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Oddpols United: New Insights Into Transcription by RNA Polymerases I and III[1]

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OVERVIEW OF THE MOST RECENT CONFERENCE

An asylum would seem to be a fitting place to assemble a group that fondly refers to itself as "oddpols," and indeed it is, once every 2 years. The most recent gathering was officially billed as the Third International Conference on Transcription by RNA Polymerases I and III, which took place once again at the Asilomar ("asylum by the sea") Conference Grounds in Pacific Grove, CA, June 5–9, 2002. Of course, asylum has multiple meanings, and Asilomar fits the more pleasant definition of "sanctuary." There among the dunes and windswept Monterey Pines, a dedicated and lively group of principal investigators, postdocs, and graduate students shared their insights into the workings of the two RNA polymerases responsible for most of the RNA synthesis that occurs in a eukaryotic nucleus. The meeting, organized by Marvin Paule (Colorado State University), Ian Willis (Albert Einstein College of Medicine), and Craig Pikaard (Washington University, St. Louis), was an international affair, bringing together leading research labs from India, Germany, France, Italy, Australia, Canada, Puerto Rico, the United Kingdom, and the United States.

RNAs, rather than proteins, are the final functional products of genes transcribed by RNA polymerases I and III (except in trypanosomes, which have evolved a clever way to use pol I to transcribe protein coding genes). RNA polymerase I transcribes the genes that encode the precursor transcript for the three largest RNAs (25-28S, 18S, 5.8S) that form the structural and catalytic core of ribosomes. RNA polymerase III transcribes small RNAs, including the fourth ribosomal RNA (5S RNA), tRNAs, U6 snRNA (and in plants, U3 snRNA), SINES (short interspersed repeats) such as Alu elements, and a variety of other short RNAs (7SL, 7SK, adenovirus VA1). 5S rRNA and tRNAs are the most abundant of the pol III transcripts and they share with pol I-transcribed rRNAs a central role in establishing the protein synthetic capacity of the cell. Highlights of the meeting included insights into how the two polymerase systems are regulated by common signaling pathways, how chromatin and chromosomal influences affect gene function, and how transcription factors accomplish such tasks as promoter recognition, transcription initiation, polymerase elongation and termination. Other highlights included new evidence that activators and transcription factors utilized by RNA polymerase II to transcribe protein-coding genes are also involved in pol I and pol III transcription.

REGULATION OF POL I BY CELL SIGNALING PATHWAYS

Data presented at the meeting leave no doubt that changes in the phosphorylation status of the pol I

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transcription machinery have profound effects on rRNA synthesis. Joost Zomerdijk (Dundee, Scotland, UK) and Tom Moss (Laval, Quebec, Canada) presented data on insulin-like growth factor signaling and MAP kinase signaling, respectively, whereas Herbert Tschochner (Heidelberg, Germany), Larry Rothblum (Danville, USA), Joe Gogain (Fort Collins, CO, USA), and Lucio Comai (Los Angeles, CA, USA) presented data concerning the effects of phosphorylation on the basal transcription machinery.

Joost Zomerdijk (Dundee, UK) showed that insulin-like growth factor 1 (IGF-1) stimulates rDNA transcription up to fourfold in human embryonic kidney cells after serum starvation. The stimulation is rapid, with 50% activation being achieved within 30 min. The signal is dependent upon phosphatidylinositol 3-kinase as demonstrated by inhibition of the IGF-1 response using the specific chemical inhibitor, LY294002. The signal is transduced through mTOR (part of a nutrient sensing pathway) and Ras-MAP kinase signaling cascades as demonstrated by the additive inhibitory effects of rapamycin and the chemical inhibitor PD98059. If amino acids are removed from the medium and autophagy (salvaging of metabolites, especially amino acids, by breaking down cellular components during starvation) is inhibited by 3-methyl adenine, no IGF-1 response is observed. These results suggest that IGF-1 stimulation requires autophagic conditions, as found in serum-starved cells. The triggers of these responses remain to be identified in future studies.

