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Meeting report from the first European OddPols meeting: Toulouse 2018

Lise Dauban*, Emilie Cerezo*, Anthony Henras & Olivier Gadal

*: participated equally to the work

Abstract:

The eleventh international conference on transcription by RNA polymerases I, III, IV and V (the OddPols) was held from June 26th to June 29th 2018 at the Museum of Natural History of Toulouse, France and organized by Anthony Henras and Olivier Gadal. The scientific committee was composed of David Engelke, Joachim Griesenbeck, Ross Hannan, Deborah Johnson, Richard Maraia, Christoph Müller, Craig Pikaard, David Schneider and Ian Willis. The organizers are grateful to the “*Centre de Biologie Intégrative de Toulouse*”, for support in the organization of the event. Participants from 13 different countries presented their newest exciting results during the scientific sessions and during extended conversation hours in the cosy atmosphere of the botanic garden of the Museum. Here we present the highlights of all oral presentations.

Introduction:

The 2018 OddPols meeting was held for the first time in Europe, at the Museum of Natural History of Toulouse, France, under the supervision of a giraffe proposed to become the meeting mascot. The meeting gathered 85 participants who shared their science through 37 oral presentations, divided in 8 general topics and 27 posters. The scientific committee awarded prizes for the best student talk and poster to encourage the upcoming generation of OddPols specialists. Congratulations to Aishwarya Iyer-Bierhoff for her talk during the session on regulation of transcription (see below). Congratulations to Marcel Böhner for his poster dealing with the characterization of genetic and epigenetic determinants of RNA Pol IV activity in *Arabidopsis thaliana*).

Plenary sessions:

Sessions 1,2. Structure and function of OddPols and transcription machineries

Carlos Fernández-Tornero (*Centro de Investigaciones Biológicas*, Spain) presented crystal and cryo-electron microscopy structures of Pol I in three different states: Pol I dimers, Pol I monomers and RRN3-associated Pol I monomers. When inactive, Pol I forms a dimer in which Rpa43 subunit C-terminus (the “connector” domain) of one monomer is inserted into the DNA-binding cleft of the other one. To prove the existence of such inactive Pol I dimers in yeast cells, they developed an elegant microscopy approach and showed a strong increase in dimer formation upon nutrient starvation, as well as upon inhibition of either ribosome maturation or protein synthesis. Using ChIP, they further show that Pol I dimerization down-regulates transcription of ribosomal DNA. As it becomes activated, structural rearrangements inside the polymerase occur that result in partial closing of the cleft. The fully activated form of the enzyme comprises Rrn3 protein bound to the stalk,

limiting its flexibility, while the Rpa43 C-terminus remains disordered.

Herbert Tschochner (*Universität Regensburg, Germany*) focused on Pol I-specific heterodimer Rpa49/Rpa34.5 activity using an *in vitro* approach. The study uncoupled the functions of Rpa49 C-terminal domain (A49-Ct), which shares homology with RNA polymerase II (Pol II) transcription factor TFIIE, and of the complex dimerization module composed of Rpa34.5 and A49 N-terminus (A34.5/A49-Nt), related to TFIIIF. When present in the polymerase, A49-Ct enhances DNA binding and promotes transcription speed but leads to introduction of more errors. In contrast, an A34/A49-Nt containing Pol I is slowed down in elongation, but enhances fidelity. Moreover A49-Ct supports the polymerase to transcribe through a nucleosomal template. Interestingly, both Pol I and Pol III are able to transcribe through nucleosomal templates *in vitro* contrary to Pol II. This property is correlated with the presence of Pol I A349/A34.5 heterodimer or with the Pol III equivalent Rpc37/Rpc53 complex, which also shares homology with TFIIE and TFIIIF.

Another Pol I specific feature, compared to Pol II and III, is its relatively high sensitivity to DNA-intercalating agents. The rRNA gene promoter sequences are not conserved across species and **Konstantin I. Panov** (Queen's University Belfast, United Kingdom) proposed that their structures and physical properties, such as bendability and meltability are conserved. He further exposed that Pol I inhibitors all share a flat structural region that can disrupt DNA 3D structure, increase DNA melting temperature and thus alter polymerase recruitment to the promoter and/or initiation events.

