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Meeting report for Odd Pols 2012

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Abstract

The Eighth International Biennial Conference on RNA polymerases I and III (the ‘Odd Pols’) was held June 7–11, 2012 at The Airlie Center in Warrenton Virginia, USA. It was sponsored by the Université Laval and the Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, and organized by RichMaraia and Tom Moss. The meeting honored the memory of Pierre Thuriaux (Jan 1, 1950–March 18, 2012) and David Schneider reminisced on the important accomplishments his mentor Masayasu Nomura (1927–2011). The goal of the conference was to bring together the world’s experts on RNA polymerase I and RNA polymerase III to highlight and share their latest results and varied experimental approaches. The meeting drew attendees from twelve countries and most contributed through oral and poster presentations. The talks were organized into several sessions subdivided into 10 distinct topics. The keynote speaker, Ian Willis, opened the meeting with his presentation entitled “New Regulators of Signaling to Odd Pols” and the closing presentation was given by Patrick Cramer with his presentation “Conservation of the RNA polymerase I, II and III transcription initiation machineries”. Here we present some of the highlights from the meeting using summaries provided by the participants.

Introduction

Oddly enough the biennial “Odd Pols” meeting is held on even years. The 2012 meeting represented the eighth time the participants gathered to share their findings on two fundamental and highly important RNA polymerases: Pol I and Pol III. The presentations at this meeting covered a large diversity of topics from detailed crystal structures to the development of new research tools and the discovery of new targets for cancer treatment. At the end of the meeting it was decided that the Ninth International Conference on RNA polymerases I and III will be held 19–22 June, 2014 at the University of Michigan in Ann

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Plenary Sessions

Keynote address

Ian Willis “New Regulators of Signaling to Odd Pols”

Ian Willis (Albert Einstein College of Medicine, NY USA) described the recent identification of LAMMER/Cdc-like and GSK-3 family kinases as downstream effectors of TOR complex 1 that are required for efficient repression of all three nuclear RNA polymerase function in ribosome and tRNA synthesis. The detailed investigation of the RNA polymerase III system revealed that the polymerase itself is an important regulatory target in the response to nutrient limitation and cellular stresses that repress transcription. Under these conditions, the LAMMER kinase Kns1 becomes hyperphosphorylated and accumulates in the nucleus where it primes the phosphorylation of Rpc53 (a TFIIF-like subunit of the Pol III) by the GSK-3 family kinase, Mck1. These changes alone are not sufficient for repression but are part of a cooperative response that includes another polymerase subunit, Rpc11. Together the data suggest that changes in RNA polymerase III function caused by Kns1 and Mck1 render the enzyme sensitive to inhibition by the global repressor Maf1.

1. Structure and enzymology

Christoph Müller (EMBL, Germany) presented recent insights into the structural organization of the Pol III enzyme and its general transcription factor TFIIC. His group studies the overall architecture of *Saccharomyces cerevisiae* TFIIC and has obtained crystal structures of several TFIIC subcomplexes. One of these subcomplexes is formed by two β -propeller proteins, while another subcomplex is related to the Pol II-specific general transcription factor TFIIF. A detailed cryo-EM reconstruction of the Pol III enzyme at 10 Å resolution was presented. Comparison with the cryo-EM structure of elongating Pol III bound to a DNA/RNA transcription bubble shows the rearrangement of two Pol III-specific subcomplexes that enclose incoming DNA.

Sébastien Fribourg (IECB, Inserm and Universite de Bordeaux, France) showed data demonstrating that RNA polymerases (Pol) share a common core structure comprised of multiple subunits, which has evolved with greater complexity from bacteria to eukaryotes; where only 5 subunits form the bacterial Pol whereas 17 subunits constitute Pol III. Through the structure and function analysis of human RPC62, one of the Pol III-specific subunits is involved in initiation, Fribourg's group demonstrated its structural relationship with Pol II transcription factor TFIIEalpha. The data provided showed that RPC62 binds single-stranded DNA in a sequence-independent manner while RPC39 binds double-stranded DNA. These data allow for a better understanding of the involvement of these two subunits in Pol III transcription initiation.

