effects. Interestingly, extension of silent domains reveals subtelomeric domains that possess consistent chromatin signature and define slightly different subtelomeres than synteny does. Accordingly we observed that the syntenic chromosome core is accessible to the SIR complex at 12 subtelomeres (SupFigS5). We recently showed that chromatin state impact on repair efficiency and outcome (Batte et al, 2017). This raises the question of whether the specific chromatin associated with subtelomeric domains that we uncovered in this study, contributes to the particular evolution of those regions.

Contribution of telomere proximity to subtelomeric properties

A central question of the biology of subtelomeres is to which extent the properties of subtelomeres are due to their proximity to telomeres or mere consequence of their gene content. Several studies demonstrated that the SIR complex contributes to the localization of enzymes to subtelomeres. For example, subtelomeric localization of the Okazaki fragment processing protein Dna2 is severely reduced in *sir* mutants (Choe et al. 2002). In addition, the kinase Tel1 responsible for H2A phosphorylation in subtelomeric regions is present at telomeres but H2AP levels depend mainly on the integrity of the SIR complex (Tasuku Kitada et al. 2011). Interestingly Sir3 stabilizes this mark even at regions where Sir3 is not detectable by CHIP in wild-type cells, suggesting that either Sir3 act remotely, or is binding these regions at least transiently in wild-type. Intriguingly, regions enriched for H2AP coincide with ESD leading to the hypothesis that overexpressing Sir3 stabilizes these transient interactions. Accordingly, profiling of Sir3 binding in G1 arrested cells demonstrated extended Sir3 binding domains at a subset of subtelomeres (Mitsumori et al. 2016) and Dam-ID profiling of Sir3 binding in *set1/htz1* double mutants uncovered binding of Sir3 that were unnoticeable by CHIP (Venkatasubrahmanyam et al. 2007).

Conclusion

By taking the opposite approach to knock down or knock out studies, our work describes the dose dependency of budding yeast heterochromatin. In the presence of a large excess of silencing factors, ectopic nucleation of heterochromatin remains limited and does not impact euchromatic transcription. In contrast we observed the extension of subtelomeric silent domains and characterized their maximal extension along with the antagonistic factors that have been overcome, such as H2Az or H3K79me. The use of chromatin binding protein to scan chromatin properties enabled to uncover major subtelomeric histone mark transition zones, which functionally protect euchromatin from the spread of silencing. The long-term contribution of heterochromatin to the peculiar properties of subtelomeres will require further study.

Material and methods:

Media and Growth conditions: Yeast cells were grown on YP with 2% glucose, raffinose or galactose. All the strains used in this study were grown at 30 °C with shaking at 250rpm.

Yeast transformation protocol

Cells were seeded on liquid medium and grown to $0,8 < OD_{600} < 1,2$. 3 ODs ($\sim 3 \times 10^7$ yeast cells) of cells were taken and washed with 1X TEL (10mM EDTA pH 8, 100mM Tris pH8, 1M Lithium Acetate), then 3 μ l of SSDNA (Sigma ref: D9156-5ML), DNA template (0,5 μ l if plasmid DNA, 5 μ l of digested plasmid or PCR product), 300 μ l of 1X TEL and 45% PEG-4000 solution were added. The mix was put 30 min at 30 °C and heat shocked at 42°C for 15 minutes. Lastly, cells were plated on appropriate selective medium.

Drop Assays

Yeast cells were transformed using the conventional protocol expect that plating was made on selection plates supplemented with 5mM NAM. Pre-cultures were also made in YPD 5mM NAM. 5X serial dilutions are shown. Plates were grown for 2-3 days at the indicated temperature. When temperature is not shown it is 30°C.

RNA extraction and reverse transcription

RNA extraction was carried following RNeasy Mini kit instructions with DNase treatment and using glass beads acid-washed for the mechanistic lysis. Total RNA integrity was assessed using nanodrop. 250ng or 500ng of total RNA was used as a substrate for reverse transcription by Super Script III enzyme using poly-A primers. Each experiment was made of 2-5 biological replicates.

Pellet preparation for CHIP

A total of 20 O.D equivalent of exponentially growing cells were fixed in 20 mL with 0.9 % formaldehyde for 15 min at 30°C, quenched with 0.125 M glycine and washed twice in cold TBS

1x pH 7.6. Pellets were suspended in 1mL TBS 1X, centrifuged and frozen in liquid nitrogen for -80°C storage.

Chromatin immunoprecipitation

All following steps were done at 4°C unless indicated. Pellets were re-suspended in 500 µL of lysis buffer (0.01% SDS, 1.1% TritonX-100, 1.2 mM EDTA pH 8, 16.7 mM Tris pH8, 167 mM NaCl, 0.5 % BSA, 0.02 g.L⁻¹ tRNA and 2.5 µL of protease inhibitor from SIGMA P1860) and mechanically lysed by three cycles of 30 s with 500 µm zirconium/silica beads (Biospec Products) using a Fastprep instrument (MP Biomedicals). Each bead beating cycle was followed by 5 min incubation on ice. The chromatin was fragmented to a mean size of 500 bp by sonication in the Bioruptor XL (Diagenode) for 14 min at high power with 30 s on / 30 s off and centrifuged 5 min at 13 000 rpm. 10 µL were kept to be used as Input DNA. Cleared lysate was incubated overnight with 1 µL of polyclonal antibody anti-Sir3 (Agro-bio). 50 µL of magnetic beads protein A (NEB) were added to the mixture and incubated for 4h at 4°C. Magnetic beads were washed sequentially with lysis buffer, twice with RIPA buffer (0.1% SDS, 10mM Tris pH7.6, 1mM EDTA pH8, 0,1% sodium deoxycholate and 1% TritonX-100), twice with RIPA buffer supplemented with 300 mM NaCl, twice in LiCl buffer (250 mM LiCl, 0.5% NP40, 0.5 % sodium deoxycholate), with TE 0.2% TritonX-100 and with TE. Input were diluted 10x with elution buffer (50mM Tris, 10mM EDTA pH8, 1%SDS) and beads were re-suspended in 100 µL elution buffer. A reversal cross-linking was performed by heating samples overnight at 65°C. Proteins were digested with proteinase K in presence of glycogen and the remaining DNA was purified on QIAquick PCR purification columns. Finally, samples were treated with 29 µg.mL⁻¹ RNase A 30 min at 37°C.

ChIP-qPCR

1.2 µL out of the 50 µL of eluate and 2.4 out of 50 were used for qPCR reactions for the IP and the Input fractions respectively. qPCR reactions and analysis were done as in (Ruault et al., 2011). Values were either normalized by the enrichment at the OGG1 locus or at 0.2 kb from TELVIR. Error bars correspond to the standard deviation.

ChIP-chip preparation and hybridation

Samples used for ChIP-chip have all been analysed by qPCR prior to microarray hybridization. For microarray hybridization 4/5 of the immunoprecipitated DNA and of the DNA from the input were ethanol precipitated and re-suspended in 10 μ L of water (Gibco). Purified material was amplified, incorporating amino-allyl-dUTP using as described in (Guidi et al. 2015). The size of amplified fragments (~500 bp) was assessed by gel electrophoresis. For each sample 1.5 μ g of amplified DNA was coupled either with Cy5 (immunoprecipitated sample) or Cy3 (input sample) and hybridized on 44k yeast whole genome tiling array (Agilent) as described in (Guidi et al. 2015)

Microarray data acquisition, analysis and visualization

Microarray was imaged using a Agilent DNA microarray scanner and quantified using GenePix Pro6.1 as described in (Guidi et al. 2015).

Data analysis

All dataset were lifted over to Saccar3 when required. Histone marks data were obtained from (Weiner et al. 2015). Sir3 binding in H3 tail mutants from (Sperling and Grunstein 2009), nucleosome turnover from (Dion et al. 2007). Transcriptome data were downloaded from the website supporting the publication (Kemmeren et al. 2014). Subtelomere definition was obtained from (Yue et al. 2017). Zscores were computed using the R scale function.

Downsampling of Sperling data for figure 4 was done using R, visual inspection of the data confirmed that downsampling occurred without error. Average telomeric profiles were done by computing the mean of the signal over 10 kb windows separated by 10 bp.

The limits of Extended silent domains were computed as the first probes possessing 5 neighboring probes that have Zscore inferior to 1, starting from the telomere.

Fitting of the data was done using Matlab fitting toolbox using Bisquare robustness option. The function used is $f(x)=K/(1+\exp(-r*(-x+t_0)))+1$, with the following fitting parameters for K,r, and t0

: lower bounds : [10 0.0001 1000], Starting point : [10 0.0001 1000], upper bounds: [200 0.01 40000].

Area under the curve was exactly computed on the fitted signal of Sir3 binding in strains overexpressing SIR2 and SIR3, 10kb before the end of silent domains and 5 kb after.

Mutants showing localized effects were identified with using the hypergeometric distribution, function phyper with bonferroni correction for multiple testing (n=703).

Rap1 foci analysis:

The image analysis is performed with a slightly modified version of the dedicated tool from (Guidi et al. 2015). These modifications regard the quantification of foci and aim at providing a more accurate estimation of the quantity of fluorescence held inside each focus. The gaussian fitting approach has been replaced by a template matching framework with a bank of 100 symmetric 2D gaussian kernels with standard deviations ranging from 0.5 to 7 pixels. The position of each template is determined as the maximum of normalized cross correlation whereas the most suitable template for a single focus is selected by minimizing the sum of square differences between the gaussian template and the data within a circular mask of radius twice the standard deviation. The foci are then defined as spherical objects with radii of two times the standard deviations of the matched templates. All foci that could not be fitted were considered as a cube of dimension 5*5*5. Variation of the box size did not affect overall results. The foci intensity can thus be measured as the sum of the fluorescence signal inside its sphere. Furthermore, the proportion of intensity from a nucleus held inside each of its foci is also computed.

RNAseq

Total RNA from a 25mL culture of exponentially growing yeasts were extracted using phenol-chloroform. Banks were constructed using the kit SOLiD Total RNA-Seq, with minor modifications : RNA are Zinc fragmented and fragments with size ranging from 100 to 200 nt selected by gel purification. After reverse transcription only fragment of size > 150nt are kept.

Paired end (50 + 35) sequencing was done by the Institut Curie platform. Differential expression was called using EdgeR, with a false discovery rate inferior to 0.1.

Author contributions :

A.H carried the ChIP experiments, ChIP-chip hybridization and all the data analysis except RNA-seq read mapping (M.D) and upgrade of qFoci software (M.G). M.D designed the primary RNAseq analysis. M.R imaged most Rap1-GFP images. A.H. and M.R generated yeast strains. A.M co-designed the transcriptome experiment and participated in the elaboration of the project. A.H. and A.T. drafted the figures. A.H, A.T and M.R designed and interpreted experiments, and wrote the manuscript.

Acknowledgments :

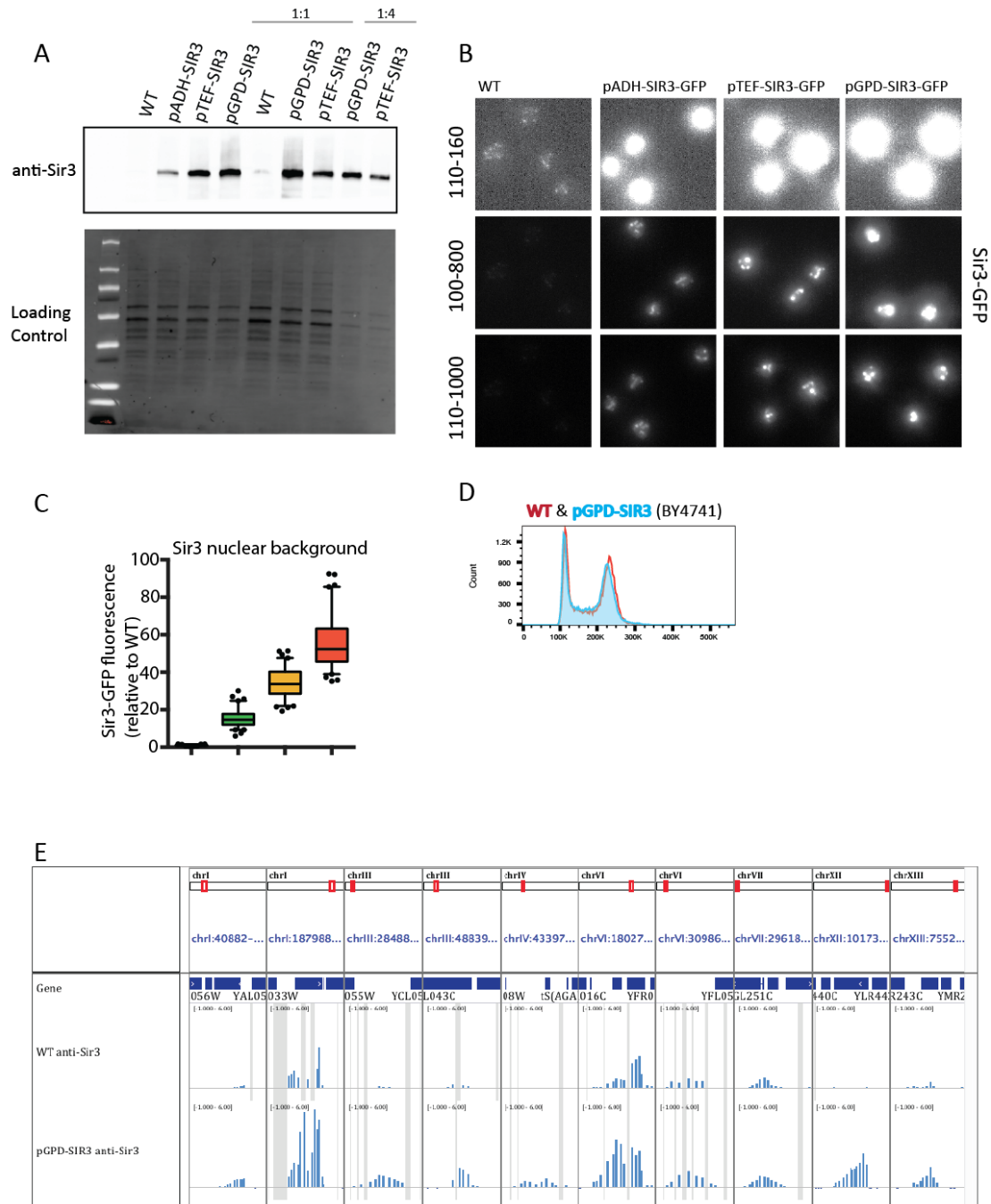
We thank T. Rio Frio, S. Baulande and P. Legoix-Né (NGS platform, Institut Curie), A. Lermine (bioinformatics platform, Institut Curie), P.Lebaccon (PICT@Pasteur microscopy platform) for support. We also thank Camille Gautier for help with multiple mapping reads analysis and Marta Kwapisz for RNAseq library preparation. We thank Valérie Borde for help with microarray experiments, David Sitbon, Valérie Borde and Arnaud De Muyt for fruitful discussions, David Sitbon and Ann Ehrenhofer-Murray for critical reading of the manuscript. This work has benefited from the facilities and expertise of the NGS platform of Institut Curie (supported by the Agence Nationale de la Recherche [ANR-10-EQPX-03, ANR10-INBS-09-08] and the Canceropôle Ile-de-France), and of the high-throughput sequencing platform of IMAGIF (<http://www.i2bc.paris-saclay.fr>). A. Morillon's lab is supported by the Agence Nationale de la Recherche (DNAlife) and the European Research Council (EpincRNA starting grant, DARK consolidator grant). This work was supported by funding from the Labex DEEP (ANR-11-LABX-0044_DEEP and ANR-10-IDEX-0001-02 PSL) and from the ANR DNA-Life (ANR-15-CE12-0007). AH was supported by fellowships from the ENS PhD program and the FRM (DEP20131128535).

Strain table

ID	matingT.	genotype	background
191	alpha	ade2-1::ADE2 adh4::URA3-4xUASG-(C1-3A)n ppr1Δ::HIS3 rap1::GFP-RAP1(LEU2)	(W303)
1254	alpha	ade2-1::ADE2 adh4::URA3-4xUASG-(C1-3A)n ppr1Δ::HIS3 rap1::GFP-RAP1(LEU2) sir3::GPD-SIR3(NAT)	(W303)
1256	alpha	ade2-1::ADE2 adh4::URA3-4xUASG-(C1-3A)n ppr1Δ::HIS3 rap1::GFP-RAP1(LEU2) sir3::GPD-sir3-A2Q(NAT)	(W303)
2487	a	ade2-1::ADE2 hmlΔ::HPH rap1::RAP1-GFP(LEU2)	(W303)
2627	a	ade2-1::ADE2 hmlΔ::HPH rap1::RAP1-GFP(LEU2) sir3::pADH- SIR3(NAT)	(W303)
2629	a	ade2-1::ADE2 hmlΔ::HPH rap1::RAP1-GFP(LEU2) sir3::pTEF-SIR3(NAT)	(W303)
2554	a	ade2-1::ADE2 hmlΔ::HPH rap1::RAP1-GFP(LEU2) sir3::GPD-Sir3(NAT)	(W303)
1667	a	RAD5+ rap1::RAP1-GFP(LEU2) RDN1::ADE2 sir2::GPD-SIR2(KanMX)	(W303)
1668	a	RAD5+ rap1::RAP1-GFP(LEU2) RDN1::ADE2 sir2::GPD- SIR2(KanMX)sir3::GPD-SIR3(NAT)	(W303)
779	a	ade2-1::ADE2 sir3::SIR3-GFP(LEU2)	(W303)
3441	a	ade2-1::ADE2 sir3::(KAN) pADH-SIR3-GFP(LEU2)	(W303)
3442	a	ade2-1::ADE2 sir3::(KAN)pTEF-SIR3-GFP(LEU2)	(W303)
3443	a	ade2-1::ADE2 sir3::(KAN) pGPD-SIR3-GFP(LEU2)	(W303)
2056	a	can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[HHTS-HHFS]*- URA3 where H4WT	BY4733
2986	a	can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[HHTS-HHFS]*- URA3 where H3Δ4-30 Rap1-GFP(LEU2)	BY4733
2987	a	can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[HHTS-HHFS]*- URA3 where H3Δ4-30 Rap1-GFP(LEU2) pGPD-SIR3(NAT)	BY4733

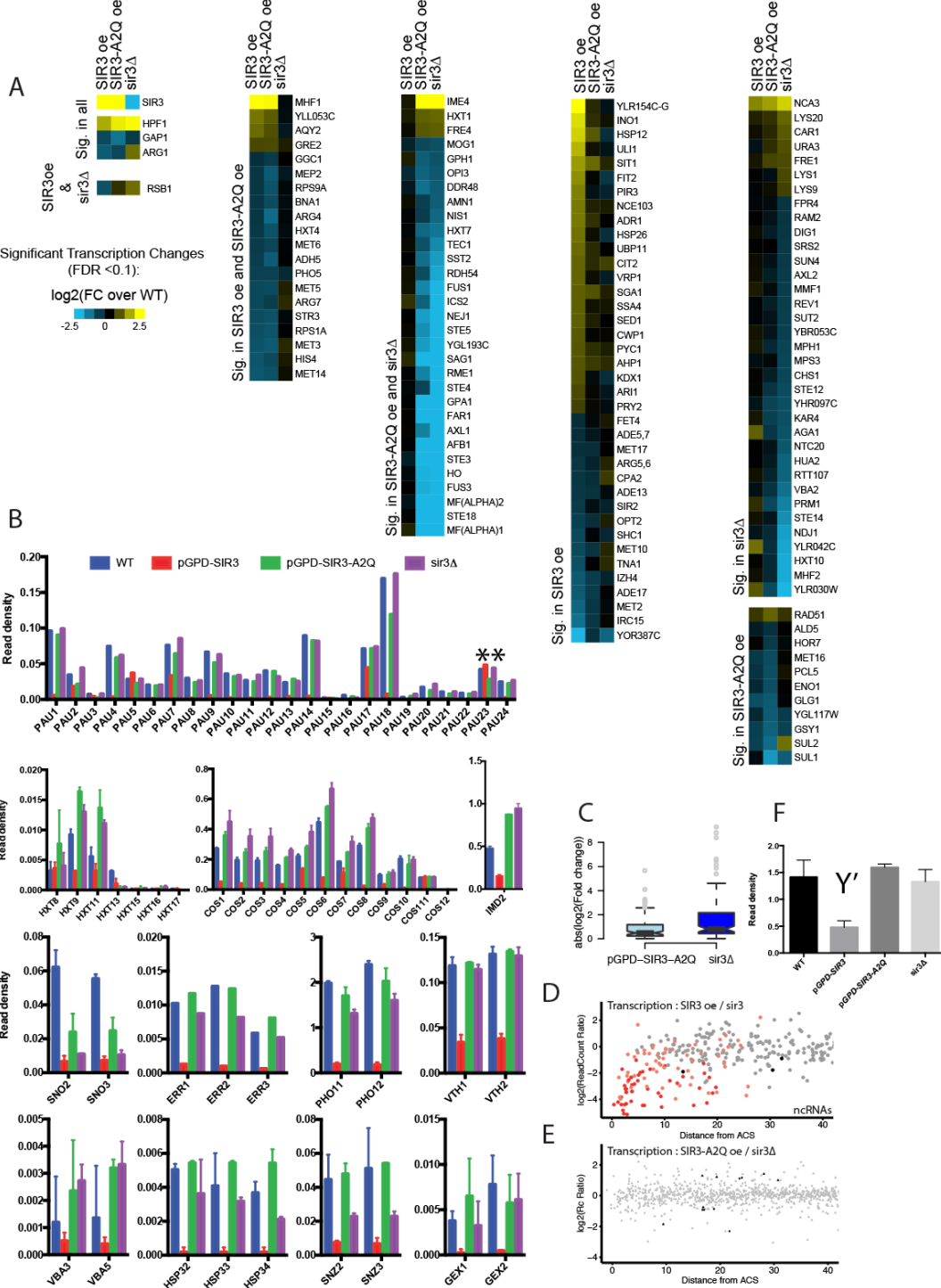
2476	a	rap1::GFP-RAP1(LEU2) sir3::GPD-SIR3(NAT)	BY4741
3004	alpha	ade2-1::ADE2 adh4::URA3-4xUASG-(C1-3A)n ppr1Δ::HIS3 rap1::GFP-RAP1(LEU2) bre1Δ::KanMx	(W303)
3123	alpha	ade2-1::ADE2 adh4::URA3-4xUASG-(C1-3A)n ppr1Δ::HIS3 rap1::GFP-RAP1(LEU2) bre1Δ::KanMx SIR3::pGPD-SIR3 (NatMx)	(W303)
3180	alpha	ade2-1::ADE2 adh4::URA3-4xUASG-(C1-3A)n ppr1Δ::HIS3 rap1::GFP-RAP1(LEU2) dot1Δ::KanMx	(W303)
3181	alpha	ade2-1::ADE2 adh4::URA3-4xUASG-(C1-3A)n ppr1Δ::HIS3 rap1::GFP-RAP1(LEU2) set1Δ::KanMx	(W303)
3182	alpha	ade2-1::ADE2 adh4::URA3-4xUASG-(C1-3A)n ppr1Δ::HIS3 rap1::GFP-RAP1(LEU2) dot1Δ::KanMx pGPD-SIR3-A2Q (NAT)	(W303)
3183	alpha	ade2-1::ADE2 adh4::URA3-4xUASG-(C1-3A)n ppr1Δ::HIS3 rap1::GFP-RAP1(LEU2) dot1Δ::KanMx pGPD-SIR3 (NAT)	(W303)
3184	alpha	ade2-1::ADE2 adh4::URA3-4xUASG-(C1-3A)n ppr1Δ::HIS3 rap1::GFP-RAP1(LEU2) set1Δ::KanMx pGPD-SIR3(NAT)	(W303)
2838	alpha	ade2-1::ADE2 adh4::URA3-4xUASG-(C1-3A)n ppr1Δ::HIS3 rap1::GFP-RAP1(LEU2) rpd3Δ::KanMx	(W303)
2841	alpha	ade2-1::ADE2 adh4::URA3-4xUASG-(C1-3A)n ppr1Δ::HIS3 rap1::GFP-RAP1(LEU2) rpd3Δ::KanMx pGPD-SIR3(NAT)	(W303)
3301	1N	ade2-1::ADE2 adh4::URA3-4xUASG-(C1-3A)n ppr1Δ::HIS3 rap1::GFP-RAP1(LEU2) set2Δ::KanMx	(W303)
3333	1N	ade2-1::ADE2 adh4::URA3-4xUASG-(C1-3A)n ppr1Δ::HIS3 rap1::GFP-RAP1(LEU2) set2Δ::KanMx sir3::GPD-SIR3(NAT)	(W303)

Supplementary figures

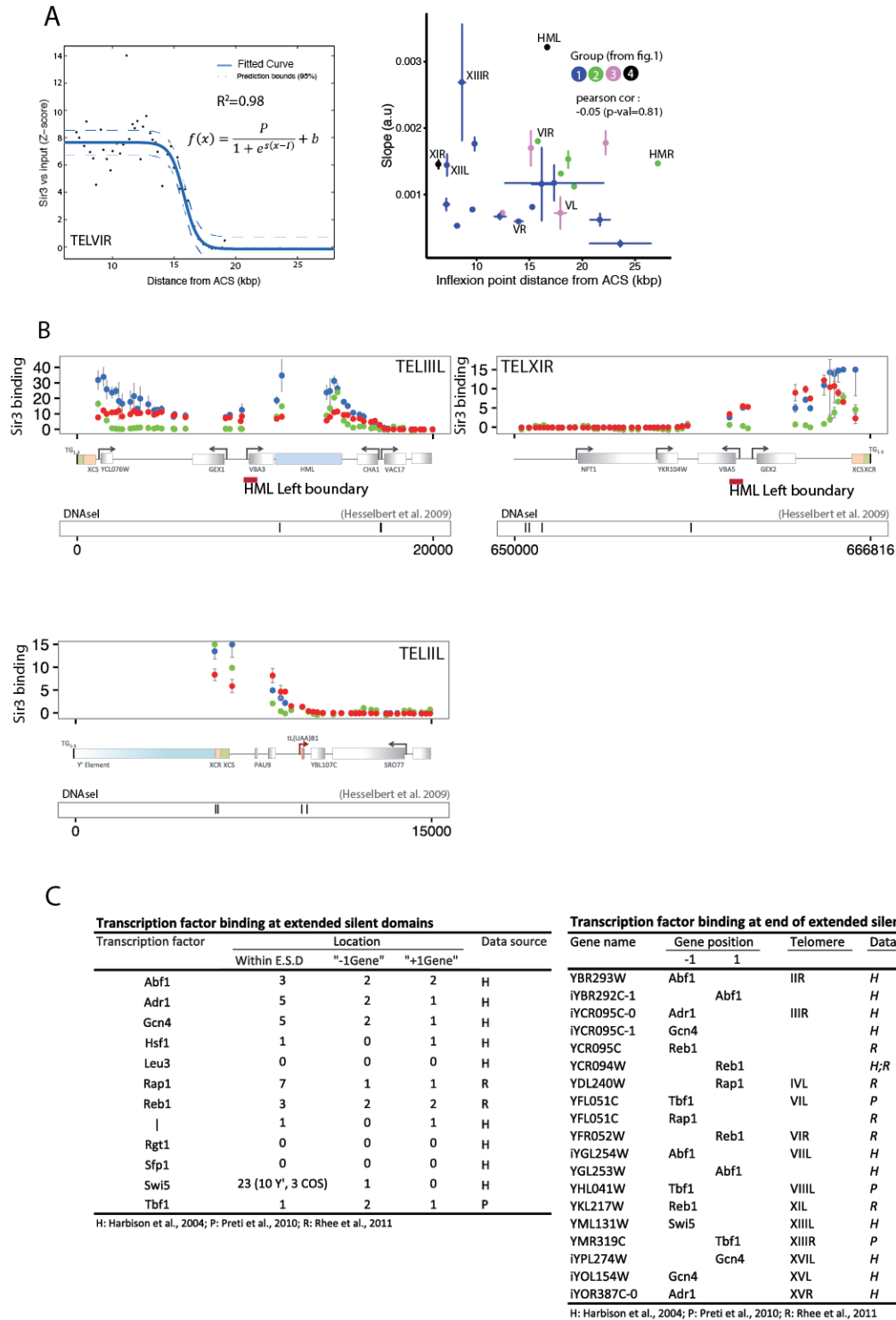


Supplementary Figure 1 (A) Western Blot anti-Sir3 in the strains used in Figure one for ChIP-chip. (B) representative examples of Sir3 fluorescence in strains overexpressing Sir3-GFP (C) Quantification of Sir3-GFP nuclear background (D) FACS profile of exponentially growing WT and pGPD-SIR3 strains (E) Representative images of loci bound by Sir3 within euchromatin.

