



HAL
open science

Heterochromatin replication goes hand in hand with telomere protection

Aaron Mendez-Bermudez, M.J. Giraud-Panis, Jing Ye, Eric Gilson

► **To cite this version:**

Aaron Mendez-Bermudez, M.J. Giraud-Panis, Jing Ye, Eric Gilson. Heterochromatin replication goes hand in hand with telomere protection. *Nature Structural and Molecular Biology*, 2020, 27 (4), pp.313-318. 10.1038/s41594-020-0400-1 . hal-03013735

HAL Id: hal-03013735

<https://hal.science/hal-03013735v1>

Submitted on 24 Nov 2020

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.

Heterochromatin replication goes hand in hand with telomere protection

Aaron Mendez-Bermudez^{1,2}, Marie-Josèphe Giraud-Panis¹, Jing Ye^{2*} and Eric Gilson^{1,2,3*}

¹Côte-d'Azur University, Institute for Research on Cancer and Aging, Nice (IRCAN), CNRS UMR 7284 - INSERM U1081, France.

²International Research Laboratory "Hematology, Cancer and Aging", Shanghai Ruijin Hospital affiliated to Shanghai Jiaotong University, CNRS, Inserm, Côte-d'Azur University, Shanghai, China.

³CHU Nice, Department of Genetics, France

- Correspondence: eric.gilson@unice.fr (E.G.), yj11254@rjh.com.cn (J.Y.)

Abstract

Telomeres arose from the need to stabilize natural chromosome ends, resulting in terminal chromatin structures with specific protective functions. Their constituent proteins also execute global functions within heterochromatin, where they mediate late replication and facilitate fork progression. Emerging insights into the mechanisms governing heterochromatin replication suggest concerted actions between telomeres and heterochromatin during development and aging. They also suggest a common evolutionary origin of these two chromosome regions during eukaryogenesis.

Introduction

During evolution, chromosomal DNA molecules have adopted two types of geometry: linear for all eukaryotes and circular for most prokaryotes, with the exception of some bacteria and bacteriophages. Chromosome linearity imposes two molecular problems: first, the inherent difficulty of replicating chromosomal termini and second, the potential deleterious activation of the DNA damage response (DDR) due to the presence of DNA ends. Our growing knowledge of the comparative biology of telomeres between organisms reveals a wide variety of specialized nucleoprotein organizations that function to compensate for replicative erosion (e.g. telomerase) and to protect from DDR processes (e.g. shelterin) ¹. In many organisms, telomeric DNA forms a 3' overhang that can invade internal homologous duplex DNA sequences to form a protective terminal chromatin loop (t-loop).

In this Perspective, we discuss a wealth of recent studies showing that some of the specialized telomere protective proteins also regulate heterochromatin replication. These findings reveal unexpected genome-wide concerted actions between telomeres and heterochromatin, and invite us to consider the hypothesis of a common evolutionary origin between telomeres and heterochromatin.

Heterochromatin and telomeres, old acquaintances

If the original definition of heterochromatin is a form of chromatin that remains condensed throughout the cell cycle ², a more molecular definition is preferred today that considers heterochromatin as regions enriched in specific post-translational histone modifications,

including H3K9me3 and H3K27me3³. A general property of heterochromatin is its ability to repress the expression of genes located in its vicinity in a variegated manner⁴.

The first functional indication of the heterochromatic character of telomeres was the demonstration of variegated expression of genes inserted in their proximity in budding yeast, a phenomenon named TPE for Telomere Position Effect⁵. In fact, constitutive heterochromatin in budding yeast is localized within the telomeric regions, and is generated by the formation of a long array of Rap1 proteins on the telomeric DNA that serves as a platform for the loading and spreading of the Silent Information Regulator (SIR) complex⁶.

Since its initial discovery, TPE was considered an ancient mechanism of gene expression regulation⁵ and opened the way to the idea that the telomeric state can remotely influence gene expression⁷. However, telomere protection does not necessarily rely on heterochromatin formation, since telomeres that have lost their heterochromatic character are nonetheless propagated in budding yeast⁸ or in mammalian cells⁹ without any apparent effect on their stability. Similarly, telomeres of many human cell types do not exhibit characteristic heterochromatin marks^{9,10}. Nevertheless, in some circumstances, the formation of terminal heterochromatin may serve as a protective mechanism, as in fission yeast strains that lack telomerase¹¹ {Jain, 2010 #4609} and in *Drosophila*¹².

In agreement with the intimate links between telomere and heterochromatin, several general heterochromatin factors are involved in telomere protection³. In the following sections, we consider reciprocal relationships of specialized telomere protective factors and specific regulators of heterochromatin replication genome-wide that have recently been reported.

Telomeric proteins as regulators of late replicating DNA regions

Heterochromatic domains are generally replicated late during S-phase. Such a timed process is of paramount importance for the regulation of transcriptional programs and for chromosome maintenance during the cell cycle, as well as differentiation and development ¹³. In fission yeast, Taz1 is a key shelterin subunit that specifically binds telomeric DNA through a C-terminal Telobox domain (Figure 1a) ¹⁴. Taz1 was found to delay replication firing of roughly half of *S. pombe* late origins by associating with short stretches of nearby telomeric DNA repeats ¹⁵. An independent study reported that Taz1 also associates with a subset of late origins to assemble heterochromatin by recruiting Ccq1, another shelterin subunit that interacts with the histone H3K9 methyltransferase complex CLRC ¹⁶. Thus, Taz1 functions with other shelterin subunits to couple telomere protection, heterochromatin assembly, late replication firing and gene silencing at several internal regions of fission yeast chromosomes **(Figure 1a)**.

