The 'Odd Pols' are even when it comes to controlling cell function

Conference on RNA Polymerases I and III

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The Sixth International Conference on RNA Polymerases I and III (the 'Odd Pols') took place between 4 and 8 June 2008, at Station Touristique Duchesnay near Québec, Canada, and was organized by T. Moss, M. Paule, C. Pikaard, R. White & I. Willis. The Canadian Institutes of Health Research sponsored the meeting.

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See Glossary for abbreviations used in this article.

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Introduction

The RNA polymerase (RNAP) I and RNAP III transcriptional machineries can be thought of as distinct system modules in cellular physiology. The RNAP I module produces the three largest ribosomal RNAs (rRNAs), whereas the RNAP III module produces small non-coding RNAs (ncRNAs), including 5S rRNA, the transfer RNAs (tRNAs) and U6 small nuclear RNA (snRNA). These modules, although distinct in terms of the 'Odd Pol' RNAs that they produce, have extensive connections to one another and to other system modules in the cell (Fig 1). Work reported during the Sixth International Conference on RNA Polymerases I and III, which was held in June 2008, advanced our understanding of the biological processes within such modules and the nature of the connectivity between them.

Nuclear organization

The cross-talk between the RNAP I and RNAP III system modules is being revealed by studies of nuclear organization. The perinucleolar compartment (PNC) is enriched in a RNAP III transcript required for pre-rRNA processing. S. Huang (Chicago, IL, USA) reported that PNC prevalence correlates with malignancy and metastatic behaviour of human breast and prostate cells. As cancer cells generally produce ribosomes at a higher rate than normal cells, this increased frequency of PNCs in cancer cells might reflect their higher rate of pre-rRNA processing.

The nucleolar localization of tRNA genes in budding yeast also connects the RNAP I and RNAP III system modules at the level of nuclear organization. D. Engelke (Ann Arbor, MI, USA) reported that the nucleolar localization of tRNA genes depends on microtubules and that tRNA genes cluster together. Surprisingly, this clustering requires condensin, which is best known as a modulator of mitotic chromosome architecture and sister-chromatid resolution during anaphase. Condensin is not required for transcription, but it is concentrated at tRNA and some other RNAP III-transcribed genes, with a bias towards the region bound by TFIIC—a core RNAP III transcription factor. Collectively, these results suggest a role for condensin in genome organization that is intimately connected to the behaviour of the RNAP III transcriptional machinery (D'Ambrosio *et al*, 2008; Haeusler *et al*, 2008). The Odd Pol–condensin link was also explored

in work presented by C.-K. Tsang (Piscataway, NJ, USA), who showed a role for condensin in rDNA condensation in yeast upon starvation or inhibition of the TOR protein kinases (Tsang *et al.*, 2007). Tsang reported that the absence of condensin during starvation leads to elevated extra-chromosomal ribosomal DNA circles (ERCs), which suggests that condensin helps to maintain rRNA gene stability. Consistent with this was the observation that condensin-mediated rDNA compaction prevents the incursion of a strand-exchange protein, Rad52, into the rDNA during stress, thereby preventing ERC formation.

A crucial role for RNAP I activity in the coordination of all stages of ribosome biogenesis was recently discovered (Laferté *et al.*, 2006). By using a genetic approach in yeast, N. Ayoub (Gif-sur-Yvette, France) showed that the constitutive activation of RNAP I leads to the concomitant accumulation of 5S rRNA, messenger RNAs (mRNAs) encoding ribosomal proteins and fully assembled ribosomes. Subsequent studies have shown that nucleolar structure and RNAP I activity—two processes long thought to be intimately connected—might sometimes be uncoupled. Which molecular mechanisms underlie the coupling of nucleolar structure and RNAP I transcription when it does occur? Work in humans is shedding new light on this issue. Nucleolar organizer regions (NORs)—comprising arrays of rRNA genes—and their surrounding sequences have been omitted from all drafts of the human genome. B. McStay (Dundee, UK) described the sequencing of the boundaries of human NORs. By using three-dimensional DNA-immuno-FISH (fluorescence *in situ* hybridization), the distal junctions of NORs were shown to be located within the heterochromatin surrounding nucleoli. The anchoring of these sequences provides an explanation for the nucleolar segregation observed upon RNAP I inhibition with actinomycin D. Further characterization of the chromosomal context of NORs will be crucial to understanding how they are organized within interphase nuclei and how multiple NORs associate with mature nucleoli.