Christoph W. Müller (European Molecular Biology Laboratory, Germany) compared Pol I and Pol II mechanistic, based on the analysis of their respective atomic structures. One striking difference is the path the DNA adopts when engaged with the RNA polymerase. While the DNA fibre remains almost straight when transcribed by Pol I, it is bent when engaged within Pol II. In Pol I, this is explained by the presence of the Pol I transcription factor Rrn7 cyclin folds which bind further upstream on the DNA compared to those of TFIIB and by the absence of TBP bound to the Pol I core promoter.

As Pol I and Pol II are quite divergent, Pol III is closer to Pol II. Indeed **Matthias K. Vorländer** (from Christoph Muller's lab, EMBL, Germany) presented 3.7 Å to 5.5 Å resolution structures obtained by cryo-electron microscopy of yeast Pol III Pre-Initiation Complex (PIC) comprising Pol III bound to U6 promoter DNA and TFIIB (composed of Brf1, Bdp1 and TBP). The group observed that TFIIB binds to DNA on top of the polymerase cleft, with Brf1 contacting directly the polymerase as TFIIB does in Pol II. They could also determine that TFIIB is tightly bound to DNA, forming a cage around the promoter, thus creating a stable platform capable of sustaining Pol III high initiation rate. Within the pre-initiation complex, TFIIB contacts transcription factor-like polymerase subunits Rpc37 and Rpc34 and stabilizes the transcription bubble.

Type 1 and type 2 Pol III promoters are peculiar in that they contain intragenic promoter elements. Type 3 Pol III promoter architecture is similar to snRNA gene promoters transcribed by Pol II. **Jerome Gouge** (The Institute of Cancer Research, United Kingdom) presented a study focused on Brf2, a transcription factor specific for these type III promoters. Very interestingly, they showed that Brf2 can be oxidised, and in turn modulates Pol III recruitment in a redox sensitive manner (Gouge et al., 2015). Under prolonged oxidative conditions, Brf2 impedes transcription of seleno-cysteine tRNAs required for translation of

detoxifying enzymes. Accumulation of reactive oxygen species then leads to cell apoptosis. Alessandro Vannini's fellow proposed that Brf2 activation in cancer cells lead to sustained detoxification and apoptosis evasion.

Seok-Kooi Khoo (Institute of Molecular Biology, Taiwan) focused on the functional characterization of Rpc82/Rpc34/Rpc31 Pol III subcomplex. Rpc82 subunit, which is Pol II TFIIE paralogue, contains a structurally disordered insertion in the third Winged-Helix domain, conserved in fungi and required for optimal cell growth and transcription initiation *in vitro*. Using site-specific photo-crosslinking analysis to explore Rpc82 function, the group showed that it interacts with both TFIIB-related Brf1 protein in the PIC and DNA upstream of the transcription start site. Rpc82 also contacts Pol III Rpc31 and Rpc34 subunits on the protrusion and wall domains of the cleft.

Session 3. Regulation of transcription: activators and repressors

Aishwarya Iyer-Bierhoff (German Cancer Research, Germany), from Ingrid Grummt's group, presented data on the regulation of fibrillarin (FBL), an enzyme that catalyzes methylation of both rRNA and nucleolar histone H2A. Glutamine methylation of histone H2A is exclusively present at the rDNA locus and correlates with Pol I transcription (Tessarz et al., 2014). The group identified FBL as a direct target of the NAD⁺ dependent deacetylase, SIRT7. They showed that acetylation and deacetylation of FBL by CBP and SIRT7 respectively, impact on H2A methylation and Pol I transcription without altering pre-rRNA methylation or processing. The study reveals that modulation of FBL acetylation and histone H2A glutamine methylation contributes to cell cycle-dependent fluctuations in Pol I transcription. In interphase, SIRT7 dependent deacetylation of FBL promotes H2A methylation and high levels of Pol I transcription, whereas hyperacetylation of FBL attenuates H2A methylation during mitosis and correlates with repression of Pol I transcription.