Another protein initially identified as a telomere-specific factor, Rif1, was subsequently shown to be a general regulator of late replication timing. In budding yeast, Rif1 was discovered as a Rap1 interacting factor that negatively regulates TPE and telomere elongation ¹⁷. These roles of Rif1 are dependent on its interaction with Protein Phosphatase 1 (PP1), which inhibits the recruitment and activation of Tel1 (ATM, ataxia-telangiectasia mutated, in mammals), a kinase crucial for telomerase recruitment to short telomeres ¹⁸. In fission yeast, Rif1 also binds telomeres indirectly through the shelterin subunit Taz1, and contributes to telomere length regulation ¹⁹. In mammals, Rif1 does not appear to be directly recruited to telomeres, but rather exerts its telomere maintenance functions by controlling subtelomeric heterochromatin and the expression of genes involved in telomere length regulation ²⁰. Overall, it appears that Rif1 is widely conserved through eukaryotic evolution connecting telomere length regulation and heterochromatin formation **(Figure 1a)**.

Rif1 also exhibits extratelomeric functions by scheduling late replication origin firing through the recruitment of PP1, which dephosphorylates and inhibits the Dbf4-dependent cdc7 kinase (DDK), a crucial factor for origin firing that phosphorylates the replicative minichromosome maintenance complex (MCM) DNA helicase. PP1 also dephosphorylates components of the MCM complex, thus counteracting the effects of DDK. This conserved function of Rif1 was documented in fission yeast ²¹; budding yeast ^{22,23,24}, *Drosophila* ²⁵ and vertebrates ^{26,27,28,29}.

In addition to its local role as PP1 recruiter to late origins, Rif1 controls and limits the number of late replicating domains as well as their spatial organization (**Figure 1b**) ^{27,30,31,32}. In budding and fission yeasts, only a subset of the Rif1-controlled origins are generated by Rif1 recruitment through Rap1 and Taz1 respectively ^{15,33}, implying that Rif1 can control the structure of late replication domains through pathways independent from the binding to telomeric factors. Among the known properties of Rif1, multimerization ³⁴, as well as binding to particular DNA conformations ^{35,36} or heterochromatin factors ²⁰ {Dan, 2014 #4249} may play important roles in its chromatin domain-organizing role. The crosstalk between telomeres and heterochromatin has important implications, since the telomere changes that occur during development and aging can impact heterochromatin replication genome-wide. For instance, in fission yeast, mutations that over-elongate telomeres lead to accelerated replication at internal late origins due to the sequestration of PP1 at telomeres in a Taz1-Rif1 dependent manner ³⁷. In a similar way, the telomeres of budding yeast concentrate most of the cellular Rif1 protein explaining why the Rif1 action is largely restricted to subtelomeric origins in this organism ³⁸. There are also examples where telomere and heterochromatin behave in a concerted manner. For instance, replication stress in fission yeast triggers a vast chromosome spatial rearrangement leading to the telomeric association of the subset of late origins controlled by Taz1, a process that requires the two shelterin subunits Rap1 and Ccq1

³⁹. During *Drosophila* development, Rif1 expression is regulated to allow late replication to emerge at the mid-blastula transition by delaying replication at heterochromatin ²⁵. Finally, in mouse embryonic stem cells, Rif1 modulates the expression of the subtelomeric gene *Zscan4* involved in telomere length elongation ²⁰.

Telomere proteins facilitate fork progression through hard-to-replicate regions

A characteristic, shared feature of telomeres and heterochromatin is their ability to hinder replication fork progression, leading to frequent paused or stalled forks that reduce replication speed and trigger topological stress ⁴⁰. The obstacles that block fork progression in these regions are only partially understood, but include DNA secondary structures such as G quadruplexes (G4), condensed and looped chromatin, transcription of non-coding RNA forming R-loops, attachment to the nuclear matrix and envelope and, for telomeres, t-loops. In both prokaryotes and eukaryotes, excessive topological constraints at stalled forks promote their reversal, which leads to the conversion of a typical replication fork (three-way junction) into a four-way junction (resembling a Holiday junction) by the annealing of the two newly synthesized strands, forming the regressed arm, and the re-annealing of the parental strands ⁴¹ (**Figure 2a**). This process is believed to decrease supercoiling constraints and to transiently protect the forks to allow more time to relieve the replication block, repair the DNA lesions and resume fork progression ⁴².

Amazingly, the telomeric protective factors shown to be involved in the control of late replication timing (TRF2, Taz1 and Rif1) are also required to facilitate fork progression through hard-to-replicate regions at telomeres and at heterochromatin. The first indication came from studies of the fission yeast Taz1, which was shown to block the progression of replication forks through a stretch of telomeric DNA sequences artificially inserted inside a yeast chromosome

⁴³. The mammalian Taz1 orthologs, TRF1 and TRF2, were also shown to have replicative functions. Specifically, TRF1 prevents telomere replication fork stalling and ATR activation by recruiting the Bloom helicase (BLM), most likely to remove secondary structures formed by the G-rich strand of telomeres ⁴⁴. For its part, TRF2 is involved in the progression of the replisome through telomeric chromatin by relieving torsional stress ⁴⁵ and by recruiting the BUB1-BUB3 complex, a component of the spindle assembly checkpoint that acts in synergy with TRF1 and BLM, suggesting a coordination between TRF1 and TRF2 to cope with replication stress at telomeres ⁴⁶.