Hung-Ta Chen (Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan) summarized the study of Pol III structural organization. In collaboration with Patrick Cramer

and Ruedi Aebersold, the research groups have combined cross-linking and hydroxyl radical probing to position the C82/34/31 subcomplex around the Pol III active center cleft. The data demonstrate that the extended winged helix (WH) domains 1 and 4 of C82 localize to the polymerase clamp head and clamp core, respectively, and the two WH domains of C34 span the polymerase cleft from the coiled-coil region of the clamp to the protrusion. The WH domains of C82 and C34 apparently cooperate with other mobile regions flanking the cleft during promoter DNA binding, opening, and loading. Together with the published data, the presented results provide a more complete subunit architecture of Pol III, and indicate that all TFIIE-related components of eukaryotic and archaeal transcription systems adopt an evolutionarily conserved location in the upper part of the cleft that supports their functions in open promoter complex formation and stabilization.

2. Basic mechanisms: initiation

Transcription initiation by Pol II and Pol III, as well as the archaeal RNA polymerase, is known to require a TFIIB-related general transcription factor that operates at multiple steps during initiation, such as Pol recruitment, pre-initiation complex formation, start site selection, promoter opening, and abortive initiation. Until now, transcription by Pol I seemed unique, as none of its basal transcription factors were known to share homology with TFIIB or TFIIB-related factors. *Joost Zomerdijs* (University of Dundee, UK) reported that human TAF1B (hTAF_I63), a subunit of the Pol I basal transcription factor SL1, which drives pre-initiation complex formation, is the structural and functional counterpart of TFIIB in Pol I transcription. TAF1B is structurally similar to TFIIB, having the signature N-terminal Zn-ribbon and core domain containing two direct repeats with the potential to form two cyclin-like folds. Furthermore, as with TFIIB/TFIIB-like proteins (such as Pol III transcription factor TFIIB subunit Brf1/2 and Archaeal TFB), TAF1B interacts with TBP, binds promoter DNA sequences and is involved in polymerase recruitment (perhaps in part through its interaction with Pol I-associated factor RRN3) and has a post-recruitment role in transcription.

Bruce A. Knutson (Fred Hutchinson Cancer Research Center, Seattle, USA) provided independent findings showing that the yeast core factor subunit Rrn7, a TAF1B ortholog, is also a paralog of TFIIB and shares many basic functions similar to their Pol II and III counterparts. Analogous to the mechanism of TFIIB–Pol II binding, the Rrn7 N-terminal Zn ribbon domain binds Pol I in a manner dependent on the Rrn7 zinc binding residues and the Pol I dock domain. Although the TFIIB-related domains of Rrn7 and TAF1B are distantly related, TAF1B functions in yeast when the human TAF1B carboxyl-terminal domain (CTD) is replaced by the Rrn7-specific CTD, revealing their core functions are conserved. Finally, further domain-swapping experiments revealed that the Rrn7 Zn ribbon domain is interchangeable with the analogous ribbon of Brf1 but not TFIIB, suggesting that Rrn7 is most closely related to the Pol III general factor Brf1. This conservation of TFIIB-like proteins from yeast to humans and across eukaryotic Pol I, Pol II and Pol III transcription indicates that the fundamental processes leading to the initiation of transcription and the key elements of the basal transcription machinery that direct them have remained essentially unaltered despite specialization of the multisubunit polymerase systems.

The Pol I enzyme is one of the three nuclear RNA polymerases that has 12 structurally and functionally conserved subunits. Pol I has two additional associated proteins/subunits, CAST (hPAF49) and PAF53, with homology to TFIIF and TFIIE. CAST and PAF53 interact with one another to form a heterodimer and colocalize in the nucleolus. *Manjula Nagala* (postdoctoral fellow from the Zomerdijk lab, University of Dundee, UK) exploited systems for CAST- and PAF53-depletion from cells and expression of tagged wildtype (WT) and mutant subunits. This approach was used to assess the functional interactions of CAST and PAF53 with Pol I and other components of the transcription machinery, the interdependence of these proteins and their requirement in nucleolar localization, Pol I association and transcription. Interestingly, depletion of CAST (or PAF53) led to a differential defect in the serum-induced re-activation of rDNA transcription following serumstarvation of cells, suggesting an important role for CAST-PAF53 in the up-regulation of rDNA transcription and in cell proliferation.