Transcription initiation by Pol III requires the activity of TFIIB transcription factor. **Guillermo Abascal-Palacios** from the group of Alessandro Vannini (The Institute of Cancer Research, United Kingdom) reported the cryo-EM structure of Pol III pre-initiation complex at 3.4-4.0 Å and apo-Pol III at 3.1-3.4 Å resolution. These structures revealed a function of TFIIB in the rearrangement of Rpc34 and Rpc37 Pol III specific subunits to facilitate DNA opening during Pol III transcription initiation.

Magdalena Boguta (Institute of Biochemistry and Biophysics, Poland) highlighted a dual role of a second major Pol III transcription factor, TFIIC. This complex activates Pol III transcription by binding to the intragenic promoter elements of tRNA genes and allowing TFIIB recruitment. The group reported that, depending on growth conditions, TFIIC also functions as a tDNA transcription repressor. Under repressive conditions, interaction between TFIIC subunit Tfc4 and TFIIB subunits Brf1 and Bdp1 is strengthened. This leads to TFIIB sequestration away from the tDNA transcription start site (TSSs), failure in Pol III recruitment and impairment in transcription initiation.

Session 4. Regulation of transcription: elongation and termination

Richard Maraia (National Institutes of Health, MD, USA) presented a new activity of Rpc37/Rpc53 Pol III subunits during termination which occurs at an oligo(T) tract on the non-template strand; oligo(rU:dA) hybrid on the template strand. The Rpc37/Rpc53 heterodimer promotes Pol III elongation in the proximal part of this tract by maintaining the RNA 3' end in the active site, ensuring processivity until sufficient instability of the (rU:dA) RNA:DNA hybrid results in transcript release (Mishra and Maraia, 2018).

David A. Schneider (University of Alabama, AL, USA) presented a study focused on Pol I subunit Rpa12 required for hydrolytic cleavage of the nascent rRNA within backtracked or arrested transcription complexes. The results show that Rpa12 also participates in transcription elongation. Using *in vitro* transient-state kinetic analyses, they showed that Pol I lacking Rpa12 displays a dramatic defect in nucleotide addition during transcription elongation, suggesting that Rpa12 takes part directly in nucleotide addition mechanism. Authors also found that polymerases lacking Rpa12 are more stably associated with DNA template *in vitro* and are more resistant to high salt concentrations, indicating that Rpa12 influences the structure of the elongating polymerase. This new destabilization role of Rpa12 is also in agreement with data suggesting its involvement in transcription termination.

Work from **Tommy Darriere** from Olivier Gadal's group (Centre de Biologie Intégrative de Toulouse, France) demonstrated that point mutations in the two largest Pol I subunits can bypass the need for Rpa49 specific subunit in living yeast cells. Moreover, these mutations were all localized in a very specific area, near Rpa12 subunit, at the interface between the jaw and the lobe. Authors also showed that one of the mutations, in an otherwise WT context, allows overproduction of rRNAs, leading to the formation of a putative super-Pol I.

Tom Moss (Laval University, Quebec, Canada) reported a combination of ChIP-seq data using Mouse Embryonic Fibroblast (MEFs) and Embryonic Stem Cells (ESCs) conditional for the transcription factors UBF, RRN3 and TAF1B. Analysis of these data suggests that UBF, in collaboration with SL1, is a key factor in establishing and maintaining rDNA repeat activity. RRN3 release from elongating transcription complex is stochastic and depends on cell type and possibly initiation rate. Furthermore, a peak of arrested Pol I is observed at the "spacer promoter" immediately upstream of the enhancer repeats and the gene body. This peak is positioned just downstream of a unique chromatin boundary consisting of CTCF and cohesin complexes. Active chromatin marks and phased nucleosomes are detected adjacent to this boundary, but not elsewhere in the rDNA repeat.

Session 5. Chromatin effect on transcription

Konstantin I. Panov (Queen's University, Belfast, United Kingdom) presented a study focused on the epigenetic regulation of rDNA chromatin in response to stress in human cells. The group discovered a new link between stress and chromatin epigenetic changes at the rDNA locus involving histone demethylase KDM4A. Upon stress, such as starvation, DNA damage or heat shock, KDM4A dissociates from rDNA. This correlates with down-regulation of Pol I transcription and closing of rRNA genes as visualized by ATAC-seq method. Conversely, KDM4A is required for recovery after stress since upon its re-localization to nucleoli, rRNA genes adopt an open conformation and rRNA synthesis resumes. Association of KDM4A with rDNA in starved-refed cells depends on PI3K/SGK1

signaling pathway but other non-identified stress-dependent signalling pathways are involved in the cases of other stresses.