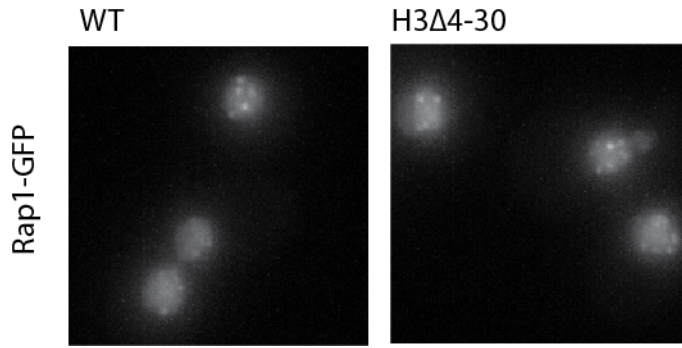
Sup fig2



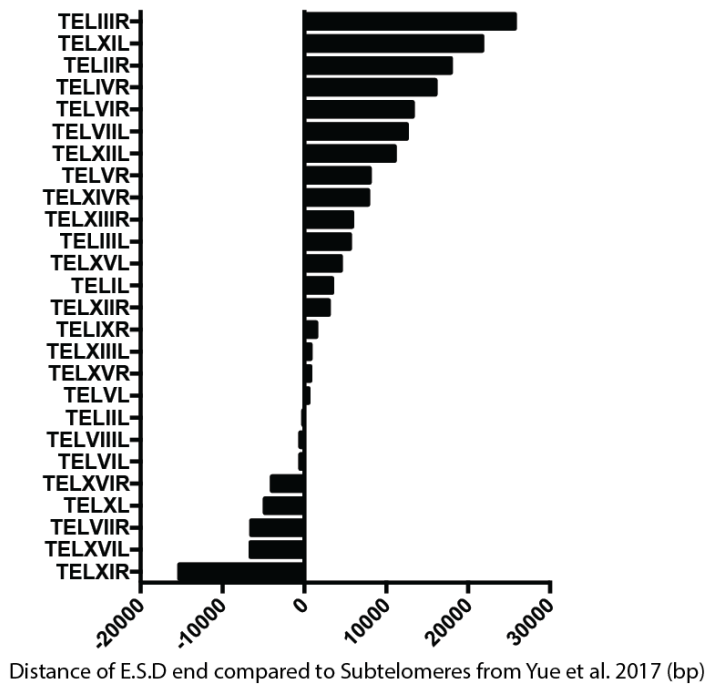
Supplementary Figure 2 : (A) All transcriptional changes coined significant by EdgeR within euchromatin, color code indicate $\log_2(\text{FC})$. (B) Transcriptional changes of genes from subtelomeric families.(C) absolute fold change of genes associated to pseudo-diploid signature.(D) Transcription of ncRNA within subtelomere, color code is identical as in the main figure.(E) Transcriptional changes in SIR3-A2Q mutants versus sir3 mutants. (F) Average Read density at Y' elements.



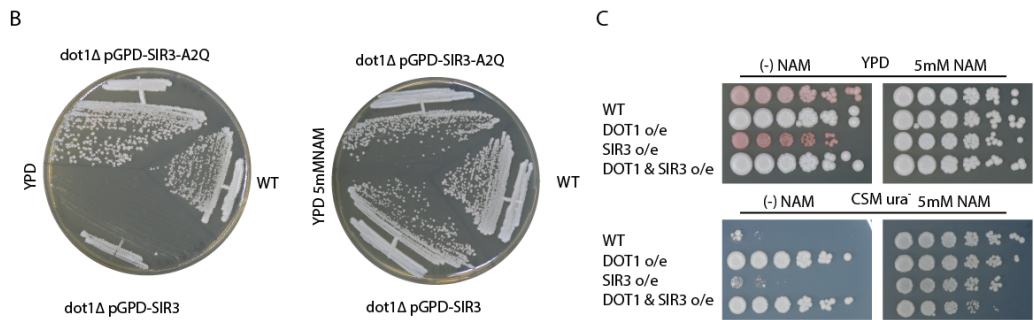
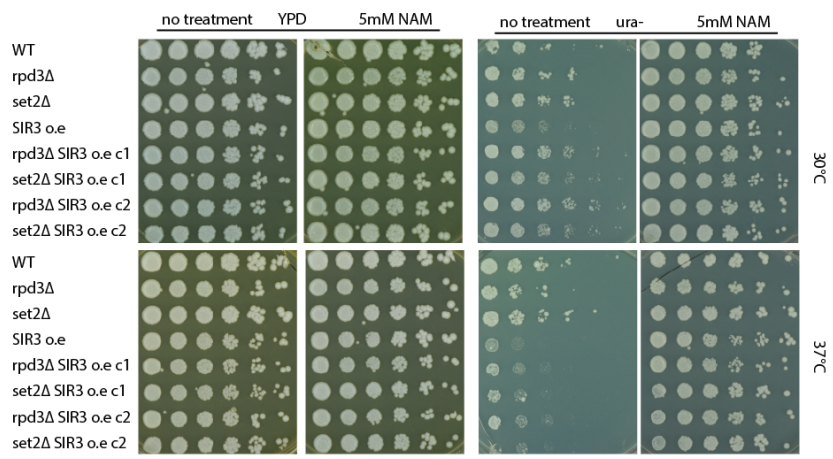
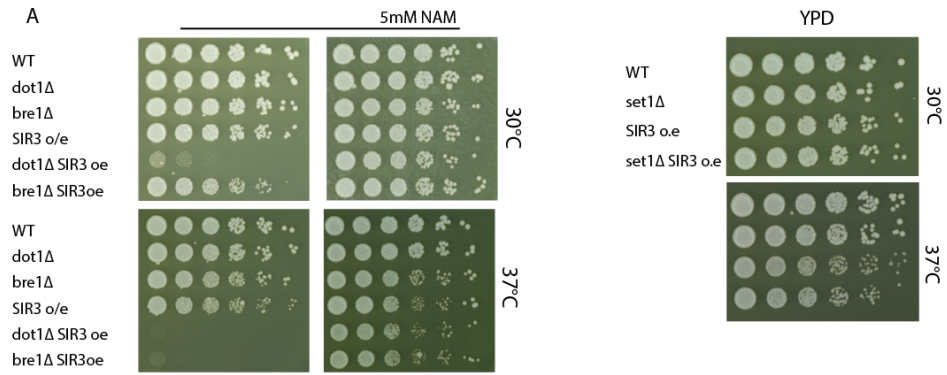
Supplementary Figure 3: (A) Example of fitting of the ChIP-chip data, function used is shown on the graph. Right: Inferred slope versus position of inflexion point. (B) Examples of identified barrier at three subtelomeres at which Sir3 spreading does not extent when Sir3 dosage is increased. (C) List of transcription factors possessing a barrier property and present within E.S.D (left) or at the boundary of E.S.D (right)



Supplementary Figure 4 : Maximal projections of Rap1-GFP images in WT and H3Δ4-30 strain, grown to exponential phase



Supplementary Figure 5: Comparison of the location of the End of extended silent domains with subtelomeres ends as defined by (Yue et al. 2017) Positive distance implies that the E.S.D end further within the core chromosome that the subtelomere as defined by synteny.



Supplementary Figure 6 (A) Drop assays probing viability in the presence of absence of 5mM NAM. Protocol is identical to the one shown on the main figure. (B) dot1 mutants overexpressing Sir3-A2Q are viable. (C) Dot1 overexpression counteracts Sir3 overexpression.

Results, part II: Subtelomeric properties and Sir3 recruitment at internal loci

A. Causality and subtelomeres

This second part was largely motivated by my interest on subtelomeres, which emerged following the work on subtelomeric silent domains expansion. Here we asked a rather simple question: to which extend the functional properties of subtelomeres depend on their proximity from the end of the chromosome?

To address this question we took advantage of a set of yeast strains developed in the laboratory of Stephane Marcand. Those strains were originally built to study the breakage of dicentric chromosomes. In those strains chromosomes VI and VII are fused and the centromere of chromosome VI can be removed at will. To assess the influence of Rap1 binding, we compared two strains differing for the presence of 302bp of TG₁₋₃ interstitial telomeric sequences (ITS) at their original position (**Figure 1**). Those strains were built by homologous recombination between two telomeres followed by the disruption of centromere VI. Consequently the domains previously corresponding to subtelomeres are internal to the chromosome VI-VII in those strains. This experimental setup theoretically allows us to determine to which extend subtelomeres are influenced by their location at the physical ends of chromosomes.

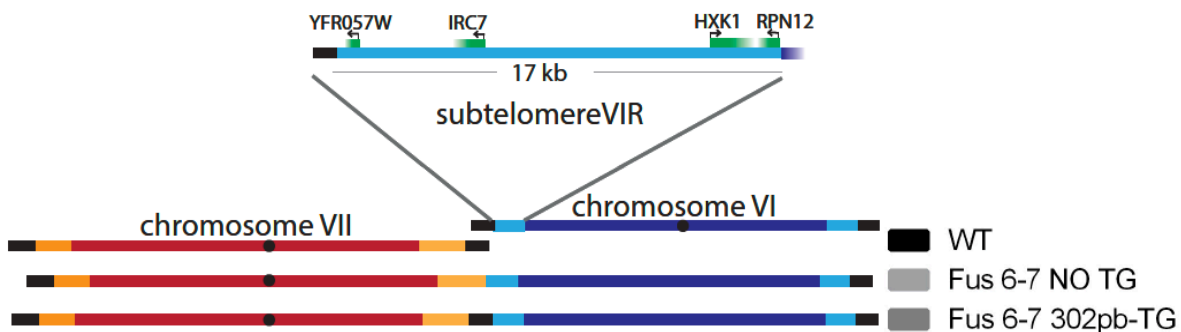


Figure 1 Schematic representation of the chromosome fusion used.

In budding yeast, ITS can act as silencers in a mechanism that involves Rap1 binding and the SIR complex, just like TPE (Stavenhagen 1994, Aksenova 2015). This phenomenon is referred to as C1-3A silencing. C1-3A silencing is affected by the proximity of the ITS to the telomere and by the length of the ITS. Thus C1-3A silencing share several properties with E silencers which efficiency is also affected by proximity to chromosome end. However, C1-3A silencing is Sir1 independent (Stavenhagen 1994). C1-3A silencing is not detected when one or two 270bp C1-3A tracks are present but only becomes apparent when three 270bp C1-3A tracks are inserted proximal to a reporter gene (Stavenhagen 1994). We hypothesized that the requirement for very long ITS might be different depending on the chromosomal location, and particularly at subtelomeres. As mentioned earlier, subtelomeres are relatively gene poor compared to the core chromosome, and the genes presents in subtelomeres are generally lowly expressed. Thus, this experimental setup offers the possibility to study the impact of ITS when they are present in a domain that can be silenced by the SIR complex.

Silencing at interstitial telomeric track:

We did not observe SIR dependent silencing of YFR057W in the strains containing 302bp ITS (**Figure 2A**). In contrast, the expression of SIR independent subtelomeric genes were unaffected by their new chromosomal location (**Figure 2A & B**). We observed relatively large variation in the basal level of *HXK1* expression but the average difference of expression was not significant; while it is tempting to attribute those variations to the internalization of the gene, a previous study reported that this gene is highly variable in its uninduced state, preventing further conclusion. We conclude that in glucose rich, unstressful conditions SIR based silencing is the only apparent contribution of telomeres to the transcriptional regulation of subtelomeres VI and VII.

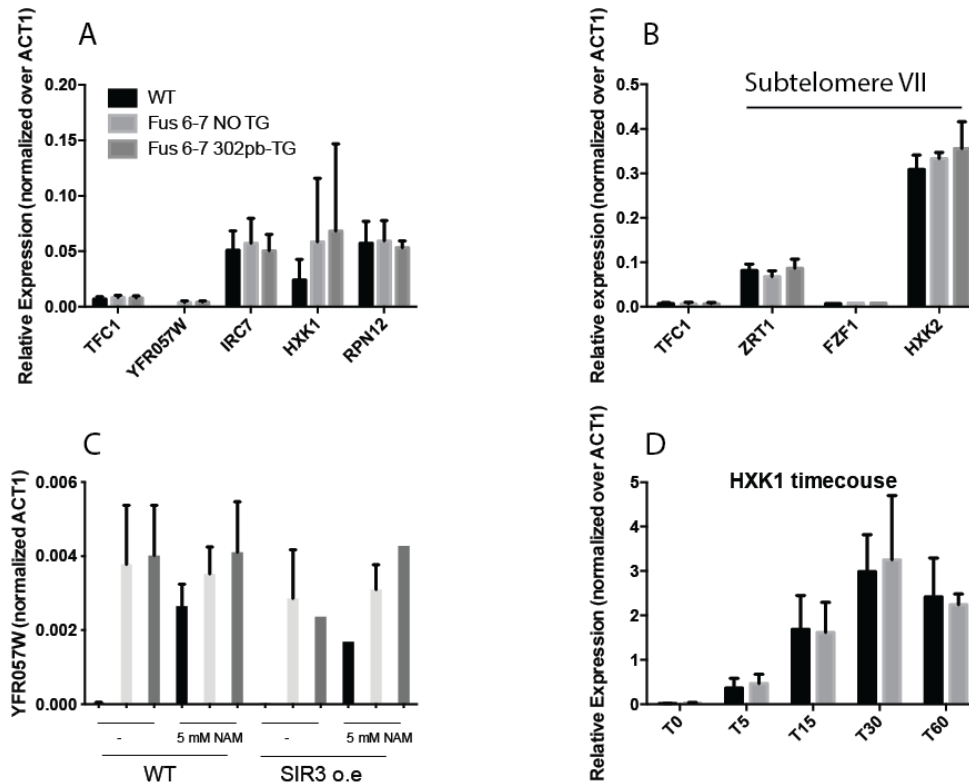


Figure 2 Subtelomeric transcription is not affected by chromosomal location: (A) transcription of genes along subtelomere VII, HXK1 apparent differences were not significant. (B) Idem over subtelomere VIII. (C) Focus on the YFR057W gene. (D) HXK1 expression upon growth in the absence of glucose. Time in minutes.

Factor limiting silencing establishment

We reasoned that the absence of silencing at the internalized subtelomeres could be due to a limiting concentration of Sir3p in the subnuclear environment of the internalized telomere. Consequently we overexpressed Sir3 in the fused telomere strains. We controlled for the increased silencing in those strains by monitoring the expression of *GIT1*, a gene located close to the HMR locus. In addition we verified that telomere clustering was increased in those strains by monitoring Rap1-GFP fluorescence in live cells. To our surprise, there was no silencing even when Sir3p was in large excess (**Figure 2C**), indicating that another factor was limiting heterochromatin nucleation at internalized subtelomeres.

Transcriptional activation of *HXK1*

In fungi, subtelomeric genes are often induced in response to adverse environmental conditions or when alternative carbon source are to be used. Even if proximity from telomere did not affect the basal levels of the genes under study, we hypothesized that the transcriptional regulation of an inducible gene might be affected by its subtelomeric location. To address this question we turned our attention to the glucose-repressed subtelomeric *HXK1* gene. The regulation of this gene has been extensively studied and offers a strong point of comparison (Rodriguez et al. 2001; Taddei et al. 2006). Time course experiments demonstrated that it is strongly activated within 30 minutes of growth in the absence of glucose. Upon growth in glucose-depleted medium (YP-Raff 2%), we observed similar de-repression of *HXK1* in WT as in (Rodriguez et al. 2001) and telomere fused strains (**Figure 2D**). We conclude that subtelomeric location of *HXK1* is not required for its transcriptional regulation.

Chromatin landscape at internalized subtelomeres

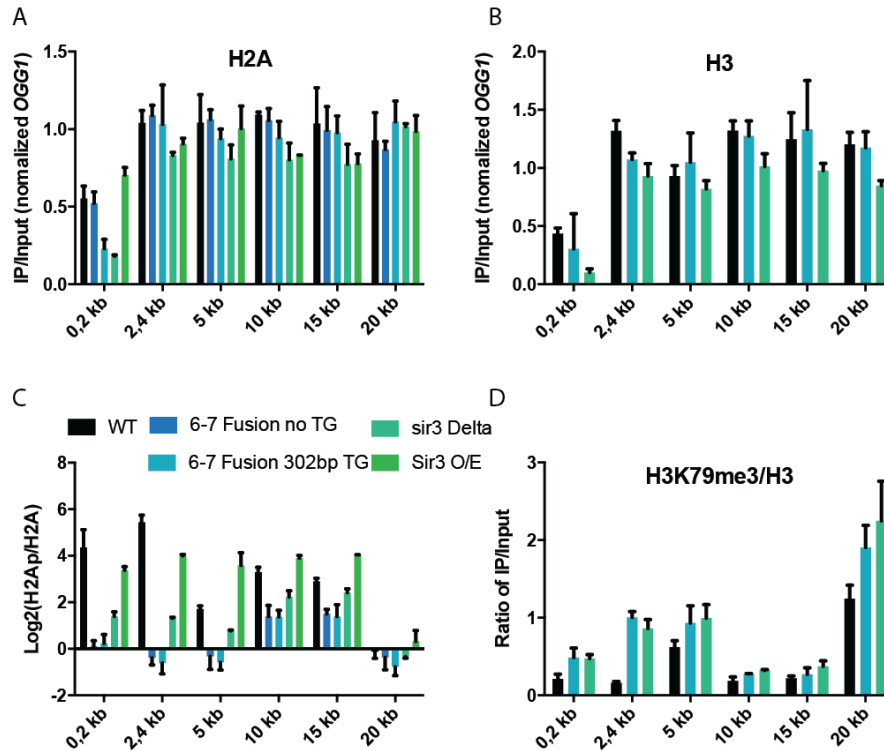


Figure 3 Histone occupancy and Histone marks along subtelomere VI. Sample colors are identical in all graphs. (A) ChIP anti- H2A normalized over OGG1, of note in the absence of TG histone occupancy at 0.2kb goes back to WT while it is lower when TG are present but Sir3 is not. (B) ChIP anti- H3 (C) H2AS129 phosphorylation, shown using a log scale for visualization purposes, as differences span orders of magnitude. Signal is lost in fused strains (D) H3K79me3 is increased in mutants that do not recruit Sir3.

Next we probed H2A phosphorylation and H3K79 tri-methylation at internalized subtelomeres (**Figure 3**). Given that Sir3 bound to the internalized subtelomeres we used a *sir3Δ* mutant as a control. Consistent with previous reports (Tasuku Kitada et al. 2011), we find that the absence of Sir3 abolishes a large part of subtelomeric H2Ap. However the residual levels of H2Ap present in *sir3Δ* mutants is further diminished at fused subtelomeres indicating that H2AP in subtelomeric regions depends on the proximity to the chromosome end and that this mark is stabilize by Sir3 binding. Consistently, H2Ap increased within ESD upon *SIR3* overexpression.

Interestingly, we observed that H2A occupancy is decreased in the absence of Sir3 or at the vicinity of ITS and is similar to wild type in the absence of ITS (**Figure 3 A & B**). This suggests that the native sequences flanking telomeres are not favorable to histone incorporation and that Sir3 presence maintains nucleosomes in place.

In contrast, we observed no differences when we compared the tri-methylation of H3K79 in *sir3Δ* mutants and fused subtelomeres. In both strains H3K79me3 is increased in the at the loci that were previously bound by Sir3. In addition, we observed increased tri-methylation of H3K79 at *RPN12* in *sir3Δ* mutants and in fused subtelomere strains (**Figure 3D**), which is unexpected given the absence of Sir3 at this gene when probed by ChIP-qPCR.

Discussion

Here we studied the impact of the end-to-end fusion of two chromosomes on the transcription of previously-subtelomeric genes and on two chromatin marks normally depleted or enriched within those domains. We observed that the presence of a 302bp ITS does not lead to the formation of a detectable silent domain in our experimental conditions. It appears that the ITS was able to recruit Sir3 but spreading of the SIR complex did not occur. Interestingly the presence of ITS correlated with changes in histone occupancy, which might have functional consequences on the stability of the ITS. Consistent with previous studies we report that the majority of H2A phosphorylation is lost when Sir3 doesn't spread on subtelomeric chromatin. However we were able to show that the relatively small fraction of H2Ap that is Sir3 independent is abolished when subtelomeric sequences of chromosome VI are internal to the chromosome.

Absence of silencing at ITS

Previous studies of C1-3A based silencing reported that a 276 bp ITS track is not sufficient to induce the silencing of a *URA3* gene when inserted at the *LYS2* locus. FOA resistant colonies only appear when triple 276 ITS are inserted proximal to the *URA3* gene. The configuration is slightly different as in our case the 302 bp ITS is located proximal to the X element of ex-telomere VIR. X elements contain an ARS that has been shown to improve TPE. Despite the presence of the X

element we did not observe silencing or *YFR057W*. Intriguingly, ChIP directed against Sir3 detected a 10 fold enrichment at a probe located 200 bp from the ITS (not shown). Given that sonicated DNA has an average length of 500bp in our conditions we interpret this enrichment as the sign of Sir3 binding to the ITS. If we hypothesize that Sir3 is indeed binding at ITS sequences then the question is what prevents the spread of the SIR complex? We demonstrated that it is not Sir3p concentration. In Addition the de-localization of telomeres at the nuclear interior upon overexpression of Sir3 is not sufficient to implement silencing at the ITS. While there is no reason to think that the interplay between the Rif proteins and the Sir is different at ITS from at telomeres, the Ku70/80 heterodimer is likely absent from ITS. One possibility is that ~300bp ITS do not recruit sufficient Sir4 to initiate spreading, while triple ITS do. However it remains surprising that the sole absence of the Ku70/80 heterodimer prevents silencing as additional deletion of *RIF1* in a ku70 mutant restores silencing. Another possibility is that the local structure of repeated Rap1 binding sites influences its ability to implement silencing. It is intriguing that such long arrays of telomeric repeats are required to get detectable C1-3A silencing. It could be that a specific conformation, similar to telomere clusters, would only be allowed by long internal telomeric tracks and be required for silencing. Future experiment will localize the ITS with the use of the LacO system to probe and probe whether anchoring ITS to the nuclear periphery can induce silencing.

SIR independent subtelomeric transcription is unaffected by internalization

Here we report that the transcription of SIR independent genes present on subtelomere VI and VII are independent of their distance from the telomere. It indicates that subtelomeric location of *HXK1*, which association to nuclear pore is required for rapid de-repression (Taddei et al. 2006), does not affect its transcriptional regulation.

H2A phosphorylation at subtelomeres

The strong correlation between H2A phosphorylation and the distance from the telomere was first noticed by Szilard *et al.*. This study shows that phosphorylation is independent of the presence of Y' element as it is present at the subtelomere VIR which only contains a X element. Later studies showed that a large fraction of this signal is Sir3 dependent. Here we showed that

when the subtelomere VI is internally located -including the X element, further loss of H2A phosphorylation is observed. This suggest that part of the H2A phosphorylation emerges from the telomeric structure itself and not from the X element or the [TG]₁₋₃ sequences. Interestingly, tethering of the ATM kinase Tel1, which is normally present at telomeres (Takata et al. 2004) favors heterochromatin formation (Kirkland et al. 2015). The function of H2A phosphorylation at subtelomeres however, remains unknown.

Perspectives and initial aim

There is a lot to do for this project to be complete. First, additional subtelomeres should be studied especially one containing members of repeated gene families such as COS, PAU or FLO genes. Third probing H3K18 acetylation within a HAST domain (such as the one present within subtelomere VIR) would enable to state whether those domains are influenced by their proximity to the telomere. Ultimately this would enable to understand which properties generally associated to subtelomeres are the consequence of the clustering of particular genes within subtelomeres, a structural property that likely emerges over relatively long periods of time and which properties are directly influenced by the proximity to telomere, such as SIR silencing.

In the eventuality of successful implementation of silencing, either using longer ITS or rif mutants, one of the initial aim of this project was to probe Rap1 binding signal. The reason for this is that Rap1 binding signal is dependent of Sir3 spreading when probed by CHIP. I hypothesized the particular conformation of the telomeric structure might be the cause of this signal, and that it could be abolished when silencing emerges from an ITS.

B Nucleation of the SIR complex at non-canonical sites

Several studies reported the detection of Sir3 binding within euchromatin (Lieb et al. 2001)(Radman-Livaja et al. 2011; Sperling and Grunstein 2009). In particular, Sir3 binding upon activation of GAL genes was further studied by ChIP-qPCR(Radman-Livaja et al. 2011). In contrast to its binding at subtelomeres, Sir3 binding to euchromatin could never be associated to transcriptional repression (Marchfelder, Rateitschak, and Ehrenhofer-Murray 2003; Radman-Livaja et al. 2011). A study initially aiming at studying a potential euchromatic function of the Sir complex revealed the existence of an artifact common to ChIP experiment (L Teytelman et al. 2013). The authors convincingly show that ChIP inherently give rise to false positive signal over several loci, including highly transcribed genes, casting doubt on the actual existence of Sir binding within euchromatin. Indeed expression of a nuclear localized GFP protein gave rise to signal similar to the one of the Sirs at highly transcribed genes and other loci. It turns out that at the time this paper was published we already had evidence indicating that in some cases Sir3 binding signal within euchromatin could be modulated, arguing that it might not be artifactual. Importantly, we did find signs of the artifact demonstrated by Teytelman at highly transcribed genes -not shown-. In this part we will briefly describe data pointing toward the binding of Sir3 within euchromatin in specific contexts.

As shown on **(figure 4)**, low magnitude signal is present in wild type cells at some euchromatic loci. Upon overexpression of *SIR3* we observed at some of those loci binding signal is increased and qualitatively changes of shape, as for *YAT1*, *GLK1* or *ACS1*. In addition we also observed binding signal over loci that were previously at background levels. Over-abundance of Sir3 led to apparent Sir3 binding at *SPB1*, *YDLC007C-A* but also at *SIR3*, as we will discuss later on. Local depletion of H4K16 and H3K79me3 appeared consistent with the binding of Sir3. Interestingly similar increases in Sir3 binding were observed when we analyzed Sir3 binding data from Sperling et al. In *gcn5Δ* mutants or in H3 tail mutants the same loci exhibited increased binding signal compared to WT. As reported by others we generally did not observed transcriptional repression associated to Sir3 binding, to the exception of a ncRNA located within *YAT1* CDS that was significantly repressed in strains overexpressing *SIR3* compared to *sir3* mutants.

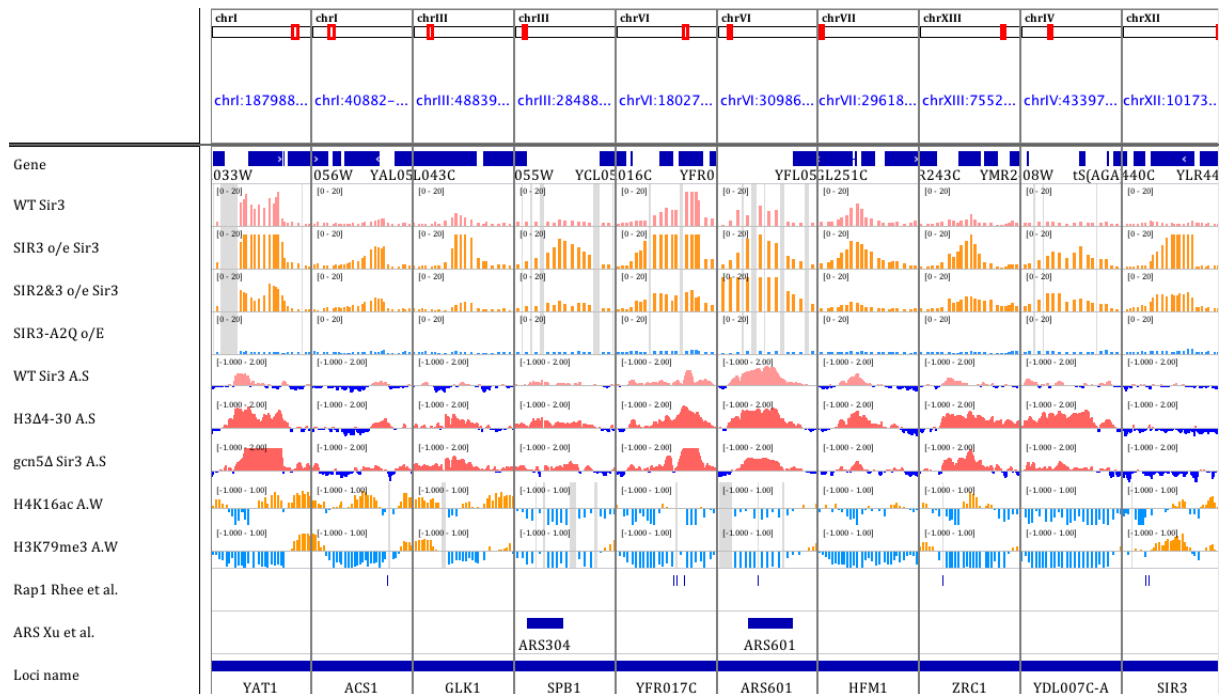


Figure 4 Examples of euchromatic loci at which Sir3 is recruited in wild type cells, upon overexpression or/ and in H3 tail acetylation mutants. Note that Sir3 recruitment at SIR3 only occurs in strains overexpressing SIR3 and that the sequence of its promoter (pGPD) is not present on this representation. External data source :A.S : Adam Sperling Data : Sperling et al 2009. A.W : Assaf Weiner data : Weiner et al. 2015, ARS form Xu et al. 2006

We decided to study in more details Sir3 recruitment at two euchromatic loci, *YAT1* and *SIR3* (**Figure 5**). Those loci were chosen for their singularity: *YAT1* is the most GC rich gene of budding yeast genome, it requires the THO/TREX complex for efficient transcription (Chávez et al. 2001) and its sequence impairs nucleosome positioning (Jimeno-González et al. 2006). Furthermore, *YAT1* is a unusually large double-strand break hot spot during meiosis (Pan et al. 2011). *SIR3* was chosen because we suspected that recruitment of Sir3 at *SIR3* might be the cause of the genetic instability of strains overexpressing *SIR3*. Briefly, previous work from our laboratory (Myriam Ruault, A. Counillon and myself) established that when loss of silencing gives a strong fitness advantage, frequent suppressors are observed in a population of yeast strongly overexpressing *SIR3*. In most cases studied those suppressors carried mutations within the *SIR3* gene -not

shown-. Using CHIP-qPCR, a quantification technique that is less prone to artifacts, we confirmed the recruitment of Sir3 at *YAT1*. Upon overexpression of *SIR3* we observed a ~3 fold increase in Sir3 binding signal. Deletion of *SIR2* abolished most of Sir3 binding signal at *YAT1* (**Figure 5A**).

