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MEETING REVIEW

Polymerase I transcription, termination, and processing

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The second meeting devoted exclusively to transcription by eukaryotic RNA polymerase I (pol I) was held August 6–9, 1992, in Lake Bluff, Illinois. A dozen years ago the development of in vitro transcription systems led to a general understanding of the mechanisms of both rDNA initiation and regulation. However, surprising new findings suggest the need for reevaluation of some of these established concepts. This review will attempt to highlight new information revealed at the meeting, and to put it into the context of material previously published or in press.

Transcription factors

Three proteins, pol I, UBF, and TIF-IB, are known to serve important and possibly essential roles in the transcription of ribosomal RNA genes in all eukaryotes. However, the exact number and mechanistic role of specific transcription factors in initiation and regulation of ribosomal RNA transcription continues to be an area of controversy. One problem for the casual reader is the lack of unified nomenclature for the various factors. Table 1 lists the names given to the purified factors or partially purified fractions and shows the probable relationships among them. In the table, related factors are grouped under the single term which will be used in this review.

TIF-IB is the factor that confers species specificity on ribosomal RNA gene transcription (Grummt et al., 1982). The ribosomal DNA promoter and TIF-IB must be from the same species for correct initiation. The core promoter, which binds TIF-IB, extends from approximately 50 base pairs upstream (−50) to a few nucleotides downstream of the transcription initiation site and is the minimal sequence needed for correct transcription initiation. TIF-IB also binds to the upstream control element (UCE), normally found between about −75 and −150. The UCE stimulates the core promoter. Promoter elements are reviewed by Reeder (1989).

TIF-IB is believed to be the fundamental transcription factor for RNA pol I. That is, it appears to serve a role akin to TFIIIB for RNA pol III (Paule 1990) and TFIID for RNA pol II (Pugh and Tjian, 1992). Supporting this notion, several groups—Comai et al. (1992), Grummt (Heidelberg), McStay (Seattle), Paule (Fort Collins)—reported that highly purified TIF-IB contains the subunit TBP, originally identified in the pol II transcription factor TFIID, the TATA box-binding factor. The minimum complex that commits an rRNA gene to transcription contains TIF-IB bound to the core promoter. Geiss (Fort Collins) reported that this committed complex could be super-shifted in an electrophoretic mobility retardation assay by the addition of antibody against Acanthamoeba TBP. A similar result has been obtained for the committed complex for yeast pol III transcription containing only TFIIIB (E. P. Geiduschek, personal communication); thus this subunit is present in the fundamental transcription complex of all three eukaryotic polymerases.

Despite the presence of TBP-containing factors, TBP is assembled differently in the three

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Table 1. Ribosomal DNA transcription factor nomenclature.

Core promoter/UCE binding	
TIF-IB (probably the fundamental transcription factor)	
TIF-I (usually abbreviated TIF)[1]	SL1[9]
TIF-IB[2]	SL-1[6]
Factor D[4,3]	RIB1[7]
TFID[4]	CPBF[8]
UBF	
UBF[7,9]	
Other factors reportedly required for initiation	
TIF-IC[2]	
TIF-IA[2]	
Enhancer-binding factors	
p16[6]	
E_1BF[8]	
RIB1[7]	
UBF[7]	
REB1[10]	
Termination factor	
TTF-I[2]	
RIB2[7]	
(REB1)[7,10]	
Regulatory components	
Active RNA pol I (Pol_A)/encystment RNA pol I (Pol_E)[1]	
TIF-IA[2]	
Factor C (pol I + C*)[3,4]	
TFIC[5]	
UBF[2,6]	