A complementary study presented by **Ann-Kristin Östlund Farrants** (Stockholm University, Sweden) highlighted the crucial role of B-WICH complex (SNF2h, WSTF, NM1) in Pol I transcription. Following glucose stimulation, B-WICH is recruited at rDNA locus where it is implicated in chromatin opening. Indeed, in absence of B-WICH subunit WSTF, rDNA remains in a repressive chromatin state despite glucose stimulation. This prevents association of c-Myc and histone deacetylase SIRT7. Furthermore dissociation from the promoter of the NuRD silencing complex component CDH4, is impaired in absence of WSTF. Therefore, upon glucose stimulation, B-WICH is required to counteract the repressive poised state induced by NuRD complex.

To understand whether rRNA gene expression is differentially regulated depending on growth, **Jean-Clément Mars** (Moss group, Laval University, Quebec, Canada) studied rDNA activity in mouse cell lines displaying different proliferation rates. In contrast to MEFs, fast growing ESCs have almost all their rRNA genes transcriptionally active. The authors characterized the distribution of histone modifications across the rDNA as well as the presence of a CTCF boundary adjacent to the spacer promoter (Herdman et al., 2017). Histone modifications associated with gene activity were all detected at this boundary, but not elsewhere. Different distributions of RRN3 and Pol I/RPI indicated a distinct regulation of transcription in ESCs as compared to MEFs. Moreover, ESCs displayed a lower per gene density of engaged Pol I as compared to MEFs, suggesting that in MEFs the reduced number of active genes is compensated by an increase in polymerase density.

Martina Dvorackova (Masaryk University, Czech Republic) uses *Arabidopsis thaliana* to better decipher the role of CAF-1 complex, responsible for depositing histone variant H3.1 during replication. The group showed that depleting CAF-1 subunits does not impact replication timing but results in increase in DNA damages and chromosome instability. Indeed, both telomeric and rDNA repeats are progressively lost in these mutants. In reverted plant lines telomeric and rDNA repeat number are recovered in a stochastic manner.

Session 6. Interplay between transcription, genome organization and nuclear structures

The group of **Brian McStay** (Centre for Chromosome Biology, Ireland) investigates the genomic architecture of nucleolar organizer regions (NORs) to address the conundrum on what dictates nucleolar architecture. On the short arms of acrocentric chromosomes, ribosomal gene arrays are flanked by proximal and distal sequences (relative to centromeres). The distal regions contain a large inverted repeat transcribed by Pol II. Sequencing of acrocentric chromosome distal junctions by PacBio revealed that the precise barrier between rDNA array and distal region on each acrocentric chromosome displays strong sequence identity. Recombination events occur not only in the ribosomal gene array but also in this region.

Interestingly a study presented by **Aline Marnef** (Legube group, *Centre de Biologie Intégrative de Toulouse*, France) showed that Pol I transcription inhibition and nucleolar cap formation can be uncoupled. They observed that induction of DSBs in nucleoli using the

AsiSI restriction endonuclease affects Pol I transcription in all cell cycle phases (G1, S, G2). However, Pol I transcription inhibition upon DSB induction in G1 phase does not induce nucleolar cap formation, indicating that transcription inhibition is not sufficient for nucleolar cap formation. Nucleolar DSBs can be repaired by homologous recombination at the nucleolar periphery. Moreover, CTCF and cohesin complex are required for cap formation and recombination events.

Nutrient deprivation also leads to major nucleolar rearrangements in yeast such as massive rDNA condensation. This is caused by inhibition of Pol I and Pol III through inactivation of protein kinase TOR and loading of condensin complex on rDNA, as explained by **X.F. Steven Zheng** (Rutgers Cancer Institute of New Jersey, NJ, USA). Indeed, rapamycin treatment leads to a rapid loading of condensin complex onto rDNA. Inhibition of Pol I transcription in absence of condensin results in hyper recombination at the rDNA locus correlated with increased association of Rad52. High rDNA condensation is therefore essential to prevent deleterious recombination between rDNA units and cancer cell transformation. mTOR activation leads to nucleolar hypertrophy due to an increase in both Pol I and Pol III activities. This hypertrophy is associated with elevated growth rate and genomic instability, two major actors in tumour progression.