Rrn3 functions to bridge core Pol I with the pre-initiation complex assembled on the rDNA promoter. One of the polymerase subunits involved in this interaction is rpa43. *Lawrence I. Rothblum's* group (University of Oklahoma, OK, USA) has found that an evolutionarily conserved, 22 amino acid long domain within rpa43 is both necessary and sufficient to mediate the interaction between rpa43 and Rrn3. Dr. Rothblum's group designed and tested a 22mer to use as a small molecule inhibitor of rDNA transcription which was based on the understanding of the mechanism of rDNA transcription and appears to be specific for the rDNA transcription apparatus. When added to in vitro transcription reactions, a peptide based on that sequence of rpa43 but not a mutated peptide (randomized 22mer/ Ψ) was able to inhibit rDNA transcription. The peptide (coupled to TAT peptide for transduction) inhibited rDNA transcription and cell growth in tissue culture cells, while the Ψ peptide had no effect on cell growth or rDNA transcription. When the wildtype peptide was transduced into cultured cancer cells, it induced cell death where 90% cell death occurred in some cell lines within 8 h. It was proposed that this approach represents a novel way to interfere with cell growth, and that it demonstrates a potentially novel pharmaceutical target for the treatment of cancer cells.

Topoisomerases can resolve transcription elongation-generated DNA supercoiling. *Konstantin Panov* (Queen's University, Belfast Ireland), presented their work on how DNA topoisomerase II-alpha (Top2 α) promotes activation of transcription by Pol I by facilitating pre-initiation complex formation. He presented evidence for a distinct role for topoisomerase activity in activation of transcription by RNA polymerase I (Pol I) and reported that Top2 α is an integral component of the initiation-competent Pol I β complex. Top2 α interacts directly with Pol I β -component Rrn3, which targets Pol I to rDNA promoter-bound transcription factor SL1. The data presented suggest that Top2 α facilitates rRNA transcription activation by cleavage in the promoter vicinity, converting the rDNA into a form accessible for pre-initiation complex formation. This observation is significant as topoisomerases are targets for effective anti-cancer drugs. The data showing that Top2 α inhibitors can decrease rRNA transcription activation in a p53-independent manner, suggests that inhibitors designed to target Top2 α in Pol I transcription could be useful nongenotoxic anti-cancer agents.

3. Basic mechanisms: elongation

Inactivation of the UBF gene in mouse leads to a rapid depletion of UBF protein, a shut-down of transcription of the ribosomal RNA genes by Pol I, and the arrest of mouse development and of cell proliferation. Concomitantly, major changes occurred in rRNA gene chromatin, in the structure of the nucleolus, and polymerase II gene expression. *Tom Moss* (Universite Laval, Quebec, Canada) also presented data on the fine mapping of the Nucleolar Localization Site (NoLS) and the p19-ARF tumor suppressor binding site on the Pol I transcription termination factor (TTF-I, also known as TTF1) which revealed that these sites intimately overlap, underscoring the suppressive role ARF plays in regulating ribosome biogenesis via nucleolar displacement of TTF-I.

Oliver Gadal (Universite deToulouse, France) presented studies on the phenotype of a mutant lacking the Pol I subunit Rpa34/Rpa49. Rpa49 has similarity with TFIIE and TFIIIF, and is required for high loading rate of Pol I per gene and for nucleolar assembly in vivo. Their data showed that in the absence of Rpa49, rDNA chromatin structure is modified, allowing the functional analysis of HMGB containing Pol I factors: human UBF and yeast Hmo1. Both were shown to be able to stimulate Pol I, and their role in making rDNA permissive for Pol I transcription was discussed.

4. Basic mechanisms: termination

Using an in vivo approach to test Pol I transcription termination in *S. cerevisiae*, *Herbert Tschochner's* group (Universitat Regensburg, Germany) discovered the open reading frame Ydr026c (Nsi1) as a bona fide termination factor. Ydr026c (Nsi1) is a Reb1 homologue containing three Myb1-binding sites and was very recently shown to affect rDNA stability and aging. The findings presented favor an in vivo termination model in which association of a specific protein with a cis-element is required for termination which might possibly be supported by RNA cleavage/degradation reactions. They proposed that one physiological role of Pol I termination in yeast is the maintenance of rDNA stability.

Keshab Rijal (postdoctoral fellow with Rich Maraia, NICHD, NIH, Bethesda, USA) presented data from the generation of a random mutagenic library of Rpc37/53 (C37) in *Saccharomyces pombe* and the results from the screening for mutants affecting transcription termination in vivo. The majority of C37 mutants that showed readthrough phenotypes map to a 13 amino acid C-terminal tract previously localized near Rpc2p in the active center. In addition, they demonstrated that the C37 termination defective mutants are not significantly decreased in Pol III transcription in vivo as would be expected if they were impaired for Pol III recycling.