[1] Paule (Acanthamoeba)
[2] Grummt (mouse)
[3] Sollner-Webb (mouse)
[4] Muramatsu (rat, human)
[5] Thompson (mouse)
[6] Rothblum (rat)
[7] Reeder (Xenopus)
[8] Jacob (rat)
[9] Tjian (human, mouse, rat)
[10] Warner (Saccharomyces)

systems. Schultz (Seattle) tested a series of TBP mutants obtained from Steven Hahn in yeast in vitro transcription systems. The findings are revealing, in that some TBP mutants result in significant decreases in transcription from all three polymerase systems, while other mutations affect the RNA pol II and III systems without affecting pol I transcription. In each case, the addition of wild-type TBP can rescue the transcription inhibition effects. In the pol I system, however, simple addition of TBP is not sufficient. The system had to be warmed to 30°C for several minutes before rescue could occur. Other mutations that prevent TBP from binding to the TATA box cannot rescue pol II transcription. However, they do rescue pol I and III transcription in the in vitro systems. Furthermore, some TBP mutants with the same phenotype are even able to rescue in vitro transcription of the *cyc1* pol II promoter. Therefore, TBP can be recruited into transcription complexes by a variety of mechanisms and in a variety of configurations, and its functional role may be different in the three systems.

UBF is the second transcription factor to be identified in all systems under study. After its cloning from a few vertebrate systems several years ago, this factor has been extensively examined by Tjian (Berkeley), Reeder (Seattle), Rothblum (Danville), Moss (Quebec), and Grummt (Heidelberg). UBF binds to the UCE, probably to the core promoter, and to the repeated sequence elements that occur within the intergenic spacer (IGS) of ribosomal RNA genes. The latter repeated elements serve as transcriptional enhancers (Reeder, 1984 and 1989). Thus UBF serves a dual function—both as an enhancer-binding protein and as a general transcription factor.

UBF had only been reported in a number of relatively closely related vertebrates (human, mouse, rat, and Xenopus). However, Yang and Radebaugh (Fort Collins) now report the identification of a homologue in the small free-living amoeba, Acanthamoeba castellanii. It binds to the repeated Acanthamoeba IGS elements, enhances transcription, and strongly cross-reacts with antibodies against rat UBF. It is somewhat larger (125 kDa) than the vertebrate UBFs (which range from 85 to 100 kDa) and does not bind the core promoter alone. Finding UBF in this lower eukaryote, therefore, appears to verify it as a ubiquitous component of the ribosomal gene transcription system.

Many published structure–function studies of UBF have shown it to consist of the following domains (starting at the N-terminus): a helix-gap-helix domain involved in dimerization, a basic region, a hinge region with high proline content, three to five DNA-binding domains with homology to the HMG1 DNA-binding sequence, and an acidic amino acid-rich tail containing a number of serine residues. In vertebrates, two forms of UBF have been identified (UBF1 and UBF2). These differ from one another by a small deletion within one of the HMG boxes. They are found in approximately equal amounts from most sources (however, see Regulation of rDNA Transcription below). Surprisingly, both Rothblum (Danville) and Grummt (Heidelberg) report that the smaller form, UBF2, is inactive on ribosomal RNA genes from rat and mouse, respectively. Since the two forms apparently arise by alternative splicing, and UBF is pres-

ent in the cell at a level approximately 100-fold higher than TIF-IB, one might suspect alternative functions for UBF2 in the cell. Immunological studies, however, show UBF predominantly in the nucleolus.

The mechanism by which UBF binds to DNA is under intensive study in several laboratories. Proteins containing the HMG box DNA-binding domain are believed to bind to specific DNA structures rather than specific sequences (see the review by Lilley, 1992). Hu (Seattle) presented evidence suggesting that UBF fits this model. Truncated UBF containing N-terminal sequence through the first HMG box binds to DNA. Each HMG box in the truncated UBF dimer appears capable of binding a separate double-stranded DNA molecule. Such complexes appear to require a fair amount of flexibility at the bifurcation point of the protein dimer. Substitution of the four prolines in this region with alanines, thereby allowing the formation of α-helix through this region, eliminates the formation of complexes attributable to the HMG box. A specific DNA sequence is not necessary for such binding, as demonstrated by strong binding of this truncated UBF to supercoiled nonribosomal DNAs. These presumably contain many double helical crossover points believed to be the DNA structure recognized by the HMG box (Lilley 1992). Results presented by Copenhaver and Pikaard (St. Louis) are consistent with UBF binding to double helical crossovers if one also assumes a degree of primary sequence specificity. Using an electrophoretic mobility retardation assay, Copenhaver found that long DNAs were retarded an additional increment for each pair of enhancer elements added to the DNA molecule (four enhancers gave two shifted bands, ten enhancers gave five shifted bands, etc.). This is consistent with the notion that two enhancers can form a double-stranded crossover point and bind an additional UBF.