The role of TRF2 in fork progression is not limited to telomeres. Indeed, in human cancer cells, TRF2 also binds and protects the pericentromeric heterochromatin DNA sequences from DDR activation (**Figure 2a**) ^{40,47}. One of the mechanisms by which TRF2 facilitates pericentromeric replication is through recruitment of the helicase RTEL1, which relieves heterochromatin replication blockade ⁴⁰. The effects of TRF2-RTEL1 on heterochromatin replication are most likely linked to G4-like conformations, as they are modulated by G4 ligands and since RTEL1 is known to unwind G4 structures efficiently. These G4-related structures may constitute one of the barriers impeding replication fork progression at pericentromeric heterochromatin. TRF2 recruitment at replicating pericentromeres does not depend upon its telomeric sequence-specific DNA binding but on its capacity to bind several structural features of topologically constrained reversed stalled forks, particularly four-way DNA junctions and positive supercoils (**Figure 2a**) ^{40,48,49}.

Like TRF2 and Taz1, Rif1 also controls replication fork progression, as shown in both yeast ³³ and mammals, where its interaction with PP1 dephosphorylates the DNA2-WRN complex and consequently prevents the degradation of nascent DNA at stalled forks ^{50,51,52}.

Was the ancestral telomere born out of a regressed replication fork?

An intriguing implication of the fork reversal process is the formation of a DNA terminus at the regressed arm that has to be protected during replication, a situation bearing striking similarities with the need to protect telomeric DNA from unwanted DDR. Indeed, regressed forks and telomeres exhibit numerous similarities (**Figure 2b and Figure 3**), and regressed forks share several mechanisms of formation with t-loops^{49,53,54,55}. Moreover, regressed fork and telomeres are protected by common factors^{33,40,50,51,52,56,57,58,59,60}. Not only are formation and protection similar between telomeres and regressed forks, but also the way that their DNA termini are processed via a controlled 5' resection reaction involving the nucleases Apollo^{61,62} and Exo1⁶³. It is also noteworthy that SLX4, which acts as a scaffold to coordinate the recruitment of various structure-specific endonucleases involved in the processing of stalled forks, interacts with telomeres via TRF2⁶⁴. Finally, telomeric DSBs and regressed forks can be healed by telomerase in yeast and mammalian cells^{65,66}.

Together, these findings suggest that the mechanisms that were implemented during early eukaryogenesis to protect regressed forks and telomeres were intertwined and possibly of common origin (**Figure 3**). We propose that, during eukaryogenesis, the evolution of condensed heterochromatin-like structures led to massive replicative and topological stresses that required robust mechanisms to be put in place to allow the DNA to be faithfully replicated. In the light of our present knowledge of the common mechanisms protecting telomeres and regressed forks discussed above, these ancestral mechanisms might include proteins that recognize positive supercoils, such as the TRFH domain of the shelterin complex, and that promote four-way DNA junction formation, such as the basic domain of TRF2, to

sense and remodel topologically blocked forks. In this hypothesis, the origin of ancestral telomeres would stem from replication difficulties at the time of heterochromatin invention. In favor of the scenario that regressed forks can be a source of free chromosome ends, replicative stress in *E. coli* can generate cells with a heritable but unstable chromosome carrying a broken end⁶⁷. Thus, the early evolution of factors protecting DNA double-strand breaks (DSBs) of the regressed arm of regressed forks could have been employed as a “first aid” to protect the free ends of primitive linearized genomes.

A prior model of telomere invention during eukaryogenesis proposed that the invasion of ancestral circular genomes by group II introns would have created conditions that stabilize DNA termini by allowing the formation of primitive t-loops⁶⁸. It is quite conceivable that group II intron repeats would have also favored heterochromatin formation to limit their propagation as it is observed today for retrotransposons and consistent with the demonstrated toxicity conferred by an experimental invasion of group II introns in *E. coli*⁶⁹. Therefore, the group II intron model of telomere invention by t-loop formation is compatible with the one proposed here based on replication accident at ancestral heterochromatin.

Conclusions

A direct link between telomeres and heterochromatin replication genome-wide represents an important breakthrough in our understanding of the specific mechanisms of heterochromatin replication and how telomeres and heterochromatin act in concert to control chromosome maintenance and function. Moreover, such findings invite us to envisage a common evolutionary origin between telomeres and heterochromatin.

We believe that the genome-wide roles of key telomere protective proteins in heterochromatin replication (reviewed here) and in transcriptional regulation⁷⁰, reflect a

fundamental need to couple the telomeric state to cell fate. It is therefore quite conceivable that in the course of evolution, the original relationships between telomeres, heterochromatin replication and transcriptional regulation genome-wide have been preserved and diversified. Such mechanisms are expected to provide an explanation for the broad contributions of telomeres to development as well as normal and pathological ageing.

Acknowledgments

This work in the EG lab was supported by the cross-cutting Inserm program on aging (AGEMED), the ANR program TELOCHROM, the Sino-French PHC Cai Yuanpei program and the “Investments for the Future” LABEX SIGNALIFE (reference ANR-11-LABX-0028-01). We apologize to all authors whose publications have not been cited due to space limitation.

Figure 1. Telomere proteins and late replicating domains.

a) Schematic representation comparing the DNA-protein organization of telomeres, subtelomeres and heterochromatin (late replication) domains in *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and mammals. Heterochromatin domains are boxed in grey rectangles.

b) Higher-order chromatin structures controlled by Rif1. In *S. cerevisiae*, telomeres are clustered at the nuclear periphery, forming a heterochromatic late-replicating domain where the Rif1 proteins are enriched. In mammals, Rif1 determines the formation of late-replicating heterochromatin domains. The divergent arrows within the late replicating domains indicate the ability of Rif1 to assemble and limit the 3D organization of these domains in both yeast and mammalian nuclei.