The regulation of rRNA gene structure was examined by G. Langst (Ragensburg, Germany), who reported on NoRC, a multifunctional chromatin-dependent regulator of rRNA that has a role in nucleosomal positioning, transcriptional repression, epigenetic silencing and replication timing. DNA-binding experiments revealed that the four AT-hook domains in TIP5, and a newly described extended AT-hook domain also in the TIP5 subunit of NoRC, cooperate to anchor NoRC and the rRNA genes to the nuclear matrix *in vivo*.

Novel genes and gene structure

The intense interest in RNAP III-dependent expression of U6 snRNA has extended to a related molecule, U6atac, which is a component of the minor spliceosome responsible for the excision of U12-type introns. B.-J. Benecke (Bochum, Germany) showed that RNAP III transcription of the human *U6atac* gene depends on a TATA box, and proximal and distal sequence elements, as is the case for U6. This work sets the stage for studies aimed at understanding how the differential transcription of these genes might contribute to higher steady-state expression of U6 over U6atac snRNA. C. Marck (Gif-sur-Yvette, France) reported that many 5S rRNA genes in the yeast *Yarrowia lipolytica* are fused to the 3' ends of tRNA genes, and yield a precursor transcript that includes the tRNA and the 5S rRNA. Although tRNA and 5S rRNA genes have internal promoters, the transcription of dicistronic tRNA-5S genes seems to depend solely on initiation from the tRNA promoter. S.-Y. Choi (Seoul, Korea) showed that RNAP III transcribes a new gene in the long terminal repeats of M-MuLV, the transcript of which is called Let. It will be

Glossary

ARF	alternate reading frame
BRF1	B-related factor 1
Dst1	DNA strand transferase 1
FACT	facilitates chromatin transcription
HMG	high mobility group
Hmo1	high mobility group box protein
HOT1	hotspot 1
M-MuLV	Moloney murine leukaemia virus
NoRC	nucleolar remodelling complex
PC4	positive cofactor 4
Rad	radiation sensitive
RHEB	Ras homologue enriched in brain
Rpa43	RNA polymerase I subunit A43
RRM	RNA recognition motif
Rrn3p	ribosomal RNA-synthesis defective protein
siRNA	small-interfering RNA
SL-1	selectivity factor-1
SNR6	small-nuclear RNA 6
Sub1	suppressor of transcription factor IIB mutations
TF	transcription factor
Tif-1A	transcription-initiation factor IA
TIP5	TIF-interacting protein 5
topo II	topoisomerase II
TOR	target of rapamycin
tRNA ^{iMet}	transfer RNA initiator methionine
t-Utp	t-U three protein
UBF	upstream binding factor
UtpA	U three protein A

important to understand the regulation of Let synthesis, as it is a crucial determinant of leukemogenicity.

Signalling

Studies in various systems continue to expand our understanding of the signalling mechanisms that control Odd Pol transcription. M. Schultz (Edmonton, Canada) reported the repression of yeast tRNA gene transcription by the replication inhibitor hydroxyurea. This regulation involves a conserved checkpoint kinase, Rad53, and a universal repressor of the RNAP III transcriptional apparatus, Maf1. As tRNA transcription into replication forks slows fork progression—because active RNAP III seems to inhibit converging replication—it is possible that Rad53 signalling to the RNAP III transcriptional machinery influences the pattern of replication.