Rothblum (Danville) reviewed data showing that half-helical and full-helical turn spacing changes between the core promoter element and UCE lead to cyclical inhibition of transcription. This suggests a need for loops or bends to form in the DNA, allowing proteins bound to one helical face of the UCE to contact proteins bound to an appropriately juxtaposed helical face of the core promoter. Similar data has been obtained by Sollner-Webb (Baltimore) when transcribing the Xenopus gene in an in vitro transcription system from mouse. If UBF can bind two double-stranded regions of the same DNA molecule, perhaps it can induce DNA loops.

One functional role of UBF1 is to augment the binding of TIF-IB to the core promoter. However, whether it is a mandatory assembly factor for TIF-IB or only stimulates its association is controversial. Rothblum (Danville) and Grummt (Heidelberg) presented convincing arguments that in the rat and mouse, respectively, UBF is not required for specific transcription. The validity of these experiments depends upon assurance that UBF does not contaminate any other fraction that is utilized to assemble the transcription system, and this is difficult since UBF commonly contaminates pol I preparations (see below). Rothblum has tested the rat pol I fraction for contaminating UBF utilizing a sensitive antibody that can detect as little as 2.7 fmoles of UBF. None was detected. Further, Jacob (North Chicago) has shown that a fraction from rat that is devoid of UBF can direct rDNA transcription in vitro. In contrast, it is argued from similar studies that UBF is absolutely required for TIF-IB binding in human (Bell et al., 1988), Xenopus (Reeder, Seattle), and Acanthamoeba (Paule, Fort Collins). These latter experiments can be criticized because low DNA concentrations were utilized for gel shift and footprinting, and the equilibrium would have been shifted away from TIF-IB–DNA complex formation. Thus, the question of whether UBF is mandatory remains unanswered. It is critical, for reasons discussed below, that this be determined.

Recruitment of pol I to the promoter is directed by specific protein–protein interactions with the components in the committed complex (Kownin et al., 1987), and it is not known whether these involve TIF-IB alone, or UBF as well. Grummt (Heidelberg) reported that columns containing immobilized pol I will retain UBF preferentially. Radiolabeled UBF can be immunoprecipitated along with pol I using antibodies against pol I. Riva (Saclay) used radiolabeled UBF to show that it binds to yeast RNA pol I subunit A34.5 and yeast RNA pol III subunit C53 when these proteins have been blotted to nitrocellulose from an SDS polyacrylamide gel and renatured (Far Western blot). The

A34.5 and C53 subunits have no known homology or similar amino acid sequences. Thus, the functional significance of these interactions is not clear, but they could signal UBF–pol I interactions in the initiation complex.

An understanding of the exact mechanism by which UBF affects the initiation complex assembly process and whether UBF is present in the committed complex in all species is important because of the potential role of UBF in regulating ribosomal RNA gene transcription. Reeder was the first to elaborate on the notion that ribosomal RNA gene transcription is potentially regulated at more than one step, i.e., during committed complex formation and during initiation of each round of transcription. The assembly of the committed complex is mediated by ribosomal DNA enhancers and their binding protein, UBF. Regulatory mechanisms involving RNA pol I or a tightly associated factor are well documented (see below), so possible involvement of both mechanisms must be considered.