Tom Moss (Laval, Canada) presented data on MAP kinase signaling. Treatment of a human neuroepithelioma cell line (SKF-5) with epidermal growth factor (EGF) results in a 2.5-fold stimulation of rRNA synthesis. Again, this is rapid, reaching full stimulation by 30 min. Use of MEK inhibitors demonstrates that EGF signals through the MAP kinase (ERK1/2) cascade. This result is also observed in NIH3T3 cells containing a RAF:estrogen receptor fusion construct in which the MAPK cascade is activated by 4-hydroxytamoxifen. Specific threonines in HMG boxes 1 and 2 of the pol I transactivator UBF (upstream binding factor) are the targets for phosphorylation by ERK1/2 in vitro and in vivo. By comparison with the known structure of other HMG box DNA binding motifs, it would be expected that phosphorylation of T117/201 would negatively influence DNA binding. Indeed, this seems to be the case with the effect being more pronounced on promoter DNA than on cruciform DNA (a generic HMG box binding substrate).

Rrn3p/TIF-IA mediates growth-dependent control of rRNA synthesis. Rrn3p/TIF-IA appears to have a role in both pol I recruitment to the promoter, by bridging between pol I and promoter-bound factor TIF-IB/SL-1 (mammals) or CF (core factor; yeast), and in some subsequent step in promoter clearance. The quantitative importance of these roles differs between species. In humans, TIF-IA/Rrn3p is required for recruitment, but in yeast, pol I interaction with CF does not require Rrn3p/TIF-IA. Radebaugh (Fort Collins, CO, USA) presented two-hybrid and GST-pulldown data supporting an alternative recruitment interaction between the largest yeast pol I subunit and the Rrn7p subunit of CF. The labs of Herbert Tschochner (Heidelberg, Germany) and Larry Rothblum (Danville, USA) presented data on how phosphorylation regulates the interaction of Rrn3p/TIF-IA with pol I in yeast and mammalian cells, respectively. In yeast, Rrn3p is predominantly phosphorylated when not associated with pol I. Nonphosphorylated Rrn3p produced in bacteria can productively interact with pol I in vitro, but phosphorylation of pol I was found to be a prerequisite for this interaction to occur. Both Tschochner and Gogain (Fort Collins, USA) note that dephosphorylation of pol I leads to complex effects on initiation and on elongation, complicating interpretation of simple phosphatase experiments in the pol I system.

Numerous labs have demonstrated that cycloheximide treatment of mammalian cells results in rapid cessation of rRNA synthesis. Ingrid Grummt's lab previously demonstrated that cycloheximide-inhibited cell extracts could be rescued by recombinant baculovirus-produced Rrn3p/TIF-IA. The Rothblum lab presented data that cycloheximide inhibits phosphorylation of Rrn3p/TIF-IA and its interaction with mammalian pol I subunit RPA43. Furthermore, baculovirus-produced (phosphorylated) but not bacterially produced (nonphosphorylated) Rrn3p/TIF-IA can rescue an extract from cycloheximide-treated cells. Thus, in contrast to yeast, it would appear that phosphorylated rather than nonphosphorylated Rrn3p/TIF-IA interacts with pol I in mammals. Whatever the identity of the kinases or phosphatases that regulate the interaction of pol I and Rrn3p/TIF-IA, this interaction is likely to be a target for signaling cascades.

Other kinases/phosphatases implicated in regulation of pol I include PP1α protein phosphatase 1 alpha) and the TBP-associated factor, TAF1 (see below). Zomerdijk showed that PP1α is enriched in the nucleolus and cofractionates with the active subform of pol I. Mechanistic studies demonstrated that PP1α is required for multiple cycles of transcription to occur on templates in vitro.

PHYSIOLOGICAL REGULATION OF THE POL III TRANSCRIPTIONAL MACHINERY

The pol III machinery, like its pol I counterpart, is tightly regulated. One of the classical problems in pol

III regulation is how cells switch from expression of oocyte-type to somatic-type 5S rRNA genes during development in *Xenopus laevis.* New work on TFIIIA has revealed that its phosphorylation at serine 16 by protein kinase CK2 is critical for switching. Specifically, Huber and coworkers (South Bend, IN, USA) find that stable recruitment of CK2-phosphorylated TFIIIA to oocyte-type genes generates a repressed state favoring expression of somatic-type genes in embryos. Therefore, frog cells use phosphorylation of TFIIIA by CK2 to convert TFIIIA from an activator to a repressor of transcription of oocyte-type 5S rRNA genes. Work from Michael Schultz and colleagues (Edmonton, Alberta, Canada) has shown that CK2 also regulates pol III transcription in yeast, where it controls repression of TFIIIB in response to DNA damage signals. DNA damage signals additionally repress synthesis of the large rRNAs in vivo by a CK2-dependent mechanism, although this repression evidently does not act at the level of pol I initiation. Synthesis of the pol I and pol III gene products in a human cell line was also shown to be coordinately downregulated by UV irradiation. In the context of published evidence that CK2 controls pol I and pol III transcription in all eukaryotes tested so far, this observation raises the possibility that repression of ribosomal and tRNA synthesis is a universal feature of DNA damage responses controlled by CK2.