In striking contrast, deletion of *SIR2* had no effect on Sir3 binding signal at *SIR3*. Given that nicotinamide treatment abolished Sir3 recruitment at *SIR3*, we hypothesized that another NAD dependent HDAC was responsible for Sir3 recruitment at *SIR3*. To further characterize the mechanism of recruitment of Sir3 we overexpressed *SIR3* in all NAD dependent HDAC mutants. As shown on figure 3B, Sir3 binding to *SIR3* was reduced to background level in *hst1Δ SIR3 o/e* strains (**Figure 5B**). In contrast, no changes were observed in all others NAD dependent HDAC mutants strains. What could be the function of Sir3 at *SIR3*? As Sir3-A2Q is not binding to *SIR3* when overexpressed and is expressed at similar levels than *SIR3*, we ruled out an effect on transcription. We began to investigate a potential role of *SIR3* in transcription-associated mutagenesis. Our attempts to measure *SIR3* mutation rate in *hst1Δ SIR3 o.e* strains by measuring the frequency of silencing loss were not successful. We built strains to study the influence of Sir3 on the mutation of the Canavanine gene but did not study them yet.

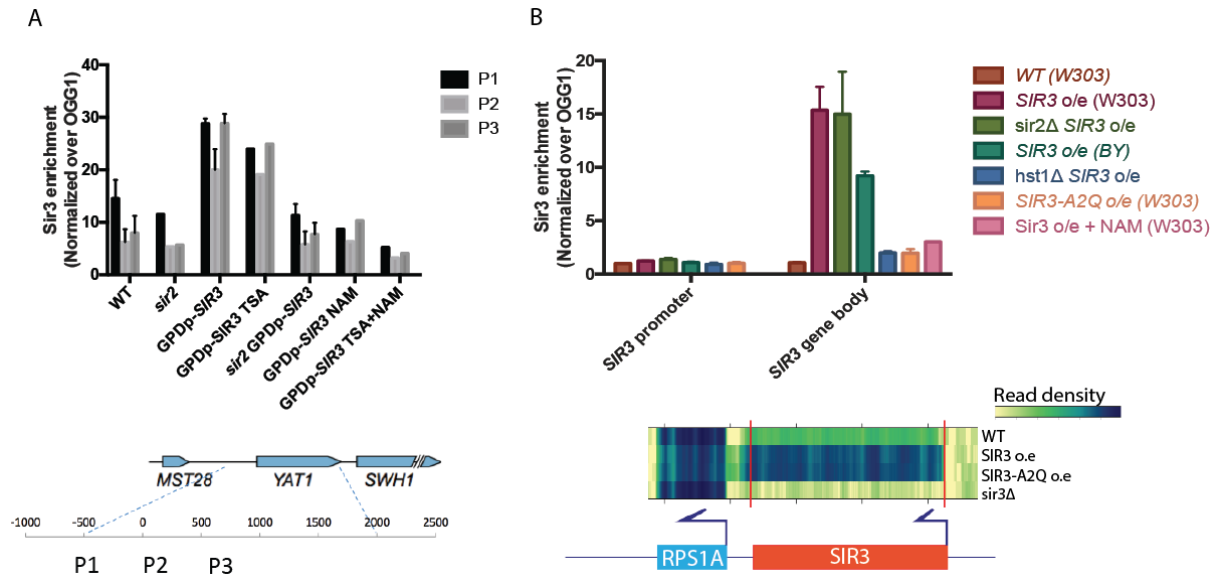


Figure 25 Sir3 recruitment at *YAT1* is Sir2 dependent but Hst1 dependent at *SIR3* (A) Binding of Sir3 probed by ChIP-qPCR in the indicated strains, no error bar indicate a single experiment. (B) Binding of Sir3 probed at *SIR3* by ChIP-qPCR and screenshot representing transcription of the *SIR3* gene in different mutants. Read density color scale is in log, from Marc Describes visualization tool.

H3 acetylation and Sir3 binding to non-RPG Rap1 binding sites

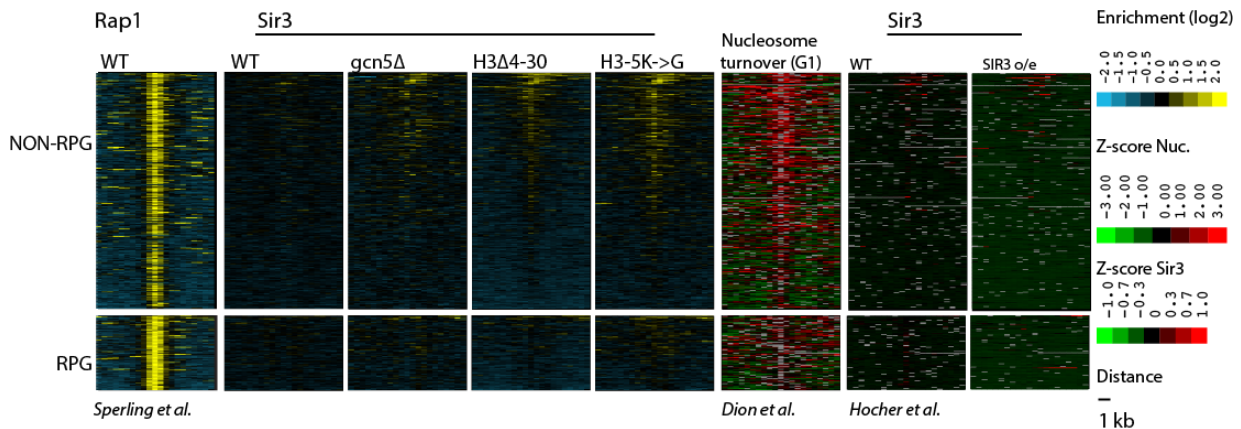


Figure 26 Sir3 is recruited to NON-RPG Rap1 binding sites in H3 tail mutants. Data source is indicated at the bottom of the heatmap graphs. For all heatmaps, loci are ordered according to the sum of the signal of Sir3 binding in H3Δ4-30 mutants. Rap1 binding sites where obtained from Rhee et al. 2009.

During the course of our analysis of euchromatic recruitment of Sir3 we noticed the specific presence of new recruitment sites in mutants defective for H3 acetylation. We were intrigued

by the fact that Sir3 was not recruited to those loci upon overexpression and decided to investigate this further. Visual inspection of the data enabled to notice the frequent colocalization of those recruitment sites with Rap1. In details, we report that Sir3 is recruited to Rap1 binding sites of non-Ribosomal Protein Genes (RPG) in mutants contexts associated with perturbation of H3 tail. As shown on **(figure 6)** alignment of Sir3 binding signal over Rap1 binding sites reveals that non-RPG Rap1 binding sites recruit Sir3 in *gcn5*, H3 Δ 4-30 and H3-5K>G mutants. Interestingly, recruitment of Sir3 at non-RPG Rap1 binding sites correlates with a high nucleosome turn-over during the G1 phase of the cell cycle.

Discussion

The absence of Sir3 at *SIR3* in *hst1* mutants could be due to decreased transcription of *SIR3* in *hst1* mutants. RT-qPCR experiments are required to probe transcription of *SIR3* in *hst1* Δ *pGPD-SIR3* strains. If we confirm that transcription is comparable to *pGPD-SIR3* strains, we will investigate if Hst1 also modulate Sir3 binding signal upon *GAL* gene activation. The potential role of Sir3 at those internal loci will be discussed in the next result part.

Additional material and methods

Media and Growth conditions

Yeast cells were grown on YP with 2% glucose, raffinose or galactose. All the strains used in this study were grown at 30 °C with shaking at 250rpm.

Chromatin immunoprecipitation

20 O.D equivalent of exponential cells were used, the immunoprecipitation was then performed as described in the first result part. Antibodies used: H2A (3µl, #3235) and γH2A (1µl, ab15083), H3 (Abcam), H3K79me3 (Abcam) 2µL. Each experiment was made of 2 biological replicates.

Data Analysis

Rap1 binding sites were obtained from (Rhee and Pugh 2012). Replication independent nucleosome turnover was obtained from (Dion et al. 2007). Data from (Sperling and Grunstein 2009) was processed as in result part I. Figure 4 was realized using Integrated Genome Viewer. Ribosomal protein genes list was obtained from the ribosomal protein genes database. For the analysis shown on figure 6, all Rap1 binding sites annotated as "telomeric" were excluded. To avoid confounding effect due to increased spreading in mutants, all Rap1 sites located within the extended silent domains defined in the results part I were excluded. For each Rap1 binding site, binding signal was averaged over 400 bp windows and aligned centered on Rap1 binding site. Visual inspection confirmed that data analysis did not affect the original data. Data visualization was done using Java Treeview. I wrote all scripts using R, "very secure dishes" version.

Strain table

191	alpha	ade2-1::ADE2	adh4::URA3-4xUASG-(C1-3A)n	ppr1Δ::HIS3	(W303)
		rap1::GFP-RAP1(LEU2)			
1254	alpha	ade2-1::ADE2	adh4::URA3-4xUASG-(C1-3A)n	ppr1Δ::HIS3	(W303)
		rap1::GFP-RAP1(LEU2)	sir3::GPD-SIR3(NAT)		

1256	alpha	ade2-1::ADE2 adh4::URA3-4xUASG-(C1-3A)n ppr1Δ::HIS3 rap1::GFP-RAP1(LEU2) sir3::GPD-sir3-A2Q(NAT)	(W303)
1289	alpha	ade2-1::ADE2 adh4::URA3-4xUASG-(C1-3A)n ppr1Δ::HIS3 rap1::GFP-RAP1(LEU2) sir2Δ::KanMX sir3::GPD-SIR3(NAT)	(W303)
3305	a	loxP-NAT-CEN6-URA3-loxP lys2::GAL-CRE rap1::GFP-RAP1(LEU2)	(W303)
3306	a	cen6-Δ ChrVI TEL6R-7L-noTG rap1::GFP-RAP1(LEU2)	(W303)
3307	a	cen6-Δ ChrVI TEL6R-7L-noTG rap1::GFP-RAP1(LEU2)	(W303)
3308	a	cen6-Δ ChrVI TEL6R-7L-302 pb de TG1-3 rap1::GFP-RAP1(LEU2)	(W303)
3309	a	cen6-Δ ChrVI TEL6R-7L-302 pb de TG1-3 rap1::GFP-RAP1(LEU2)	(W303)
3340	a	cen6-Δ ChrVI TEL6R-7L-noTG rap1::GFP-RAP1(LEU2) sir3::pGPD-SIR3(KAN)	(W303)
3343	a	cen6-Δ ChrVI TEL6R-7L-302 pb de TG1-3 rap1::GFP-RAP1(LEU2) sir3::pGPD-SIR3(KAN)	(W303)
2471	a	hst1Δ::KanMx rap1::GFP-RAP1(LEU2) sir3::GPD-SIR3(NAT)	(BY4741)
2472	a	hst2Δ::KanMx rap1::GFP-RAP1(LEU2) sir3::GPD-SIR3(NAT)	(BY4741)
2473	a	hst3Δ::KanMx rap1::GFP-RAP1(LEU2) sir3::GPD-SIR3(NAT)	(BY4741)
2474	a	hst4Δ::KanMx rap1::GFP-RAP1(LEU2) sir3::GPD-SIR3(NAT)	(BY4741)
2475	a	hos2Δ::KanMx rap1::GFP-RAP1(LEU2) sir3::GPD-SIR3(NAT)	(BY4741)
2476	a	rap1::GFP-RAP1(LEU2) sir3::GPD-SIR3(NAT)	(BY4741)

Results part III: Screening viability upon overexpression of *SIR3*

In the first part of this thesis we established that Dot1 is required for viability upon overexpression of Sir3. In the second part we saw one example of Hst1 dependent recruitment of Sir3 at non-canonical locus. To search for factors required to limit silencing spreading upon Sir3 overexpression and for potential factors involved in another function of Sir3 we set up a genetic screen based on the Synthetic Gene Array technology in collaboration with the Institut Curie's dedicated platform. In this part we will go through the preliminary results obtained during the course of this screen.

Screen design and rationale

Because we noticed that strains overexpressing *SIR3* have strong meiosis defects leading to aberrant segregation, we built a query strain in which *SIR3* expression is inducible. As *SIR3* mutant cannot mate they are not suitable for SGA based screen, which requires the formation of diploids. For those reasons we built a strain in which an additional copy of *SIR3* driven by the inducible GAL promoter was inserted at the *TRP1* locus. In addition, Rap1 was tagged with GFP in order to monitor the clustering of telomeres. We crossed those strains to four different mutant collections:

- Single deletions collection (DMA)
- Decreased mRNA collection (DAMP)
- Temperature sensitive alleles Collection (ts)
- H3 and H4 point mutants collection (HPM)

The screen is based on a simple design, which uses four different growth medium. We could probe four different situations:

-WT amounts of Sir3 (SC 2 % Glu) -WT amounts of Sir3, no silencing (SC 2% Glu 5mM NAM)

-Sir3 ++ (SC 2 % Gal) -Sir3 ++, no silencing (SC 2 % Gal 5mM NAM)

Briefly, we excluded potential false negatives, which growth is impaired or enhanced in galactose compared to glucose by analyzing single mutants growth (**figure 1A**). To enrich our results, we merged this screen with protein localization and telomere length when available. We selected candidates based on their normalized growth score, the output of the SGAtools pipeline analysis. For each condition (no NAM and 5mM NAM) this score was obtained by comparison of growth on glucose and growth on galactose. We chose to only investigate mutants that have an $|\text{Score}| > 0.2$ (coloured dots on **figure 1 B, Figure 3B**), as this threshold gave enough candidates to work on and included our positive control *dot1*. We note however that this threshold might be slightly too low for the analysis of temperature sensitive mutants which showed stronger growth defect than most DMA candidates.

Results

For the purpose of concision, we restricted the results presented here to mutants that were synthetic sick upon overexpression of *SIR3* but not in presence of 5mM NAM. Importantly Nicotinamide inhibits all NAD dependent deacetylases. A table listing results for the DMA and ts collections is showed on the next page (**Figure 1**).

DMA : Nuclear proteins, Sickness rescued by Nicotinamide

Gene	30 °C no NAM	30 °C 5mM nAM	Telo. Length	Localization	brief description (SGD)
ASF1*	-0.23	-0.16		nucleus (m)	Nucleosome assembly factor
BRE1	-0.26	0.03		nucleus (m)	E3 ubiquitin ligase
DOT1	-0.24	-0.04		nucleus (m)	Nucleosomal histone H3-Lys79 methylase
IES1	-0.34	-0.10		nucleus (m)	Subunit of the INO80 chromatin remodeling complex
MED1	-0.23	-0.07		nucleus	Subunit of the RNA polymerase II mediator complex
MFT1	-0.28	-0.17	ss	nucleus	Subunit of the THO complex
PIP2	-0.22	-0.16		nucleus	Autoregulatory, oleate-activated transcription factor
PRE9	-0.22	-0.11		nucleus	Alpha 3 subunit of the 20S proteasome
RDS2	-0.21	0.01		nucleus	Transcription factor involved in regulating gluconeogenesis
RPL40A	-0.20	-0.17		nucleus:cytosol	Ubiquitin-ribosomal 60S subunit protein L40A fusion protein
RXT2	-0.22	-0.10		nucleus	Component of the histone deacetylase Rpd3L complex
SAP30	-0.23	-0.07	S	nucleus (m)	Component of Rpd3L histone deacetylase complex
SAS4	-0.23	0.15		nucleus (m)	Subunit of the SAS complex (Sas2p, Sas4p, Sas5p)
SAS5	-0.29	0.19		nucleus (m)	Subunit of the SAS complex (Sas2p, Sas4p, Sas5p)
SDC1	-0.21	-0.06		nucleus	Subunit of the COMPASS (Set1C) complex
SDS3	-0.26	-0.10		nucleus	Component of the Rpd3L histone deacetylase complex
SEM1	-0.24	-0.08		nucleus	19S proteasome regulatory particle lid subcomplex component
SLT2*	-0.35	-0.18		nucleus:cytosol	Serine/threonine MAP kinase
THP3	-0.25	-0.10		nucleus	Protein that may have a role in transcription elongation

Ts : Nuclear proteins, Sickness at 37°C rescued by Nicotinamide

Allele	37 °C no NAM	37 °C 5mM nAM	Telo. Length	Localization	brief description (SGD)
abd1-8	-0.21	-0.09		nucleus	Methyltransferase
cdc45-27	-0.57	0.09		nucleus	DNA replication initiation factor
crm1-1	-0.26	-0.18		nucleus	Major karyopherin
dna2-1	-0.21	0.02		nucleus	Tripartite DNA replication factor
glc7-12	-0.51	-0.17		puncate:nucleus	Type 1 S/T protein phosphatase (PP1) catalytic subunit
nop2-9	-0.35	-0.04		nucleus	rRNA m5C methyltransferase
pop4-ph	-0.20	-0.20		nucleus	Subunit of both RNase MRP and nuclear RNase P
rpn5-ph	-0.62	0.10		nucleus	Subunit of the CSN and 26S proteasome lid complexes
yju2-ph	-0.57	-0.05		nucleus	Essential protein required for pre-mRNA splicing

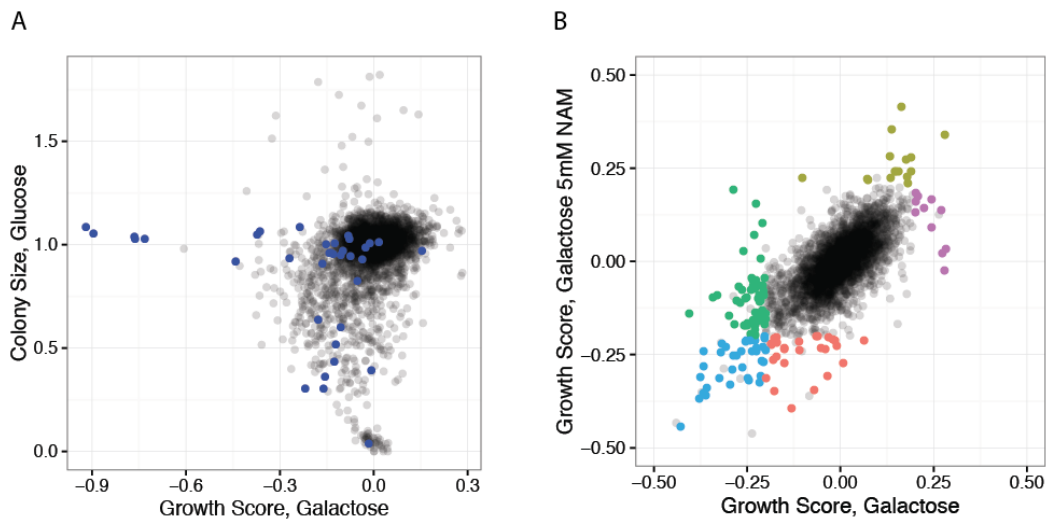


Figure 1 DMA and ts collections results. (Table) DMA and ts collection results, restricted to genes encoding nuclear proteins and genes which sickness is rescued by 5 mM NAM treatment. When available, telomere length as well as details on nuclear localization is shown. Nuclear(m) signifies manual annotation of the localization. * indicate DMA genes that were present in the ts collection (A) Growth score was largely independent of the colony size on glucose; blue dots represent mutants which growth is affected (either positively or negatively) by growth on a medium containing glucose as main carbon source. (B) Example of the genes selected for analysis. Coloured dots correspond to genes that pass our p-value and Score threshold

requirements. The different colors classify the type of response, in green are the mutant synthetic sick with overexpression of Sir3 and rescued by NAM addition.

Satisfyingly, growth defects of *dot1* and *bre1* mutants were rescued by nicotinamide, consistent with the results presented on part1. Similarly H3K79R was the sole histone point mutant showing growth defects that could be rescued by 5 mM NAM treatment (**Figure 3A**).

Ontology analysis indicated enrichment for genes dedicated to histone modification and chromatin silencing at telomeres, two categories somehow expected here. Our results show that deletion of member of the SAS-I complex as well as Rpd3I complex leads to growth defect that are rescued by 5 mM NAM. While we did not investigate how SAS-I mutant resist *SIR3* overexpression in the first part, we had previously showed that *rpd3Δ* mutants overexpressing *SIR3* are viable.

Interestingly, H4K16R mutant had a growth score below our threshold (**figure 3 C**), consistent with a rather subtle influence of H4K16 on the maximal extent of silencing upon overexpression of *SIR3*. Nevertheless, the interaction between SAS-I and *SIR3* overexpression should be investigated more carefully as it is difficult to conciliate with the absence of phenotype of H4K16R. Similarly, we were surprised to find that *SDC1* exhibited growth defect upon overexpression of *SIR3*, as deletion of *SET1* did not led to major defects in W303 background.

Intrigued by those apparent discrepancies, we searched for all the mutants corresponding to RPD3 and COMPASS complex that were present in our screen. It comes out that while Rpd3I deletions show growth defects to various extends, no growth defects were observed in the absence of the 3 proteins common to Rpd3I and Rpd3s: Rpd3, Sin3, Ume1. Unfortunately, *set1* was absent from our collection, nevertheless we observed limited growth defects for mutants of other members of COMPASS and no growth defect for H3K4 point mutants (**figure 3 C**).

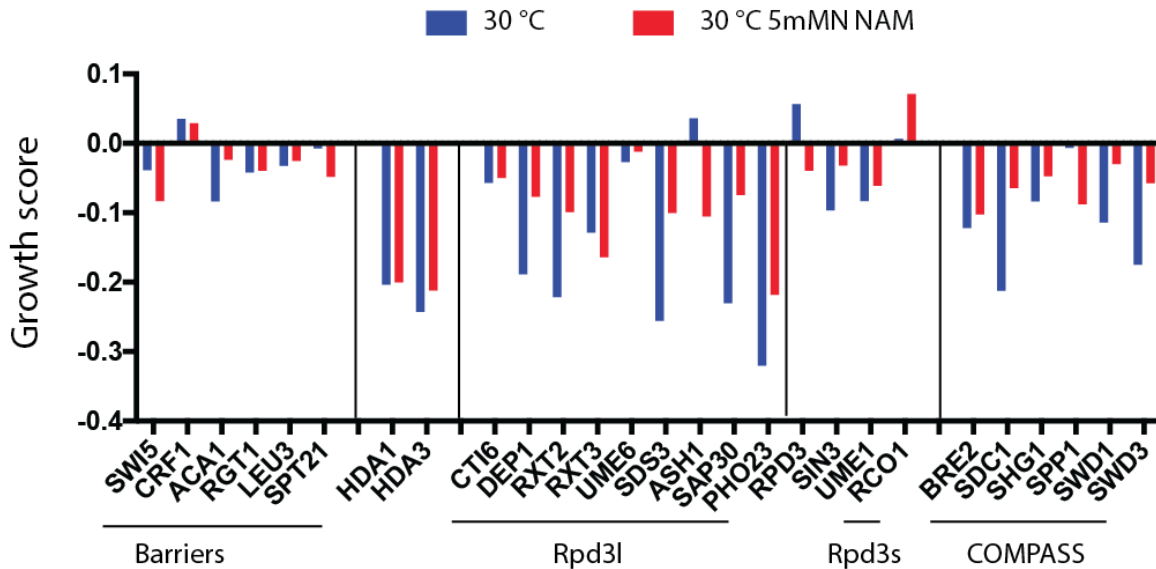


Figure 2 Growth Score of selected mutants: Barrier TF, RPD3, HDA, RPD3, COMPASS

We took the opportunity of this screen to verify that the prediction we made in part I concerning TF having a barrier property was correct. While we could only study 6 of such mutants, none of them had detectable defects (**Figure2**). Those results demonstrate that those transcription factors are indeed not required to limit toxic spreading of Sir3 in vivo.

SIR3, transcription and replication

SIR3 had synthetic interactions with the THO complex gene *MFT1* and with the Tho Related Protein 3 gene *THP3* (**Figure 1**). The THO complex is comprised of Tho2, Hpr1, Mft1 and Thp2 and is required for the transcription of 'difficult' templates, such as genes containing tandemly repeated sequences(Chávez et al. 2000)(Chávez et al. 2001). Deletion of *THO2* or *HPR1* affect telomere length by reducing the expression of RIF1, however mft1 mutants have WT telomeres and don't affect the expression of Rif1 (T. Y. Yu, Wang, and Lin 2012). Thp3 is recruited to transcribed genes and its absence leads to defects in transcription elongation; furthermore, thp3 mutation rescues the gene expression phenotype of THO mutants (Jimeno et al. 2011). Future work will investigate whether this interaction is related to the recruitment of *SIR3* at Sir3 upon overexpression. Selective inhibition of Sir2 with the use of splitomycin (Bedalov et al.

2001) instead of nicotinamide would shed light on the potential NAD dependent HDAC required for this process.

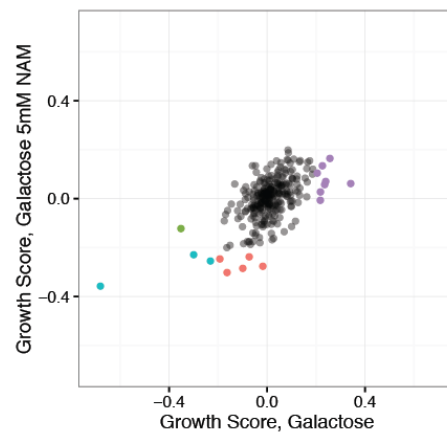
We found that at least three genes involved in replication or replication regulation are synthetic sick upon *SIR3* overexpression. Dna2 has been shown to physically interact with Sir3 and its presence at telomere depends of Sir3 (Choe et al. 2002). Thus the cause of synthetic sickness could be direct in this case. This intriguing link between Sir3 dosage and replication associated proteins is reinforced with the finding that Glc7 and Cdc45 alleles have strong growth defects when Sir3 is overexpressed.

A

H3 & H4 point mutants, all results

ArrayName	30 °C no NAM	30 °C 5mM nAM	Behavior
H3Δ5-24	0.20	0.10	Silencing_Rescue
H3Δ21-24	-0.16	-0.30	NAM_Sick
H3S22A	-0.02	-0.27	NAM_Sick
H3D77N	-0.30	-0.23	Silencing_Indep_Sick
H3G90A	0.24	0.07	Silencing_Rescue
H3K79R	-0.35	-0.12	NAMrescued
H4Δ1-24	-0.68	-0.36	Silencing_Indep_Sick
H4Δ5-8	0.23	0.14	Silencing_Rescue
H4K5Q	0.34	0.06	Silencing_Rescue
H4K8R	0.22	0.03	Silencing_Rescue
H4G11A	0.26	0.17	Silencing_Rescue
H4K12A	0.22	-0.01	Silencing_Rescue
H4A38S	-0.19	-0.25	NAM_Sick
H4E53A	-0.07	-0.24	NAM_Sick
H4K91A	-0.23	-0.25	Silencing_Indep_Sick
H4G94A	0.23	0.06	Silencing_Rescue

B



C

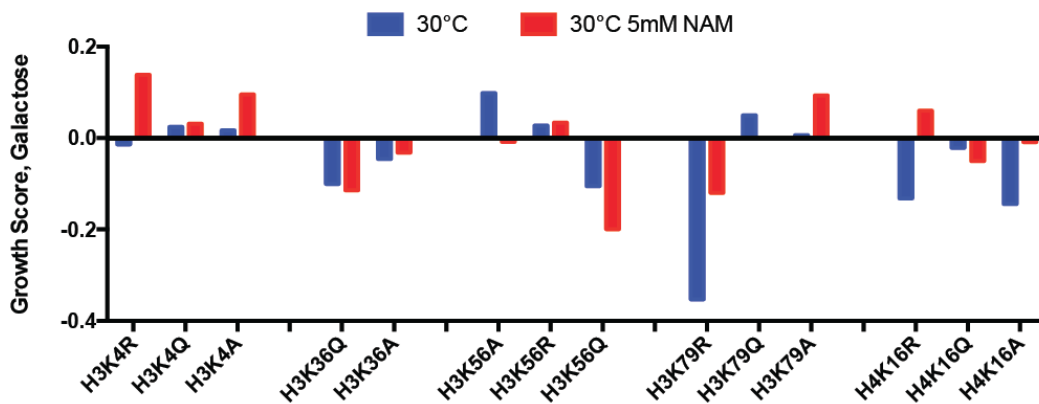


Figure 3 *SIR3* synthetic interactions with Histone Point Mutants: (A) Table listing all results obtained during the course of this screen, filtered for mutants sensitive to galactose. (B) Results visualization, colored dots are mutants that passed our thresholds. (C) Selected histone point mutants for comparison.

At last, the histone point mutants collection results revealed that the H3D77N mutations is synthetic sick with overexpression of *SIR3*. This interaction was not affected by our nicotinamide treatment (**Figure 3A**). Interestingly, this mutation is predicted to increase Sir3 binding to nucleosomes (Armache et al. 2011). It would be interesting to investigate the propagation of Sir3 in this context.

To confirm that Sir3 was overexpressed in the screen strains we chose few mutants and probed Sir3 levels by Western blot and Rap1-GFP clustering (**figure 4**).