Several recent observations by Rothblum and by Grummt suggest an as yet undiscovered role of UBF in regulation. UBF exists in both phosphorylated and dephosphorylated forms (O'Mahony et al., 1992; Voit et al., 1992). The phosphorylated form predominates in actively proliferating cells, and both forms bind DNA. Only the phosphorylated form is active in stimulating ribosomal RNA gene transcription in vitro. Thus, phosphorylated UBF is clearly associated with active transcription, and phosphorylation levels might alter committed complex levels. However, the finding that the number of ribosomal RNA gene copies assembled into committed complexes does not change between rapidly proliferating and stationary cells shows that this mechanism is not involved in growth rate-dependent regulation (see below). Furthermore, Paule (Fort Collins) reported that the source of the UBF-containing fraction, transcriptionally active vegetative or inactive cysts of Acanthamoeba, has no effect upon transcription efficiency in vitro. In contrast, the activity of the pol I fraction parallels in vivo transcription rates. Therefore, phosphorylated UBF does not appear to function in a regulatory manner at this level.

UBF is phosphorylated in three regions: two sites near the N-terminus and multiple sites within the acidic tail. The kinase which phosphorylates the acidic tail and one of the N-terminal sites has characteristics of casein kinase II (5,6-dichloro-1-β-D-ribofuranosylbenzimidazole [DRB] and heparin sensitivity; ATP and GTP serve as substrates). Pikaard (St. Louis) presented preliminary evidence that UBF might also be able to phosphorylate itself. Xenopus UBF cloned and expressed in E. coli autophosphorylates, even following resolution from contaminants on SDS-polyacrylamide gels, blotting to nitrocellulose, and renaturation on the filter. The preparation does not phosphorylate added casein or histone H1, suggesting a lack of contamination with general protein kinases. The kinase activity is DRB-sensitive. If UBF can self-phosphorylate, and if it is present in the committed complex, one might speculate that a UBF phosphorylation event occurs during each round of initiation. This might be similar to phosphorylation of the C-terminal domain in pol II and could lead to increases in phosphorylated UBF in active cells. This is unlikely, because Paule (Fort Collins) presented data clearly showing that phosphorylation does not occur during multiple rounds of transcription by pol I.

In addition to phosphorylation state changes, the level of UBF and the ratio of UBF1 to UBF2 vary. In L6 cells undergoing differentiation from myoblasts into myotubes, ribosomal RNA gene transcription is drastically reduced. Surprisingly, Rothblum (Danville) reported that the amount of UBF detected in extracts of cells undergoing this transition also decreases drastically. Such changes in the amount of UBF do not normally occur in cells entering stationary phase, such as CHO cells. Grummt (Heidelberg) reported changes in the ratio of UBF1 to UBF2 mRNAs. Both entry of cultured mouse cells into stationary phase and progression from ten to seventeen days of mouse embryogenesis resulted in a change in ratio from approximately 1:1 to 1:3.5.

Repressor proteins and chromatin

Another puzzle that is beginning to be resolved concerns why ribosomal DNA enhancers exhibit great stimulation (100X) in vivo, but exhibit considerably less stimulation in vitro, especially in highly purified systems. Kuhn (Heidelberg) has identified a mouse transcriptional repressor consisting of 91 and 74 kDa polypeptides that might compete with TIF-IB for the