Figure 2. Telomere proteins and heterochromatin replication elongation.

a) TRF2 is required to protect stalled forks formed in pericentromeric heterochromatin and to facilitate replication fork progression by recruiting the helicase RTEL1 and blunting ATM activation. TRF2 can bind stalled forks within heterochromatin through the positively supercoiled DNA that accumulates ahead of the stalled replication fork or the four-way DNA junctions of the reversed fork.

b) Similarities between the protective mechanisms at play at telomeres (left) and at reverse stalled forks (right). Common factors and their context-dependent functions are indicated.

Figure 3. Model of telomere evolution.

At the early stages of eukaryogenesis, we hypothesise that the appearance of heterochromatin-like structures within circular chromosomes triggered a high level of replicative and topological stress, leading to the formation of reversed stalled forks at a high rate and, consequently, the requirement for potent mechanisms of reversed fork protection and maturation. This, in turn, may have favoured stabilization of linearized chromosomes resulting either from stalled fork breakage or chromosome instability generated, for instance, by group II intron invasion, high levels of ultraviolet irradiation and oxidative stress, or frequent desiccation periods.

References

1. Giraud-Panis, M.J. et al. One identity or more for telomeres? *Front Oncol* **3**, 48 (2013).
2. Heitz E. Das Heterochromatin der Moose. *Jarhb. Wiss. Bot.* **69**, 762-818 (1928).
3. Nishibuchi, G. & Dejardin, J. The molecular basis of the organization of repetitive DNA-containing constitutive heterochromatin in mammals. *Chromosome Res* **25**, 77-87 (2017).

4. Müller H.J. The remaking of chromosomes. *The Collecting Net* **13**, 182-198 (1938).
5. **Gottschling D.E., Aparicio O.M., Billington B.L. & Zakian, V.A. Position effect at *S. cerevisiae* telomeres : reversible repression of Pol II transcription. *Cell* **63**, 751-762 (1990).**
This paper reveals that gene expression can be repressed due to telomere proximity
6. Gasser, S.M. & Cockell, M.M. The molecular biology of the SIR proteins. *Gene* **279**, 1-16 (2001).
7. Maillet, L. et al. Evidence for silencing compartments within the yeast nucleus: a role for telomere proximity and Sir protein concentration in silencer-mediated repression. *Genes Dev* **10**, 1796-811 (1996).
8. Palladino F. et al. SIR3 and SIR4 proteins are required for the positioning and integrity of yeast telomeres. *Cell* **75**, 543-555 (1993).
9. Gauchier, M. et al. SETDB1-dependent heterochromatin stimulates alternative lengthening of telomeres. *Sci Adv* **5**, eaav3673 (2019).
10. Cubiles, M.D. et al. Epigenetic features of human telomeres. *Nucleic Acids Res* **46**, 2347-2355 (2018).
11. **Jain, D., Hebden, A.K., Nakamura, T.M., Miller, K.M. & Cooper, J.P. HAATI survivors replace canonical telomeres with blocks of generic heterochromatin. *Nature* **467**, 223-7 (2010).**
This paper provides evidence in fission yeast for the use of heterochromatic blocks to replace telomeres and to provide end protection in the absence of telomerase.
12. Fanti, L., Giovanazzo, G., Berloco, M. & Pimpinelli, S. The heterochromatin protein 1 prevents telomere fusions in *Drosophila*. *Mol Cell* **2**, 527-38 (1998).
13. Zhao, P.A., Rivera-Mulia, J.C. & Gilbert, D.M. Replication Domains: Genome Compartmentalization into Functional Replication Units. *Adv Exp Med Biol* **1042**, 229-257 (2017).
14. Cooper J P., Nimmo E R., Allshire R C. & Cech, T.R. Regulation of telomere length and function by a Myb-domain protein in fission yeast. *Nature* **385**, 744-747 (1997).
15. **Tazumi, A. et al. Telomere-binding protein Taz1 controls global replication timing through its localization near late replication origins in fission yeast. *Genes Dev* **26**, 2050-62 (2012).**
This paper shows the Taz1 replication timing control of a subset of late origins. This is mediated by the direct binding of Taz1 to telomeric repeats found proximal to origins.
16. **Zofall, M., Smith, D.R., Mizuguchi, T., Dhakshnamoorthy, J. & Grewal, S.I.S. Taz1-Shelterin Promotes Facultative Heterochromatin Assembly at Chromosome-Internal Sites Containing Late Replication Origins. *Mol Cell* **62**, 862-874 (2016).**
This work shows that Shelterin subunits assemble facultative heterochromatin at internal chromosomal sites containing late replication origins.
17. Hardy C.F.J., Balderes D. & Shore, D. Dissection of a carboxy-terminal region of the yeast regulatory protein RAP1 with effects on both transcriptional activation and silencing. *Mol. Cell. Biol.* **12**, 1209-1217 (1992).
18. Kedziora, S. et al. Rif1 acts through Protein Phosphatase 1 but independent of replication timing to suppress telomere extension in budding yeast. *Nucleic Acids Res* **46**, 3993-4003 (2018).
19. Miller, K.M., Ferreira, M.G. & Cooper, J.P. Taz1, Rap1 and Rif1 act both interdependently and independently to maintain telomeres. *EMBO J* **24**, 3128-35 (2005).
20. Dan, J. et al. Rif1 maintains telomere length homeostasis of ESCs by mediating heterochromatin silencing. *Dev Cell* **29**, 7-19 (2014).