The TOR kinases control RNAP I and RNAP III transcription in all eukaryotes. S. Zheng (Piscataway, NJ, USA) reported that TOR complex 1 localizes to the nucleolus, and interacts directly with the 35S and 5S promoters in a rapamycin-dependent and starvation-dependent manner in yeast (Li *et al.*, 2006). The interaction of TOR with these promoters is important for the transcription of RNAP I- and RNAP III-dependent genes and, in the case of the 5S gene, is required for Maf1 phosphorylation, which promotes repression through a currently unknown mechanism. R. White (Glasgow, UK) described new insights about the regulation of RNAP III transcription by human TOR. The GTPase RHEB has been added to the list of mammalian TOR-pathway components that control tRNA and 5S rRNA gene transcription. Mammalian TOR itself cross-links to tRNA and 5S rRNA genes, and Maf1 is required for repression by the TOR kinase-inhibitor rapamycin. Surprisingly, in contrast to the

Fig 1 | Partial interactome of budding yeast *RPC40*. *RPC40* encodes the shared 40 kDa subunit of RNAP I and RNAP III. *RPC40* and its protein product are connected to components of diverse system modules (only a subset is shown). The edges denote interactions collated in the Biological General Repository for Interaction Datasets (BioGRID) database at the *Saccharomyces* Genome Database (<http://yeastgenome.org>). RNAP, ribonucleic acid polymerase.

situation in yeast, Maf1 interacts directly with human TFIIIC and its cross-linking to RNAP III-transcribed genes is not rapamycin sensitive.

S. Grewal (Calgary, Canada) described genetic studies showing that *Drosophila* Tor controls ribosome biogenesis (Grewal et al., 2007). The localization of *Drosophila* Tif-1A—the fly homologue of the yeast RNAP I transcription-initiation factor Rrn3p—to the rRNA gene promoter is regulated by Tor, and Tif-1A overexpression can maintain high levels of rRNA synthesis when Tor activity is reduced. Clearly the *Drosophila* system has the potential to provide important information about the molecular mechanisms by which Tor regulates Rrn3 in multicellular organisms.

Chromatin modifications and epigenetics

Studies of the U6 snRNA gene are revealing how chromatin regulation affects RNAP III transcription. Chromatin immunoprecipitation (ChIP) mapping of histone (H)3/H4 acetylation and methylation marks has revealed that some paradigms of chromatin regulation at RNAP II-transcribed genes do not apply at the yeast *SNR6* U6 gene (P. Bhargava, Hyderabad, India). For example, tail acetylation of H3/H4 at the *SNR6* promoter nucleosomes increases in the repressed state, whereas increased acetylation is associated with RNAP II activation. It will be fascinating to determine the molecular differences between the

RNAP II and RNAP III transcriptional machineries that underlie their divergent regulation by chromatin. By contrast, the regulation of gene expression by the human retinoblastoma protein (RB) seems to be similar for RNAP II and RNAP III target genes. T. Selvakumar (East Lansing, MI, USA) showed that RB directs DNA methyltransferase, chromatin remodelling and histone deacetylase activities to the U6 promoter in order to establish a chromatin state that inhibits RNAP III transcription; events that are also necessary for the repression of RNAP II-transcribed genes by the canonical RB pathway.

The mechanisms that regulate rRNA gene silencing in mammals are unclear. R. Hannan (Melbourne, Australia) shed new light on this issue by showing that depletion of the RNAP I transcription factor UBF leads to stable and reversible silencing of rRNA genes. This is achieved by promoting H1-induced assembly of transcriptionally inactive chromatin without changes in rRNA gene methylation. These data indicate that UBF is required to regulate dynamically the open chromatin structure found in active rRNA genes during development. Studies of gene silencing in *Arabidopsis* reported by C. Pikaard (St Louis, MO, USA) reveal that rRNA genes and some RNAP III-transcribed genes are controlled by siRNA-directed DNA methylation. This regulation involves RNAP IV and RNAP V (Pol IVb), which are enzymes specific to higher plants. siRNA-directed DNA methylation switches off the expression of RNAP III-transcribed short-interspersed nuclear elements, and is also implicated in controlling the dosage of active 45S and 5S rRNA genes. Fundamental insights into gene regulation will probably originate from future work on siRNA-dependent control of RNAP III-transcribed genes.