promoter. The repressor binds near the transcription initiation site, protecting position +6 and creating a hypersensitive site at −9 in DNase I footprinting. Addition of UBF to the assembly mix prevents or overcomes the repression, so the difference between basal level and activated transcription approaches that seen in vivo. Both electrophoretic mobility retardation and UV cross-linking suggest that the mouse inhibitor is related to the rat factor E_1BF characterized by Jacob (North Chicago). This protein can either stimulate or, at high concentrations, inhibit pol I transcription. Li (Fort Collins) has also identified an inhibitory protein whose DNase footprint (−60 to −90) overlaps the region protected in the committed complex of Acanthamoeba (−12 to −67). In addition, Paule's group in collaboration with Georgel and Van Holde (Corvallis) has identified a strong nucleosome positioning sequence in the core promoter of the Acanthamoeba ribosomal RNA gene. The positioning sequence is strong enough to compete effectively with nucleosome positioning sequences present in the 5S RNA gene of L. variegatus placed in multiple copy immediately downstream of the promoter. RNA pol I, initiated at this promoter and stalled at +8 before nucleosome assembly, was shown capable of reading through downstream nucleosomes. Under these conditions, the nucleosomes are properly positioned on the L. variegatus 5S RNA genes. However, the placement of a nucleosome directly over the promoter is expected to repress preinitiation complex formation. Thus, the repressor proteins and nucleosomes decrease basal level transcription by a mechanism that may be modulated by UBF.

These results indirectly support a regulatory mechanism modulating the number of ribosomal RNA genes assembled into committed complexes. Regulation at this level would be modulated by the amount of UBF present in the cells, by a change in the phosphorylation state of UBF, or by alteration of repressor levels. However, this notion contrasts with studies of the chromatin state of ribosomal RNA genes in proliferating versus stationary cells. Increased susceptibility to psoralen cross-linking and nuclease sensitivity is believed to reflect active assembly. Estimates of the number of active genes show no variation with growth rate (Conconi et al., 1989; Conconi et al., 1992). This contrasts with regulation in pol II systems in which turning on or off individual genes is the norm. In rRNA gene transcription, all of the factors, and even pol I itself, are dedicated solely to expression of rRNA. Therefore, regulation can target any of the components without affecting other genes.

Yeast enhancer

It is informative to compare the role of UBF, which binds to the enhancer elements of higher organisms, with proteins that bind to the enhancer element of yeast. The yeast ribosomal DNA enhancer is a region of approximately 200 base pairs that stimulates transcription from both upstream and downstream ribosomal RNA genes (Elion and Warner, 1986). Warner (Albert Einstein, Bronx) pointed out that this contrasts with pol II enhancers of yeast, which stimulate only downstream genes. Warner reported that the rDNA enhancer is required for rDNA transcription to respond to an upshift in growth conditions. The enhancer binds at least two proteins: REB1 and ABF1. Warner reported that REB1 also binds strongly to an element immediately adjacent to or part of the rDNA promoter. Warner has cloned the gene for REB1 (Ju et al., 1990). It is unrelated to UBF. Two tryptophane-rich regions with strong sequence similarities to the *myb* oncogene are apparently required for enhancer binding. REB1 has been identified independently as an RNA pol II transcription factor (GRF2) and shown to block the formation of nucleosomes over its binding site. Planta (Amsterdam) has developed an in vivo assay using a construct containing two copies of a sequence-tagged ribosomal RNA gene transcription unit flanking an intergenic spacer. The tagged rRNAs allow analysis of their transcriptional activity without interference from the endogenous genes. This construct was integrated into the ribosomal DNA repeats so that transcription could be analyzed in the chromosomal context. Under these conditions, the integrated ribosomal RNA genes exhibited activities approximately equaling those of the natural genes. Deletion of either of the REB1 binding sites or of the enhancer led to a significant decrease in the transcriptional activity of both the upstream and downstream genes. Deletion of any other portion of the IGS had no effect, indicating that only the enhancer and the REB1 binding sites are transcriptional regulatory ele-

ments in the intergenic spacer. Planta suggested that the role of these binding sites is to form DNA loops which juxtapose the promoter of one ribosomal DNA transcription unit with the terminator for another. (Termination also occurs within the yeast 200 bp enhancer element.) However, Schultz (Seattle) reported that the yeast enhancer can be separated endonucleolytically from the promoter following committed complex assembly in vitro without decreasing enhancement of subsequent transcription. This seems inconsistent with a mechanism involving terminator/promoter juxtaposition, but it may reflect the difference between in vitro and in vivo assays. Alternatively, the enhancer may function by several mechanisms.