Phosphorylation of another initiation factor, Brf1, is critical for induction of pol III transcription in mitogenically stimulated mammalian cells. In this case the effector kinase is ERK, a component of the p42/44 MAP-kinase pathway that also plays a role in hormonal activation of pol I transcription via effects on UBF. Pamela Scott (Glasgow, Scotland, UK) presented evidence for a model in which ERK phosphorylates and activates Brf1. A Brf1–ERK2 complex could be recovered from cells, and its amount increased upon mitogenic stimulation. Considering previous evidence that ERK activates pol I transcription in serum-stimulated cells, it is reasonable to conclude that the ERK pathway coordinately regulates synthesis of the pol I and pol III gene products in response to cell proliferation signals.

The mechanisms responsible for regulating transcription by pols I and III during the cell cycle in metazoans have been described in considerable detail in recent years. Oddly, in yeast, evidence for this type of regulation has been lacking until now. Work in the Willis laboratory (Bronx, NY, USA) has shown that precursor tRNA synthesis fluctuates during the cell cycle, peaking at the G_1 to S phase transition and that these changes exhibit an unusual dependence on G_1 cyclins: the level of pol III transcription in G_1 phase is correlated with the abundance of G_1 cyclins but does not require Cdc28 kinase.

INSIGHTS INTO THE POL III TRANSCRIPTION CYCLE

The mechanistic details of pol III transcription continue to be intensively studied. In metazoans, pol III genes encoding U6 and 7SK RNA utilize a gene-external proximal sequence element (PSE) and a TATA box to direct transcription. In humans, binding of the PSE by the five-subunit SNAPc complex cooperatively stimulates the assembly of a TFIIIB complex comprising TBP, Brf2, and Bdp1, on the TATA box. Work from the Hernandez laboratory (Cold Spring Harbor, NY, USA) has shown that multiple rounds of transcription on a U6 snRNA template can be accomplished using entirely recombinant transcription factors and a highly purified pol III fraction of defined subunit composition. Their analysis of human pol III has identified homologs for all 17 subunits of yeast pol III, demonstrating the high degree of structural conservation of this enzyme.

In addition to its role in pol III transcription, the SNAPc complex is also required for transcription of other small nuclear RNA genes (e.g., U1 and U2 snRNA genes) by pol II. Studies in both human and *Drosophila* systems have been investigating the basis for the assembly of polymerase-specific transcription complexes on U1/U2 and U6 snRNA genes. Interestingly, the mechanisms that are used in flies versus humans appear to be different. In flies, a small number of point mutations in the PSE of the U1 and U6 snRNA genes are able to switch polymerase specificity whereas in humans the presence of a TATA box in the U6 promoter and its absence in the U2 promoter is the key determinant. Extensive photo-cross-linking of *Drosophila* PBP (the orthologous complex to SNAPc in flies) to the U1 and U6 snRNA promoters has revealed significant differences downstream of the PSE. Based on these data, Bill Stumph (San Diego, CA, USA) proposed at the meeting that conformational differences in the structure of PBP on the two promoters are responsible for the recruitment of the appropriate polymerase-specific factors.

Changes in protein conformation are an integral part of TFIIIC-mediated complex assembly and provide a mechanistic explanation for the ability of certain dominant mutations in the TFIIIB assembly subunit of TFIIIC (TFIIIC131) to stimulate pol III transcription. New studies presented from the Willis laboratory (Bronx, NY, USA) indicate that a mutation (*PCF1-1*) in the second of 11 tetratricopeptide repeats in TFIIIC131 facilitates complex assembly by relieving autoinhibition of Brf1 binding. The mutation is thought to achieve this effect by stabilizing an alternative conformer of TFIIIC131 that promotes its interaction with Brf1.