Transcription, RNA processing and regulation

The coupling of RNAP I transcription and processing is an emerging theme in the ribosome-biogenesis field. The t-Utp subcomplex, UtpA, is a small subunit processome component of the pre-18S rRNA processing machinery that also localizes to rRNA gene chromatin. S. Baserga (New Haven, CT, USA) reported that t-Utps can be co-immunoprecipitated with short sense ncRNAs transcribed by RNAP I from the start of the rRNA gene. As depletion of t-Utp decreases transcription of the short ncRNAs and pre-rRNA, the ncRNAs are probably crucial for RNAP I transcription, in addition to pre-rRNA processing. Moreover, HOT1 sequence elements within the non-coding region of the rDNA repeats are sufficient to recruit t-Utps, providing a mechanism for the physical linkage of transcription and pre-rRNA processing.

Ribosome synthesis is tightly adjusted to nutrient availability, although the mechanisms of this regulation are unclear. H. Tschochner (Regensburg, Germany) reported that repression of rRNA gene transcription in yeast in response to nutrient depletion is associated with proteasome-dependent degradation of the initiation factor Rn3p. However, reduced transcription initiation did not fully explain the inactivation of rRNA transcription; the dominant effect on ribosome synthesis after acute nutrient depletion was actually a result of defects in rRNA processing and maturation. These findings are consistent with the emerging model that growth-dependent regulation of rRNA synthesis occurs on several levels, including the formation of initiation complexes, elongation and rRNA processing.

As deregulation of RNAP I and RNAP III has long been associated with malignant cell transformation, many laboratories continue to work on the Odd Pol connections to cancer. The ARF tumour suppressor has been shown to regulate ribosome biogenesis, in part through the modulation of pre-rRNA processing, which involves nucleophosmin/B32. T. Moss (Québec, Canada) described

an alternative mechanism by which ARF regulates processing by interacting directly with the RNAP I transcription termination factor 1 (TTF-1). When ARF binds to TTF-1, the nucleolar localization domain of the latter is masked, thereby excluding it from the nucleolus and leading to a defect in processing. The knockdown of TTF-1 inhibits pre-rRNA processing, which suggests that ARF mediates pre-rRNA processing through its interaction with TTF-1.

c-Myc has been shown to activate RNAP I gene transcription directly in mammalian cells, although the molecular mechanisms by which this activation occurs are unclear. A. Wright (Huddinge, Sweden) presented evidence from chromatin conformation-capture experiments showing that rat c-Myc induces higher-order gene-loop structures in rRNA gene chromatin that juxtapose upstream and downstream rRNA gene sequences. Temporal analysis of looping indicates that it might be responsible for the reprogramming of rDNA transcription of quiescent cells as they re-enter the cell cycle.

The cancer connection was extended from RNAP I to the other Odd Pol by L. Marshall (Glasgow, UK), who presented strong evidence that the induction of the RNAP III transcription factor BRF1 can be sufficient to increase proliferation and cause oncogenic transformation (Marshall *et al*, 2008). Overexpression of the RNAP III product tRNA^{iMet}—which initiates polypeptide synthesis—was also sufficient to stimulate cell proliferation and to promote tumour formation in mice. These studies provide the first evidence that dysregulation of RNAP III transcription might contribute to tumour development in humans.

New twists for familiar factors

Some familiar factors continue to be studied intensively with respect to their functions at specific steps in Odd Pol biogenesis. P. Cramer (Munich, Germany) described an analysis of the functional architecture of RNAP I and, by comparison with RNAP II, revealed RNAP I-specific features that match the unique nature of rRNA transcription (Kuhn *et al*, 2007). In RNAP I, the A14/A43 subcomplex, the clamp and the dock domain contribute to a unique surface that interacts with initiation factors required for RNAP I recruitment to the rRNA promoter. Furthermore, the built-in elongation-stimulatory Pol I-specific subcomplex A49/A34.5 can explain the efficient and processive nature of rRNA transcription during cell growth. Finally, Cramer showed that, in contrast to RNAP II, RNAP I has intrinsic RNA-cleavage activity that facilitates rRNA 3' trimming and proofreading, thereby preventing the formation of erroneous rRNAs and catalytically deficient ribosomes.