During his plenary lecture, **Craig Pikaard** (Howard Hughes Medical Institute at Indiana University, IN USA) gave a retrospective account of the 20-year history of multisubunit RNA Polymerases IV and V, beginning with his identification of their atypical catalytic subunits in preliminary sequence data for the first plant genome (that of *Arabidopsis thaliana*). He highlighted key discoveries made in his and other laboratories that revealed distinct roles for Pols IV and V in siRNA-directed DNA methylation and transposon silencing and that identified their transcripts, subunit compositions and interacting partners, leading to current models for the RNA-directed DNA methylation pathway. The biochemical activities of Pol IV and Pol V are still not well defined, such that more studies are needed to understand their mechanisms of action to the same extent as for Pols I, II and III.

Session 7. Mechanisms of gene silencing

Later on during the meeting, **Craig Pikaard** exposed his work based on the development of in vitro systems to study the first steps of RNA-directed DNA methylation and gene silencing in plants. They recapitulated the synthesis of 24 nt siRNAs from template DNA using purified Pol IV, RNA-dependent RNA polymerase 2 (RDR2) and the dicer endonuclease, DCL3. Pol IV transcribes the single-stranded DNA template and terminates soon after it encounters the non-template strand of double-stranded DNA. The resulting termination complex enables RDR2, which needs a free RNA 3' end to function, to initiate its transcription, converting the Pol IV RNA strand into a dsRNA which is then processed by DCL3 into 24 nt siRNAs.

Todd Blevins (*Institut de Biologie Moléculaire des Plantes*, France) presented a talk about the mechanism of Pol IV silencing of transposable elements (TE) in plants. Using partial loss-of-function alleles of Pol IV subunits in *Arabidopsis*, his lab discovered that the Pol IV enzyme itself could trigger an initial wave of TE silencing without generating high levels of siRNAs. A second wave, involving amplification of the siRNAs, is then needed to complete transposable element silencing.

Gene silencing in plants involves another DNA-dependent RNA polymerase, namely Pol V. Studies in the group of **Andrzej T. Wierzbicki** (University of Michigan, MI, USA) aim at understanding the role of this polymerase. Loci to be silenced need to be transcribed by Pol V, this enzyme is therefore an important decision maker in the silencing process. While transcribing, Pol V recruits chromatin remodelers such as SWI/SNF complex. Nucleosome positioning and epigenetic landscape of target genes are thus affected, enhancing their silencing. Fine-tuning this mechanism is fundamental to prevent heterochromatin spreading to neighbouring genes. However, Pol V transcripts are hardly detectable. Pol V transcribes loci harbour CG methylations but this mark is not the only determinant in the recruitment of the polymerase, CHH methylation also contributes to some extent. Using a RIP-seq approach, they captured and sequenced Pol V transcripts and detected transcription start sites (TSSs) all over the transcribed regions. The presence of CHG methylation around these TSSs suggests that this mark could be involved in Pol V initiation.

Apart from gene silencing mechanism, **Charlotte Montacié** (Sáez-Vásquez group, *Laboratoire Génome et Développement des Plantes*, France) presentation dealt with the influence of iron level on rDNA organisation and ribosome biogenesis in plants. Plant nucleoli contain high iron concentrations. To assess the impact of iron on ribosome biogenesis they studied nicotinamide synthase (*nas*) mutant plants characterized by reduced iron levels in nucleoli. In these plants, an increased number of fibrillar centres is observed but no evidence for changes in ribosome synthesis levels could be obtained. Remarkably, iron reduction led to nucleolar rearrangement with reactivation of the normally silenced NOR2, now present in the nucleolus of plant cells and supporting expression of rDNA variant 1. This reactivation may be caused by epigenetic modifications since changes in CHG and CHH methylation are observed. These data are in agreement with those showing that high iron amounts in animal cells cause rDNA hyper methylation and oxidation.