G. Aneeshkumar Arimbasseri (postdoctoral fellow with Rich Maraia NICHD, NIH, Bethesda, USA) showed biochemical results suggesting that Pol III can terminate transcription by two mechanisms: (i) a core mechanism that works in the absence of C53/37/11 but requires transcripts with a longU tract, and (ii) a holoenzyme mechanism that requires C11/53/37 which prevents terminator arrest and promotes release of RNAs with fewer Us.

5. Regulation and control pathways

In budding yeast, tRNA gene transcription is repressed by the replication stress checkpoint pathway during normal cycling, and when cells are exposed to genotoxins that cause replication interference. Repression is mediated by core components of the replication stress checkpoint pathway: the upstream sensor/signal initiator kinases Mec1 and Tel1, the adaptor protein Mrc1, and the transducer kinase Rad53. Although the tRNA genes are not responsive to genotoxins that directly cause chromosome double strand breaks (DSBs), evidence presented by *Michael (Mike) Schultz* (University of Alberta, Canada) and colleagues suggests that tRNA gene transcription is also downregulated by three well-characterized components of the DSB checkpoint system. These proteins – the MRX complex which processes DNA ends, the adaptor Rad9, and the transducer kinase Chk1 – have not yet been ordered in a pathway that controls tRNA gene expression. Nonetheless, the emerging data suggest that tRNA genes are regulated by a much more diverse repertoire of DNA structure abnormality signaling molecules than previously suspected.

Pol III-transcribed noncoding RNAs (ncRNAs) of the Alu/B2 family are cytotoxic and thus are found only in trace levels in cultured mammalian cells. In mammalian macrophages, these ncRNAs are induced during extensive phagocytosis of cellular debris or parasites. Alu/B2 RNAs activate the inflammasome and ultimately cause the death of the macrophages engaged in the phagocytosis. *G. Chaudhuri* (Meharry Medical College, Nashville, TN, USA) presented evidence that during the infection of macrophages by the obligate macrophage parasite *Leishmania*, the biosynthesis of these cytotoxic ncRNAs are inhibited, serving to increase the life span of the infected macrophages and support parasite growth. He reported the mechanism of this downregulation. They found that *Leishmania* through its surface protease activates the thrombin receptor PAR1 in the macrophages. This activation of PAR1 raised the cytosolic level of Ca^{2+} thereby activating the Ca^{2+} -dependent protease $\mu(\text{mu})$ -calpain. Mu-calpain then degraded TFIIC110 to inhibit the transcription from the B-box-containing promoters of the Pol III-transcribed genes. This mechanism of controlling the levels of cytotoxic ncRNAs in macrophages could also be operative in other macrophage-associated diseases including cancer and atherosclerosis.

To gain insight into the regulation of 5S rRNA transcription, *Sylvette Tourmente's* group (Clermont Universite, France) has investigated the expression of the exon-skipped (ES) and exon-including (EI) TFIIA transcripts. These two transcript isoforms result from alternative splicing of the TFIIA pre-mRNA, and affect the TFIIA protein amounts relative to the levels of its target transcription product, 5S rRNA, during development of *Arabidopsis thaliana*. The ES transcript encodes the TFIIA protein, whereas the EI transcript was degraded by the NMD (nonsense mediated decay) pathway. 5S rRNA quantities were regulated through distinct, but complementary mechanisms operating both through a transcriptional and post-transcriptional (alternative splicing) control of TFIIA transcripts as well as at the post-translational level through proteolytic cleavage of the TFIIA protein. During the reproductive phase of *Arabidopsis thaliana*, high expression of the TFIIA gene and regulated splicing together with low proteolytic cleavage contributed to the accumulation of functional, full-length TFIIA protein, resulting in appropriate 5S rRNA accumulation in the seed. In contrast, just after germination, amounts of TFIIA-coding

transcripts were low and stable. The TFIID protein was completely cleaved, therefore full-length TFIID protein was undetectable, and 5S rRNA stored in the embryo progressively decreased. After day 4, cleavage of TFIID protein decreased; therefore full-length TFIID protein that possessed transcriptional activity accumulated to accommodate reorganization of 5S rDNA chromatin to a mature state, and consequently permitted de novo transcription of 5S rRNA.