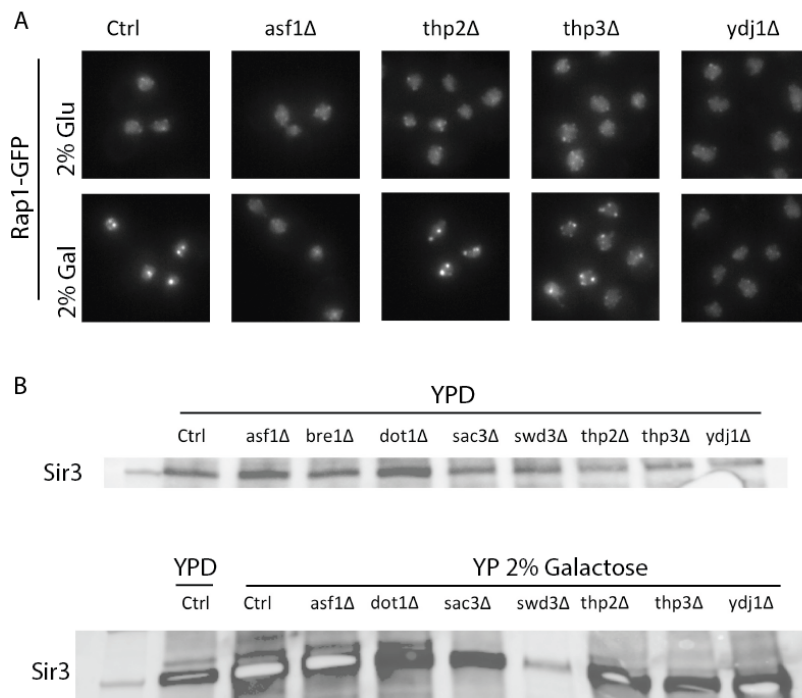


Figure 4 *asf1* and *ydj1* mutants are unable to mediate increased clustering upon *SIR3* o.e (A) Live microscopy images of indicated strains growing exponentially (OD <1). (B) Western blot using antibodies against native Sir3 in the indicated conditions.

While most mutants had the expected behavior, Sir3 expression was not increased in *sac3Δ* or *swd3Δ*, indicating that we might have some false positive. Interestingly, we observed that increase of Sir3 levels had quasi no-effect on the clustering of telomeres in *asf1* and *ydj1* mutants (**Figure 4A**). *asf1* mutants have been reported to have telomere clustering defect during S phase, and defaults in HM clustering (S. I. Hiraga, Botsios, and Donaldson 2008; Miele, Bystricky, and Dekker 2009). We show here that those defects are not due to limiting Sir3 levels. In addition, as H3K56 point mutants mediate better telomere clustering than *asf1* mutants, the

implication of Asf1 in the acetylation of H3K56 residues is not the cause of the telomeric defect of *asf1* mutants.

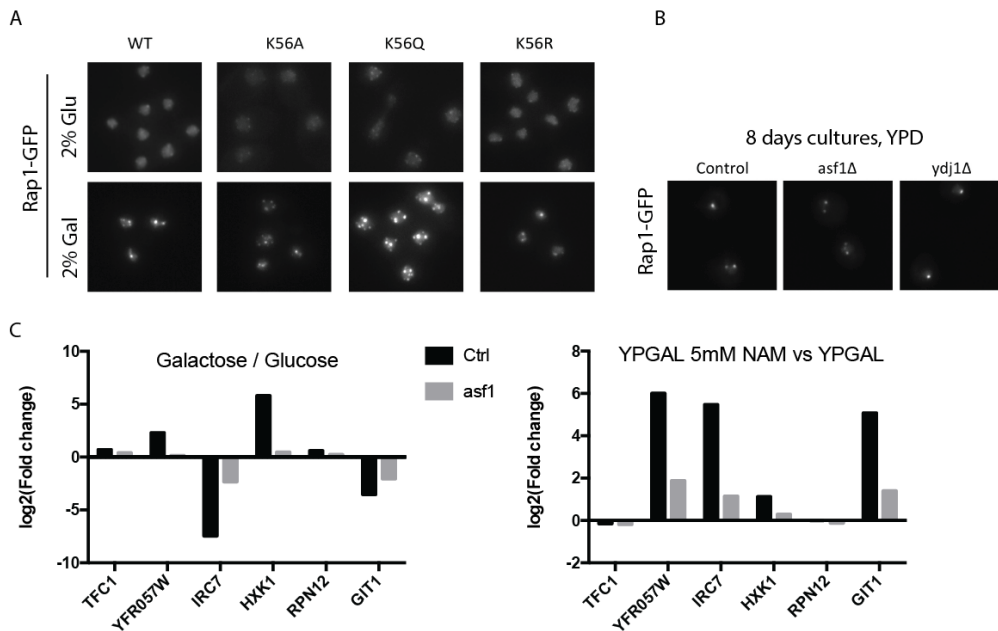


Figure 5 Preliminary investigation of Asf1 influence on clustering and silencing. (A) Live microscopy images of indicated strains growing exponentially ($OD < 1$). (B) Indicated strains were grown to stationary phase in YPD for 8 days, and Rap1-GFP was imaged on live cells. (C) Transcription changes probed by RT-qPCR in *asf1* mutants compared to WT. TFC1 is a second internal control, GIT1 is a gene present on chromosome III, it is silenced upon overexpression of SIR3 (see results part I).

In contrast, those strains were able to cluster telomeres during growth on quiescence (**Figure 5B**). Comparison of subtelomere VI and *GIT1* (subtelomere III) expression indicated that additional genes are silenced upon overexpression of *SIR3* in *asf1* mutant -experiment done once- (**Figure 5C**). This indicates that *asf1* mutants are able to implement silencing- albeit inefficiently- but not to increase the clustering of telomeres. Those results are preliminary and require additional experiments.

Conclusion

By conducting a high throughput genetic screen we could uncover synthetic dosage interactions that might associate Sir3 with processes such as transcription elongation or replication. Furthermore, preliminary results suggests that in some mutant contexts overexpression of Sir3 leads to Sir3 spreading without concomitant increase in telomere clustering, a situation that has not been observed before.

Additional material and methods and authors contribution:

The strains used for this screen were built during my supervision of a Master student, Layla El Mossadeq, and the screen was realized by the SGA platform of the Institut Curie, run by Petra Kaferle. Layal El Mossadeq conducted all the western blots and microscopy experiments, under my supervision. I carried data analysis. Protocols for RT-qPCR and microscopy are as in part I.

Culture conditions for stationary phase experiments

Yeast were grown overnight in YPD then suspended in 5 mL YPD at a OD ~0.2 and left for 8 days in a shacking incubator 225rpm at 30°C. Cells were washed in water before microscopy.

Plasmid construction for SGA:

We constructed a cassette containing *HPH* upstream a *SIR3* gene driven by pGAL1 promoter. Bot fragment were amplified from genomic DNA using Q5 polymerase from NEB following manufacturer's instructions. This cassette was inserted within the *TRP1* gene using NEBuilder HiFi DNA assembly cloning kit following manufacturer's instructions. The plasmid and yeasts strains were verified by sequencing *SIR3*, microscopy and enzymatic digestion (using PmlI + BsgI, hindIII and BamHI).

Systematic Genetic Array procedure:

SGA experiment was adapted from (Tong et al. 2001). Colonies were grown for 3 days at 30 or 37 °C in dedicated incubators. Screen was performed by SGA platform at Institut Curie. All the

steps were carried out using automated replicator BM3-BC (S&P Robotics, Canada).

Data analysis:

All SGA pictures were analyzed using SGAtools (<http://sgatools.cabr.utoronto.ca/>). Images were uploaded and results were retrieved by P.Karferle. Galactose sensitivity was attributed to genes which | Score | > 0.2. Candidates genes were filtered for p-value < 0.001 and | Score | > 0.2.

Gene product location was downloaded from LoQate website (www.weizmann.ac.il/molgen/loqate) (Breker et al. 2014). Telomere length when available was collected from (Askree et al. 2004). All scripts were written in R version 3.2.4 "very secure dishes". Data visualization was done using ggplot2. Figures were assembled using Adobe Illustrator. Gene names and ontology was obtained from yeastmine.org

Strains:

Name	Mating Type	Genotype	Alias	Auxotrophies
yAT1949	alpha	<i>rap1::RAP1-GFP</i> (LEU2)	SGA parent1	(BY4742)
yAT3302	alpha	<i>can1D::STE2pr-his5</i>	SGA parent 2	his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 lyp1Δ can1Δ
yAT3303	alpha	<i>can1D::STE2pr-his5 rap1::RAP1-GFP</i> (LEU2)	SGA parent 2 <i>RAP1-GFP</i>	his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 lyp1Δ can1Δ

Not listed: all indicated mutants resulting from the SGA cross and selected on medium lacking leucine for Rap1-GFP (LEU2) presence.

General discussion

In this work we explored the plasticity of silent domains in budding yeast. Our work shows that the various mechanisms restricting the spread of silencing efficiently protects the genome from ectopic silencing, even when silencing factors are in large excess. We characterized the extent of silent domains when Sir2 and Sir3 are not limiting the spread of silencing. This enabled us to probe the permissivity of subtelomeric chromatin to Sir binding. Doing so we realized that extended silent domains end at a major zone of histone marks transition, which could delimitate other processes. To further detail the functional determinants of subtelomeric properties we initiated a series of experiments that will potentially help to understand which subtelomeric properties are directly associated to the proximity from telomeres. Preliminary results demonstrated that some subtelomeric chromatin marks are influenced by the SIR complex presence but also by the proximity from the telomere in the absence of the SIR complex. Lastly, we opened perspectives concerning the understanding of Sir3 functions by revealing genetic links with genes implicated in transcription and in replication.

To conclude this thesis, we will discuss the flexibility of the structures associated with the SIR complex and underline what we think could bring a better understanding of silencing in budding yeast. Then we will underline the implication of our results on our understanding of the links between SIR spreading and spatial clustering of silenced loci. We will sum-up the evidence pointing toward a role of Sir3 in clustering independent from silencing and suggest experiments that could be informative to this respect. The very last words will be an opportunity to discuss the link between heterochromatin and telomeres, and the potential influence of chromatin based silencing on subtelomeric biology.

Studies of the SIR complex brought unvaluable knowledge concerning the process of gene silencing and the mechanism of heterochromatin propagation. The level of details of our understanding of this complex is incomparable with other heterochromatin systems and offers the possibility to ask numerous questions. As we have seen throughout this work, the SIR complex can function with a relative flexibility. Different structures can nucleate the complex, which propagation is sensitive to the dose of Sir3 as long as the chromatin substrate allows its binding. For this reason the SIR complex has to be considered with all the proteins that contributes to its limitation. For example, while over-abundancy of Sir3 is lethal to cell lacking Dot1, it is not even sufficient to implement silencing in cells overexpressing *DOT1*. In addition, changes in the environment can impact the spreading of Sir3. For example we noticed that the left barrier of HMR is specifically changed upon growth in galactose (Batté et al. 2017) (Data from Annex 2, figure not shown)

All those parameters constitute a multi-dimensional phase space, which our work contributed to explore. Looking back after completion of this work, I think that the approach undertaken should be extended systematically. Using combinations of carbon source independent tunable promoters such as the beta oestradiol system (Ottoz, Rudolf, and Stelling 2014; Quintero et al. 2007; C. Y. Gao and Pinkham 2000) and copper inducible promoters one could study the impact of modulating the dose of Sir2, Sir3, Sir4, Sas2, Dot1 on Sir3 binding and on silencing. It is interesting to consider where our laboratory strains stand within this phase space. It is likely that the conditions that permit the variegation of telomeric gene expression represent a restricted volume of the phase space.

Since a few years various groups undertook to set up interactive models of the spreading of the heterochromatin. Those studies can be separated into two categories: probabilistic models (Mukhopadhyay and Sengupta 2013; Dayarian and Sengupta 2013; David-Rus et al. 2009) and polymer-physics models (Jost et al. 2014; Jost, Vaillant, and Meister 2017). Models describing the SIR complex are in majority based on a dynamic systems framework (Mukhopadhyay and Sengupta 2013). Our work would help significantly to confront the predictions of models with experimental observations. For example we clearly showed that changing the dose of Sir2 is not equivalent to changing the dose of Sir3, something that is not currently accounted for. Moreover the perspective of using polymer models might

enable to efficiently take into account the influence of spatial proximity on the silencing process and to compare it with the Hi-C data that we produced (Guidi et al, and data not shown). Hopefully working on those models will pay-back and help us to understand the current grey areas concerning SIR propagation and silencing. Among those stand the mechanism by which tight binding of proteins to DNA can induce silencing (Dubarry et al. 2011) or the mechanism of silencing inheritance (Jeffery et al. 2013).

The presence of active mechanisms reducing the heritability of the silenced state in *S.pombe* is inspiring (Ragunathan, Jih, and Moazed 2014). While we are chasing examples of epigenetic memories and associate those memories with beneficial processes, fungi actively erase them. It would be fascinating to study the functional consequences of the active erasure of epigenetic silencing at the population scale.

On the link between spatial clustering of telomere and silencing propagation

A fascinating property of silenced domains is their tendency to cluster in space. Spatial proximity has been suggested to help the nucleation and the propagation of silencing by increasing the local concentration of silencing factors. However this hypothesis is difficult to address. Previous work from our laboratory demonstrated that the spatial clustering of telomere can be separated from silencing (Ruault et al. 2011b). Furthermore we established that when cells enter quiescence telomeres cluster together at the nuclear interior. In this configuration however Sir3 dosage is wild type, and binding of Sir3 along subtelomeres was similar to that of exponentially growing cells (Guidi et al. 2015) (Annexe1). In specific mutant context such as the H3 Δ 4-30 mutants Sir3 was shown to spread further toward the centromeres (Sperling and Grunstein 2009). When we probed telomere clustering in those mutants we observed that it is largely comparable to wild type cells. However when Sir3 is overexpressed in those strains we observed telomere hyperclusters (not shown). In *asf1* mutants Sir3 binding is similar to WT at most telomeres and extended at some (Lu and Kobor 2014), but silencing is partially compromised. Clustering of HM loci is lost in *asf1* mutants but silencing is only partially lost (Miele, Bystricky, and Dekker 2009). Our preliminary results showed that overexpression of Sir3 doesn't induce telomere clustering

but apparently leads to increased silencing at subtelomeres. In striking contrast *asf1* mutants could mediate clustering upon entry into quiescence. Additional work is required to confirm those results and to dig further in the implication of this histone chaperone in the spatial clustering of silent domains. Irrespective of the mechanistic details of those processes, it suggests that Sir3 binding to telomeres or to chromatin is not sufficient to trigger the clustering of silencing loci. The absence of telomere in a histone chaperone mutant overexpressing Sir3 is particularly surprising as histones are absent from telomeric tracks and clustering of Rap1-foci can occur when the Sir3-A2Q mutants is only bound to TG₁₋₃ tracks (Ruault et al. 2011; PartI). At last we also observed deficient telomere clustering in exponential phase but not during quiescence in the *ydj1* mutant which is involved in protein folding and has a role in H3 eviction (Qiu et al. 2016). Somehow those observation are reminiscent of a study showing that an imbalance in the Sir3/H4 ratio affects telomere length and Y' recombination (Venditti et al. 1999).

What could be the missing link?

We established a collaboration with Luciana Lazar-Stefanita from the group of R.Kozul from the Institut Pasteur in order to find out if Sir3 bound to chromatin can mediate trans interactions. The results are not presented here because the analysis of the Hi-C data is not completed yet. By comparing the contact maps of strains overexpressing Sir3 to strains overexpressing Sir3-A2Q we should be able to answer our question. Mechanistically there are reasons to think that the ability of Sir3 to mediate trans-interaction might differ at nucleation sites and on chromatin. The main argument for this is that Sir3 uses different surfaces to interact with histones or to interact with Rap1, which can lead to different positioning of the C-terminal dimerization domain. One hypothesis explaining *asf1* mutants phenotype could be that histone chaperones are involved in the maturation step of silencing and that their action changes the ability of the SIR complex to mediate trans-interaction. Alternatively, the effects of *asf1* or *ydj1* deletion might be indirect, but in this case transcriptome comparison should help to uncover potential factor(s) of interest.

Potential other roles of Sir3

First indications of a potential role of Sir3 independent of the SIR complex came from early experiments showing that increasing Sir2, Sir3 or Sir2 and Sir3 dosage in a *sir4* Δ mutant background leads to genomic instability (Holmes et al. 1997). Together with our observation of decreased Sir3 binding at overexpressed *SIR3* in *hst1* mutants and the identification of synthetic dosage lethality of Sir3 with genes involved in transcription or replication, a potential role of Sir3 independent of the SIR complex is plausible. In accordance with the well-known properties of the Sir3 protein, if any other function of Sir3 exists it likely involves chromatin recognition and it might be restricted to the same histone marks as silencing is. We will conduct experiments to probe the influence of Sir3 on transcription associated mutagenesis (Sue and Bhagwat 2014; T. Takahashi et al. 2011; Lippert et al. 2011). Future experiments will include the measurement of *SIR3* expression in *hst1 GPD-SIR3* strains. If we confirm that Sir3 is recruited at *SIR3* in a *hst1* dependent manner, the next step will be to see if Sir3 binding signal at the highly expressed GAL genes upon transcriptional activation is affected by *hst1* deletion. In addition, experiments probing Sir3 localization by DAM-ID in a *sir4* mutant background could help to uncover potential localization of Sir3 that might not be captured by ChIP. In parallel, potential unknown interactors of Sir3 might be revealed by mass-spectrometry profiling of Sir3 interactors in a *sir4* Δ or in a *sir2* Δ background. Comparison of the results in the two mutant backgrounds could also help to detect Sir3 binding partners that are specific to the rDNA loci, as Sir3 is bound at the rDNA array in a *sir4* mutant (Ruault et al, unpublished).

Telomeres, subtelomeres and heterochromatin

The transition from circular to linear chromosomes has been proposed to emerge after the genome invasion by spliceosomal introns (Koonin 2006; Garavís, González, and Villasante 2013). Those newcomers destabilized the circular chromosomes leading to frequent double strand breaks. One possible scenario postulates that the mobilisation of non-LTR retrotransposition to double strand breaks might have enabled the formation of prototelomeres at DSB (Garavís, González, and Villasante 2013). Much like what is seen at

Drosophila telomeres, the heterochromatin present at those prototelomeres might have contributed to their capping, but also insured the role of centromeres (Villasante, Abad, and Méndez-Lago 2007)(Agudo et al. 1999), albeit this hypothesis is controversial (Cavalier-Smith 2010). In this scenario one expects that telomeres might have kept segregation functions. Several evidence point toward this direction. In *S.pombe*, deletion of a centromere leads to the formation of a neo-centromere within subtelomeres (Kojiro Ishii et al. 2008). In *D.melanogaster*, overexpression of CENP-A leads to the formation of neo-centromeres that are preferentially located close to subtelomeric heterochromatin boundaries (Olszak et al. 2011). Lastly, a recent study showed that telomeres and centromeres have interchangeable roles in promotion of meiotic spindle formation (Fennell et al. 2015).

It is striking that those two structures are often associated to repeated elements and heterochromatin structures. Interestingly, when telomeric or centromeric are inserted in a plasmid, they are stably propagated in *S.cerevisiae* (M S Longtine et al. 1992). In detail, telomeric track improves plasmid partitioning by recruiting Sir4. Sir4 interaction with Esc1 though its PAD domains is required in this process (Andrulis et al. 2002). The tethering of plasmids to segregating structures such as the nuclear envelope or telomeres improves their partitioning during budding yeast asymmetric division (Gehlen et al. 2011). However, when both telomeric and centromeric are presents the same plasmid becomes unstable. This phenomenon is termed CEN-TEL antagonism and requires Rap1 and the SIR complex (M S Longtine et al. 1993; M S Longtine et al. 1992; Enomoto, Longtine, and Berman 1994).

Thus the link between telomere and heterochromatin silencing is likely an old one. In this work we explored the maximal extent subtelomeric silent domains and found that it correlates with major transitions of subtelomeric chromatin properties. While our experiments are done in a synthetic context in which Sir3 is overabundant they might give useful information in certain contexts. As subtelomeric content is known to be highly variable, rearrangements can potentially position the telomeres in close proximity to the end of extended silent domains. In this case, we can predict that silencing propagation will be blocked. Therefore our experiments potentially identified the location of functional safeguards that could jugulate the variation of subtelomere size. In the future we will

continue to investigate the impact of the Sir complex on subtelomeric chromatin. We hypothesize that its influence might go beyond its location as probed by ChIP. To investigate this possibility we will probe Sir3 binding by DAM-ID.

Last, it would be worth investigating the role of the SIR complex in telomerase mutants survivors. It has been shown that heterochromatin can contribute to telomere capping functions and that telomerase survivor can adapt to a loss of the normally essential capping protein Cdc13 (Larrivé and Wellinger 2006). Thus it would be interesting to probe the influence of the SIR complex on the spatial organization of telomeres in telomerase survivors and test if the SIR complex is required for viability in the absence of telomerase and of Cdc13.

Bibliography

- Agudo, Marta, Ana Losada, José P. Abad, Sergio Pimpinelli, Pedro Ripoll, and Alfredo Villasante. 1999. "Centromeres from Telomeres? The Centromeric Region of the Y Chromosome of *Drosophila Melanogaster* Contains a Tandem Array of Telomeric HeT-A- and TART-Related Sequences." *Nucleic Acids Research* 27 (16): 3318–24. doi:10.1093/nar/27.16.3318.
- Ai, W, P G Bertram, C K Tsang, T F Chan, and X F Zheng. 2002. "Regulation of Subtelomeric Silencing during Stress Response." *Mol Cell* 10 (6): 1295–1305. doi:S1097276502006950 [pii].
- Allshire, Robin C., Jean Paul Javerzat, Nicola J. Redhead, and Gwen Cranston. 1994. "Position Effect Variegation at Fission Yeast Centromeres." *Cell* 76 (1): 157–69. doi:10.1016/0092-8674(94)90180-5.
- Altaf, M, R T Utley, N Lacoste, S Tan, S D Briggs, and J Cote. 2007. "Interplay of Chromatin Modifiers on a Short Basic Patch of Histone H4 Tail Defines the Boundary of Telomeric Heterochromatin." *Mol Cell* 28 (6): 1002–14. doi:S1097-2765(07)00827-1 [pii]10.1016/j.molcel.2007.12.002.
- Andrulis, E D, D C Zappulla, A Ansari, S Perrod, C V Laiosa, M R Gartenberg, and R Sternglanz. 2002. "Esc1, a Nuclear Periphery Protein Required for Sir4-Based Plasmid Anchoring and Partitioning." *Mol Cell Biol* 22 (23): 8292–8301.
- Ansari, A, and M R Gartenberg. 1997. "The Yeast Silent Information Regulator Sir4p Anchors and Partitions Plasmids." *Molecular & Cellular Biology* 17 (12): 7061–68.
- Aparicio, O M, and D E Gottschling. 1994. "Overcoming Telomeric Silencing: A Trans-Activator Competes to Establish Gene Expression in a Cell Cycle-Dependent Way." *Genes Dev* 8 (10): 1133–46.
- Aparicio, Oscar M., Barbara L. Billington, and Daniel E. Gottschling. 1991. "Modifiers of Position Effect Are Shared between Telomeric and Silent Mating-Type Loci in *S. Cerevisiae*." *Cell* 66 (6): 1279–87. doi:10.1016/0092-8674(91)90049-5.
- Armache, K.-J., J. D. Garlick, D. Canzio, G. J. Narlikar, and R. E. Kingston. 2011. "Structural Basis of Silencing: Sir3 BAH Domain in Complex with a Nucleosome at 3.0 Å Resolution." *Science* 334 (6058): 977–82. doi:10.1126/science.1210915.
- Arnaudo, N, I S Fernandez, S H McLaughlin, S Y Peak-Chew, D Rhodes, and F Martino. 2013. "The N-Terminal Acetylation of Sir3 Stabilizes Its Binding to the Nucleosome Core Particle." *Nat Struct Mol Biol* 20 (9): 1119–21. doi:10.1038/nsmb.2641.
- Askree, Syed H, Tal Yehuda, Sarit Smolnikov, Raya Gurevich, Joshua Hawk, Carrie Coker, Anat Krauskopf, Martin Kupiec, and Michael J McEachern. 2004. "A Genome-Wide Screen for *Saccharomyces Cerevisiae* Deletion Mutants That Affect Telomere Length." *Proceedings of the National Academy of Sciences of the United States of America* 101 (23): 8658–63. doi:10.1073/pnas.0401263101.
- Axel, Richard. 2005. "Scents and Sensibility: A Molecular Logic of Olfactory Perception (Nobel Lecture)." In *Angewandte Chemie - International Edition*, 44:6111–27. doi:10.1002/anie.200501726.
- Barr, ML, and G.E Bertram. 1949. "A Morphological Distinction between Neurones of the Male and Female, and the Behaviour of the Nucleolar Satellite during Accelerated Nucleoprotein Synthesis." *Nature*.
- Barry, C E, S F Hayes, and T Hackstadt. 1992. "Nucleoid Condensation in *Escherichia Coli* That Express a Chlamydial Histone Homolog." *Science (New York, N.Y.)* 256 (5055): 377–79. doi:10.1126/science.256.5055.377.
- Batté, Amandine, Clémentine Brocas, Hélène Bordelet, Antoine Hoher, Myriam Ruault, Adouda Adjiri, Angela Taddei, and Karine Dubrana. 2017. "Recombination at Subtelomeres Is Regulated by Physical Distance, Double-strand Break Resection and Chromatin Status." *The EMBO Journal*, e201796631.

doi:10.15252/embj.201796631.