Session 8,9. OddPols involvement in development, genetic disorders, cancer and diseases

Nadine Hein and Ross Hannan (John Curtin School of Medical Research, Australia) presented studies on the development of drugs targeting Pol I to treat cancer. **Ross Hannan** presented advanced studies on CX-5461, the first Pol I inhibitor to enter clinical trials in Australia and Canada. Investigations to understand the mechanism of action of CX-5461 are crucial to reveal additional activities, which can contribute to efficacy, toxicity and mechanisms of acquired resistance. The gene encoding TOP2 α is frequently mutated in cells with acquired resistance to CX-5461. Extracts from resistant cells show a decrease in TOP2 α activity in vitro and TOP2 α knock down is sufficient to cause resistance to CX-5461. As CX-5461, TOP2 α inhibits PIC formation and transcription initiation but not elongation. CX-5461 induces a DNA damage response restricted to nucleoli characterized by a strong increase in DSBs at rDNA promoters. In addition to Pol I, CX-5461 also inhibits TOP2 α , which may impair the DNA Damage Repair response in nucleoli and increase rDNA instability.

In parallel, **Nadine Hein** is working with Pimera, Inc (San Diego, CA) on the development of a second generation Pol I inhibitors that could be used in combination therapies to avoid acquired resistances. Authors showed preliminary studies on a new selective inhibitor of Pol I, PMR-116. Cell treatment with PMR-116 impairs Pol I recruitment to the rDNA in a similar fashion as CX-5461 and does not affect Pol II transcription. PMR-116

inhibits Pol I transcription both in cancer and primary cells but these latter do not display a cytotoxic response. The main cellular response to PMR-116 is cell death by apoptosis. PMR-116 induces phosphorylation and accumulation of p53 but does not activate CHK1 or CHK2 as CX-5461. In clinical terms, PMR-116 is a promising drug since it increases survival compared to CX-5461, outperforms CX-5461 therapeutic window in Acute Myeloid Leukaemia (AML) and prostate cancers and displays good pharmacological properties.

Marikki Laiho (Johns Hopkins University School of Medicine, MD, USA) presented the newest results on another Pol I inhibitor, BMH-21, a potential systemic cancer therapeutic. BMH-21 is a selective inhibitor of Pol I that impacts Pol I transcription cycle by causing transcription blocks. BMH-21 treatment induces specific degradation of Pol I subunit RPA194. This degradation requires the presence of transcribing Pol I on rDNA as shown by the requirement of UBF, TAF1C, and RRN3. The design and characterization of BMH-21 derivatives allowed to show that DNA intercalation *per se* is not the only determinant of RPA194 degradation. The ongoing studies in the Laiho group also aim at developing patient selection strategies by analyzing the oncogenic drivers or genetic alterations that upregulate Pol I transcription in cancers. A new rRNA probe detecting the 45S rRNA precursor has also been designed to study Pol I activity in cancer specimens.

Bruce A. Knutson (SUNY Upstate Medical University, NY, USA) talked about Treacher Collins Syndrome (TCS). This craniofacial disorder is characterized by facial bone malformations resulting from defects in neural crest cell migration during pharyngeal arches development. POLR1C or POLR1D genes encode subunits conserved in Pol I and Pol III (Rpac40 and Rpac19 in yeast, respectively) and are often found mutated in affected patients, as well as TCOF1, encoding a factor involved in rRNA synthesis and processing. As Treacher Collins mutations target highly conserved residues within yeast and human proteins, authors explored the molecular basis leading to the disease in *Saccharomyces cerevisiae*. Most of the TCS mutations are believed to affect dimerization of these subunits. One particular mutation (T71I) does not affect dimerization. It induces a cryo-sensitive phenotype associated with a defect in the production of the 18S and 25S rRNAs. Impaired recruitment of both Pol I and Pol III to rDNA is observed in this mutant and co-IP experiments showed that Rpac19 and Rpac40 no longer interact with other Pol I or Pol III subunits. Altogether, their results show that Treacher Collins mutations affect both Pol I and Pol III machineries (Walker-Kopp et al., 2017).