6. Regulation and control pathways: Maf1

Maf1 protein is a global negative regulator of Pol III transcription conserved from yeast to man that was discovered by *Magdalena Boguta* (Polish Academy of Sciences, Poland) who reported that Maf1 is phosphorylated by protein kinase CK2, a highly evolutionarily conserved eukaryotic kinase, and is required for efficient Pol III transcription. Upon transfer of *S. cerevisiae* from repressive to favorable growth conditions, CK2 activity was required for the release of Maf1 from Pol III complex and from the tDNA gene. The innovative mechanism proposed is control of Pol III recycling through CK2-mediated phosphorylation of Maf1, occurring directly on tDNA chromatin.

7. Pol III & cancer

Most human tumors examined express abnormally high levels of $\text{tRNA}_i^{\text{Met}}$, the initiator tRNA that starts each new polypeptide. To model this situation, *Robert (Bob) White's* lab (Beatson Institute for Cancer Research, UK) has constructed transgenic mice carrying an extra copy of the $\text{tRNA}_i^{\text{Met}}$ gene, transcribed from its own promoter. *Kirsteen Campbell* described the phenotype of these mice. She reported that the mice are healthy and viable, but show abnormally elevated numbers of several cell types and are pre-disposed to cancer.

These properties agree well with published observations that elevated $\text{tRNA}_i^{\text{Met}}$ can promote proliferation and oncogenic transformation of cultured mouse embryonic fibroblasts. New evidence was presented that $\text{tRNA}_i^{\text{Met}}$ can also promote the proliferation of a human melanoma cell line. Furthermore, *Joanna Birch* showed that migration of these and other human cells can be enhanced by raising $\text{tRNA}_i^{\text{Met}}$ levels. Two other members of White's lab, *Dritan Liko* and *Louise Mitchell*, described a mouse line in which part of the Brf1 gene can be deleted in vivo in a spatially-and temporally-controlled manner. This revealed that embryos do not develop beyond the morula stage in the absence of Brf1. Furthermore, deletion of Brf1 in the intestines of adult mice triggers apoptosis. These data confirm the importance of Brf1 in living mice, which is consistent with its essential role in recruiting Pol III to many genetic targets, including tRNA and 5S rRNA. Finally, *Sarah Dowding* from White's lab presented evidence that the response of Pol III transcription to activation of p53 is very dependent on cellular context. For example, when U2OS cells are subjected to oncogenic stress, tRNA expression undergoes p53-dependent repression. In contrast, tRNA levels increased when the same cells are exposed to UV-C irradiation, despite induction of p53. Such differential responses may reflect variations in the level and post-translational modification of p53, as well as the involvement of alternative regulatory pathways.

8. Pol I and cancer

Morphologic abnormalities of the nucleolus, the site of transcription of the ribosomal genes (rDNA) by Pol I, have been recognized as diagnostic for cancer for over a century. Nevertheless, an unresolved question has been whether the accelerated ribosome biogenesis responsible for the nucleolar changes is required for maintenance of the malignant phenotype. To address this, *Ross Hannan's* group (Melbourne University, Australia) used genetic manipulation and a selective small molecule inhibitor of Pol I transcription, CX-5461 (Cylene Pharmaceuticals) to provide definitive evidence that accelerated rDNA transcription and nucleolar integrity are necessary for oncogenic activity. Further, they demonstrated that Pol I transcription can be targeted in vivo to therapeutically treat lymphoma and leukemia tumors in vivo through the non-genotoxic activation of p53-dependent apoptosis, while sparing normal cells. Mechanistically they demonstrated that the therapeutic effect of Pol I inhibition was not due to ribosome insufficiency but instead was due to acute activation of nucleolar stress that leads to the release of ribosomal proteins (RPs) from the nucleolus into the nucleoplasm where they can sequester the p53 inhibitory protein MDM2, causing activation of p53 and induction of apoptosis. In summary, the presented studies demonstrated for the first time that selective inhibition of Pol transcription, can serve as a therapeutic strategy for the treatment of cancer. Dr Hannan and his clinical colleagues are conducting the first in human trial of the Pol I inhibitor CX-5461 in patients with hematologic malignancies at the Peter MacCallum Cancer Centre in Melbourne which started in 2012.

rRNA synthesis by Pol I is a key and rate limiting stage of ribosome biogenesis which is up-regulated in cancers. *William J. Andrews* (PhD student with Dr. Panov, Queen's University, Belfast, Ireland) presented work showing that ellipticines selectively inhibit Pol I dependent transcription at a nanomolar range both in vitro and in cells in a p53, ATM/ATR and topoisomerase-II independent manner without affecting Pol II and Pol III transcription. They concluded that the interactions of the essential transcription factor SL1 with the promoter are the primary target of the drugs. The presented study revealed a novel class of Pol I inhibitors and their mechanism of action.