- Baumann, P, and T R Cech. 2001. "Pot1, the Putative Telomere End-Binding Protein in Fission Yeast and Humans." *Science (New York, N.Y.)* 292 (5519): 1171–75. doi:10.1126/science.1060036.
- Baur, J A, Y Zou, J W Shay, and W E Wright. 2001. "Telomere Position Effect in Human Cells." *Science* 292 (5524): 2075–77.
- Bedalov, A, T Gathbonton, W P Irvine, D E Gottschling, and J A Simon. 2001. "Identification of a Small Molecule Inhibitor of Sir2p." *Proc Natl Acad Sci U S A* 98 (26): 15113–18.
- Bell, Stephen P., Jay Mitchell, Jess Leber, Ryuji Kobayashi, and Bruce Stillman. 1995. "The Multidomain Structure of Orc1 P Reveals Similarity to Regulators of DNA Replication and Transcriptional Silencing." *Cell* 83 (4): 563–68. doi:10.1016/0092-8674(95)90096-9.
- Bernard, P, J F Maure, J F Partridge, S Genier, J P Javerzat, R C Allshire, K. J. Dej, et al. 2001. "Requirement of Heterochromatin for Cohesion at Centromeres." *Science (New York, N.Y.)* 294 (5551): 2539–42. doi:10.1126/science.1064027.
- Bernstein, B E, J K Tong, and S L Schreiber. 2000. "Genomewide Studies of Histone Deacetylase Function in Yeast." *Proceedings of the National Academy of Sciences of the United States of America* 97 (25): 13708–13. doi:10.1073/pnas.250477697.
- Bi, X, M Braunstein, G J Shei, and J R Broach. 1999. "The Yeast HML I Silencer Defines a Heterochromatin Domain Boundary by Directional Establishment of Silencing." *Proc Natl Acad Sci U S A* 96 (21): 11934–39. <http://www.ncbi.nlm.nih.gov/pubmed/10518554>.
- Bi, X, and J R Broach. 1999. "UASrpg Can Function as a Heterochromatin Boundary Element in Yeast." *Genes Dev* 13 (9): 1089–1101. <http://www.ncbi.nlm.nih.gov/pubmed/10323861>.
- . 2001. "Chromosomal Boundaries in *S. Cerevisiae*." *Curr Opin Genet Dev* 11 (2): 199–204. <http://www.ncbi.nlm.nih.gov/pubmed/11250144>.
- Bi, X, Q Yu, J J Sandmeier, and S Elizondo. 2004. "Regulation of Transcriptional Silencing in Yeast by Growth Temperature." *J Mol Biol* 344 (4): 893–905. doi:S0022-2836(04)01270-7 [pii]10.1016/j.jmb.2004.10.002.
- Bi, X, Q Yu, J J Sandmeier, and Y Zou. 2004. "Formation of Boundaries of Transcriptionally Silent Chromatin by Nucleosome-Excluding Structures." *Mol Cell Biol* 24 (5): 2118–31. <http://www.ncbi.nlm.nih.gov/pubmed/14966290>.
- Biamonti, Giuseppe, and Claire Vourc'h. 2010. "Nuclear Stress Bodies." *Cold Spring Harbor Perspectives in Biology*. doi:10.1101/cshperspect.a000695.
- Biswas, Moumita, Nazif Maqani, Ragini Rai, Srikala P Kumaran, Kavitha R Iyer, Erdem Sendinc, Jeffrey S Smith, and Shikha Laloraya. 2009. "Limiting the Extent of the RDN1 Heterochromatin Domain by a Silencing Barrier and Sir2 Protein Levels in *Saccharomyces Cerevisiae*." *Molecular and Cellular Biology* 29 (10): 2889–98. doi:10.1128/MCB.00728-08.
- Blackburn, Elizabeth H. 2010. "Telomeres and Telomerase: The Means to the End (Nobel Lecture)." *Angewandte Chemie (International Ed. in English)* 49: 7405–21. doi:10.1002/anie.201002387.
- Blackburn, Elizabeth H., and Joseph G. Gall. 1978. "A Tandemly Repeated Sequence at the Termini of the Extrachromosomal Ribosomal RNA Genes in *Tetrahymena*." *Journal of Molecular Biology* 120 (1): 33–53. doi:10.1016/0022-2836(78)90294-2.
- Brachmann, C. B., J. M. Sherman, S. E. Devine, E. E. Cameron, L. Pillus, and J. D. Boeke. 1995. "The SIR2 Gene

- Family, Conserved from Bacteria to Humans, Functions in Silencing, Cell Cycle Progression, and Chromosome Stability." *Genes and Development* 9 (23): 2888–2902. doi:10.1101/GAD.9.23.2888.
- Brangwynne, C. P., C. R. Eckmann, D. S. Courson, A. Rybarska, C. Hoegel, J. Gharakhani, F. Julicher, et al. 2009. "Germline P Granules Are Liquid Droplets That Localize by Controlled Dissolution/Condensation." *Science* 324 (5935): 1729–32. doi:10.1126/science.1172046.
- Brangwynne, Clifford P, Timothy J Mitchison, and Anthony A Hyman. 2011. "Active Liquid-like Behavior of Nucleoli Determines Their Size and Shape in *Xenopus Laevis* Oocytes." *Proc. Natl. Acad. Sci.* 108 (11): 4334–39. doi:10.1073/pnas.1017150108.
- Breker, Michal, Melissa Gymrek, Ofer Moldavski, and Maya Schuldiner. 2014. "LoQAtE - Localization and Quantitation ATlas of the Yeast proteome. A New Tool for Multiparametric Dissection of Single-Protein Behavior in Response to Biological Perturbations in Yeast." *Nucleic Acids Research* 42 (D1). doi:10.1093/nar/gkt933.
- Brown, C A, A W Murray, and K J Verstrepen. 2010. "Rapid Expansion and Functional Divergence of Subtelomeric Gene Families in Yeasts." *Curr Biol* 20 (10): 895–903. doi:10.1016/j.cub.2010.04.027.
- Brown, Spencer W. 1966. "Heterochromatin." *Science*.
- Bupp, J M, A E Martin, E S Stensrud, and S L Jaspersen. 2007. "Telomere Anchoring at the Nuclear Periphery Requires the Budding Yeast Sad1-UNC-84 Domain Protein Mps3." *J Cell Biol* 179 (5): 845–54. doi:jcb.200706040 [pii]10.1083/jcb.200706040.
- Burgess, Rebecca J., Hui Zhou, Junhong Han, Qing Li, and Zhiguo Zhang. 2012. "The SCFDia2 Ubiquitin E3 Ligase Ubiquitylates Sir4 and Functions in Transcriptional Silencing." *PLoS Genetics* 8 (7). doi:10.1371/journal.pgen.1002846.
- Cam, Hugh P, Tomoyasu Sugiyama, Ee Sin Chen, Xi Chen, Peter C FitzGerald, and Shiv I S Grewal. 2005. "Comprehensive Analysis of Heterochromatin- and RNAi-Mediated Epigenetic Control of the Fission Yeast Genome." *Nature Genetics* 37 (8): 809–19. doi:10.1038/ng1602.
- Carlson, M, J L Celenza, and F J Eng. 1985. "Evolution of the Dispersed SUC Gene Family of *Saccharomyces* by Rearrangements of Chromosome Telomeres." *Molecular and Cellular Biology* 5 (11): 2894–2902. doi:10.1128/MCB.5.11.2894.
- Carrozza, Michael J., Bing Li, Laurence Florens, Tamaki Saganuma, Selene K. Swanson, Kenneth K. Lee, Wei Jong Shia, et al. 2005. "Histone H3 Methylation by Set2 Directs Deacetylation of Coding Regions by Rpd3S to Suppress Spurious Intragenic Transcription." *Cell* 123 (4): 581–92. doi:10.1016/j.cell.2005.10.023.
- Cavalier-Smith, T. 2010. "Origin of the Cell Nucleus, Mitosis and Sex: Roles of Intracellular Coevolution." *Biology Direct* 5: 7. doi:10.1186/1745-6150-5-7.
- Celic, Ivana, Hiroshi Masumoto, Wendell P. Griffith, Pamela Meluh, Robert J. Cotter, Jef D. Boeke, and Alain Verreault. 2006. "The Sirtuins Hst3 and Hst4p Preserve Genome Integrity by Controlling Histone H3 Lysine 56 Deacetylation." *Current Biology* 16 (13): 1280–89. doi:10.1016/j.cub.2006.06.023.
- Chakraborty, Sangita A., Robert T. Simpson, and Sergei A. Grigoryev. 2011. "A Single Heterochromatin Boundary Element Imposes Position-Independent Antisilencing Activity in *Saccharomyces Cerevisiae* Minichromosomes." *PLoS ONE* 6 (9). doi:10.1371/journal.pone.0024835.
- Chang, Chun Hsiang, and Donal S. Luse. 1997. "The H3/H4 Tetramer Blocks Transcript Elongation by RNA Polymerase II in Vitro." *Journal of Biological Chemistry* 272 (37): 23427–34. doi:10.1074/jbc.272.37.23427.
- Chang, J F, B E Hall, J C Tanny, D Moazed, D Filman, and T Ellenberger. 2003. "Structure of the Coiled-Coil

- Dimerization Motif of Sir4 and Its Interaction with Sir3." *Structure* 11 (6): 637–49. doi:S0969212603000935 [pii].
- Chávez, S, T Beilharz, A G Rondón, H Erdjument-Bromage, P Tempst, J Q Svejstrup, T Lithgow, and A Aguilera. 2000. "A Protein Complex Containing Tho2, Hpr1, Mft1 and a Novel Protein, Thp2, Connects Transcription Elongation with Mitotic Recombination in *Saccharomyces Cerevisiae*." *The EMBO Journal* 19 (21): 5824–34. doi:10.1093/emboj/19.21.5824.
- Chávez, S, M García-Rubio, F Prado, and A Aguilera. 2001. "Hpr1 Is Preferentially Required for Transcription of Either Long or G+C-Rich DNA Sequences in *Saccharomyces Cerevisiae*." *Molecular and Cellular Biology* 21 (20): 7054–64. doi:10.1128/MCB.21.20.7054-7064.2001.
- Chen, L, and J Widom. 2005. "Mechanism of Transcriptional Silencing in Yeast." *Cell* 120 (1): 37–48. doi:10.1016/j.cell.2004.11.030.
- Chen, Lingyi, and Jonathan Widom. 2005. "Mechanism of Transcriptional Silencing in Yeast." *Cell* 120 (1): 37–48. doi:10.1016/j.cell.2004.11.030.
- Chen, Q, A Ijima, and C W Greider. 2001. "Two Survivor Pathways That Allow Growth in the Absence of Telomerase Are Generated by Distinct Telomere Recombination Events." *Molecular and Cellular Biology* 21 (5): 1819–27. doi:10.1128/MCB.21.5.1819-1827.2001.
- Chen, Yong, Rekha Rai, Zi-Ren Zhou, Junko Kanoh, Cyril Ribeyre, Yuting Yang, Hong Zheng, et al. 2011. "A Conserved Motif within RAP1 Has Diversified Roles in Telomere Protection and Regulation in Different Organisms." *Nature Structural & Molecular Biology* 18 (2): 213–21. doi:10.1038/nsmb.1974.
- Chiu, Ya Hui, Qun Yu, Joseph J. Sandmeier, and Xin Bi. 2003. "A Targeted Histone Acetyltransferase Can Create a Sizable Region of Hyperacetylated Chromatin and Counteract the Propagation of Transcriptionally Silent Chromatin." *Genetics* 165 (1): 115–25.
- Choe, W, M Budd, O Imamura, L Hoopes, and J L Campbell. 2002. "Dynamic Localization of an Okazaki Fragment Processing Protein Suggests a Novel Role in Telomere Replication." *Mol Cell Biol* 22 (12): 4202–17. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12024033.
- Churchman, L Stirling, and Jonathan S Weissman. 2011. "Nascent Transcript Sequencing Visualizes Transcription at Nucleotide Resolution." *Nature* 469 (7330): 368–73. doi:10.1038/nature09652.
- Cockell, M M, S Perrod, and S M Gasser. 2000. "Analysis of Sir2p Domains Required for rDNA and Telomeric Silencing in *Saccharomyces Cerevisiae*." *Genet* 154 (3): 1069–83.
- Connelly, J J, P Yuan, H C Hsu, Z Li, R M Xu, and R Sternglanz. 2006. "Structure and Function of the *Saccharomyces Cerevisiae* Sir3 BAH Domain." *Mol Cell Biol* 26 (8): 3256–65. doi:26/8/3256 [pii]10.1128/MCB.26.8.3256-3265.2006.
- Cremer, T, and C Cremer. 2001. "Chromosome Territories, Nuclear Architecture and Gene Regulation in Mammalian Cells." *Genetics* 2 (April): 292–301. doi:10.1038/35066075.
- Cubizolles, F, F Martino, S Perrod, and S M Gasser. 2006. "A Homotrimer-Heterotrimer Switch in Sir2 Structure Differentiates rDNA and Telomeric Silencing." *Mol Cell* 21 (6): 825–36. doi:S1097-2765(06)00086-4 [pii]10.1016/j.molcel.2006.02.006.
- Cui, Tailin, Naoki Moro-oka, Katsufumi Ohsumi, Kenichi Kodama, Taku Ohshima, Naotake Ogasawara, Hirota Mori, Barry Wanner, Hironori Niki, and Takashi Horiuchi. 2007. "Escherichia Coli with a Linear Genome." *EMBO Reports* 8 (2): 181–87. doi:10.1038/sj.embor.7400880.

- Dan, Jiameng, Yifei Liu, Na Liu, Maria Chiourea, Maja Okuka, Tao Wu, Xiaoying Ye, et al. 2014. "Rif1 Maintains Telomere Length Homeostasis of ESCs by Mediating Heterochromatin Silencing." *Developmental Cell* 29 (1): 7–19. doi:10.1016/j.devcel.2014.03.004.
- David-Rus, Diana, Swagatam Mukhopadhyay, Joel L. Lebowitz, and Anirvan M. Sengupta. 2009. "Inheritance of Epigenetic Chromatin Silencing." *Journal of Theoretical Biology* 258 (1): 112–20. doi:10.1016/j.jtbi.2008.12.021.
- Dayarian, Adel, and Anirvan M Sengupta. 2013. "Titration and Hysteresis in Epigenetic Chromatin Silencing." *Physical Biology* 10 (3): 036005. doi:10.1088/1478-3975/10/3/036005.
- De Jong, R. N., and P. C. Van Der Vliet. 1999. "Mechanism of DNA Replication in Eukaryotic Cells: Cellular Host Factors Stimulating Adenovirus DNA Replication." *Gene*. doi:10.1016/S0378-1119(99)00249-8.
- De Koning, Leanne, Armelle Corpet, James E Haber, and Geneviève Almouzni. 2007. "Histone Chaperones: An Escort Network Regulating Histone Traffic." *Nature Structural & Molecular Biology* 14 (11): 997–1007. doi:10.1038/nsmb1318.
- de Lange, Titia. 2004. "T-Loops and the Origin of Telomeres." *Nature Reviews. Molecular Cell Biology* 5 (4): 323–29. doi:10.1038/nrm1422.
- De Rubertis, F, D Kadosh, S Henchoz, D Pauli, G Reuter, K Struhl, and P Spierer. 1996. "The Histone Deacetylase RPD3 Counteracts Genomic Silencing in *Drosophila* and Yeast." *Nature* 384 (6609): 589–91.
- Dekker, J, K Rippe, M Dekker, and N Kleckner. 2002. "Capturing Chromosome Conformation." *Science* 295 (5558): 1306–11.
- Denison, C, D S Kirkpatrick, and S P Gygi. 2005. "Proteomic Insights into Ubiquitin and Ubiquitin-like Proteins." *Curr Opin Chem Biol* 9 (1): 69–75. doi:10.1016/j.cbpa.2004.10.010.
- Dion, Michael F, Steven J Altschuler, Lani F Wu, Oliver J Rando, Steven J Altschuler, Oliver J Rando, and Michael F Dion. 2005. *Genomic Characterization Reveals a Simple Histone H4 Acetylation Code. Proc Natl Acad Sci USA*. Vol. 102. doi:10.1073/pnas.0500136102.
- Dion, Michael F, Tommy Kaplan, Minkyu Kim, Stephen Buratowski, Nir Friedman, and Oliver J Rando. 2007. "Dynamics of Replication-Independent Histone Turnover in Budding Yeast." *Science (New York, N.Y.)* 315 (5817): 1405–8. doi:10.1126/science.1134053.
- Dodson, Anne E., and Jasper Rine. 2015. "Heritable Capture of Heterochromatin Dynamics in *Saccharomyces Cerevisiae*." *eLife* 4: e05007. doi:10.7554/eLife.05007.
- Donze, D., and R. T. Kamakaka. 2001. "RNA Polymerase III and RNA Polymerase II Promoter Complexes Are Heterochromatin Barriers in *Saccharomyces Cerevisiae*." *EMBO Journal* 20 (3): 520–31. doi:10.1093/emboj/20.3.520.
- Donze, David, Christopher R. Adams, Jasper Rine, and Rohinton T. Kamakaka. 1999. "The Boundaries of the Silenced HMR Domain in *Saccharomyces Cerevisiae*." *Genes and Development* 13 (6): 698–708. doi:10.1101/gad.13.6.698.
- Donze, David, and Rohinton T. Kamakaka. 2002. "Braking the Silence: How Heterochromatic Gene Repression Is Stopped in Its Tracks." *BioEssays*. doi:10.1002/bies.10072.
- Downey, Michael, Britta Knight, Ajay A. Vashisht, Charles A. Seller, James A. Wohlschlegel, David Shore, and David P. Toczyski. 2013. "Gcn5 and Sirtuins Regulate Acetylation of the Ribosomal Protein Transcription Factor ifh1." *Current Biology* 23 (17): 1638–48. doi:10.1016/j.cub.2013.06.050.
- Dubarry, M, I Loiodice, C L Chen, C Thermes, and A Taddei. 2011. "Tight Protein-DNA Interactions Favor Gene

- Silencing." *Genes Dev* 25 (13): 1365–70. doi:10.1101/gad.611011.
- Ehrentraut, Stefan, Markus Hassler, Mariano Oppikofer, Stephanie Kueng, Jan M. Weber, Jonathan W. Mueller, Susan M. Gasser, Andreas G. Ladurner, and Ann E. Ehrenhofer-Murray. 2011. "Structural Basis for the Role of the Sir3 AAA + Domain in Silencing: Interaction with Sir4 and Unmethylated Histone H3K79." *Genes and Development* 25 (17): 1835–46. doi:10.1101/gad.17175111.
- Ehrentraut, Stefan, Jan M Weber, J Nikolaj Dybowski, Daniel Hoffmann, and Ann E Ehrenhofer-Murray. 2010. "Rpd3-Dependent Boundary Formation at Telomeres by Removal of Sir2 Substrate." *Proceedings of the National Academy of Sciences of the United States of America* 107 (12): 5522–27. doi:10.1073/pnas.0909169107.
- Ekwall, K, E R Nimmo, J P Javerzat, B Borgstrom, R Egel, G Cranston, and R Allshire. 1996. "Mutations in the Fission Yeast Silencing Factors *clr4+* and *rik1+* Disrupt the Localisation of the Chromo Domain Protein Swi6p and Impair Centromere Function." *J Cell Sci* 109 (Pt 11): 2637–48.
- Ellahi, Aisha, Deborah M. Thurtle, and Jasper Rine. 2015. "The Chromatin and Transcriptional Landscape of Native *Saccharomyces Cerevisiae* Telomeres and Subtelomeric Domains." *Genetics* 200 (2): 505–21. doi:10.1534/genetics.115.175711.
- Emil, Heitz. 1928. "Das Heterochromatin Der Moose." *Jahrbücher Für Wissenschaftliche Botanik* 69: 762–818.
- Enomoto, S, M S Longtine, and J Berman. 1994. "TEL+CEN Antagonism on Plasmids Involves Telomere Repeat Sequences Tracts and Gene Products That Interact with Chromosomal Telomeres." *Chromosoma* 103 (4): 237–50. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7988285.
- Enomoto, S, P McCune-Zierath, M Geraminejad, M Sanders, and J Berman. 1997. "Rfl2, a Subunit of Yeast Chromatin Assembly Factor-I, Is Required for Telomeric Chromatin Function in Vivo." *Genes Dev.* 11: 358–63.
- Falahati, Hanieh, Bobbie Pelham-Webb, Shelby Blythe, and Eric Wieschaus. 2016. "Nucleation by rRNA Dictates the Precision of Nucleolus Assembly." *Current Biology* 26 (3): 277–85. doi:10.1016/j.cub.2015.11.065.
- Fennell, Alex, Alfonso Fernández-álvarez, Kazunori Tomita, and Julia Promisel Cooper. 2015. "Telomeres and Centromeres Have Interchangeable Roles in Promoting Meiotic Spindle Formation." *Journal of Cell Biology* 208 (4): 415–28. doi:10.1083/jcb.201409058.
- Fingerman, Ian M., Chia Ling Wu, Bradley D. Wilson, and Scott D. Briggs. 2005. "Global Loss of Set1-Mediated H3 Lys4 Trimethylation Is Associated with Silencing Defects in *Saccharomyces Cerevisiae*." *Journal of Biological Chemistry* 280 (31): 28761–65. doi:10.1074/jbc.C500097200.
- Fourel, G, E Lebrun, and E Gilson. 2002. "Protosilencers as Building Blocks for Heterochromatin." *Bioessays* 24 (9): 828–35.
- Fourel, G, E Revardel, C E Koering, and E Gilson. 1999. "Cohabitation of Insulators and Silencing Elements in Yeast Subtelomeric Regions." *EMBO J* 18 (9): 2522–37. doi:10.1093/emboj/18.9.2522.
- Fox, C. a., Ann E Ehrenhofer-Murray, S Loo, and J Rine. 1997. "The Origin Recognition Complex, SIR1, and the S Phase Requirement for Silencing." *Science* 276 (5318): 1547–51. doi:10.1126/science.276.5318.1547.
- Frederiks, Floor, Manuel Tzouros, Gideon Oudgenoeg, Tibor van Welsem, Maarten Fornerod, Jeroen Krijgsveld, and Fred van Leeuwen. 2008. "Nonprocessive Methylation by Dot1 Leads to Functional Redundancy of Histone H3K79 Methylation States." *Nature Structural & Molecular Biology* 15 (6): 550–57. doi:10.1038/nsmb.1432.

- Friedman, R, and A. L Hughes. 2001. "Gene Duplication and the Structure of Eukaryotic Genomes." *Genome Research* 11 (3): 373–81. doi:10.1101/gr.155801.
- Fulcher, Nick, Elisa Derboven, Sona Valuchova, and Karel Riha. 2014. "If the Cap Fits, Wear It: An Overview of Telomeric Structures over Evolution." *Cellular and Molecular Life Sciences*. doi:10.1007/s00018-013-1469-z.
- Gaglio, Davide, Anna D'Alfonso, and Giorgio Camilloni. 2013. "Functional Complementation of sir2 Δ Yeast Mutation by the Human Orthologous Gene SIRT1." *PLoS ONE* 8 (12). doi:10.1371/journal.pone.0083114.
- Gao, C Y, and J L Pinkham. 2000. "Tightly Regulated, Beta-Estradiol Dose-Dependent Expression System for Yeast." *BioTechniques* 29 (6): 1226–31. <http://www.ncbi.nlm.nih.gov/pubmed/11126125>.
- Gao, L, and D S Gross. 2008. "Sir2 Silences Gene Transcription by Targeting the Transition between RNA Polymerase II Initiation and Elongation." *Mol Cell Biol* 28 (12): 3979–94. doi:MCB.00019-08 [pii]10.1128/MCB.00019-08.
- Garavís, Miguel, Carlos González, and Alfredo Villasante. 2013. "On the Origin of the Eukaryotic Chromosome: The Role of Noncanonical Dna Structures in Telomere Evolution." *Genome Biology and Evolution*. doi:10.1093/gbe/evt079.
- Garcia, Jennifer F., Phillip A. Dumesic, Paul D. Hartley, Hana El-Samad, and Hiten D. Madhani. 2010. "Combinatorial, Site-Specific Requirement for Heterochromatic Silencing Factors in the Elimination of Nucleosome-Free Regions." *Genes and Development* 24 (16): 1758–71. doi:10.1101/gad.1946410.
- Gartenberg, Marc R., and Jeffrey S. Smith. 2016. "The Nuts and Bolts of Transcriptionally Silent Chromatin in *Saccharomyces Cerevisiae*." *Genetics* 203 (4): 1563–99. doi:10.1534/genetics.112.145243.
- Gehlen, L R, S Nagai, K Shimada, P Meister, A Taddei, and S M Gasser. 2011. "Nuclear Geometry and Rapid Mitosis Ensure Asymmetric Episome Segregation in Yeast." *Curr Biol*. doi:S0960-9822(10)01602-7 [pii]10.1016/j.cub.2010.12.016.
- Geissenhoner, A, C Weise, and A E Ehrenhofer-Murray. 2004. "Dependence of ORC Silencing Function on NatA-Mediated Nalpha Acetylation in *Saccharomyces Cerevisiae*." *Mol Cell Biol* 24 (23): 10300–312. doi:24/23/10300 [pii]10.1128/MCB.24.23.10300-10312.2004.
- Gendrel, Anne-Valerie, and Edith Heard. 2014. "Noncoding RNAs and Epigenetic Mechanisms During X-Chromosome Inactivation." *Annual Review of Cell and Developmental Biology* 30 (1): 561–80. doi:10.1146/annurev-cellbio-101512-122415.
- Gerhold, Joachim M., Tiina Sedman, Katarina Visacka, Judita Slezakova, Lubomir Tomaska, Jozef Nosek, and Juhan Sedman. 2014. "Replication Intermediates of the Linear Mitochondrial DNA of *Candida Parapsilosis* Suggest a Common Recombination Based Mechanism for Yeast Mitochondria." *Journal of Biological Chemistry* 289 (33): 22659–70. doi:10.1074/jbc.M114.552828.
- Ghidelli, S, D Donze, N Dhillon, and R T Kamakaka. 2001. "Sir2p Exists in Two Nucleosome-Binding Complexes with Distinct Deacetylase Activities." *EMBO J* 20 (16): 4522–35. doi:10.1093/emboj/20.16.4522.
- Gilson, E, M Roberge, R Giraldo, D Rhodes, and S M Gasser. 1993. "Distortion of the DNA Double Helix by RAP1 at Silencers and Multiple Telomeric Binding Sites." *J Mol Biol* 231 (2): 293–310. doi:S0022-2836(83)71283-0 [pii]10.1006/jmbi.1993.1283.
- Girton, Jack R, and Kristen M Johansen. 2008. "Chromatin Structure and the Regulation of Gene Expression: The Lessons of PEV in *Drosophila*." In *Advances in Genetics*, 61:1–43. doi:10.1016/S0065-2660(07)00001-6.
- Gotta, M, T Laroche, A Formenton, L Maillet, H Scherthan, and S M Gasser. 1996. "The Clustering of Telomeres

- and Colocalization with Rap1, Sir3, and Sir4 Proteins in Wild-Type *Saccharomyces Cerevisiae*." *J Cell Biol* 134 (6): 1349–63.
- Gottschling, D E, O M Aparicio, B L Billington, and V A Zakian. 1990. "Position Effect at *S. Cerevisiae* Telomeres: Reversible Repression of Pol II Transcription." *Cell* 63 (4): 751–62.
- Goudsouzian, Lara K., Creighton T. Tuzon, and Virginia A. Zakian. 2006. "S. *Cerevisiae* Tel1p and Mre11p Are Required for Normal Levels of Est1p and Est2p Telomere Association." *Molecular Cell* 24 (4): 603–10. doi:10.1016/j.molcel.2006.10.005.
- Govind, Chhabi K., Hongfang Qiu, Daniel S. Ginsburg, Chun Ruan, Kimberly Hofmeyer, Cuihua Hu, Venkatesh Swaminathan, Jerry L. Workman, Bing Li, and Alan G. Hinnebusch. 2010. "Phosphorylated Pol II CTD Recruits Multiple HDACs, Including Rpd3C(S), for Methylation-Dependent Deacetylation of ORF Nucleosomes." *Molecular Cell* 39 (2): 234–46. doi:10.1016/j.molcel.2010.07.003.
- Greider, Carol W. 2016. "Regulating Telomere Length from the inside out: The Replication Fork Model." *Genes and Development* 30 (13): 1483–91. doi:10.1101/gad.280578.116.
- Grewal, Shiv I S, and Songtao Jia. 2007. "Heterochromatin Revisited." *Nature Reviews. Genetics* 8 (1): 35–46. doi:10.1038/nrg2008.
- Griffen A, B; Stone W. 1940. "The Second Arm of Chromosome 4 in *Drosophila Melanogaster*."
- Grillo, Isabelle. 2003. "Small-Angle Neutron Scattering Study of a World-Wide Known Emulsion: Le Pastic." *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 225 (1-3): 153–60. doi:10.1016/S0927-7757(03)00331-5.
- Grunstein, M. 1997. "Molecular Model for Telomeric Heterochromatin in Yeast." *Curr Opin Cell Biol* 9 (3): 383–87. doi:S0955-0674(97)80011-7 [pii].
- Grunstein, M, and S M Gasser. 2013. "Epigenetics in *Saccharomyces Cerevisiae*." *Cold Spring Harb Perspect Biol* 5 (7). doi:10.1101/cshperspect.a017491.
- Guidi, Micol, Myriam Ruault, Martial Marbouty, Isabelle Loïodice, Axel Cournac, Cyrille Billaudeau, Antoine Hocher, Julien Mozziconacci, Romain Koszul, and Angela Taddei. 2015. "Spatial Reorganization of Telomeres in Long-Lived Quiescent Cells." *Genome Biology*. doi:10.1186/s13059-015-0766-2.
- Guillemette, Benoît, Alain R. Bataille, Nicolas Gévry, Maryse Adam, Mathieu Blanchette, François Robert, and Luc Gaudreau. 2005. "Variant Histone H2A.z Is Globally Localized to the Promoters of Inactive Yeast Genes and Regulates Nucleosome Positioning." *PLoS Biology* 3 (12): 1–11. doi:10.1371/journal.pbio.0030384.
- Hackstadt, T, W Baehr, and Y Ying. 1991. "Chlamydia Trachomatis Developmentally Regulated Protein Is Homologous to Eukaryotic Histone H1." *Proc Natl Acad Sci U S A* 88 (9): 3937–41. <http://ukpmc.ac.uk/articlerender.cgi?tool=pubmed&pubmedid=2023942&nhttp://www.pnas.org/content/88/9/3937.full.pdf>.
- Hall, I M, G D Shankaranarayana, K Noma, N Ayoub, A Cohen, and S I Grewal. 2002. "Establishment and Maintenance of a Heterochromatin Domain." *Science* 297 (5590): 2232–37.
- Hall, Ira M, Ken-Ichi Noma, and Shiv I S Grewal. 2003. "RNA Interference Machinery Regulates Chromosome Dynamics during Mitosis and Meiosis in Fission Yeast." *Proceedings of the National Academy of Sciences of the United States of America* 100 (1): 193–98. doi:10.1073/pnas.232688099.
- Hanson, Sara J., and Kenneth H. Wolfe. 2017. "An Evolutionary Perspective on Yeast Mating-Type Switching." *Genetics*. doi:10.1534/genetics.117.202036.