Studies on the structure and function of the multi-

subunit TFIIIC complex are currently limited by the availability of material. Efforts to change this situation are progressing well in the Sentenac laboratory (Saclay, France), who reported the reconstitution of two subcomplexes of the factor using a baculovirus expression system: the τB subcomplex (τ138, τ91, and τ60) that binds specifically to the B block element in tRNA-type promoters and the τA subcomplex (τ131, τ95, and τ55), which has nonspecific DNA binding activity but is able to supershift the τB–tDNA complex.

Studies of pol III transcription in plants have been hampered by the lack of an efficient, easily prepared in vitro transcription system. This limitation has now been overcome. Kenzior (Columbia, MO, USA) reported on his work in the Folk lab in which he has used immobilized templates to capture the transcription machinery from crude extracts of *Arabidopsis thaliana* suspension culture cells. This machinery supports specific initiation and termination. For plant researchers, the stage is now set for detailed biochemical studies of the pol III transcriptional machinery.

TERMINATION AND PROCESSING

Walter Lang (Memphis, TN, USA) reported on his lab's efforts to decipher the mechanism of RNA polymerase I transcription termination in yeast. A 40-basepair region has been shown to include the termination site as well as sequence elements that differentially affect polymerase pausing and transcript release. Lang proposed that polymerase backtracking along the template at the terminator causes DNA–RNA mismatches and that loss of precise basepairing may play a role in transcript release. Interestingly, footprinting data suggest that the polymerase remains at the terminator long after transcripts are released, suggesting that a conformational change might need to occur and could be subject to regulation.

Transcription termination is important for initiation by pol III because the terminator facilitates fast polymerase recycling to the promoter after transcript release. Giorgio Dieci and colleagues (Parma, Italy) have studied the conformation of the polymerase at the terminator by in vitro transcription of templates with a peptide nucleic acid (PNA) roadblock placed in the vicinity of the terminator. The results presented at the meeting suggest a model of termination and polymerase recycling in which pol III adopts a "strained" conformation upon recognizing the terminator that is relieved after transcript release and prior to reinitiation.

Newly completed pol III transcripts are bound by La protein predominantly via their UUU-OH 3′ end. The importance of La protein for proper trafficking and processing of tRNA precursors has been demonstrated in the Rich Maraia laboratory (Bethesda, MD, USA). They have identified a conserved nuclear retention element in the C-terminus of La. Deletion of this element causes the inappropriate cytoplasmic accumulation of La protein and the appearance of nonfunctional spliced tRNA precursors that retain their 5′ and 3′ flanking sequences. Interestingly, the same precursors have recently been found by the Geiduschek laboratory to accumulate in a mutant of the Bdp1 subunit of TFIIIB, indicating a link between the transcription machinery and the proper trafficking of pol III transcripts.

ROLE OF CHROMATIN IN POL I AND III TRANSCRIPTION

The role of chromatin has become a major focus of research in the transcription field, and pol I and III have not escaped this trend. Deciphering the chromatin state of rRNA genes in their on and off states is fraught with difficulty. Because they are multicopy genes, with only a fraction being active, nuclease accessibility or in vivo footprinting data cannot be interpreted unambiguously because both active and inactive genes contribute to the signals obtained. Electron microscopy can be used to examine the active gene repeats but the inactive copies cannot be distinguished from the bulk DNA. Nonetheless, electron microscopy and studies of accessibility to psoralen photo-cross-linking support the deduction that active rRNA genes are probably devoid of canonical nucleosomes, at least within their transcribed portions. Those histones and nucleosomes that are associated with rRNA genes are thought to occur on the inactive gene copies. An open question has been whether or not differences in chromatin structure dictate transcriptional activity or are a consequence of transcription. Marv Paule (Fort Collins, CO, USA) reported that using a highly purified reconstituted system, pol I starting at its natural promoter will transcribe through an array of at least four nucleosomes, apparently unhindered. In experiments involving templates with a single positioned nucleosome core particle, a single round of transcription by pol I was shown to be capable of stripping the core particle from the template. Furthermore, using the psoralen photo-cross-linking technique in live yeast, Paule reported that rRNA genes transcribed by pol I are accessible to cross-linking and are thus interpreted to be stripped of nucleosomes. Using a strain mutant for one of the pol I transcription factors, UAF (upstream activation factor), which causes pol I transcription to cease and rRNA transcription to occur by pol II from a normally cryptic promoter, the rRNA gene repeats

remain packaged in chromatin and are psoralen inaccesible. Thus, Paule and his colleagues conclude that it is pol I transcription, and not transcription in general, that strips nucleosome core particles from an rRNA gene template.