The presentation by **Martin Teichmann** (University of Bordeaux, France) dealt with the regulation of Pol III transcription during tumorigenesis. The group is studying the relationship between tumor promoting factors and Pol III transcription regulation during human IMR90 fibroblast transformation. This model system allows to recapitulate full tumoral transformation *in vitro*. Their data revealed that among Pol III components, only a strong increase of POLR3G and SNAPC1 expression at the mRNA levels was observed with progression of transformation. No deregulation of Pol I and Pol II components could be observed. Strikingly, several Pol III subunits and Pol III transcription factors (TFIIIB and TFIIIC) are up-regulated at the protein level. This increase is accompanied by a 2-fold increase of a subset of Pol III-synthesized RNAs such as 7SK, MRP or U6 snRNA. Collectively, these results indicate that overexpression of only a subset of the Pol III machinery components is sufficient to sustain transformation and tumor formation.

Shuping Zhong (University of Southern California, CA, USA) is interested in pathways leading to alcohol-associated cancer development. The group reported previously that alcohol stimulates JNK1 signaling, which in turn activates Estrogen Receptor α (ER α) and results in increase in Brf1 expression and activation of Pol III transcription. In the present study, they investigate the role of Runx2 (Runt-related transcription factor 2), a downstream target of the ER pathway, in Brf1 expression and Pol III gene activation in the development of breast cancer. Runx2 is overexpressed in human biopsies of breast cancer suggesting a role in tumorigenesis. Alcohol induces Runx2 overexpression in an ER positive breast cancer cell line (MCF-7) and this activation is mediated by ER α . RNAi-mediated knock down of Runx2 following alcohol treatment prevents Brf1 expression and Pol III gene activation. The group showed that the anti-tumor compound Tamoxifen reduces alcohol-mediated increase in Brf1 expression of breast cancer cells.

Deborah L. Johnson (Baylor College of Medicine, Texas, USA) described the regulation of the key repressor of Pol III transcription, MAF1, by proteasomal degradation. MAF1 protein has a short half-life and it is regulated through proteasome-mediated degradation. This occurs through both ubiquitin-dependent and independent pathways. mTOR-induced phosphorylation of MAF1 promotes its ubiquitination by the Cullin2-RING E3 ligase and this enhances its degradation. The deubiquitination enzyme, USP10, deubiquitinates MAF1 to increase its stability. These results are consistent with the previously observed decrease in USP10 expression in liver cancers and the loss of MAF1 protein with enhanced Pol III transcription.

Session 10. Novel links with Physiology and immunity

When the *MAF1* gene is knocked out in mice (*MAF1* KO mice), the resulting phenotype is quite unexpected: mice are lean and remain so even on a high fat diet. They display a reduced metabolic efficiency and have increased energy expenditure but no change in locomotor activity. **Ian Willis** (Albert Einstein College of Medicine, NY, USA) reported that in these mice, an energy sink is provided by constantly active Pol III transcription in all tested tissues leading to an energy-consuming, futile RNA cycle (Willis et al., 2018). To feed the energy demand, mice exhibit high levels of lipid and protein catabolism. Metabolite profiling revealed that the *MAF1* KO impacts central metabolism in the mouse liver. They display an increased activity of two pathways linked to the TCA cycle: the urea cycle and the purine nucleotide cycle. The metabolomic data also point to increased nucleotide synthesis and turnover. These data suggest that constitutively high Pol III transcription in *MAF1* KO mice reprograms central metabolic pathways and wastes metabolic energy through a futile RNA cycle.

SINE elements such as Alu or MIR are transposable elements, the transcription of which is assumed by RNA Pol III: their left arm encompasses A and B boxes driving Pol III transcription initiation. To ensure genome stability, these sequences have been domesticated by epigenetic silencing mechanisms involving DNA and histone methylation. However, they are found expressed at higher levels when cells are exposed to stress and notably in cancer cells. **Daide Carnevali** from the group of Giorgio Dieci (University of Parma, Italy) developed a bioinformatic pipeline (Carnevali and Dieci, 2017) to identify genuine Pol III-derived SINE RNAs expressed at single-locus resolution and distinguish them

from those that are merely ‘passengers’ of longer Pol II-transcribed genes. Using RNA-seq libraries derived from normal or Acute Myeloid Leukemia (AML) affected patient cell lines, they determined whether Alu expression is deregulated in AML. The group observed a very significant increase in the expression of many Alu elements in these cancer cells whose expression pattern seems to correlate with tumour subtype, opening the possibility to use them as a molecular biomarker.

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