- Hardy, C F, D Balderes, and D Shore. 1992. "Dissection of a Carboxy-Terminal Region of the Yeast Regulatory Protein RAP1 with Effects on Both Transcriptional Activation and Silencing." *Molecular and Cellular Biology* 12 (3): 1209–17. doi:10.1128/MCB.12.3.1209.Updated.
- Harouz, Habiba, Christophe Rachez, Benoit M Meijer, Benoit Marteyn, Françoise Donnadiou, Florence Cammas, Christian Muchardt, Philippe Sansonetti, and Laurence Arbibe. 2014. "Shigella Flexneri Targets the HP1 γ Subcode through the Phosphothreonine Lyase OspF." *The EMBO Journal* 33 (22): 2606–22. doi:10.15252/embj.201489244.
- Hayflick, L. 1965. "The Limited in Vitro Lifetime of Human Diploid Cell Strains." *Experimental Cell Research* 37 (3): 614–36. doi:10.1016/0014-4827(65)90211-9.
- Hecht, A, S Strahl-Bolsinger, and M Grunstein. 1996. "Spreading of Transcriptional Repressor SIR3 from Telomeric Heterochromatin." *Nature* 383 (6595): 92–96.
- Hediger, F, A S Berthiau, G van Houwe, E Gilson, and S M Gasser. 2006. "Subtelomeric Factors Antagonize Telomere Anchoring and Tel1-Independent Telomere Length Regulation." *EMBO J* 25 (4): 857–67.
- Hediger, F, F R Neumann, G Van Houwe, K Dubrana, and S M Gasser. 2002. "Live Imaging of Telomeres. yKu and Sir Proteins Define Redundant Telomere-Anchoring Pathways in Yeast." *Curr Biol* 12 (24): 2076–89.
- Heise, Franziska, Ho Ryun Chung, Jan M. Weber, Zhenyu Xu, Ludger Klein-Hitpass, Lars M. Steinmetz, Martin Vingron, and Ann E. Ehrenhofer-Murray. 2012. "Genome-Wide H4 K16 Acetylation by SAS-I Is Deposited Independently of Transcription and Histone Exchange." *Nucleic Acids Research* 40 (1): 65–74. doi:10.1093/nar/gkr649.
- Hickman, M A, and L N Rusche. 2010. "Transcriptional Silencing Functions of the Yeast Protein Orc1/Sir3 Subfunctionalized after Gene Duplication." *Proc Natl Acad Sci U S A* 107 (45): 19384–89. doi:10.1073/pnas.1006436107.
- Hiraga, S, S Botsios, and A D Donaldson. 2008. "Histone H3 Lysine 56 Acetylation by Rtt109 Is Crucial for Chromosome Positioning." *J Cell Biol* 183 (4): 641–51. doi:jcb.200806065 [pii]10.1083/jcb.200806065.
- Hiraga, Shin Ichiro, Sotirios Botsios, and Anne D. Donaldson. 2008. "Histone H3 Lysine 56 Acetylation by Rtt109 Is Crucial for Chromosome Positioning." *Journal of Cell Biology* 183 (4): 641–51. doi:10.1083/jcb.200806065.
- Holmes, S G, A B Rose, K Steuerle, E Saez, S Sayegh, Y M Lee, and J R Broach. 1997. "Hyperactivation of the Silencing Proteins, Sir2p and Sir3p, Causes Chromosome Loss." *Genetics* 145 (3): 605–14. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9055071.
- Hoppe, G J, J C Tanny, A D Rudner, S A Gerber, S Danaie, S P Gygi, and D Moazed. 2002. "Steps in Assembly of Silent Chromatin in Yeast: Sir3-Independent Binding of a Sir2/Sir4 Complex to Silencers and Role for Sir2-Dependent Deacetylation." *Mol Cell Biol* 22 (22): 4167–80.
- Horowitz, H, P Thorburn, and J E Haber. 1984. "Rearrangements of Highly Polymorphic Regions near Telomeres of Saccharomyces Cerevisiae." *Molecular and Cellular Biology* 4 (11): 2509–17. doi:10.1128/MCB.4.11.2509.Updated.
- Hoze, N, M Ruault, C Amoruso, A Taddei, and D Holcman. 2013. "Spatial Telomere Organization and Clustering in Yeast Saccharomyces Cerevisiae Nucleus Is Generated by a Random Dynamics of Aggregation-Dissociation." *Mol Biol Cell* 24 (11): 1791–1800, S1–10. doi:10.1091/mbc.E13-01-0031.
- Hsu, Hao Chi, Chia Lin Wang, Mingzhu Wang, Na Yang, Zhi Chen, Rolf Sternglanz, and Rui Ming Xu. 2013. "Structural Basis for Allosteric Stimulation of Sir2 Activity by sir4 Binding." *Genes and Development* 27 (1): 64–73. doi:10.1101/gad.208140.112.

- Huang, Fu, Saravanan Ramakrishnan, Srijana Pokhrel, Christian Pflueger, Timothy J. Parnell, Margaret M. Kasten, Simon L. Currie, et al. 2015. "Interaction of the Jhd2 Histone H3 Lys-4 Demethylase with Chromatin Is Controlled by Histone H2A Surfaces and Restricted by H2B Ubiquitination." *Journal of Biological Chemistry* 290 (48): 28760–77. doi:10.1074/jbc.M115.693085.
- Hyman, Anthony A., Christoph A. Weber, and Frank Jülicher. 2014. "Liquid-Liquid Phase Separation in Biology." *Annual Review of Cell and Developmental Biology* 30 (1): 39–58. doi:10.1146/annurev-cellbio-100913-013325.
- Irlbacher, H, J Franke, T Manke, M Vingron, and A E Ehrenhofer-Murray. 2005. "Control of Replication Initiation and Heterochromatin Formation in *Saccharomyces Cerevisiae* by a Regulator of Meiotic Gene Expression." *Genes Dev* 19 (15): 1811–22. doi:19/15/1811 [pii]10.1101/gad.334805.
- Ishii, K, G Arib, C Lin, G Van Houwe, and U K Laemmli. 2002. "Chromatin Boundaries in Budding Yeast: The Nuclear Pore Connection." *Cell* 109 (5): 551–62.
- Ishii, Kojiro, and Ulrich K. Laemmli. 2003. "Structural and Dynamic Functions Establish Chromatin Domains." *Molecular Cell* 11 (1): 237–48. doi:10.1016/S1097-2765(03)00010-8.
- Ishii, Kojiro, Yuki Ogiyama, Yuji Chikashige, Saeko Soejima, Fumie Masuda, Tatsuyuki Kakuma, Yasushi Hiraoka, and Kohta Takahashi. 2008. "Heterochromatin Integrity Affects Chromosome Reorganization after Centromere Dysfunction." *Science (New York, N.Y.)* 321 (5892): 1088–91. doi:10.1126/science.1158699.
- Jacob, François, and Jacques Monod. 1961. "Genetic Regulatory Mechanisms in the Synthesis of Proteins." *Journal of Molecular Biology* 3 (3): 318–56. doi:10.1016/S0022-2836(61)80072-7.
- Jambunathan, Nithya, Adam W. Martinez, Elizabeth C. Robert, Nneamaka B. Agochukwu, Megan E. Ibos, Sandra L. Dugas, and David Donze. 2005. "Multiple Bromodomain Genes Are Involved in Restricting the Spread of Heterochromatic Silencing at the *Saccharomyces Cerevisiae* HMR-tRNA Boundary." *Genetics* 171 (3): 913–22. doi:10.1534/genetics.105.046938.
- Janke, C, M M Magiera, N Rathfelder, C Taxis, S Reber, H Maekawa, A Moreno-Borchart, et al. 2004. "A Versatile Toolbox for PCR-Based Tagging of Yeast Genes: New Fluorescent Proteins, More Markers and Promoter Substitution Cassettes." *Yeast* 21 (11): 947–62. doi:10.1002/yea.1142.
- Jeffery, Daniel C B, Brandon A. Wyse, Muhammad Attiq Rehman, Geoffrey W. Brown, Zhiying You, Roxanne Oshidari, Hisao Masai, and Krassimir Y. Yankulov. 2013. "Analysis of Epigenetic Stability and Conversions in *Saccharomyces Cerevisiae* Reveals a Novel Role of CAF-I in Position-Effect Variegation." *Nucleic Acids Research* 41 (18): 8475–88. doi:10.1093/nar/gkt623.
- Jia, Songtao, Ken-ichi Noma, and Shiv I S Grewal. 2004. "RNAi-Independent Heterochromatin Nucleation by the Stress-Activated ATF/CREB Family Proteins." *Science (New York, N.Y.)* 304 (5679): 1971–76. doi:10.1126/science.1099035.
- Jia, Songtao, Takatomi Yamada, and Shiv I.S. Grewal. 2004. "Heterochromatin Regulates Cell Type-Specific Long-Range Chromatin Interactions Essential for Directed Recombination." *Cell* 119 (4): 469–80. doi:10.1016/j.cell.2004.10.020.
- Jiang, Jun, Yuanchun Jing, Gregory J Cost, Jen-Chieh Chiang, Heather J Kolpa, Allison M Cotton, Dawn M Carone, et al. 2013. "Translating Dosage Compensation to Trisomy 21." *Nature* 500 (7462): 296–300. doi:10.1038/nature12394.
- Jimeno, Sonia, Cristina Tous, María L García-Rubio, Michael Ranes, Cristina González-Aguilera, Antonio Marín, and Andrés Aguilera. 2011. "New Suppressors of THO Mutations Identify Thp3 (Ypr045c)-Csn12 as a Protein Complex Involved in Transcription Elongation." *Molecular and Cellular Biology* 31 (4): 674–85. doi:10.1128/MCB.01188-10.

- Jimeno-González, Silvia, Fernando Gómez-Herreros, Paula M Alepuz, and Sebastián Chávez. 2006. "A Gene-Specific Requirement for FACT during Transcription Is Related to the Chromatin Organization of the Transcribed Region." *Molecular and Cellular Biology* 26 (23): 8710–21. doi:10.1128/MCB.01129-06.
- Jin, Chunyuan, and Gary Felsenfeld. 2007. "Nucleosome Stability Mediated by Histone Variants H3.3 and H2A.Z." *Genes and Development* 21 (12): 1519–29. doi:10.1101/gad.1547707.
- Joshi, Amita A., and Kevin Struhl. 2005. "Eaf3 Chromodomain Interaction with Methylated H3-K36 Links Histone Deacetylation to Pol II Elongation." *Molecular Cell* 20 (6): 971–78. doi:10.1016/j.molcel.2005.11.021.
- Jost, Daniel, Pascal Carrivain, Giacomo Cavalli, and Cédric Vaillant. 2014. "Modeling Epigenome Folding: Formation and Dynamics of Topologically Associated Chromatin Domains." *Nucleic Acids Research* 42 (15): 9553–61. doi:10.1093/nar/gku698.
- Jost, Daniel, Cédric Vaillant, and Peter Meister. 2017. "Coupling 1D Modifications and 3D Nuclear Organization: Data, Models and Function." *Current Opinion in Cell Biology*. doi:10.1016/j.ceb.2016.12.001.
- K??nig, Peter, and Daniela Rhodes. 1997. "Recognition of Telomeric DNA." *Trends in Biochemical Sciences*. doi:10.1016/S0968-0004(97)01008-6.
- Kanoh, Junko, Mahito Sadaie, Takeshi Urano, and Fuyuki Ishikawa. 2005. "Telomere Binding Protein Taz1 Establishes Swi6 Heterochromatin Independently of RNAi at Telomeres." *Current Biology* 15 (20): 1808–19. doi:10.1016/j.cub.2005.09.041.
- Kasinsky, H E, J D Lewis, J B Dacks, and J Ausio. 2001. "Origin of H1 Linker Histones." *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology* 15 (1): 34–42. doi:10.1096/fj.00-0237rev.
- Katan-Khaykovich, Y, and K Struhl. 2005. "Heterochromatin Formation Involves Changes in Histone Modifications over Multiple Cell Generations." *EMBO J* 24 (12): 2138–49.
- Kehayova, Polina D., and David R. Liu. 2007. "In Vivo Evolution of an RNA-Based Transcriptional Silencing Domain in *S. Cerevisiae*." *Chemistry and Biology* 14 (1): 65–74. doi:10.1016/j.chembiol.2006.11.008.
- Keller, Claudia, Ricardo Adaixo, Rieka Stunnenberg, Katrina J. Woolcock, Sebastian Hiller, and Marc Bühler. 2012. "HP1 Swi6 Mediates the Recognition and Destruction of Heterochromatic RNA Transcripts." *Molecular Cell* 47 (2): 215–27. doi:10.1016/j.molcel.2012.05.009.
- Kellis, Manolis, Bruce W Birren, and Eric S Lander. 2004. "Proof and Evolutionary Analysis of Ancient Genome Duplication in the Yeast *Saccharomyces Cerevisiae*." *Nature* 428 VN - (6983): 617–24. doi:10.1038/nature02424.
- Kellum, R, and B M Alberts. 1995. "Heterochromatin Protein 1 Is Required for Correct Chromosome Segregation in *Drosophila* Embryos." *J Cell Sci* 108 (Pt 4): 1419–31.
- Kemmeren, Patrick, Katrin Sameith, Loes A L Van De Pasch, Joris J. Benschop, Tineke L. Lenstra, Thanasis Margaritis, Eoghan O'Duibhir, et al. 2014. "Large-Scale Genetic Perturbations Reveal Regulatory Networks and an Abundance of Gene-Specific Repressors." *Cell* 157 (3): 740–52. doi:10.1016/j.cell.2014.02.054.
- Keogh, Michael Christopher, Siavash K. Kurdistani, Stephanie A. Morris, Seong Hoon Ahn, Vladimir Podolny, Sean R. Collins, Maya Schuldiner, et al. 2005. "Cotranscriptional set2 Methylation of Histone H3 Lysine 36 Recruits a Repressive Rpd3 Complex." *Cell* 123 (4): 593–605. doi:10.1016/j.cell.2005.10.025.
- Kim, Jaehoon, Sandra B. Hake, and Robert G. Roeder. 2005. "The Human Homolog of Yeast BRE1 Functions as a Transcriptional Coactivator through Direct Activator Interactions." *Molecular Cell* 20 (5): 759–70.

doi:10.1016/j.molcel.2005.11.012.

- Kim, Jaehoon, Jung Ae Kim, Robert K. McGinty, Uyen T T Nguyen, Tom W. Muir, C. David Allis, and Robert G. Roeder. 2013. "The N-SET Domain of Set1 Regulates H2B Ubiquitylation-Dependent H3K4 Methylation." *Molecular Cell* 49 (6): 1121–33. doi:10.1016/j.molcel.2013.01.034.
- Kim, Seung Kyoong, Inkyung Jung, Hosuk Lee, Keunsoo Kang, Mirang Kim, Kwiwan Jeong, Chang Seob Kwon, et al. 2012. "Human Histone H3K79 Methyltransferase DOT1L Methyltransferase Binds Actively Transcribing RNA Polymerase II to Regulate Gene Expression." *Journal of Biological Chemistry* 287 (47): 39698–709. doi:10.1074/jbc.M112.384057.
- Kimura, A, T Umehara, and M Horikoshi. 2002. "Chromosomal Gradient of Histone Acetylation Established by Sas2p and Sir2p Functions as a Shield against Gene Silencing." *Nat Genet* 32 (3): 370–77. doi:10.1038/ng993ng1102-343 [pii].
- Kimura, Akatsuki, and Masami Horikoshi. 2004. "Partition of Distinct Chromosomal Regions: Negotiable Border and Fixed Border." *Genes to Cells*. doi:10.1111/j.1356-9597.2004.00740.x.
- Kirchmaier, A L, and J Rine. 2001. "DNA Replication-Independent Silencing in *S. Cerevisiae*." *Science* 291 (5504): 646–50.
- Kirchmaier, Ann L, and Jasper Rine. 2006. "Cell Cycle Requirements in Assembling Silent Chromatin in *Saccharomyces Cerevisiae*." *Molecular and Cellular Biology* 26 (3): 852–62. doi:10.1128/MCB.26.3.852-862.2006.
- Kirkland, Jacob G., Misty R. Peterson, Christopher D. Still, Leo Brueggeman, Namrita Dhillon, and Rohinton T. Kamakaka. 2015. "Heterochromatin Formation via Recruitment of DNA Repair Proteins." *Molecular Biology of the Cell* 26 (7): 1395–1410. doi:10.1091/mbc.E14-09-1413.
- Kirkman, Laura A., and Kirk W. Deitsch. 2012. "Antigenic Variation and the Generation of Diversity in Malaria Parasites." *Current Opinion in Microbiology*. doi:10.1016/j.mib.2012.03.003.
- Kitada, T, B G Kuryan, N N Tran, C Song, Y Xue, M Carey, and M Grunstein. 2012. "Mechanism for Epigenetic Variegation of Gene Expression at Yeast Telomeric Heterochromatin." *Genes Dev* 26 (21): 2443–55. doi:10.1101/gad.201095.112.
- Kitada, Tasuku, Thomas Schleker, Adam S. Sperling, Wei Xie, Susan M. Gasser, and Michael Grunstein. 2011. "H2A Is a Component of Yeast Heterochromatin Required for Telomere Elongation." *Cell Cycle* 10 (2): 293–300. doi:10.4161/cc.10.2.14536.
- Kobryn, Kerri, Julien Briffotiaux, and Victor Karpov. 2009. "Holliday Junction Formation by the *Borrelia burgdorferi* Telomere Resolvase, ResT: Implications for the Origin of Genome Linearity." *Molecular Microbiology* 71 (5): 1117–30. doi:10.1111/j.1365-2958.2008.06584.x.
- Koering, Catherine Elaine, Alessandra Pollice, Mia Pia Zibella, Serge Bauwens, Alain Puisieux, Michele Brunori, Christine Brun, et al. 2002. "Human Telomeric Position Effect Is Determined by Chromosomal Context and Telomeric Chromatin Integrity." *EMBO Reports* 3 (11): 1055–61. doi:10.1093/embo-reports/kvf215.
- König, Peter, Rafael Giraldo, Lynda Chapman, and Daniela Rhodes. 1996. "The Crystal Structure of the DNA-Binding Domain of Yeast RAP1 in Complex with Telomeric DNA." *Cell* 85 (1): 125–36. doi:10.1016/S0092-8674(00)81088-0.
- Koonin, Eugene V. 2006. "The Origin of Introns and Their Role in Eukaryogenesis: A Compromise Solution to the Introns-Early versus Introns-Late Debate?" *Biology Direct* 1: 22. doi:10.1186/1745-6150-1-22.
- Kornberg, R D. 1974. "Chromatin Structure: A Repeating Unit of Histones and DNA." *Science* 184 (139): 868–71.

- Kouzarides, Tony. 2007. "Chromatin Modifications and Their Function." *Cell*. doi:10.1016/j.cell.2007.02.005.
- Kowalik, Katarzyna Maria, Yukiko Shimada, Valentin Flury, Michael Beda Stadler, Julia Batki, and Marc Bühler. 2015. "The Paf1 Complex Represses Small-RNA-Mediated Epigenetic Gene Silencing." *Nature* 520 (7546): 248–52. doi:10.1038/nature14337.
- Kristjuhan, A, B O Wittschieben, J Walker, D Roberts, B R Cairns, and J Q Svejstrup. 2003. "Spreading of Sir3 Protein in Cells with Severe Histone H3 Hypoacetylation." *Proc Natl Acad Sci U S A* 100 (13): 7551–56. doi:10.1073/pnas.13322991001332299100 [pii].
- Kristjuhan, Arnold, Jane Walker, Noriyuki Suka, Michael Grunstein, Douglas Roberts, Bradley R. Cairns, and Jesper Q. Svejstrup. 2002. "Transcriptional Inhibition of Genes with Severe Histone H3 Hypoacetylation in the Coding Region." *Molecular Cell* 10 (4): 925–33. doi:10.1016/S1097-2765(02)00647-0.
- Krogan, Nevan J., Jim Dover, Adam Wood, Jessica Schneider, Jonathan Heidt, Marry Ann Boateng, Kimberly Dean, et al. 2003. "The Paf1 Complex Is Required for Histone H3 Methylation by COMPASS and Dot1p: Linking Transcriptional Elongation to Histone Methylation." *Molecular Cell* 11 (3): 721–29. doi:10.1016/S1097-2765(03)00091-1.
- Kusch, T, and J L Workman. 2007. "Histone Variants and Complexes Involved in Their Exchange." *Subcell Biochem* 41: 91–109. <http://www.ncbi.nlm.nih.gov/pubmed/17484125>.
- Kyrion, G., K. Liu, C. Liu, and A. J. Lustig. 1993. "RAP1 and Telomere Structure Regulate Telomere Position Effects in *Saccharomyces Cerevisiae*." *Genes and Development* 7 (7 A): 1146–59. doi:10.1101/gad.7.7a.1146.
- Ladurner, A G, C Inouye, R Jain, and R Tjian. 2003. "Bromodomains Mediate an Acetyl-Histone Encoded Antisilencing Function at Heterochromatin Boundaries." *Mol Cell* 11 (2): 365–76.
- Larin, Michelle L., Katherine Harding, Elizabeth C. Williams, Noel Lianga, Carole Doré, Sophie Pilon, Éric Langis, Corey Yanofsky, and Adam D. Rudner. 2015. "Competition between Heterochromatic Loci Allows the Abundance of the Silencing Protein, Sir4, to Regulate de Novo Assembly of Heterochromatin." *PLoS Genetics* 11 (11). doi:10.1371/journal.pgen.1005425.
- Larrivé, Michel, and Raymund J Wellinger. 2006. "Telomerase- and Capping-Independent Yeast Survivors with Alternate Telomere States." *Nature Cell Biology* 8 (7): 741–47. doi:10.1038/ncb1429.
- Larson, Adam G., Daniel Elnatan, Madeline M. Keenen, Michael J. Trnka, Jonathan B. Johnston, Alma L. Burlingame, David A. Agard, Sy Redding, and Geeta J. Narlikar. 2017. "Liquid Droplet Formation by HP1 α Suggests a Role for Phase Separation in Heterochromatin." *Nature*. doi:10.1038/nature22822.
- Lau, Anna, Hannah Blitzblau, and Stephen P. Bell. 2002. "Cell-Cycle Control of the Establishment of Mating-Type Silencing in *S. Cerevisiae*." *Genes and Development* 16 (22): 2935–45. doi:10.1101/gad.764102.
- Lenstra, Tineke L., Joris J. Benschop, TaeSoo Kim, Julia M. Schulze, Nathalie A C H Brabers, Thanasis Margaritis, Loes A L van de Pasch, et al. 2011. "The Specificity and Topology of Chromatin Interaction Pathways in Yeast." *Molecular Cell* 42 (4): 536–49. doi:10.1016/j.molcel.2011.03.026.
- Lescasse, Rachel, Sabrina Pobiega, Isabelle Callebaut, and Stéphane Marcand. 2013. "End-Joining Inhibition at Telomeres Requires the Translocase and polySUMO-Dependent Ubiquitin Ligase Uls1." *EMBO J* 32 (6): 805–15. doi:emboj201324 [pii]\r10.1038/emboj.2013.24.
- Levis, Robert W., Robin Ganesan, Kathleen Houtchens, Leigh Anna Tolar, and Fang miin Sheen. 1993. "Transposons in Place of Telomeric Repeats at a *Drosophila* Telomere." *Cell* 75 (6): 1083–93. doi:10.1016/0092-8674(93)90318-K.
- Li, Qing, Hui Zhou, Hugo Wurtele, Brian Davies, Bruce Horazdovsky, Alain Verreault, and Zhiguo Zhang. 2008.

- "Acetylation of Histone H3 Lysine 56 Regulates Replication-Coupled Nucleosome Assembly." *Cell* 134 (2): 244–55. doi:10.1016/j.cell.2008.06.018.
- Li, Y, D A Kirschmann, and L L Wallrath. 2002. "Does Heterochromatin Protein 1 Always Follow Code?" *Proc Natl Acad Sci U S A* 99 Suppl 4: 16462–69. doi:10.1073/pnas.162371699.
- Liaw, H, and A J Lustig. 2006. "Sir3 C-Terminal Domain Involvement in the Initiation and Spreading of Heterochromatin." *Mol Cell Biol* 26 (20): 7616–31. doi:MCB.01082-06 [pii]10.1128/MCB.01082-06.
- Lieb, J D, X Liu, D Botstein, and P O Brown. 2001. "Promoter-Specific Binding of Rap1 Revealed by Genome-Wide Maps of Protein-DNA Association." *Nat Genet* 28 (4): 327–34. doi:10.1038/ng569ng569 [pii].
- Lin, Yu yi, Jin ying Lu, Junmei Zhang, Wendy Walter, Weiwei Dang, Jun Wan, Sheng Ce Tao, et al. 2009. "Protein Acetylation Microarray Reveals That NuA4 Controls Key Metabolic Target Regulating Gluconeogenesis." *Cell* 136 (6): 1073–84. doi:10.1016/j.cell.2009.01.033.
- Liou, G G, J C Tanny, R G Kruger, T Walz, and D Moazed. 2005. "Assembly of the SIR Complex and Its Regulation by O-Acetyl-ADP-Ribose, a Product of NAD-Dependent Histone Deacetylation." *Cell* 121 (4): 515–27. doi:S0092-8674(05)00354-5 [pii]10.1016/j.cell.2005.03.035.
- Lippert, Malcolm J, Nayun Kim, Jang-Eun Cho, Ryan P Larson, Nathan E Schoenly, Shannon H O'Shea, and Sue Jinks-Robertson. 2011. "Role for Topoisomerase 1 in Transcription-Associated Mutagenesis in Yeast." *Proceedings of the National Academy of Sciences of the United States of America* 108 (2): 698–703. doi:10.1073/pnas.1012363108.
- Lloyd, Neil R, Thayne H. Dickey, Robert A. Hom, and Deborah S. Wuttke. 2016. "Tying up the Ends: Plasticity in the Recognition of Single-Stranded DNA at Telomeres." *Biochemistry* 55 (38): 5326–40. doi:10.1021/acs.biochem.6b00496.
- Lomberk, Gwen, Debora Bensi, Martín E Fernandez-Zapico, and Raul Urrutia. 2006. "Evidence for the Existence of an HP1-Mediated Subcode within the Histone Code." *Nature Cell Biology* 8 (4): 407–15. doi:10.1038/ncb1383.
- Longtine, M S, S Enomoto, S L Finstad, and J Berman. 1992. "Yeast Telomere Repeat Sequence (TRS) Improves Circular Plasmid Segregation, and TRS Plasmid Segregation Involves the RAP1 Gene Product." *Mol Cell Biol* 12 (5): 1997–2009.
- . 1993. "Telomere-Mediated Plasmid Segregation in *Saccharomyces Cerevisiae* Involves Gene Products Required for Transcriptional Repression at Silencers and Telomeres." *Genetics* 133 (2): 171–82. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=8436267.
- Longtine, Mark S., Nancy Maxfield Wilson, Marie E. Petracek, and Judith Berman. 1989. "A Yeast Telomere Binding Activity Binds to Two Related Telomere Sequence Motifs and Is Indistinguishable from RAP1." *Current Genetics* 16 (4): 225–39. doi:10.1007/BF00422108.
- Loo, Stephen, and Jasper Rine. 1994. "Silencers and Domains of Generalized Repression." *Science (New York, N.Y.)* 264 (5166): 1768–71. doi:10.1126/science.8209257.
- Lorch, Yahli, Janice W. LaPointe, and Roger D. Kornberg. 1987. "Nucleosomes Inhibit the Initiation of Transcription but Allow Chain Elongation with the Displacement of Histones." *Cell* 49 (2): 203–10. doi:10.1016/0092-8674(87)90561-7.
- Louis, E J. 2002. "Are *Drosophila* Telomeres an Exception or the Rule?" *Genome Biol* 3 (10): REVIEWS0007. <http://www.ncbi.nlm.nih.gov/pubmed/12372147>.
- Louis, E J, and J E Haber. 1992. "The Structure and Evolution of Subtelomeric Y' Repeats in *Saccharomyces*

- Cerevisiae." *Genetics* 131 (3): 559–74. <http://www.ncbi.nlm.nih.gov/pubmed/1628806>.
- Louis, E J, E S Naumova, A Lee, G Naumov, and J E Haber. 1994. "The Chromosome End in Yeast: Its Mosaic Nature and Influence on Recombinational Dynamics." *Genetics* 136 (3): 789–802. <http://www.ncbi.nlm.nih.gov/pubmed/8005434>.
- Louis, EJ, and MM Becker. 2014. "Subtelomeres." <http://link.springer.com/content/pdf/10.1007/978-3-642-41566-1.pdf>.
- Lu, Phoebe Y T, and Michael S. Kobor. 2014. "Maintenance of Heterochromatin Boundary and Nucleosome Composition at Promoters by the Asf1 Histone Chaperone and SWR1-C Chromatin Remodeler in *Saccharomyces Cerevisiae*." *Genetics* 197 (1): 133–45. doi:10.1534/genetics.114.162909.
- Luger, K, A W Mader, R K Richmond, D F Sargent, and T J Richmond. 1997. "Crystal Structure of the Nucleosome Core Particle at 2.8 Å Resolution [see Comments]." *Nature* 389 (6648): 251–60.
- Luijsterburg, Martijn S., Malcolm F. White, Roel van Driel, and Remus Th. Dame. 2008. "The Major Architects of Chromatin: Architectural Proteins in Bacteria, Archaea and Eukaryotes." *Critical Reviews in Biochemistry and Molecular Biology* 43 (6): 393–418. doi:10.1080/10409230802528488.
- Lundblad, Victoria, and Elizabeth H. Blackburn. 1993. "An Alternative Pathway for Yeast Telomere Maintenance Rescues est1- Senescence." *Cell* 73 (2): 347–60. doi:10.1016/0092-8674(93)90234-H.
- Lundblad, Victoria, and Jack W. Szostak. 1989. "A Mutant with a Defect in Telomere Elongation Leads to Senescence in Yeast." *Cell* 57 (4): 633–43. doi:10.1016/0092-8674(89)90132-3.
- Luo, K, M A Vega-Palas, and M Grunstein. 2002. "Rap1-Sir4 Binding Independent of Other Sir, yKu, or Histone Interactions Initiates the Assembly of Telomeric Heterochromatin in Yeast." *Genes Dev* 16 (12): 1528–39.
- Lynch, Patrick J, and Laura N Rusche. 2009. "A Silencer Promotes the Assembly of Silenced Chromatin Independently of Recruitment." *Molecular and Cellular Biology* 29 (1): 43–56. doi:10.1128/MCB.00983-08.
- Magklara, Angeliki, Angela Yen, Bradley M. Colquitt, E. Josephine Clowney, William Allen, Eirene Markenscoff-Papadimitriou, Zoe A. Evans, et al. 2011. "An Epigenetic Signature for Monoallelic Olfactory Receptor Expression." *Cell* 145 (4): 555–70. doi:10.1016/j.cell.2011.03.040.
- Maltby, Vicki E, Benjamin J E Martin, Julie Brind'Amour, Adam T Chruscicki, Kristina L McBurney, Julia M Schulze, Ian J Johnson, et al. 2012. "Histone H3K4 Demethylation Is Negatively Regulated by Histone H3 Acetylation in *Saccharomyces Cerevisiae*." *Proceedings of the National Academy of Sciences of the United States of America* 109 (45): 18505–10. doi:10.1073/pnas.1202070109.
- Marcand, S, V Brevet, C Mann, and E Gilson. 2000. "Cell Cycle Restriction of Telomere Elongation." *Current Biology* 10 (8): 487–90.
- Marcand, S, S W Buck, P Moretti, E Gilson, and D Shore. 1996. "Silencing of Genes at Nontelomeric Sites in Yeast Is Controlled by Sequestration of Silencing Factors at Telomeres by Rap 1 Protein." *Genes Dev.* 10 (11): 1297–1309.
- Marcand, S, E Gilson, and D Shore. 1997. "A Protein-Counting Mechanism for Telomere Length Regulation in Yeast." *Science* 275 (5302): 986–90. <http://www.ncbi.nlm.nih.gov/pubmed/9020083>.
- Marcand, S, B Pardo, A Gratias, S Cahun, and I Callebaut. 2008. "Multiple Pathways Inhibit NHEJ at Telomeres." *Genes Dev* 22 (9): 1153–58. doi:10.1101/gad.455108.
- Marchfelder, U, K Rateitschak, and A E Ehrenhofer-Murray. 2003. "SIR-Dependent Repression of Non-Telomeric Genes in *Saccharomyces Cerevisiae*?" *Yeast* 20 (9): 797–801. doi:10.1002/yea.1001.