Topoisomerases are abundant non-histone chromatin proteins that appear to have a role in transcription of nucleosomal rRNA gene templates. Kostya Panov (Dundee, Scotland, UK) reported that human topoisomerase II alpha stimulated pol I transcription through a pair of nucleosomes but has no affect on transcription of naked templates. Consistent with this observation, topo II alpha inhibitors reduce transcription of chromatin templates, but not naked DNA templates. Furthermore, topo II alpha is found associated with the initiation competent form of Pol I, apparently via an interaction with Rrn3p/TIF-IA.

In yeast, Georgio Camillioni (Rome, Italy) found that topoisomerase I (top1) is associated with specific regions of yeast rRNA repeats, just upstream and downstream of the 35S rRNA primary transcript. The distribution of topoisomerase I is altered in strains mutated for subunits of the pol I transcription factor UAF, leading them to propose that top1p may associate with UAF.

Chromatin modifications including DNA methylation and histone acetylation play pivotal roles in a number of rDNA-associated phenomena. When certain species are crossed, the rRNA genes from only one parental species are actively transcribed in the hybrid; the others are silenced. This epigenetic phenomenon is called nucleolar dominance. Craig Pikaard (St. Louis, MO, USA) reported on the roles of methylation and histone deacetylation in the mechanism of nucleolar dominance. Pikaard's lab has shown that CpG methylation and histone deacetylation are partners in rRNA gene silencing. Either aza-deoxycytosine (an inhibitor of cytosine methylation) or trichostatin A (an inhibitor of histone deacetylation) will derepress the silenced set of rRNA genes in hybrid Brassica or Arabidopsis plants. Chromatin immunoprecipitation data show that the intergenic spacers (including the promoters) of active rRNA genes are hyperacetylated on histone H4; however, there is no analogous correlation between cytosine methylation density (at Hpa II sites) and silencing. In fact, analysis of natural variation for nucleolar dominance and cytosine methylation shows that uniparental silencing can occur among genes that have little or no methylation. These data suggest that histone acetylation status may be more important than cytosine methylation density in determining rRNA gene activity in nucleolar dominance.

Using chromatin immunoprecipitation (ChIP) assays, Ingrid Grummt (Heidelberg, FRG) reported that acetylated histone H4 is found associated with rRNA genes. In a clever approach, Grummt and her colleagues used the differential DNA methylation of the rRNA genes to distinguish between active and inactive copies. Her laboratory did the standard ChIP, but before amplifying the precipitated rDNA, they cut the promoter DNA with a methylation-sensitive restriction endonuclease. If cutting occurs at a site between the two PCR primers, no amplification results. Inactive rDNA copies were shown to be hypermethylated and endonuclease resistant at this promoter site whereas active copies were hypomethylated, allowing active and inactive promoters to be distinguished in the ChIP assays. They found that the acetylated H4 was associated with the active rDNA copies. Thus, at least one histone (H4) is associated with the promoter of active genes, though whether it does so as a subunit of a nucleosome remains unclear.

Grummt also provided insight into how histone and DNA modifications might be established and coordinated at rRNA gene promoters. Grummt's lab has identified a novel mammalian nucleolar chromatin remodeling complex (NoRC) that associates preferentially with inactive rDNA repeats methylated at a critical site in the promoter. Tip5p, the large subunit of NoRC, interacts with both DNA methylytransferase (Dnmt 1) and with a histone deacetylase (HDAC1), as well as with the transcription termination factor TTF-1. The latter interaction is thought to recruit NoRC to the rRNA promoter region via the promoter-proximal TTF-1 binding site such that Dnmt1 and HDAC1 might then accomplish the DNA and histone modifications needed to silence the promoters. Coupled with studies of nucleolar dominance and published work from the Moss and Rothblum laboratories showing a mutually exclusive recruitment of CBP (a histone acetyltransferase) and retinoblastoma protein (Rb, a transcriptional repressor) by UBF, these investigations provide a foundation for understanding the role of chromatin modifications in rRNA transcription.