9. Factors & mechanisms shared by Pols III and/or II and/or I

William (Bill) Stumph (San Diego State University, USA) reported on his lab's continuing work on the fruit fly small nuclear RNA activating protein complex DmSNAPc), a heterotrimeric transcription factor required for U6 snRNA transcription (and a number of other small stable RNAs) by Pol III, as well as U1–U5 snRNAs by Pol II. Previous work using site-specific protein-DNA photo-cross-linking assays had indicated that the three subunits of DmSNAPc contact the DNA of U6 and U1 promoters differently. By combining the cross-linking with site-specific protein cleavage, the Stumph lab showed now that different domains of the two smaller subunits, DmSNAP50 and DmSNAP43, contact the DNA at different nucleotide positions in U6 versus U1 promoter sequences. The largest subunit, DmSNAP190, uniquely contains 4.5 tandem Myb repeats. Recent work has demonstrated that these Myb repeats contact the U1 promoter with the most C-terminal repeat farthest from the transcription start site and the more N-terminal repeats closer to the

start site. The different conformations of DmSNAPc are believed to be responsible for the different RNA polymerase specificities of the U1 and U6 promoters.

The transcription and DNA repair factor TFIIF, found mutated in either cancer-prone (Xeroderma Pigmentosum) or premature aging (Trichothiodystrophy) patients, plays a role in ribosomal RNAs (rRNAs) transcription. The nature of this function in ribosomal biogenesis together with a possible biological relevance for TFIIF-related diseases remains to be explored. *Julie Nonnekens* (Universite Toulouse, France) presented studies on the involvement of TFIIF in the whole process of ribosome biogenesis, from transcription to maturation of rRNAs. The results showed that TFIIF binds all along the rDNA, confirming a role of this factor during the elongation step. Polymerase binding to the rDNA was disturbed specifically in some TFIIF mutants, which concomitantly showed a severe reduction of Pol I transcription. Additionally, a defect in the 40S rRNAs maturation pathway found specifically in XPD^{TTD} ES cells and XPD^{TTD} murine brain advocate a new possible explanation of the dichotomy cancer/premature ageing observed in TFIIF affected patients.

10. Chromatin modifications and epigenetics

The *Engelke* lab (University of Michigan, MI, USA) provided an update on the mechanism of tRNA gene-mediated (tgm) silencing. While previous work showed that clustering of the scattered tRNA genes at the nucleolus was required for the silencing of nearby Pol II transcription, Engelke's group demonstrated that nucleosome modification and remodeling are also essential. Although condensin bound at each tRNA gene was needed for clustering of the tRNA genes, communication with the local chromatin structure appeared to require several other factors in cis at the tRNA genes, including MafI protein, the tRNA transcript, and the Mod5 protein.

Understanding the complex process of eukaryotic transcription requires detailed knowledge about both the transcriptional machineries and the natural templates, the chromatin. To derive a molecular description of chromatin transcribed by the Pol I and Pol III, *Stephan Hamperl's* group (Universitat Regensburg, Germany) has developed a technique for native isolation of chromatin segments from the yeast ribosomal DNA locus carrying the Pol I transcribed 35S ribosomal RNA (rRNA) gene and the Pol III transcribed 5S rRNA gene. This allowed the mass spectrometric analysis of proteomes co-purifying with the respective chromatin domains and the identification of covalent histone modifications. Electron microscopy further allowed investigating chromatin structure on a single molecule level. Initial experiments indicated that these findings with ex vivo purified chromatin have relevance in vivo, and that this approach will provide deep insights in natural templates of Pol I and Pol III.