- Marenduzzo, Davide, Kieran Finan, and Peter R. Cook. 2006. "The Depletion Attraction: An Underappreciated Force Driving Cellular Organization." *Journal of Cell Biology*. doi:10.1083/jcb.200609066.
- Mariño-Ramírez, Leonardo, Maricel G Kann, Benjamin A Shoemaker, and David Landsman. 2005. "Histone Structure and Nucleosome Stability." *Expert Review of Proteomics* 2 (5): 719–29. doi:10.1586/14789450.2.5.719.
- Martin, A M, D J Pouchnik, J L Walker, and J J Wyrick. 2004. "Redundant Roles for Histone H3 N-Terminal Lysine Residues in Subtelomeric Gene Repression in *Saccharomyces Cerevisiae*." *Genetics* 167 (3): 1123–32. doi:10.1534/genetics.104.026674167/3/1123 [pii].
- Martin, S G, T Laroche, N Suka, M Grunstein, and S M Gasser. 1999. "Relocalization of Telomeric Ku and SIR Proteins in Response to DNA Strand Breaks in Yeast." *Cell* 97 (5): 621–33.
- Martina, Marina, Diego Bonetti, Matteo Villa, Giovanna Lucchini, and Maria Pia Longhese. 2014. "Saccharomyces Cerevisiae Rif1 Cooperates with MRX-Sae2 in Promoting DNA-End Resection." *EMBO Reports* 15 (6): 695–704. doi:10.1002/embr.201338338.
- Martino, F, S Kueng, P Robinson, M Tsai-Pflugfelder, F van Leeuwen, M Ziegler, F Cubizolles, M M Cockell, D Rhodes, and S M Gasser. 2009. "Reconstitution of Yeast Silent Chromatin: Multiple Contact Sites and O-AADPR Binding Load SIR Complexes onto Nucleosomes in Vitro." *Mol Cell* 33 (3): 323–34. doi:10.1016/j.molcel.2009.01.009.
- Matot, B??atrice, Yann Va?? Le Bihan, Rachel Lescasse, Javier P??rez, Simona Miron, Gabriel David, Bertrand Castaing, et al. 2012. "The Orientation of the C-Terminal Domain of the *Saccharomyces Cerevisiae* Rap1 Protein Is Determined by Its Binding to DNA." *Nucleic Acids Research* 40 (7): 3197–3207. doi:10.1093/nar/gkr1166.
- Matsuda, Atsushi, Yuji Chikashige, Da-Qiao Ding, Chizuru Ohtsuki, Chie Mori, Haruhiko Asakawa, Hiroshi Kimura, Tokuko Haraguchi, and Yasushi Hiraoka. 2015. "Highly Condensed Chromatins Are Formed Adjacent to Subtelomeric and Decondensed Silent Chromatin in Fission Yeast." *Nature Communications* 6: 7753. doi:10.1038/ncomms8753.
- Mattarocci, Stefano, Maksym Shyian, Laure Lemmens, Pascal Damay, Dogus Murat Altintas, Tianlai Shi, Clinton R. Bartholomew, Nicolas H. Thom??, Christopher F J Hardy, and David Shore. 2014. "Rif1 Controls DNA Replication Timing in Yeast through the PP1 Phosphatase Glc7." *Cell Reports* 7 (1): 62–69. doi:10.1016/j.celrep.2014.03.010.
- Matzat, Leah H., Ryan K. Dale, Nellie Moshkovich, and Elissa P. Lei. 2012. "Tissue-Specific Regulation of Chromatin Insulator Function." *PLoS Genetics* 8 (11). doi:10.1371/journal.pgen.1003069.
- McClintock, Barbara. 1950. "The Origin and Behavior of Mutable Loci in Maize." *Genetics* 36 (6): 344–55. doi:10.1073/pnas.36.6.344.
- McGhee, James D., and Gary Felsenfeld. 1980. "The Number of Charge-Charge Interactions Stabilizing the Ends of Nucleosome DNA." *Nucleic Acids Research* 8 (12): 2751–70. doi:10.1093/nar/8.12.2751.
- Mefford, Heather C, and Barbara J Trask. 2002. "The Complex Structure and Dynamic Evolution of Human Subtelomeres." *Nature Reviews. Genetics* 3 (2): 91–102. doi:10.1038/nrg727.
- Meijsing, S. H., and A. E. Ehrenhofer-Murray. 2001. "The Silencing Complex SAS-I Links Histone Acetylation to the Assembly of Repressed Chromatin by CAF-I and Asf1 in *Saccharomyces Cerevisiae*." *Genes and Development* 15 (23): 3169–82. doi:10.1101/gad.929001.
- Meneghini, M D, M Wu, and H D Madhani. 2003. "Conserved Histone Variant H2A.Z Protects Euchromatin from the Ectopic Spread of Silent Heterochromatin." *Cell* 112 (5): 725–36.

- Miele, A, K Bystricky, and J Dekker. 2009. "Yeast Silent Mating Type Loci Form Heterochromatic Clusters through Silencer Protein-Dependent Long-Range Interactions." *PLoS Genet* 5 (5): e1000478. doi:10.1371/journal.pgen.1000478.
- Millar, C B, F Xu, K Zhang, and M Grunstein. 2006. "Acetylation of H2AZ Lys 14 Is Associated with Genome-Wide Gene Activity in Yeast." *Genes Dev* 20 (6): 711–22. doi:10.1101/gad.1395506.
- Millar, Catherine B, and Michael Grunstein. 2006. "Genome-Wide Patterns of Histone Modifications in Yeast." *Nature Reviews. Molecular Cell Biology* 7 (9): 657–66. doi:10.1038/nrm1986.
- Milliman, Eric J, Neelu Yadav, Yin Chu Chen, Bhavana Muddukrishna, Sheelarani Karunanithi, and Michael C. Yu. 2012. "Recruitment of Rpd3 to the Telomere Depends on the Protein Arginine Methyltransferase Hmt1." *PLoS ONE* 7 (8). doi:10.1371/journal.pone.0044656.
- Mills, K D, D A Sinclair, and L Guarente. 1999. "MEC1-Dependent Redistribution of the Sir3 Silencing Protein from Telomeres to DNA Double-Strand Breaks." *Cell* 97 (5): 609–20.
- Mitsumori, Risa, Tomoe Ohashi, Kazuto Kugou, Ayako Ichino, Kei Taniguchi, Kunihiro Ohta, Hiroyuki Uchida, and Masaya Oki. 2016. "Analysis of Novel Sir3 Binding Regions in *Saccharomyces Cerevisiae*." *Journal of Biochemistry* 160 (1): 11–17. doi:10.1093/jb/mvw021.
- Mittwoch, Ursula. 1964. "Sex Chromatin." *J. Med. Genetic.*
- Moazed, D, A Kistler, A Axelrod, J Rine, and A D Johnson. 1997. "Silent Information Regulator Protein Complexes in *Saccharomyces Cerevisiae*: A SIR2/SIR4 Complex and Evidence for a Regulatory Domain in SIR4 That Inhibits Its Interaction with SIR3." *Proceedings of the National Academy of Sciences of the United States of America* 94 (6): 2186–91. doi:10.1073/pnas.94.6.2186.
- Moretti, P, K Freeman, L Coodly, and D Shore. 1994. "Evidence That a Complex of SIR Proteins Interacts with the Silencer and Telomere-Binding Protein RAP1." *Genes Dev* 8 (19): 2257–69.
- Moretti, P, and D Shore. 2001. "Multiple Interactions in Sir Protein Recruitment by Rap1p at Silencers and Telomeres in Yeast." *Mol Cell Biol* 21 (23): 8082–94. doi:10.1128/MCB.21.23.8082-8094.2001.
- Moretti, Paolo, Katie Freeman, Lavanya Coodly, and David Shore. 1994. "Evidence That a Complex of SIR Proteins Interacts with the Silencer and Telomere-Binding Protein RAP1." *Genes and Development* 8 (19): 2257–69. doi:10.1101/gad.8.19.2257.
- Moser, Bettina A, and Toru M Nakamura. 2009. "Protection and Replication of Telomeres in Fission Yeast." *Biochemistry and Cell Biology = Biochimie et Biologie Cellulaire* 87 (5): 747–58. doi:10.1139/O09-037.
- Mukhopadhyay, Swagatam, and Anirvan M. Sengupta. 2013. "The Role of Multiple Marks in Epigenetic Silencing and the Emergence of a Stable Bivalent Chromatin State." *PLoS Computational Biology* 9 (7). doi:10.1371/journal.pcbi.1003121.
- Muller, H. 1938. "The Remaking of Chromosomes." *Collecting Net (Woods Hole)*.
- Muller, H. J. 1930. "Types of Visible Variations Induced by X-Rays in *Drosophila*." *Journal of Genetics* 22 (3): 299–334. doi:10.1007/BF02984195.
- Murphy, G A, E J Spedale, S T Powell, L Pillus, S C Schultz, and L Chen. 2003. "The Sir4 C-Terminal Coiled Coil Is Required for Telomeric and Mating Type Silencing in *Saccharomyces Cerevisiae*." *J Mol Biol* 334 (4): 769–80. <http://www.ncbi.nlm.nih.gov/pubmed/14636601>.
- Nagai, Shigeki, Ralph E Davis, Pierre Jean Mattei, Kyle Patrick Eagen, and Roger D Kornberg. 2017. "Chromatin Potentiates Transcription." *Proceedings of the National Academy of Sciences of the United States of America* 114 (7): 1536–41. doi:10.1073/pnas.1620312114.

- Nakanishi, Shima, Shin Lee Jung, Kathryn E. Gardner, Jennifer M. Gardner, Yoh Hei Takahashi, Mahesh B. Chandrasekharan, Zu Wen Sun, et al. 2009. "Histone H2BK123 Monoubiquitination Is the Critical Determinant for H3K4 and H3K79 Trimethylation by COMPASS and Dot1." *Journal of Cell Biology* 186 (3): 371–77. doi:10.1083/jcb.200906005.
- Ng, Huck Hui, Rui Ming Xu, Yi Zhang, and Kevin Struhl. 2002. "Ubiquitination of Histone H2B by Rad6 Is Required for Efficient Dot1-Mediated Methylation of Histone H3 Lysine 79." *Journal of Biological Chemistry* 277 (38): 34655–57. doi:10.1074/jbc.C200433200.
- Nimmo, E R, G Cranston, and R C Allshire. 1994. "Telomere-Associated Chromosome Breakage in Fission Yeast Results in Variegated Expression of Adjacent Genes." *EMBO J* 13 (16): 3801–11.
- Nislow, C, E Ray, and L Pillus. 1997. "SET1, a Yeast Member of the Trithorax Family, Functions in Transcriptional Silencing and Diverse Cellular Processes." *Molecular Biology of the Cell* 8 (12): 2421–36. doi:10.1091/mbc.8.12.2421.
- Nora, Elphège P., Anton Goloborodko, Anne Laure Valton, Johan H. Gibcus, Alec Uebersohn, Nezar Abdennur, Job Dekker, Leonid A. Mirny, and Benoit G. Bruneau. 2017. "Targeted Degradation of CTCF Decouples Local Insulation of Chromosome Domains from Genomic Compartmentalization." *Cell* 169 (5): 930–44.e22. doi:10.1016/j.cell.2017.05.004.
- Norris, A, M A Bianchet, and J D Boeke. 2008. "Compensatory Interactions between Sir3p and the Nucleosomal LRS Surface Imply Their Direct Interaction." *PLoS Genet* 4 (12): e1000301. doi:10.1371/journal.pgen.1000301.
- Norwood, Laura E., Stephanie K. Grade, Diane E. Cryderman, Karrie A. Hines, Nicholas Furiasse, Rafael Toro, Yuhong Li, et al. 2004. "Conserved Properties of HP1Hsa." *Gene* 336 (1): 37–46. doi:10.1016/j.gene.2004.04.003.
- O'Sullivan, J M, S M Tan-Wong, A Morillon, B Lee, J Coles, J Mellor, and N J Proudfoot. 2004. "Gene Loops Juxtapose Promoters and Terminators in Yeast." *Nat Genet* 36 (9): 1014–18.
- Oki, Masaya, Lourdes Valenzuela, Tomoko Chiba, Takashi Ito, and Rohinton T Kamakaka. 2004. "Barrier Proteins Remodel and Modify Chromatin to Restrict Silenced Domains." *Molecular and Cellular Biology* 24 (5): 1956–67. doi:10.1128/MCB.24.5.1956-1967.2004.
- Olins, A L, and D E Olins. 1974. "Spheroid Chromatin Units (v Bodies)." *Science* 183 (122): 330–32.
- Olovnikov, A.M. 1973. "A Theory of Marginotomy." *Journal of Theoretical Biology* 41 (1): 181–90. doi:10.1016/0022-5193(73)90198-7.
- Olovnikov, Alexey M. 1996. "Telomeres, Telomerase, and Aging: Origin of the Theory." *Experimental Gerontology* 31 (4): 443–48. doi:10.1016/0531-5565(96)00005-8.
- Olszak, Agata M, Dominic van Essen, António J Pereira, Sarah Diehl, Thomas Manke, Helder Maiato, Simona Saccani, and Patrick Heun. 2011. "Heterochromatin Boundaries Are Hotspots for de Novo Kinetochores Formation." *Nature Cell Biology* 13 (7): 799–808. doi:10.1038/ncb2272.
- Onishi, M, G G Liou, J R Buchberger, T Walz, and D Moazed. 2007. "Role of the Conserved Sir3-BAH Domain in Nucleosome Binding and Silent Chromatin Assembly." *Mol Cell* 28 (6): 1015–28. doi:10.1016/j.molcel.2007.12.004.
- Oppikofer, M, S Kueng, J J Keusch, M Hassler, A G Ladurner, H Gut, and S M Gasser. 2013. "Dimerization of Sir3 via Its C-Terminal Winged Helix Domain Is Essential for Yeast Heterochromatin Formation." *EMBO J* 32 (3): 437–49. doi:10.1038/emboj.2012.343.
- Oppikofer, M, S Kueng, F Martino, S Soeroes, S M Hancock, J W Chin, W Fischle, and S M Gasser. 2011. "A Dual

- Role of H4K16 Acetylation in the Establishment of Yeast Silent Chromatin." *EMBO J* 30 (13): 2610–21. doi:10.1038/emboj.2011.170.
- Osada, Shigehiro, Ann Sutton, Nemone Muster, Christine E. Brown, John R. Yates, Rolf Sternglanz, and Jerry L. Workman. 2001. "The Yeast SAS (something about Silencing) Protein Complex Contains a MYST-Type Putative Acetyltransferase and Functions with Chromatin Assembly Factor ASF1." *Genes and Development* 15 (23): 3155–68. doi:10.1101/gad.907201.
- Osborne, E A, S Dudoit, and J Rine. 2009. "The Establishment of Gene Silencing at Single-Cell Resolution." *Nat Genet* 41 (7): 800–806. doi:10.1038/ng.402.
- Osborne, E A, Y Hiraoka, and J Rine. 2011. "Symmetry, Asymmetry, and Kinetics of Silencing Establishment in *Saccharomyces Cerevisiae* Revealed by Single-Cell Optical Assays." *Proc Natl Acad Sci U S A* 108 (4): 1209–16. doi:10.1073/pnas.1018742108.
- Ottoz, Diana S M, Fabian Rudolf, and Joerg Stelling. 2014. "Inducible, Tightly Regulated and Growth Condition-Independent Transcription Factor in *Saccharomyces Cerevisiae*." *Nucleic Acids Research* 42 (17). doi:10.1093/nar/gku616.
- Oudet, P, M Gross-Bellard, and P Chambon. 1975. "Electron Microscopic and Biochemical Evidence That Chromatin Structure Is a Repeating Unit." *Cell* 4 (4): 281–300.
- Palladino, F, T Laroche, E Gilson, A Axelrod, L Pillus, and S M Gasser. 1993. "SIR3 and SIR4 Proteins Are Required for the Positioning and Integrity of Yeast Telomeres." *Cell* 75 (3): 543–55.
- Pan, Jing, Mariko Sasaki, Ryan Kniewel, Hajime Murakami, Hannah G. Blitzblau, Sam E. Tischfield, Xuan Zhu, et al. 2011. "A Hierarchical Combination of Factors Shapes the Genome-Wide Topography of Yeast Meiotic Recombination Initiation." *Cell* 144 (5): 719–31. doi:10.1016/j.cell.2011.02.009.
- Park, Y, J Hanish, and A J Lustig. 1998. "Sir3p Domains Involved in the Initiation of Telomeric Silencing in *Saccharomyces Cerevisiae*." *Genetics* 150 (3): 977–86.
- Pedram, Mehrdad, Carl N Sprung, Qing Gao, Anthony W I Lo, Gloria E Reynolds, and John P Murnane. 2006. "Telomere Position Effect and Silencing of Transgenes near Telomeres in the Mouse." *Molecular and Cellular Biology* 26 (5): 1865–78. doi:10.1128/MCB.26.5.1865-1878.2006.
- Peng, Jing, and Jin Qiu Zhou. 2012. "The Tail-Module of Yeast Mediator Complex Is Required for Telomere Heterochromatin Maintenance." *Nucleic Acids Research* 40 (2): 581–93. doi:10.1093/nar/gkr757.
- Pich, Uta, and Ingo Schubert. 1998. "Terminal Heterochromatin and Alternative Telomeric Sequences in *Allium Cepa*." *Chromosome Research* 6 (4): 315–21. doi:10.1023/A:1009227009121.
- Pillus, L, and J Rine. 1989. "Epigenetic Inheritance of Transcriptional States in *S. Cerevisiae*." *Cell* 59 (4): 637–47.
- Pobiega, Sabrina, and Stéphane Marcand. 2010. "Dicentric Breakage at Telomere Fusions." *Genes and Development* 24 (7): 720–33. doi:10.1101/gad.571510.
- Pokholok, Dmitry K., Christopher T. Harbison, Stuart Levine, Megan Cole, Nancy M. Hannett, Ihn Lee Tong, George W. Bell, et al. 2005. "Genome-Wide Map of Nucleosome Acetylation and Methylation in Yeast." *Cell* 122 (4): 517–27. doi:10.1016/j.cell.2005.06.026.
- Pryde, F E, and E J Louis. 1997. "Saccharomyces Cerevisiae Telomeres. A Review." *Biochemistry (Mosc)* 62 (11): 1232–41. <http://www.ncbi.nlm.nih.gov/pubmed/9467847>.
- . 1999. "Limitations of Silencing at Native Yeast Telomeres." *EMBO J* 18 (9): 2538–50.

- Qiu, Hongfang, Razvan V. Chereji, Cuihua Hu, Hope A. Cole, Yashpal Rawal, David J. Clark, and Alan G. Hinnebusch. 2016. "Genome-Wide Cooperation by HAT Gcn5, Remodeler SWI/SNF, and Chaperone Ydj1 in Promoter Nucleosome Eviction and Transcriptional Activation." *Genome Research* 26 (2): 211–25. doi:10.1101/gr.196337.115.
- Quintero, María J, Douglas Maya, Miguel Arévalo-Rodríguez, Angel Cebolla, and Sebastián Chávez. 2007. "An Improved System for Estradiol-Dependent Regulation of Gene Expression in Yeast. TL - 6." *Microbial Cell Factories* 6 VN - re: 10. doi:10.1186/1475-2859-6-10.
- Radman-Livaja, M, G Ruben, A Weiner, N Friedman, R Kamakaka, and O J Rando. 2011. "Dynamics of Sir3 Spreading in Budding Yeast: Secondary Recruitment Sites and Euchromatic Localization." *EMBO J* 30 (6): 1012–26. doi:10.1038/emboj.2011.30.
- Ragunathan, Kaushik, G. Jih, and D. Moazed. 2014. "Epigenetic Inheritance Uncoupled from Sequence-Specific Recruitment." *Science* 348 (6230): science.1258699 – . doi:10.1126/science.1258699.
- Ramani, Vijay, Jay Shendure, and Zhijun Duan. 2016. "Understanding Spatial Genome Organization: Methods and Insights." *Genomics, Proteomics and Bioinformatics*. doi:10.1016/j.gpb.2016.01.002.
- Ray, A, R E Hector, N Roy, J H Song, K L Berkner, and K W Runge. 2003. "Sir3p Phosphorylation by the Slr2p Pathway Effects Redistribution of Silencing Function and Shortened Lifespan." *Nat Genet* 33 (4): 522–26. doi:10.1038/ng1132ng1132 [pii].
- Recht, J, T Tsubota, J C Tanny, R L Diaz, J M Berger, X Zhang, B a Garcia, et al. 2006. "Histone Chaperone Asf1 Is Required for Histone H3 Lysine 56 Acetylation, a Modification Associated with S Phase in Mitosis and Meiosis." *Proceedings of the National Academy of Sciences of the United States of America* 103: 6988–93. doi:10.1073/pnas.0601676103.
- Reddy, Bharat D., Yu Wang, Lifang Niu, Emily C. Higuchi, Samuel B. Marguerat, Jürg Bähler, Gerald R. Smith, and Songtao Jia. 2011. "Elimination of a Specific Histone H3K14 Acetyltransferase Complex Bypasses the RNAi Pathway to Regulate Pericentric Heterochromatin Functions." *Genes and Development* 25 (3): 214–19. doi:10.1101/gad.1993611.
- Reiter, Christian, Franziska Heise, Ho Ryun Chung, and Ann E. Ehrenhofer-Murray. 2015. "A Link between Sas2-Mediated H4 K16 Acetylation, Chromatin Assembly in S-Phase by CAF-I and Asf1, and Nucleosome Assembly by Spt6 during Transcription." *FEMS Yeast Research* 15 (7). doi:10.1093/femsyr/fov073.
- Rekosh, D. M K, W. C. Russell, A. J D Bellet, and A. J. Robinson. 1977. "Identification of a Protein Linked to the Ends of Adenovirus DNA." *Cell* 11 (2): 283–95. doi:10.1016/0092-8674(77)90045-9.
- Renauld, H, O M Aparicio, P D Zierath, B L Billington, S K Chhablani, and D E Gottschling. 1993a. "Silent Domains Are Assembled Continuously from the Telomere and Are Defined by Promoter Distance and Strength, and by SIR3 Dosage." *Genes Dev* 7 (7A): 1133–45.
- Renauld, H., O. M. Aparicio, P. D. Zierath, B. L. Billington, S. K. Chhablani, and D. E. Gottschling. 1993b. "Silent Domains Are Assembled Continuously from the Telomere and Are Defined by Promoter Distance and Strength, and by SIR3 Dosage." *Genes and Development* 7 (7 A): 1133–45. doi:10.1101/gad.7.7a.1133.
- Reyes-Turcu, Francisca E, Ke Zhang, Martin Zofall, Eesin Chen, and Shiv I S Grewal. 2011. "Defects in RNA Quality Control Factors Reveal RNAi-Independent Nucleation of Heterochromatin." *Nature Structural & Molecular Biology* 18 (10): 1132–38. doi:10.1038/nsmb.2122.
- Rhee, Ho Sung, and B. Franklin Pugh. 2012. "ChiP-Exo Method for Identifying Genomic Location of DNA-Binding Proteins with near-Single-Nucleotide Accuracy." *Current Protocols in Molecular Biology*, no. SUPPL.100. doi:10.1002/0471142727.mb2124s100.
- Rine, J., and I. Herskowitz. 1987. "Four Genes Responsible for a Position Effect on Expression from HML and

- HMR in *Saccharomyces Cerevisiae*." *Genetics* 116 (1): 9–22.
- Robyr, D, Y Suka, I Xenarios, S K Kurdistani, A Wang, N Suka, and M Grunstein. 2002. "Microarray Deacetylation Maps Determine Genome-Wide Functions for Yeast Histone Deacetylases." *Cell* 109 (4): 437–46.
- Roche, B, B Arcangioli, and R A Martienssen. 2016. "RNA Interference Is Essential for Cellular Quiescence." *Science (New York, N.Y.)* 354 (6313): aah5651. doi:10.1126/science.aah5651.
- Rodriguez, A, T De La Cera, P Herrero, and F Moreno. 2001. "The Hexokinase 2 Protein Regulates the Expression of the GLK1, HXK1 and HXK2 Genes of *Saccharomyces Cerevisiae*." *Biochem J* 355 (Pt 3): 625–31.
- Romeo, Kelly, Yann Louault, Sylvain Cantaloube, Isabelle Loiodice, Geneviève Almouzni, and Jean Pierre Quivy. 2015. "The SENP7 SUMO-Protease Presents a Module of Two HP1 Interaction Motifs That Locks HP1 Protein at Pericentric Heterochromatin." *Cell Reports* 10 (5): 771–82. doi:10.1016/j.celrep.2015.01.004.
- Roth, Charles W, Frances Kobeski, Marika F Walter, and Harald Biessmann. 1997. "Chromosome End Elongation by Recombination in the Mosquito *Anopheles Gambiae*." *Molecular and Cellular Biology* 17 (9): 5176–83. doi:10.1128/MCB.17.9.5176.
- Roy, R, B Meier, A D McAinsh, H M Feldmann, and S P Jackson. 2004. "Separation-of-Function Mutants of Yeast Ku80 Reveal a Yku80p-Sir4p Interaction Involved in Telomeric Silencing." *J Biol Chem* 279 (1): 86–94.
- Ruault, M, A De Meyer, I Loiodice, and A Taddei. 2011a. "Clustering Heterochromatin: Sir3 Promotes Telomere Clustering Independently of Silencing in Yeast." *J Cell Biol* 192 (3): 417–31. doi:jcb.201008007 [pii]10.1083/jcb.201008007.
- . 2011b. "Clustering Heterochromatin: Sir3 Promotes Telomere Clustering Independently of Silencing in Yeast." *J Cell Biol* 192 (3): 417–31. doi:10.1083/jcb.201008007.
- Ruault, M, A De Meyer, I Loiodice, and A Taddei. n.d. "Clustering Heterochromatin: Sir3 Promotes Telomere Clustering Independently of Silencing in Yeast." *Journal of Cell Biology*.
- Ruben, G J, J G Kirkland, T MacDonough, M Chen, R N Dubey, M R Gartenberg, and R T Kamakaka. 2011. "Nucleoporin Mediated Nuclear Positioning and Silencing of HMR." *PLoS One* 6 (7): e21923. doi:10.1371/journal.pone.0021923.
- Rudner, A D, B E Hall, T Ellenberger, and D Moazed. 2005. "A Nonhistone Protein-Protein Interaction Required for Assembly of the SIR Complex and Silent Chromatin." *Mol Cell Biol* 25 (11): 4514–28. doi:25/11/4514 [pii]10.1128/MCB.25.11.4514-4528.2005.
- Rundlett, S E, A A Carmen, R Kobayashi, S Bavykin, B M Turner, and M Grunstein. 1996. "HDA1 and RPD3 Are Members of Distinct Yeast Histone Deacetylase Complexes That Regulate Silencing and Transcription." *Proc Natl Acad Sci USA* 93 (25): 14503–8.
- Rusche, L N, A L Kirchmaier, and J Rine. 2002. "Ordered Nucleation and Spreading of Silenced Chromatin in *Saccharomyces Cerevisiae*." *Mol Biol Cell*. 7 (13): 2207–22.
- Rusche, L N, and P J Lynch. 2009. "Assembling Heterochromatin in the Appropriate Places: A Boost Is Needed." *J Cell Physiol* 219 (3): 525–28. doi:10.1002/jcp.21749.
- Rusche, Laura N, Ann L Kirchmaier, and Jasper Rine. 2003. "The Establishment, Inheritance, and Function of Silenced Chromatin in *Saccharomyces Cerevisiae*." *Annual Review of Biochemistry* 72: 481–516. doi:10.1146/annurev.biochem.72.121801.161547.
- S, G Smith. 1944. "The Diagnosis of Sex by Means of Heteropycnosis."