Sam Jacob (Columbus, OH) presented data suggesting that human rRNA gene promoter methylation plays a role in human proliferative diseases such as cancer. There is an abundance of CpG residues in the human rRNA gene promoter compared to rodent promoters. In various human hepatocarcinoma cell lines, the rRNA genes are undermethylated relative to matching liver tissue, perhaps contributing to rRNA overexpression in cancer cells. Jacob's group used bisulfite genomic sequencing to show that two of five stretches of demethylated CpGs in tumors are located in the minimal "core" promoter region, one is located in the upstream promoter domain (UCE) and two are upstream of the UCE. RT-PCR data showed a marked correlation between elevated levels of ribosomal gene transcription and demethylation of CpGs within rRNA

promoters of hepatomas. Jacob also showed that methylation at specific sites in the promoter causes significant reductions in transcription upon transfection into cultured hepatoma cells. Using a combination of chromatin immunoprecipitation (ChIP) and DNA transfection, specific methyl C-binding proteins (MBDs) and DNA methyltransferases were implicated in regulating rRNA promoter activity.

Brian McStay (Dundee, Scotland, UK) presented evidence that the pol I transactivator protein UBF helps define the chromatin state at active or potentially active NORs in vertebrates. Using quantitative immunoblotting, McStay and colleagues have estimated that there are approximately 1 million molecules of UBF in a vertebrate nucleus, all of which appear to be associated with NORs based on antibody staining. UBF in such abundance could occupy the entire length of every rRNA gene at an NOR, including both coding and noncoding intergenic spacer sequences. Indeed, using the chromatin immunoprecipitation technique with anti-UBF antibodies, UBF was shown to be associated with all sequences of an rRNA gene repeat in both *Xenopus* and human. By contrast, subunits of the pol I transcription factor SL1/TIF-IB were only found in association with the promoter. Likewise, subunits of pol I were only found associated with the promoter and transcribed coding portions of the human rRNA gene. In human cells, not all of the 10 NORs are typically active. Silent NORs that fail to form the secondary constrictions characteristic of active NORs at metaphase are also devoid of UBF. At NORs where UBF is associated, chromatin immunoprecipitation data suggest that the density of UBF appears to be highest just upstream and downstream of the transcribed coding sequences. McStay suggested that this might be explained by overlapping signals from two classes of UBF-associated genes. A potentially active class might have UBF evenly distributed throughout its length. A second, active class, might have UBF displaced from regions transcribed by pol I but UBF occupancy of flanking sequences would persist.

Given that UBF is known to be regulated by numerous posttranslational modifications and protein–protein interactions, McStay cautioned that it will be important to determine where UBF molecules are located along the rRNA gene repeats when they experience these modifications or interactions.

CHROMOSOME POSITION EFFECTS AND BOUNDARIES INFLUENCING POL I- AND POL III-TRANSCRIBED GENES

Several talks explored the relevance of chromosomal context on gene function. Melanie Oakes (Riverside, CA, USA) reported on experiments in the Nomura lab in which a clever assortment of genetic tools were combined to eliminate the single NOR from its endogenous location in yeast and then to reestablish an NOR at new chromosomal locations. Both physiological tests and cytogenetic analyses showed that the newly established NORs were fully functional for ribosome production and nucleolus formation, suggesting that there is nothing external to the NOR at its usual location that is essential for NOR function.

Lewis and Pikaard (St. Louis, MO, USA) examined the extent of chromosome silencing in nucleolar dominance and showed that gene silencing in Arabidopsis hybrids is restricted to the NORs and does not spread to adjacent protein-coding genes as near as 3 kb. A possibility is that barrier elements at the junction between the NORs and flanking genes may play a role in limiting silencing to the NOR. To test the possibility that rRNA genes themselves are the targets of silencing, rRNA transgenes located at ectopic positions were examined in hybrids. These transgenes escaped silencing, despite the silencing of the endogenous NORs, suggesting that nucleolar dominance mechanisms act on the NOR, or its chromosomal location, rather than on individual rRNA genes.