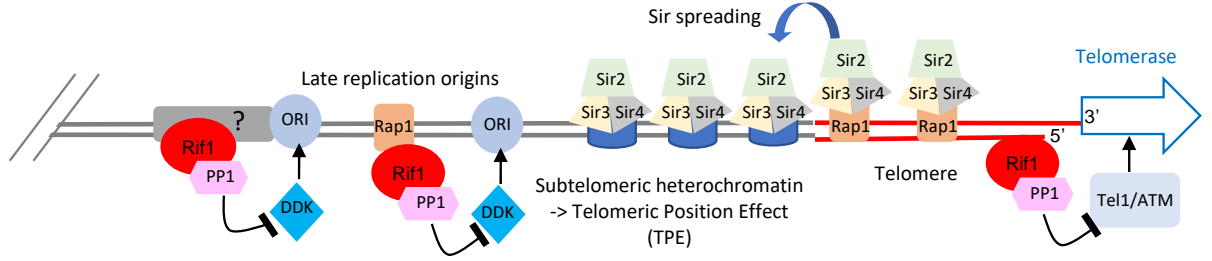
21. **Hayano, M. et al. Rif1 is a global regulator of timing of replication origin firing in fission yeast. *Genes Dev* 26, 137-50 (2012).**
This paper reveals that the global replication timing in *S. pombe* is controlled by Rif1 in a Taz1 independent fashion.
22. **Hiraga, S. et al. Rif1 controls DNA replication by directing Protein Phosphatase 1 to reverse Cdc7-mediated phosphorylation of the MCM complex. *Genes Dev* 28, 372-83 (2014).**
This paper shows that the budding yeast Rif1 controls DNA replication genome-wide and that Rif1 exerts this control through PP1-mediated dephosphorylation of the MCM complex early in the cell cycle.
23. **Mattarocci, S. et al. Rif1 controls DNA replication timing in yeast through the PP1 phosphatase Glc7. *Cell Rep* 7, 62-9 (2014).**
This work links Rif1 to the negative regulation of replication origin firing through recruitment of the Glc7 phosphatase.
24. **Dave, A., Cooley, C., Garg, M. & Bianchi, A. Protein phosphatase 1 recruitment by Rif1 regulates DNA replication origin firing by counteracting DDK activity. *Cell Rep* 7, 53-61 (2014).**
This paper shows that Rif1, regulates replication timing in budding yeas through its interaction with PP1 phosphatases.
25. **Seller, C.A. & O'Farrell, P.H. Rif1 prolongs the embryonic S phase at the Drosophila mid-blastula transition. *PLoS Biol* 16, e2005687 (2018).**
This study demonstrates the direct role played by Rif1 during development by delaying the replication of satellite sequences.
26. Cornacchia, D. et al. Mouse Rif1 is a key regulator of the replication-timing programme in mammalian cells. *EMBO J* 31, 3678-90 (2012).
27. Yamazaki, S. et al. Rif1 regulates the replication timing domains on the human genome. *EMBO J* 31, 3667-77 (2012).
28. Sukackaite, R. et al. Mouse Rif1 is a regulatory subunit of protein phosphatase 1 (PP1). *Sci Rep* 7, 2119 (2017).
29. Alver, R.C., Chadha, G.S., Gillespie, P.J. & Blow, J.J. Reversal of DDK-Mediated MCM Phosphorylation by Rif1-PP1 Regulates Replication Initiation and Replisome Stability Independently of ATR/Chk1. *Cell Rep* 18, 2508-2520 (2017).
30. **Foti, R. et al. Nuclear Architecture Organized by Rif1 Underpins the Replication-Timing Program. *Mol Cell* 61, 260-73 (2016).**
This study identifies Rif1 as the molecular link between nuclear architecture and replication-timing establishment in mammals.
31. Toteva, T. et al. Establishment of expression-state boundaries by Rif1 and Taz1 in fission yeast. *Proc Natl Acad Sci U S A* 114, 1093-1098 (2017).
32. Ogawa, S. et al. Shelterin promotes tethering of late replication origins to telomeres for replication-timing control. *EMBO J* 37, e98997 (2018).
33. Hiraga, S.I. et al. Budding yeast Rif1 binds to replication origins and protects DNA at blocked replication forks. *EMBO Rep* 20, e48152 (2019).
34. Moriyama, K., Yoshizawa-Sugata, N. & Masai, H. Oligomer formation and G-quadruplex binding by purified murine Rif1 protein, a key organizer of higher-order chromatin architecture. *J Biol Chem* 293, 3607-3624 (2018).
35. Kano, Y. et al. Rif1 binds to G quadruplexes and suppresses replication over long distances. *Nat Struct Mol Biol* 22, 889-97 (2015).
36. Masai, H. et al. Rif1 promotes association of G-quadruplex (G4) by its specific G4 binding and oligomerization activities. *Sci Rep* 9, 8618 (2019).