- Sabourin, Michelle, Creighton T. Tuzon, and Virginia A. Zakian. 2007. "Telomerase and Tel1p Preferentially Associate with Short Telomeres in *S. Cerevisiae*." *Molecular Cell* 27 (4): 550–61. doi:10.1016/j.molcel.2007.07.016.
- Sadaie, Mahito, Tetsushi Iida, Takeshi Urano, and Jun-ichi Nakayama. 2004. "A Chromodomain Protein, Chp1, Is Required for the Establishment of Heterochromatin in Fission Yeast." *The EMBO Journal* 23 (19): 3825–35. doi:10.1038/sj.emboj.7600401.
- Saint-André, Violaine, Eric Batsché, Christophe Rachez, and Christian Muchardt. 2011. "Histone H3 Lysine 9 Trimethylation and HP1 γ Favor Inclusion of Alternative Exons." *Nature Structural & Molecular Biology* 18 (3): 337–44. doi:10.1038/nsmb.1995.
- Sampath, V, P Yuan, I X Wang, E Prugar, F van Leeuwen, and R Sternglanz. 2009. "Mutational Analysis of the Sir3 BAH Domain Reveals Multiple Points of Interaction with Nucleosomes." *Mol Cell Biol* 29 (10): 2532–45. doi:MCB.01682-08 [pii]10.1128/MCB.01682-08.
- Sandman, Kathleen, and John N. Reeve. 2006. "Archaeal Histones and the Origin of the Histone Fold." *Current Opinion in Microbiology*. doi:10.1016/j.mib.2006.08.003.
- Schneider, Jessica, Pratibha Bajwa, Farley C. Johnson, Sukesh R. Bhaumik, and Ali Shilatifard. 2006. "Rtt109 Is Required for Proper H3K56 Acetylation: A Chromatin Mark Associated with the Elongating RNA Polymerase II." *Journal of Biological Chemistry* 281 (49): 37270–74. doi:10.1074/jbc.C600265200.
- Schulze, Julia M., Jessica Jackson, Shima Nakanishi, Jennifer M. Gardner, Thomas Hentrich, Jeff Haug, Mark Johnston, Sue L. Jaspersen, Michael S. Kobor, and Ali Shilatifard. 2009. "Linking Cell Cycle to Histone Modifications: SBF and H2B Monoubiquitination Machinery and Cell-Cycle Regulation of H3K79 Dimethylation." *Molecular Cell* 35 (5): 626–41. doi:10.1016/j.molcel.2009.07.017.
- Shay, J W, and W E Wright. 2000. "Hayflick, His Limit, and Cellular Ageing." *Nature Reviews. Molecular Cell Biology* 1 (October): 72–76. doi:10.1038/35036093.
- Shevtsov, Sergey P., and Miroslav Dundr. 2010. "Nucleation of Nuclear Bodies by RNA." *Nature Cell Biology* 13 (2): 167–73. doi:10.1007/978-1-62703-526-2-23.
- Shi, Tianlai, Richard D. Bunker, Stefano Mattarocci, Cyril Ribeyre, Mahamadou Faty, Heinz Gut, Andrea Scrima, et al. 2013. "XRif1 and Rif2 Shape Telomere Function and Architecture through Multivalent Rap1 Interactions." *Cell* 153 (6). doi:10.1016/j.cell.2013.05.007.
- Shogren-Knaak, Michael, Haruhiko Ishii, Jian-Min Sun, Michael J Pazin, James R Davie, and Craig L Peterson. 2006. "Histone H4-K16 Acetylation Controls Chromatin Structure and Protein Interactions." *Science* 311 (5762): 844–47. doi:10.1126/science.1124000.
- Shore, D, and K Nasmyth. 1987. "Purification and Cloning of a DNA Binding Protein from Yeast That Binds to Both Silencer and Activator Elements." *Cell* 51 (5): 721–32. doi:0092-8674(87)90095-X [pii].
- Simmer, Femke, Alessia Buscaino, Isabelle C Kos-Braun, Alexander Kagansky, Abdelhalim Boukaba, Takeshi Urano, Alastair R W Kerr, and Robin C Allshire. 2010. "Hairpin RNA Induces Secondary Small Interfering RNA Synthesis and Silencing in Trans in Fission Yeast." *EMBO Reports* 11 (2): 112–18. doi:10.1038/embor.2009.273.
- Snider, Jamie, Guillaume Thibault, and Walid A Houry. 2008. "The AAA+ Superfamily of Functionally Diverse Proteins." *Genome Biology* 9 (4): 216. doi:10.1186/gb-2008-9-4-216.
- Sobel, R E, R G Cook, C A Perry, A T Annunziato, and C D Allis. 1995. "Conservation of Deposition-Related Acetylation Sites in Newly Synthesized Histones H3 and H4." *Proc Natl Acad Sci USA* 92: 1237–41.
- Sperling, A S, and M Grunstein. 2009. "Histone H3 N-Terminus Regulates Higher Order Structure of Yeast

- Heterochromatin." *Proc Natl Acad Sci U S A* 106 (32): 13153–59. doi:0906866106 [pii]10.1073/pnas.0906866106.
- Steakley, David Lee, and Jasper Rine. 2015. "On the Mechanism of Gene Silencing in *Saccharomyces Cerevisiae*." *G3 (Bethesda, Md.)* 5 (8): 1751–63. doi:10.1534/g3.115.018515.
- Stone, E M, and L Pillus. 1998. "Silent Chromatin in Yeast: An Orchestrated Medley Featuring Sir3p [corrected]." *Bioessays* 20 (1): 30–40. doi:10.1002/(SICI)1521-1878(199801)20:1<30::AID-BIES6>3.0.CO;2-W [pii]10.1002/(SICI)1521-1878(199801)20:1<30::AID-BIES6>3.0.CO;2-W.
- Strahl-Bolsinger, S, A Hecht, K Luo, and M Grunstein. 1997. "SIR2 and SIR4 Interactions Differ in Core and Extended Telomeric Heterochromatin in Yeast." *Genes Dev* 11 (1): 83–93. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9000052.
- Strathern, Jeffrey N., Amar J S Klar, James B. Hicks, Judith A. Abraham, John M. Ivy, Kim A. Nasmyth, and Carolyn McGill. 1982. "Homothallic Switching of Yeast Mating Type Cassettes Is Initiated by a Double-Stranded Cut in the MAT Locus." *Cell* 31 (1): 183–92. doi:10.1016/0092-8674(82)90418-4.
- Stulemeijer, Iris J. E., Dirk De Vos, Kirsten van Harten, Onkar K. Joshi, Olga Blomberg, Tibor van Welsem, Marit Terweij, et al. 2015. "Dot1 Histone Methyltransferases Share a Distributive Mechanism but Have Highly Diverged Catalytic Properties." *Scientific Reports* 5: 1–11. doi:10.1038/srep09824.
- Sue, Jinks-Robertson, and Ashok S Bhagwat. 2014. "Transcription-Associated Mutagenesis." *Annu. Rev. Genet.* 48: 341–59. doi:10.1146/annurev-genet-120213-092015.
- Sugiyama, Tomoyasu, Hugh P. Cam, Rie Sugiyama, Ken ichi Noma, Martin Zofall, Ryuji Kobayashi, and Shiv I.S. Grewal. 2007. "SHREC, an Effector Complex for Heterochromatic Transcriptional Silencing." *Cell* 128 (3): 491–504. doi:10.1016/j.cell.2006.12.035.
- Suka, N, K Luo, and M Grunstein. 2002. "Sir2p and Sas2p Oppositely Regulate Acetylation of Yeast Histone H4 lysine16 and Spreading of Heterochromatin." *Nat Genet* 32 (3): 378–83.
- Sun, Zu Wen, and Michael Hampsey. 1999. "A General Requirement for the Sin3-Rpd3 Histone Deacetylase Complex in Regulating Silencing in *Saccharomyces Cerevisiae*." *Genetics* 152 (3): 921–32.
- Sun, Zu-Wen, and C David Allis. 2002. "Ubiquitination of Histone H2B Regulates H3 Methylation and Gene Silencing in Yeast." *Nature* 418 (JULY): 104–8. doi:10.1038/nature00883.
- Sussel, L., D. Vannier, and D. Shore. 1995. "Suppressors of Defective Silencing in Yeast: Effects on Transcriptional Repression at the HMR Locus, Cell Growth and Telomere Structure." *Genetics* 141 (3): 873–88.
- Tackett, Alan J., David J. Dilworth, Megan J. Davey, Michael O'Donnell, John D. Aitchison, Michael P. Rout, and Brian T. Chait. 2005. "Proteomic and Genomic Characterization of Chromatin Complexes at a Boundary." *Journal of Cell Biology* 169 (1): 35–47. doi:10.1083/jcb.200502104.
- Taddei, A, and S M Gasser. 2004. "Multiple Pathways for Telomere Tethering: Functional Implications of Subnuclear Position for Heterochromatin Formation." *Biochim Biophys Acta* 1677 (1-3): 120–28. doi:10.1016/j.bbaexp.2003.11.014S0167478103002847 [pii].
- . 2012. "Structure and Function in the Budding Yeast Nucleus." *Genetics* 192 (1): 107–29. doi:10.1534/genetics.112.140608.
- Taddei, A, G Van Houwe, F Hediger, V Kalck, F Cubizolles, H Schober, and S M Gasser. 2006. "Nuclear Pore Association Confers Optimal Expression Levels for an Inducible Yeast Gene." *Nature* 441 (7094): 774–78. doi:10.1038/nature04845.

- Taddei, A, G Van Houwe, S Nagai, I Erb, E van Nimwegen, and S M Gasser. 2009. "The Functional Importance of Telomere Clustering: Global Changes in Gene Expression Result from SIR Factor Dispersion." *Genome Res* 19 (4): 611–25. doi:gr.083881.108 [pii]10.1101/gr.083881.108.
- Tadeo, Xavier, Jiyong Wang, Scott P. Kallgren, Jinqiang Liu, Bharat D. Reddy, Feng Qiao, and Songtao Jia. 2013. "Elimination of Shelterin Components Bypasses RNAi for Pericentric Heterochromatin Assembly." *Genes and Development* 27 (22): 2489–99. doi:10.1101/gad.226118.113.
- Taggart, Andrew K. P., Shu-Chun Teng, and Virginia A Zakian. 2002. "Est1p As a Cell Cycle-Regulated Activator of Telomere-Bound Telomerase." *Science* 297 (5583): 1023–26. doi:10.1126/science.1074968.
- Takahashi, Tomio, Guenaelle Burguiere-Slezak, Patricia Auffret Van der Kemp, and Serge Boiteux. 2011. "Topoisomerase 1 Provokes the Formation of Short Deletions in Repeated Sequences upon High Transcription in *Saccharomyces Cerevisiae*." *Proceedings of the National Academy of Sciences of the United States of America* 108 (2): 692–97. doi:10.1073/pnas.1012582108.
- Takahashi, Y., G. Westfield, A. Oleskie, R. Trievel, A. Shilatifard, and G. Skiniotis. 2012. "Structural Analysis of the Core COMPASS Family of Histone H3K4 Methylases from Yeast to Human." *Proceedings of the National Academy of Sciences* 12 (1): 871–74. doi:10.1158/2159-8290.CD-12-0041.
- Takahashi, Yoh Hei, Julia M. Schulze, Jessica Jackson, Thomas Hentrich, Chris Seidel, Sue L. Jaspersen, Michael S. Kobor, and Ali Shilatifard. 2011. "Dot1 and Histone H3K79 Methylation in Natural Telomeric and HM Silencing." *Molecular Cell* 42 (1): 118–26. doi:10.1016/j.molcel.2011.03.006.
- Takata, Hideki, Yutaka Kanoh, Norio Gunge, Katsuhiko Shirahige, and Akira Matsuura. 2004. "Reciprocal Association of the Budding Yeast ATM-Related Proteins Tel1 and Mec1 with Telomeres in Vivo." *Molecular Cell* 14 (4): 515–22. doi:10.1016/S1097-2765(04)00262-X.
- Talbert, Paul B, and Steven Henikoff. 2006. "Spreading of Silent Chromatin: Inaction at a Distance." *Nature Reviews. Genetics* 7 (10): 793–803. doi:10.1038/nrg1920.
- Tan-Wong, Sue Mei, Judith B Zaugg, Jurgi Camblong, Zhenyu Xu, David W Zhang, Hannah E Mischo, Aseem Z Ansari, Nicholas M Luscombe, Lars M Steinmetz, and Nick J Proudfoot. 2012. "Gene Loops Enhance Transcriptional Directionality." *Science (New York, N.Y.)* 338 (6107): 671–75. doi:10.1126/science.1224350.
- Tanner, K G, J Landry, R Sternglanz, and J M Denu. 2000. "Silent Information Regulator 2 Family of NAD-Dependent Histone/protein Deacetylases Generates a Unique Product, 1-O-Acetyl-ADP-Ribose." *Proc Natl Acad Sci U S A* 97 (26): 14178–82. doi:10.1073/pnas.250422697.
- Tanny, J C, G J Dowd, J Huang, H Hilz, and D Moazed. 1999. "An Enzymatic Activity in the Yeast Sir2 Protein That Is Essential for Gene Silencing." *Cell* 99 (7): 735–45.
- Tanny, J C, D S Kirkpatrick, S A Gerber, S P Gygi, and D Moazed. 2004. "Budding Yeast Silencing Complexes and Regulation of Sir2 Activity by Protein-Protein Interactions." *Mol Cell Biol* 24 (16): 6931–46. doi:10.1128/MCB.24.16.6931-6946.2004.
- Tchasovnikarova, Iva, A, T Timms, Richard, J Matheson, Nicholas, Kim Wals, Robin Antrobus, Berthold Gottgens, Gordon Dougan, A. Dawson, Mark, and J. Lehner, Paul. 2015. "Epigenetic Silencing by the HUSH Complex Mediates Position-Effect Variegation in Human Cells." *Science* 348: 1481–85. doi:10.1126/science.aaa7227.
- Teixeira, M. Teresa, Milica Arneric, Peter Sperisen, and Joachim Lingner. 2004. "Telomere Length Homeostasis Is Achieved via a Switch between Telomerase-Extendible and -Nonextendible States." *Cell* 117 (3): 323–35. doi:10.1016/S0092-8674(04)00334-4.
- Teytelman, L, D M Thurtle, J Rine, and A van Oudenaarden. 2013. "Highly Expressed Loci Are Vulnerable to

- Misleading ChIP Localization of Multiple Unrelated Proteins." *Proc Natl Acad Sci U S A* 110 (46): 18602–7. doi:10.1073/pnas.1316064110.
- Teytelman, Leonid, Michael B. Eisen, and Jasper Rine. 2008. "Silent but Not Static: Accelerated Base-Pair Substitution in Silenced Chromatin of Budding Yeasts." *PLoS Genetics* 4 (11). doi:10.1371/journal.pgen.1000247.
- Thompson, J S, X Ling, and M Grunstein. 1994. "Histone H3 Amino Terminus Is Required for Telomeric and Silent Mating Locus Repression in Yeast." *Nature* 369: 245–47. doi:10.1038/369245a0.
- Thrower, D A, and K Bloom. 2001. "Dicentric Chromosome Stretching during Anaphase Reveals Roles of Sir2/Ku in Chromatin Compaction in Budding Yeast." *Mol Biol Cell* 12 (9): 2800–2812. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11553718.
- Thurtle-Schmidt, Deborah M., Anne E. Dodson, and Jasper Rine. 2016. "Histone Deacetylases with Antagonistic Roles in *Saccharomyces Cerevisiae* Heterochromatin Formation." *Genetics* 204 (1): 177–90. doi:10.1534/genetics.116.190835.
- Tomaska, Lubomir, Alexander M. Makhov, Jack D. Griffith, and Jozef Nosek. 2002. "T-Loops in Yeast Mitochondria." *Mitochondrion* 1 (5): 455–59. doi:10.1016/S1567-7249(02)00009-0.
- Tong, A H, M Evangelista, A B Parsons, H Xu, G D Bader, N Pagé, M Robinson, et al. 2001. "Systematic Genetic Analysis with Ordered Arrays of Yeast Deletion Mutants." *Science (New York, N.Y.)* 294 (5550): 2364–68. doi:10.1126/science.1065810.
- Trewick, Sarah C, Elsa Minc, Richard Antonelli, Takeshi Urano, and Robin C Allshire. 2007. "The JmjC Domain Protein Epe1 Prevents Unregulated Assembly and Disassembly of Heterochromatin." *The EMBO Journal* 26 (22): 4670–82. doi:10.1038/sj.emboj.7601892.
- Triolo, T, and R Sternglanz. 1996. "Role of Interactions between the Origin Recognition Complex and SIR1 in Transcriptional Silencing." *Nature* 381 (6579): 251–53.
- Trojer, Patrick, and Danny Reinberg. 2007. "Facultative Heterochromatin: Is There a Distinctive Molecular Signature?" *Molecular Cell*. doi:10.1016/j.molcel.2007.09.011.
- Tsukamoto, Y, J-I Kato, and H Ikeda. 1997. "Silencing Factors Participate in DNA Repair and Recombination in *Saccharomyces Cerevisiae*." *Nature* 388: 900–903.
- Vahrenholz, Carola, Gudula Riemen, Elke Pratje, Bernard Dujon, and Georg Michaelis. 1993. "Mitochondrial DNA of *Chlamydomonas Reinhardtii*: The Structure of the Ends of the Linear 15.8-Kb Genome Suggests Mechanisms for DNA Replication." *Current Genetics* 24 (3): 241–47. doi:10.1007/BF00351798.
- Van De Vosse, David W., Yakun Wan, Diego L. Lapetina, Wei Ming Chen, Jung Hsien Chiang, John D. Aitchison, and Richard W. Wozniak. 2013. "A Role for the Nucleoporin Nup170p in Chromatin Structure and Gene Silencing." *Cell* 152 (5): 969–83. doi:10.1016/j.cell.2013.01.049.
- Van Hoof, Ambro. 2005. "Conserved Functions of Yeast Genes Support the Duplication, Degeneration and Complementation Model for Gene Duplication." *Genetics* 171 (4): 1455–61. doi:10.1534/genetics.105.044057.
- Van Leeuwen, Fred, and Daniel E. Gottschling. 2002. "Genome-Wide Histone Modifications: Gaining Specificity by Preventing Promiscuity." *Current Opinion in Cell Biology*. doi:10.1016/S0955-0674(02)00393-9.
- van Welsem, T, F Frederiks, K F Verzijlbergen, A W Faber, Z W Nelson, D A Egan, D E Gottschling, and F van Leeuwen. 2008. "Synthetic Lethal Screens Identify Gene Silencing Processes in Yeast and Implicate the Acetylated Amino Terminus of Sir3 in Recognition of the Nucleosome Core." *Mol Cell Biol* 28 (11): 3861–

72. doi:MCB.02050-07 [pii]10.1128/MCB.02050-07.

- Varunan, Shalu M., Jaishree Tripathi, Sunanda Bhattacharyya, Tanvi Suhane, and Mrinal Kanti Bhattacharyya. 2013. "Plasmodium Falciparum Origin Recognition Complex Subunit 1 (PfOrc1) Functionally Complements Δ ir3 Mutant of *Saccharomyces Cerevisiae*." *Molecular and Biochemical Parasitology* 191 (1): 28–35. doi:10.1016/j.molbiopara.2013.08.004.
- Venditti, S, M A Vega-Palas, G Di Stefano, and E Di Mauro. 1999. "Imbalance in Dosage of the Genes for the Heterochromatin Components Sir3p and Histone H4 Results in Changes in the Length and Sequence Organization of Yeast Telomeres." *Mol Gen Genet* 262 (2): 367–77.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10517334.
- Venkatasubrahmanyam, S, W W Hwang, M D Meneghini, A H Tong, and H D Madhani. 2007. "Genome-Wide, as Opposed to Local, Antisilencing Is Mediated Redundantly by the Euchromatic Factors Set1 and H2A.Z." *Proc Natl Acad Sci U S A* 104 (42): 16609–14.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17925448.
- Verzijlbergen, Kitty F, Alex W Faber, Iris Je Stulemeijer, and Fred van Leeuwen. 2009. "Multiple Histone Modifications in Euchromatin Promote Heterochromatin Formation by Redundant Mechanisms in *Saccharomyces Cerevisiae*." *BMC Molecular Biology* 10: 76. doi:10.1186/1471-2199-10-76.
- Villasante, Alfredo, José P Abad, and María Méndez-Lago. 2007. "Centromeres Were Derived from Telomeres during the Evolution of the Eukaryotic Chromosome." *Proceedings of the National Academy of Sciences of the United States of America* 104 (25): 10542–47. doi:10.1073/pnas.0703808104.
- Wang, F, G Li, M Altaf, C Lu, M A Currie, A Johnson, and D Moazed. 2013. "Heterochromatin Protein Sir3 Induces Contacts between the Amino Terminus of Histone H4 and Nucleosomal DNA." *Proc Natl Acad Sci U S A* 110 (21): 8495–8500. doi:10.1073/pnas.1300126110.
- Wang, G, a Ma, C M Chow, D Horsley, N R Brown, I G Cowell, and P B Singh. 2000. "Conservation of Heterochromatin Protein 1 Function." *Molecular and Cellular Biology* 20 (18): 6970–83. doi:10.1128/MCB.20.18.6970-6983.2000.
- Wang, X, J J Connelly, C L Wang, and R Sternglanz. 2004. "Importance of the Sir3 N Terminus and Its Acetylation for Yeast Transcriptional Silencing." *Genetics* 168 (1): 547–51. doi:10.1534/genetics.104.028803168/1/547 [pii].
- Watson, J D. 1972. "Origin of Concatemeric T7 DNA." *Nature: New Biology* 239 (94): 197–201. doi:10.1038/newbio239197a0.
- Weber, Christopher M., and Steven Henikoff. 2014. "Histone Variants: Dynamic Punctuation in Transcription." *Genes and Development*. doi:10.1101/gad.238873.114.
- Weber, J M, and A E Ehrenhofer-Murray. n.d. "Design of a Minimal Silencer for the Silent Mating-Type Locus HML of *Saccharomyces Cerevisiae*." *Nucleic Acids Res*. doi:gkq689 [pii]10.1093/nar/gkq689.
- Weiner, A, T H Hsieh, A Appleboim, H V Chen, A Rahat, I Amit, O J Rando, and N Friedman. 2015. "High-Resolution Chromatin Dynamics during a Yeast Stress Response." *Mol Cell* 58 (2): 371–86. doi:10.1016/j.molcel.2015.02.002.
- Whiteway, M, R Freedman, S Van Arsdell, JW Szostak, and J Thorner. 1987. "The Yeast ARD1 Gene Product Is Required for Repression of Cryptic Mating-Type Information at the HML Locus." *Molecular and Cellular Biology* 7 (10): 3713–22. doi:10.1128/MCB.7.10.3713.
- Wijchers, Patrick J., Peter H.L. Krijger, Geert Geeven, Yun Zhu, Annette Denker, Marjon J.A.M. Verstegen,

- Christian Valdes-Quezada, et al. 2016. "Cause and Consequence of Tethering a SubTAD to Different Nuclear Compartments." *Molecular Cell* 61 (3): 461–73. doi:10.1016/j.molcel.2016.01.001.
- Wolfe, Kenneth H., and Denis C. Shields. 1997. "Molecular Evidence for an Ancient Duplication of the Entire Yeast Genome." *Nature* 387 (6634): 708–13. doi:10.1038/42711.
- Xu, Eugenia Y., Karl A. Zawadzki, and James R. Broach. 2006. "Single-Cell Observations Reveal Intermediate Transcriptional Silencing States." *Molecular Cell* 23 (2): 219–29. doi:10.1016/j.molcel.2006.05.035.
- Yamada, M., N. Hayatsu, A. Matsuura, and F. Ishikawa. 1998. "Y'-Help1, a DNA Helicase Encoded by the Yeast Subtelomeric Y' Element, Is Induced in Survivors Defective for Telomerase." *Journal of Biological Chemistry* 273 (50): 33360–66. doi:10.1074/jbc.273.50.33360.
- Yamada, T, R Fukuda, M Himeno, and K Sugimoto. 1999. "Functional Domain Structure of Human Heterochromatin Protein HP1(Hsalpha): Involvement of Internal DNA-Binding and C-Terminal Self-Association Domains in the Formation of Discrete Dots in Interphase Nuclei." *J Biochem* 125 (4): 832–37.
- Yamagishi, Yuya, Takeshi Sakuno, Mari Shimura, and Yoshinori Watanabe. 2008. "Heterochromatin Links to Centromeric Protection by Recruiting Shugoshin." *Nature* 455 (7210): 251–55. doi:10.1038/nature07217.
- Yamashita, I., T. Maemura, T. Hatano, and S. Fukui. 1985. "Polymorphic Extracellular Glucoamylase Genes and Their Evolutionary Origin in the Yeast *Saccharomyces Diastaticus*." *Journal of Bacteriology* 161 (2): 574–82.
- Yamashita, I., Y. Takano, and S. Fukui. 1985. "Control of STA1 Gene Expression by the Mating-Type Locus in Yeasts." *Journal of Bacteriology* 164 (2): 769–73.
- Yang, B, and A L Kirchmaier. 2006. "Bypassing the Catalytic Activity of SIR2 for SIR Protein Spreading in *Saccharomyces Cerevisiae*." *Mol Biol Cell* 17 (12): 5287–97. doi:E06-08-0669 [pii]10.1091/mbc.E06-08-0669.
- Yang, Dongxue, Qianglin Fang, Mingzhu Wang, Ren Ren, Hong Wang, Meng He, Youwei Sun, Na Yang, and Rui-Ming M Xu. 2013. "N α -Acetylated Sir3 Stabilizes the Conformation of a Nucleosome-Binding Loop in the BAH Domain." *Nature Structural & Molecular Biology* 20 (9): 1116–18. doi:10.1038/nsmb.2637.
- Yasuhara, Jiro C., and Barbara T. Wakimoto. 2006. "Oxymoron No More: The Expanding World of Heterochromatic Genes." *Trends in Genetics*. doi:10.1016/j.tig.2006.04.008.
- Young, Janet M, and Barbara J Trask. 2002. "The Sense of Smell: Genomics of Vertebrate Odorant Receptors." *Human Molecular Genetics* 11 (10): 1153–60. doi:10.1093/hmg/11.10.1153.
- Yu, Q, R Qiu, T B Foland, D Griesen, C S Galloway, Y H Chiu, J Sandmeier, J R Broach, and X Bi. 2003. "Rap1p and Other Transcriptional Regulators Can Function in Defining Distinct Domains of Gene Expression." *Nucleic Acids Res* 31 (4): 1224–33. <http://www.ncbi.nlm.nih.gov/pubmed/12582242>.
- Yu, Tai Yuan, Chen Yi Wang, and Jing Jer Lin. 2012. "Depleting Components of the THO Complex Causes Increased Telomere Length by Reducing the Expression of the Telomere-Associated Protein Rif1p." *PLoS ONE* 7 (3). doi:10.1371/journal.pone.0033498.
- Yue, Jia-Xing, Jing Li, Louise Aigrain, Johan Hallin, Karl Persson, Karen Oliver, Anders Bergström, et al. 2017. "Contrasting Evolutionary Genome Dynamics between Domesticated and Wild Yeasts." *Nature Genetics* 49 (6): 913–24. doi:10.1038/ng.3847.
- Zhang, Ke, Kerstin Mosch, Wolfgang Fischle, and Shiv I S Grewal. 2008. "Roles of the Clr4 Methyltransferase Complex in Nucleation, Spreading and Maintenance of Heterochromatin." *Nature Structural & Molecular Biology* 15 (4): 381–88. doi:10.1038/nsmb.1406.

- Zhang, Xinmin, Qun Yu, Lars Olsen, and Xin Bi. 2012. "Functions of Protosilencers in the Formation and Maintenance of Heterochromatin in *Saccharomyces Cerevisiae*." *PLoS ONE* 7 (5). doi:10.1371/journal.pone.0037092.
- Zhimulev, Igor F., Elena S. Belyaeva, Igor V. Makunin, Vincenzo Pirrotta, Valery F. Semeshin, Artyom A. Alekseyenko, Stepan N. Belyakin, et al. 2003. "Intercalary Heterochromatin in *Drosophila Melanogaster* Polytene Chromosomes and the Problem of Genetic Silencing." *Genetica*. doi:10.1023/A:1022912716376.
- Zhou, Jing, Bo O. Zhou, Brian A. Lenzmeier, and Jin Qiu Zhou. 2009. "Histone Deacetylase Rpd3 Antagonizes Sir2-Dependent Silent Chromatin Propagation." *Nucleic Acids Research* 37 (11): 3699–3713. doi:10.1093/nar/gkp233.
- Zhu, Lian, and Clifford P. Brangwynne. 2015. "Nuclear Bodies: The Emerging Biophysics of Nucleoplasmic Phases." *Current Opinion in Cell Biology*. doi:10.1016/j.ceb.2015.04.003.
- Zou, S, D a Wright, and D F Voytas. 1995. "The *Saccharomyces Ty5* Retrotransposon Family Is Associated with Origins of DNA Replication at the Telomeres and the Silent Mating Locus HMR." *Proceedings of the National Academy of Sciences of the United States of America* 92 (January): 920–24. doi:10.1073/pnas.92.3.920.
- Zou, Yanfei, Qun Yu, and Xin Bi. 2006. "Asymmetric Positioning of Nucleosomes and Directional Establishment of Transcriptionally Silent Chromatin by *Saccharomyces Cerevisiae* Silencers." *Molecular and Cellular Biology* 26 (20): 7806–19. doi:10.1128/MCB.01197-06.

List of abbreviations:

ChIP	chromatin immuno-precipitation
DNA	deoxyribonucleic acid
dsDNA	double stranded DNA
dNTP	deoxynucleotide
ESD	Extended silent domains
lncRNA	long non coding RNA
HAST	Hda1 de-acetylated subtelomeric
mtTBP	mitochondrial telomere binding proteins
NAP	nucleoid associated proteins
NHEJ	non-homologous end joining
PTM	Post-translational modification of histones
RBC	red blood cells
RNA	ribonucleic acid
SSB	single strand binding proteins
TP	terminal proteins
TPE	Telomere position effect