The current model proposes that the RNAP I-associated Rn3 functions as a bridge between RNAP I and transcription factors bound to the committed template. L. Rothblum (Oklahoma, OK, USA) demonstrated that Rn3 is also a DNA-binding protein. Rn3 mutants that are unable to bind to the rDNA promoter are still able to interact with Rpa43 and SL-1, but are unable to function in transcription. This suggests that DNA binding by Rn3 is essential for transcription by RNAP I. Another protein that interacts with DNA, topo II, has also emerged as an important player in RNAP I transcription. K. Panov (Belfast, Ireland) described topo II-inhibitor experiments and ChIP studies indicating that topo II contributes to transcription initiation at a step subsequent to SL-1 binding at the rDNA promoter.

The conserved La protein has been implicated in diverse aspects of RNA metabolism, including processing of RNAP III transcripts that end with its specific ligand, UUU-3'OH. R. Maraia (Bethesda, MD, USA) reported that fission yeast La distinguishes between UUU-3'OH on pre-tRNAs, the 3'-trailers released by pre-tRNA processing and mature tRNA-processing products. The concerted use of the La

motif and the RRM domain confers on La a higher affinity for pre-tRNA than for its cognate 12 nucleotide UUU-3'OH or mature tRNA-processing products. This selectivity is important for the recycling of La from released 3'-trailers to new pre-tRNAs *in vitro*, and for normal pre-tRNA accumulation and tRNA maturation *in vivo*. With many other known Odd Pol factors still poorly studied, we anticipate more exciting twists from such research in the future.

Even Pol factors with Odd Pol functions

Whether actively transcribed rRNA genes are nucleosomal is controversial. J. Griesenbeck (Regensburg, Germany) investigated the nature of 35S rDNA chromatin by using chromatin endogenous cleavage combined with psoralen cross-linking to assess the differential association of proteins with active and inactive rDNA chromatin (Merz *et al*, 2008). Active yeast rRNA genes were shown to be largely histone free and instead associated with the HMG box protein Hmo1. Similarly, J. Smith (Charlottesville, VA, USA) reported that active RNAP I transcription correlates with the removal of the H2A–H2B dimer. FACT—a chromatin remodelling complex that has been implicated in the removal of H2A–H2B dimers on RNAP II-transcribed genes—was shown to be enriched on the transcribed portion of active rRNA genes, which suggests a role for this complex in regulating histone occupancy on rDNA. Together, these data indicate that actively transcribed yeast rRNA genes are nucleosome free.

Two groups reported work in budding yeast that expands on the direct connections between the system modules that comprise the RNAP II and RNAP III transcriptional machineries. M. Werner (Gif-sur-Yvette, France) presented evidence that Dst1 (TFIIS), which is a classical RNAP II elongation factor, is also a general RNAP III transcription factor that controls start-site selection (Chavi-Helm *et al*, 2008). J. Acker (Gif-sur-Yvette, France) described work on yeast Sub1, which is the homologue of the human protein PC4 that is involved in RNAP II and RNAP III transcription. Acker presented evidence that Sub1 localizes to tRNA genes *in vivo*, and *in vitro* studies revealed that it enhances RNAP III reinitiation and the efficiency of the first transcription cycle, possibly by stabilizing protein–DNA complexes.

Studies of Maf1 also reveal the complex links between tRNA transcription and processing and other core processes in cell regulation. A high-throughput genetic analysis (I. Willis, New York, NY, USA) uncovered interactions between *MAF1* and tRNA processing, and Mediator complex genes. The latter finding spurred on work that uncovered a role for Mediator in the repression of ribosomal protein gene transcription—it was previously thought mainly to stimulate RNAP II transcription (Willis *et al*, 2008). Work on *maf1*⁺ and *tit1*⁺ in fission yeast suggests that failure to isopentylate specific tRNAs triggers a general stress response that pervasively affects tRNA gene transcription (N. Blewett, Bethesda, MD, USA). This interaction no doubt reflects the crucial role of tRNA biogenesis in overall cellular physiology.

Summary

The meeting participants reported exciting results that advance our understanding of the mechanisms and regulation of RNAP I and RNAP III transcription, and of the processing of the Odd Pol RNAs. The implications of misregulation of Odd Pol RNA expression for disease, particularly cancer, were brought into sharp focus by several of the presentations. The participants look forward to hearing about further developments in these areas at the Seventh International Conference on RNA Polymerases I and III to be held in 2010.

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