37. Hasegawa, Y., Yamamoto, M., Miyamori, J. & Kanoh, J. Telomere DNA length-dependent regulation of DNA replication timing at internal late replication origins. *Sci Rep* **9**, 9946 (2019).
38. **Hafner, L. et al. Rif1 Binding and Control of Chromosome-Internal DNA Replication Origins Is Limited by Telomere Sequestration. *Cell Rep* **23**, 983-992 (2018).**
This work shows that the global replication dynamics in budding yeast are controlled by telomeric sequestration of Rif1 by Rap1.
39. Mizuguchi, T. et al. Shelterin components mediate genome reorganization in response to replication stress. *Proc Natl Acad Sci U S A* **114**, 5479-5484 (2017).
40. **Mendez-Bermudez, A. et al. Genome-wide Control of Heterochromatin Replication by the Telomere Capping Protein TRF2. *Mol Cell* **70**, 449-461 e5 (2018).**
This paper provides evidence for the involvement of TRF2 in pericentromeric heterochromatin replication by the recruitment of the helicase RTEL1.
41. Postow, L. et al. Positive torsional strain causes the formation of a four-way junction at replication forks. *J Biol Chem* **276**, 2790-6 (2001).
42. Rickman, K. & Smogorzewska, A. Advances in understanding DNA processing and protection at stalled replication forks. *J Cell Biol* **218**, 1096-1107 (2019).
43. Miller, K.M., Rog, O. & Cooper, J.P. Semi-conservative DNA replication through telomeres requires Taz1. *Nature* **440**, 824-8 (2006).
44. Zimmermann, M., Kibe, T., Kabir, S. & de Lange, T. TRF1 negotiates TTAGGG repeat-associated replication problems by recruiting the BLM helicase and the TPP1/POT1 repressor of ATR signaling. *Genes Dev* **28**, 2477-91 (2014).
45. Ye, J. et al. TRF2 and apollo cooperate with topoisomerase 2alpha to protect human telomeres from replicative damage. *Cell* **142**, 230-42 (2010).
46. Li, F. et al. The BUB3-BUB1 Complex Promotes Telomere DNA Replication. *Mol Cell* **70**, 395-407 e4 (2018).
47. Simonet, T. et al. The human TTAGGG repeat factors 1 and 2 bind to a subset of interstitial telomeric sequences and satellite repeats. *Cell Res* **21**, 1028-38 (2011).
48. Fouche, N. et al. The basic domain of TRF2 directs binding to DNA junctions irrespective of the presence of TTAGGG repeats. *J Biol Chem* **281**, 37486-95 (2006).
49. Poulet, A. et al. TRF2 promotes, remodels and protects telomeric Holliday junctions. *EMBO J* **28**, 641-51 (2009).
50. Alabert, C. et al. Nascent chromatin capture proteomics determines chromatin dynamics during DNA replication and identifies unknown fork components. *Nat Cell Biol* **16**, 281-93 (2014).
51. Garzon, J., Ursich, S., Lopes, M., Hiraga, S.I. & Donaldson, A.D. Human RIF1-Protein Phosphatase 1 Prevents Degradation and Breakage of Nascent DNA on Replication Stalling. *Cell Rep* **27**, 2558-2566 e4 (2019).
52. Mukherjee, C. et al. RIF1 promotes replication fork protection and efficient restart to maintain genome stability. *Nat Commun* **10**, 3287 (2019).
53. Fouche, N., Ozgur, S., Roy, D. & Griffith, J.D. Replication fork regression in repetitive DNAs. *Nucleic Acids Res* **34**, 6044-50 (2006).
54. Verdun, R.E. & Karlseder, J. The DNA damage machinery and homologous recombination pathway act consecutively to protect human telomeres. *Cell* **127**, 709-20 (2006).
55. Amiard, S. et al. A topological mechanism for TRF2-enhanced strand invasion. *Nat Struct Mol Biol* **14**, 147-54 (2007).

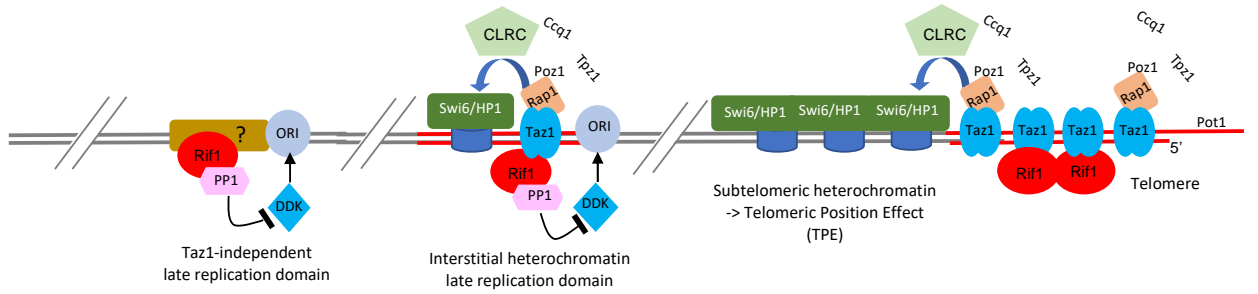
56. Sarek, G., Vannier, J.B., Panier, S., Petrini, J.H. & Boulton, S.J. TRF2 recruits RTEL1 to telomeres in S phase to promote t-loop unwinding. *Mol Cell* **57**, 622-35 (2015).
57. Jaco, I. et al. Role of mammalian Rad54 in telomere length maintenance. *Mol Cell Biol* **23**, 5572-80 (2003).
58. Badie, S. et al. BRCA2 acts as a RAD51 loader to facilitate telomere replication and capping. *Nat Struct Mol Biol* **17**, 1461-9 (2010).
59. Sfeir, A. & de Lange, T. Removal of shelterin reveals the telomere end-protection problem. *Science* **336**, 593-7 (2012).
60. Teixeira-Silva, A. et al. The end-joining factor Ku acts in the end-resection of double strand break-free arrested replication forks. *Nat Commun* **8**, 1982 (2017).
61. Lam, Y.C. et al. SNM1B/Apollo protects leading-strand telomeres against NHEJ-mediated repair. *EMBO J* **29**, 2230-41.
62. Mason, J.M. et al. The SNM1B/APOLLO DNA nuclease functions in resolution of replication stress and maintenance of common fragile site stability. *Hum Mol Genet* **22**, 4901-13 (2013).
63. Aggarwal, M., Sommers, J.A., Morris, C. & Brosh, R.M., Jr. Delineation of WRN helicase function with EXO1 in the replicational stress response. *DNA Repair (Amst)* **9**, 765-76 (2010).
64. Guervilly, J.H. & Gaillard, P.H. SLX4: multitasking to maintain genome stability. *Crit Rev Biochem Mol Biol* **53**, 475-514 (2018).
65. Myung, K., Chen, C. & Kolodner, R.D. Multiple pathways cooperate in the suppression of genome instability in *Saccharomyces cerevisiae*. *Nature* **411**, 1073-6 (2001).
66. Margalef, P. et al. Stabilization of Reversed Replication Forks by Telomerase Drives Telomere Catastrophe. *Cell* **172**, 439-453 e14 (2018).
67. Sinha, A.K. et al. Broken replication forks trigger heritable DNA breaks in the terminus of a circular chromosome. *PLoS Genet* **14**, e1007256 (2018).
68. de Lange, T. A loopy view of telomere evolution. *Front Genet* **6**, 321 (2015).
69. Lee, G. et al. Testing the retroelement invasion hypothesis for the emergence of the ancestral eukaryotic cell. *Proc Natl Acad Sci U S A* **115**, 12465-12470 (2018).
70. Ye, J., Renault, V.M., Jamet, K. & Gilson, E. Transcriptional outcome of telomere signalling. *Nat Rev Genet* **15**, 491-503 (2014).