Alu elements amplify using a Pol III generated transcript in a process termed retrotransposition. The amplification of Alu in primates has led to the accumulation of these elements, contributing to about 11% of the mass of the human genome. The vast majority of the 1.1 million Alu elements are retrotranspositionally inactive with only a few loci able to generate new inserts. However, it is unknown which specific Alu elements are active. Tissue culture studies demonstrated that active Alu elements require specific sequence features in

addition to being transcribed by Pol III. *Astrid Roy-Engel* (Tulane University, LA USA) presented the analysis of the Alu loci occupied by Pol III factors derived from three previous genome-wide studies. The findings reveal that Alu expression varies between cell types, growth conditions and transformation states. In addition, the data presented indicate that the vast majority of the Alu loci potentially transcribed by Pol III lack important sequence features for retrotransposition. The analysis hinted at a plausible general mechanism that selectively limits transcription of the retrotranspositionally competent Alu elements that serves to diminish their damage potential. Finally, it was concluded that in an in vivo scenario, the contribution of Alu activity on somatic genetic damage may significantly vary between individuals and tissues.

Nouria Hernandez (University of Lausanne, Switzerland) presented genome-wide localization of Pol III in mouse liver. As in human cell lines, the number of loci occupied by Pol III, and therefore probably actively transcribed, numbers a few hundreds. Most of these loci corresponded to annotated tRNA genes and other known RNA polymerase III genes. Some twenty corresponded to novel loci, one of which, located within an intron of the POLR3E gene, potentially plays a regulatory role in POLR3E gene expression. An intriguing observation was that like in human cells, only about 50% of the annotated tRNA genes were occupied by Pol III. Synteny relationships were established between a number of human and mouse Pol III genes, and the expression levels of these genes were significantly linked. Variations within the A and B promoter boxes, strength of the terminator sequence, as well as various genomic features as revealed by correlation studies all contributed to selective Pol III occupancy. Thus, tRNA gene occupancy by Pol III reflected both an underlying genomic organization that is conserved in dividing human culture cells and resting mouse liver cells, and the particular promoter and terminator strengths of individual genes.

Attila Németh (Universitat Regensburg, Germany) presented a novel method called ‘epi-combing’ that has been developed by his group in collaboration with Genomic Vision. The assay allows the single molecule analysis of DNA modifications on regularly stretched, megabase-sized DNA fibers genome-wide, at kilobase resolution. The Németh group investigated patterns of epigenetic marks on human genomic DNA with this approach, focusing primarily on repetitive sequences. Due to their high copy number and repetitive organization, ribosomal DNA (rDNA) units were still not fully sequenced and annotated furthermore the epigenetic status of individual repeats and repeat clusters cannot be determined with conventional methods. Epi-combing, however, revealed for the first time the nature of the epigenetic regulation of canonical and non-canonical rDNA repeats, being the latter ones stably repressed.

William (Bill) Henry (Michigan State University, MI, USA) presented the results from the exploration of the contribution of the retinoblastoma (RB) tumor suppressor to the regulation of Pol III transcription of class III genes, focusing on the human U6 snRNA, MRP, and Y families of genes. First, they showed that RB function towards class III genes was dynamic and sensitive to the growth state of the cells. For example, U6 levels were significantly more sensitive to RB influence after nocodazole treatment than in asynchronously growing cells, suggesting RB was important for coordinating a Pol III response to cellular stress. Secondly,

they observed that RB exhibited idiosyncratic targeting of class III genes, associating with only some class III genes (e.g. RNU6-1, MRP) and not with others (Y family). Moreover, they observed RB at some genes during active growth and cell cycle arrest, while RB associated with other genes only during senescence and quiescence. These data support a model wherein RB modulates Pol III activity at specific sets of genes in response to changing growth conditions. Lastly, they reported that RB expression was correlated with increased RNU6-1 promoter residence by the Brg1 ATP-dependent chromatin-remodeling factor and the HDAC2 histone deacetylase. RB also enhanced promoter association by the DNMT1 and DNMT3a DNA methyltransferases and DNA Top2 β . Finally, they proposed that RB enacts multiple levels of chromatin regulation at the DNA and histone levels to facilitate Pol III pausing during repression.

11. Closing address

Patrick Cramer “Conservation of the RNA polymerase I, II and III transcription initiation machineries”

Recent studies of the three eukaryotic transcription machineries revealed that all initiation complexes share a conserved core. This core consists of the RNA polymerase (Pol I, Pol II, or Pol III), the TATA box-binding protein (TBP), and transcription factors TFIIB, TFIIE, and TFIIIF (for Pol II) or proteins structurally and functionally related to parts of these factors (for Pol I and Pol III). The conserved core initiation complex stabilizes the open DNA promoter complex and directs initial RNA synthesis. The periphery of the core initiation complex is decorated by additional polymerase-specific factors that account for functional differences in promoter recognition and opening, and gene class-specific regulation.