Mechanistic insight into chromatin boundary function in yeast was presented by David Donze (Baton Rouge, LA, USA). Donze and Kamakaka first demonstrated the ability of certain tRNA genes to function as boundary elements to prevent the spread of transcriptionally silent heterochromatin from the silent mating type locus, HMR in yeast. New work from the Donze laboratory (Baton Rouge, LA, USA) showed that the boundary activity of the wild-type tRNAThr[AGT] gene found at HMR is strongly dependent on the HMG1-like non-histone proteins, Nhp6A and Nhp6B. This link to Nhp6 is intriguing given previous work showing that the essential function of Nhp6 in yeast is to facilitate *SNR6* transcription by pol III. Because the role of Nhp6 in *SNR6* transcription is specific and related to the suboptimally spaced promoter elements, the new observations suggest a novel function for Nhp6 in the boundary activity of tRNA genes.

INVOLVEMENT OF POL II TRANSCRIPTION FACTORS IN POL I AND POL III TRANSCRIPTION

Several well-known proteins involved in pol II transcription have emerged as players in pol I and pol III transcription. In a yeast two-hybrid screen Lucio Comai (Los Angeles, CA, USA) identified the largest human TBP-associated factor (TAF) of the pol II

transcription factor TFIID as an interacting partner with UBF. This protein, TAF1 (formerly $TAF_{II}250$), has an N-terminal kinase domain that can phosphorylate UBF in vitro. A portion of the cellular pool of TAF1 is found in the nucleolus and recombinant TAF1 protein can stimulate transcription in vitro and in transfection assays, supporting a role for this pol II factor in pol I transcription.

A recent paper resulting from a collaboration between the Grummt, Tschochner, and Egly laboratories demonstrated that the pol II transcription factor TFIIH plays an essential, if unexpected, role in activating pol I transcription. In the pol II system, TFIIH plays a role in phosphorylation of the C-terminal domain (CTD) of the largest polymerase subunit and also includes activities involved in DNA repair, probably allowing the preferential repair of the template strand following DNA damage. At the meeting, Herbert Tschochner followed up on this story with data implicating the phosphatase Fcp1p in modulating pol I phosphorylation. Interestingly, Fcp1p has been identified in yeast and mammalian cells as the phosphatase responsible for dephosphorylating the pol II CTD. In yeast, Fcp1p appears to associate with pol I and in a strain carrying a temperature-sensitive Fcp1p gene, pre-rRNA synthesis declines within 15 min upon shifting the cells to the nonpermissive temperature. Recombinant Fcp1p, but not mutant Fcp1p, can stimulate pol I activity in vitro. Collectively these data suggest that both TFIIH and Fcp1 are involved in both pol II and pol I transcription, though their mechanisms of action in the pol I system remain to be elucidated.

The involvement of a well-known pol II activator in the upregulation of tRNA gene transcription by pol III was presented by Robert White (Glasgow, UK). The proto-oncogene c-Myc is known to stimulate cell growth and cell proliferation, though it is not entirely clear how. White's lab has found that c-Myc expression causes a rapid induction of tRNA gene transcription in human fibroblasts and that targeted disruption of c-Myc has the opposite effect, downregulating tRNA genes. Chromatin immunoprecipitation experiments showed that c-Myc is localized at tRNA genes and at 5S rRNA genes, most likely via its interactions with the pol III-specific transcription factor TFIIIB. Though c-Myc can bind DNA directly, consensus binding sites for c-Myc are absent from these tRNA and 5S RNA gene promoters. White and his colleagues speculate that the increased expression of tRNAs is one way in which c-Myc stimulates cell proliferation, namely by increasing the protein synthetic capacity of the cell.

THOUGHTS FOR THE FUTURE

Considerable progress has been made in the past 2 years, especially in the areas of growth regulation, signaling, chromatin, and the involvement of transcription factors shared by polymerases I, II, and III. Most likely, these areas of endeavor will provide even greater insights in the near future. As is true for the pol II transcription field, chromatin immunoprecipitation is proving to be an invaluable technique, especially for discriminating between active and inactive genes of multicopy rRNA gene families. When RNA polymerase I and III researchers meet again at Asilomar in 2004, we can expect to learn much more about the proteins interacting with important regulatory DNA sequences and with each other.

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The authors apologize to our colleagues at the meeting whose work was not cited due to our attempt to focus on the most common or recurrent themes.