Figure 1

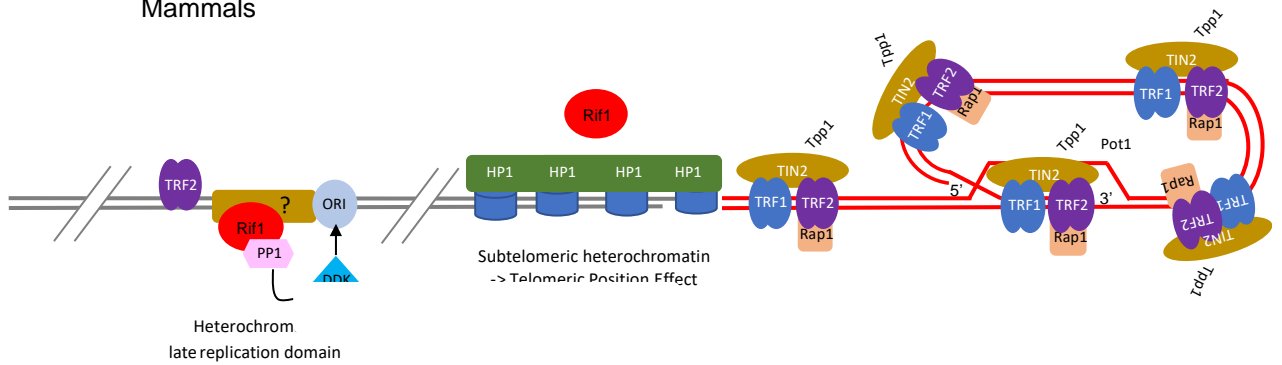
a *Saccharomyces cerevisiae*



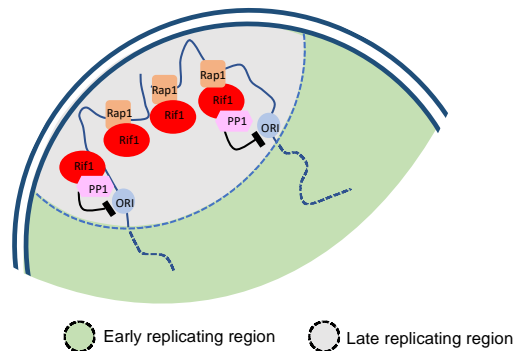
Schizosaccharomyces pombe



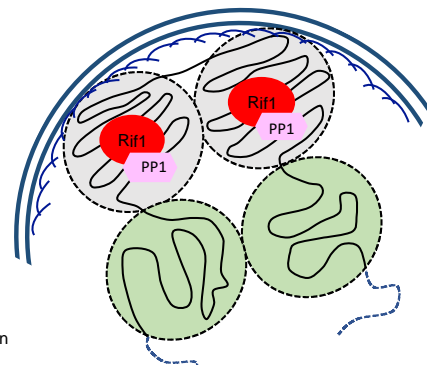
Mammals



b



S. cerevisiae



mammals

Figure 2

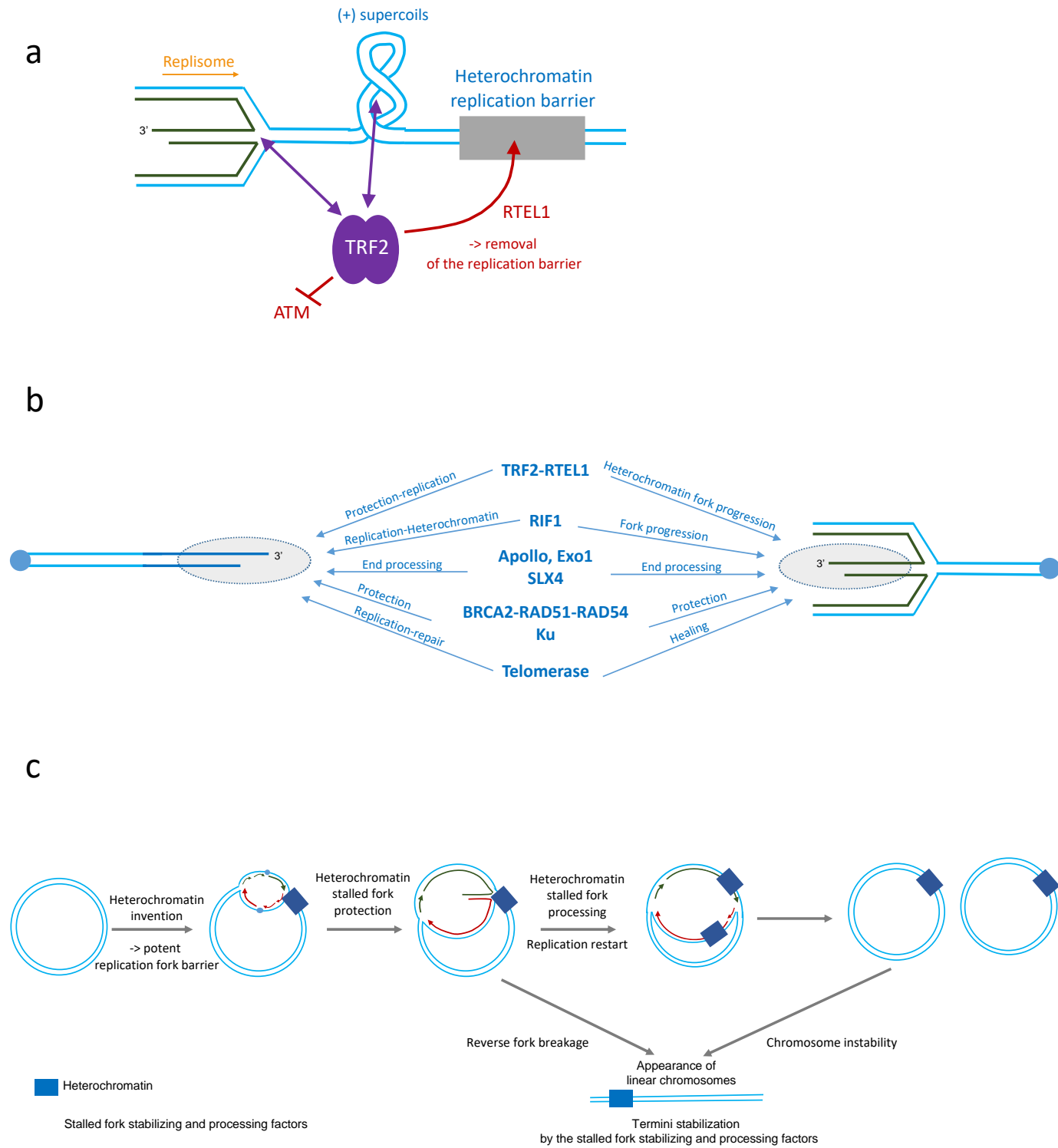


Figure 1. Telomeric-heterochromatic DNA connection.

a) *Saccharomyces cerevisiae* telomeres are coated with Rap1 proteins, serving as platform for the binding and spreading of the SIR complex to the subtelomeres. In addition, Rap1 is the binding site of Rif1, whose interaction with PP1 suppresses the firing of late origins at subtelomeres. In *Schizosaccharomyces pombe*, Taz1 is a key shelterin protein, that not only protects telomeres but also controls the firing of late origins through the recruitment of Rap1 and the Rif1-PP1 complex. Rif1-PP1 can also modulate late origins independently of Taz1-Rap1. In addition, the formation of subtelomeric heterochromatin is mediated by another shelterin protein, Ccq1, that together with the CLRC complex mediates the spreading of the heterochromatic Swi6/HP1 protein. Mammalian telomeres lack the common heterochromatic marks, however, the subtelomeric region is rich in H3K9me3, H3K20me3 and HP1. Rif1 is involved in the maintenance of subtelomeric heterochromatin as well as in the control of late replication origin timing. It is still unknown how Rif1 binds late replicating regions independently of Rap1. Grey rectangles represent heterochromatic DNA blocks.