12. Poster presentations

Tomasz Turowski presented a poster highlighting the work from the *Boguta* group that supports a model where inhibition of tRNA transcription led to stabilization of hypomodified tRNA^{Val(AAC)} due to more efficient protection by tRNA-interacting proteins. They also identified TEF1 and VAS1, encoding elongation factor eEF1-A and valyl-tRNA synthetase respectively, which likely protected hypomodified tRNA^{Val(AAC)} by direct interactions. They proposed that rate of transcription results in changed balance between tRNA and tRNA-interacting proteins.

Production of the ribosomal RNA precursor by Pol I is the first step in eukaryotic ribosome biogenesis. However, the molecular structure of Pol I remains unknown. *Christoph Engel* presented a poster with work from the *Cramer* group extending previous structural models of the polymerase and its initiation factor Rrn3. Obtaining the crystal structure of Rrn3 enabled their group to build a model for the Pol I–Rrn3 interaction, showing that Rrn3 is located between the subcomplexes A14/43 and AC40/19. Additionally, cross-linking data helped to understand architecture and function of the specific Pol I subunits A49/34.5 and A14/43. Furthermore, cross-linking showed that the C-terminus of the Pol I subunit A12.2 resembles the fold of TFIIS, explaining the intrinsic RNA cleavage activity of Pol I. Combined with a

homology model of the core factor component Rrn7 (based on TFIIB) these findings allowed for them to present a model of the minimal Pol I pre-initiation-complex.

Awards

Awards were conferred to the best graduate student and postdoctoral fellow platform and poster presentations (summarized in Table 1).

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Abbreviations

CTD	Carboxyl-terminal domain
Top2α	DNA topoisomerase IIα
Top2β	DNA topoisomerase IIβ
eEF1-A	elongation factor 1-A
ES	exon-skipped
EI	exon-including
hPAF49	human Pol I-associated factor 53
ncRNAs	noncoding RNAs
NMD	nonsense mediated decay
NoLS	Nucleolar Localization Site
PAF53	Pol I-associated factor 53
PAR1	protease activate the thrombin receptor
Pol I	RNA polymerase I
Pol II	RNA polymerase II
Pol III	RNA polymerase III
RB	Retinoblastoma
TBP	TATA box-binding protein
TAF1B	TATA box-binding protein (TBP)-associated factor 1

TTF-I	termination factor
TF	transcription factor
TFIIB	transcription factor C for Pol II
TFIIE	transcription factor E for Pol II
TFIIEalpha	transcription factor E alpha for Pol II
TFIIF	transcription factor F for Pol II
TFIIIA	transcription factor A for Pol III
TFIIIC	transcription factor C for Pol III
Tgm	tRNA gene-mediated
WT	Wildtype
WH	winged helix

Table 1

Awards presented to graduate students and postdoctoral fellows.

Award	Recipient name	Title presentation and authors
Platform presentation — graduate student	Stephan Hamperl	Compositional and structural analysis of chromatin regions within the yeast rDNA locus transcribed by RNA polymerases I, (II) and III. Stephan Hamperl, Christopher Brown, Jorge Perez-Fernandez, Astrid Bruckmann, Manuel Wittner, Katharina Huber, Ana Villar Garea, Rainer Deutzmann, Hinrich Boeger, Philipp Milkereit, Herbert Tschochner, and Joachim Griesenbeck
Platform presentation — postdoctoral fellow	Nagarajavel Vivekananthan	Genome-wide high-resolution footprinting of TFIIC complexes in <i>Saccharomyces cerevisiae</i> . Nagarajavel Vivekananthan, James Iben, Bruce H. Howard and David J. Clark
Platform presentation — postdoctoral fellow (shared)	Dritan Liko & Kirsteen Campbell	Mousemodels of Pol III transcription. Kirsteen Campbell, Dritan Liko, Louise Mitchell and Robert J. White
Poster presentation — graduate student	Philipp Merkl	Analysis of Ydr026c/Nsi1 in yeast RNA polymerase I transcription termination. Philipp Merkl, Alarich Reiter, Lydia Williams, Jorge Pérez-Fernandez, Stephan Hamperl, Philipp Milkereit, Joachim Griesenbeck and Herbert Tschochner
Poster presentation — postdoctoral fellow	Manjula Nagala	The role of the RNA polymerase I specific subunit CAST in the regulation of ribosomal RNA synthesis. Manjula Nagala, Jackie Russell and Joost Zomerdijs