b) Subnuclear organization of Rif1 domains. In *S. cerevisiae*, telomeres are clustered into few nuclear envelope foci where the Rif1 protein is mainly found. The high concentration of Rif1 in these regions creates a late replication compartment. In mammals, Rif1 modulates nuclear architecture by regulating the boundaries between euchromatin and heterochromatin creating late replicating regions.

Figure 2. Similarities between telomeres and stalled forks

a) Several proteins involved in telomere protection, repair and replication are also part of the factors involved in the protection and processing of stalled replication forks generated in difficult-to-replicate regions of the genome.

b) TRF2 is required to protect stalled forks formed in pericentromeric heterochromatin and facilitates the progression of the replication fork by recruiting the helicase RTEL1. TRF2 can bind heterochromatic DNA in several ways: through the presence of G4s, positive supercoiled DNA accumulating ahead of stalled replication fork, and four-way DNA junctions.

c) Model of telomere formation. The appearance of heterochromatin-like structures or the generation of abnormal DNA structures (e.g. by high levels of ultraviolet irradiation, high production of reactive oxygen species or frequent desiccation periods) led to huge replicative and topological stress. All this created major problems in the progression of replication forks giving rise to a high rate of reversed stalled forks and double strand breaks. The stabilization of the ancestral stalled forks may have behaved as the first mechanism of stabilization of ancestral telomeres.