The similarities between these results and those discussed above for UBF, though not compelling, should be kept in mind when devising mechanisms for ribosomal DNA enhancers. Both REB1 and UBF have multiple structural domains involved in DNA binding. Both proteins bind to sites found reiterated in the intergenic spacer and in the gene promoter. Thus DNA loops could be formed by enhancer factor binding. In contrast, UBF is able to stimulate transcription in genes containing only the core promoter, thus eliminating sequence-specific DNA loop formation. Perhaps the two enhancer binding proteins operate by different mechanisms, or it is not DNA looping per se which is important, but the resulting protein–protein interactions.

Other factors

In addition to TIF-IB and UBF, several other proteins affecting rDNA transcription have been identified. Jacob (North Chicago) described E_1BF as a factor that binds three sites: upstream and downstream of the rat transcription initiation site (−48 to +54), to a nonrepeated enhancer element (−2183 to −2357), and to a 130 bp repeated enhancer element. It is a 72–85 kDa heterodimer and is structurally distinct from rat UBF, despite their functional similarity. There is 10-fold more E_1BF in hepatoma cells than in liver, suggesting it may play a role in regulation.

E_1BF stimulates binding of another factor, CPBF, which appears to function like TIF-IB. CPBF does not appear to contain TBP, since it does not react with human anti-TBP antibodies. TBP may, however, be present via the HeLa nuclear extract used to transcribe rat rDNA. Highly purified CPBF preparations contain 130, 44, and 39 kDa polypeptides and footprint −22 to +3 in the rat promoter.

Grummt (Heidelberg) has resolved two unique factors from the mouse pol I fraction, TIF-IA and TIF-IC. Both are required for transcription initiation. They preassemble onto pol I before it joins the committed complex; alternatively, they assemble onto the initiation complex after pol I is bound. Their presence increases sarcosyl resistance from 0.005% to 0.045%. TIF-IA appears to be regulated (see below). Sollner-Webb (Baltimore) and Thompson (Galveston) have identified a fraction similar to TIF-IA, also from mouse, but TIF-IC remains unique to Grummt's laboratory.

Genetic approaches to rDNA transcription analysis

Nomura (Irvine) reported on the pioneering genetic studies being carried out in Saccharomyces (Nogi et al., 1991). The basis of these studies is a plasmid (pNOY) in which an rRNA gene is fused to the regulated GAL7 promoter (a pol II promoter). Yeast in which the gene for the second largest pol I subunit has been deleted, but which contains pNOY, can grow normally when the GAL7 promoter is induced (+ galactose), but not when it is suppressed (+ glucose). This shows that, as has been suspected for years, pol I has no other role in the cell than to transcribe rRNA genes. However, this conclusion must be modified to exclude the special case of trypanosomes (see below).

Saccharomyces mutants affecting rRNA gene transcription can be suppressed by plasmid pNOY, and Nomura and Keys (Irvine) have isolated ten such mutants. Three are pol I subunit genes (RPA190, RPA135, RPA12.2); another encodes a nuclear envelope protein; and two (*RRN*3 and *RRN*6) are potentially transcription factor genes. Extracts made from *RRN*3 or *RRN*6 mutant cells are transcriptionally inactive in vitro but can be complemented by fractions from wild-type extracts.

Muramatsu (Saitama, Japan) has cloned the cDNA for the 40 kDa subunit of pol I from mouse. The mRNA of approximately 1400 nt arises from a single gene and is 44% identical and 65% homologous to the Saccharomyces

AC40 gene. This gene encodes one of the subunits which is homologous to the α subunit of E. coli (Sawadogo and Sentenac, 1990). Surprisingly, the mouse gene can be substituted into yeast with a disrupted AC40 gene without significant effect upon growth. This shows the strong conservation of this subunit in the polymerase and opens the door to future analysis of this subunit's functions, even when isolated from sources other than yeast.

Pol I transcribes protein genes in trypanosomes

Trypanosomes, a group of insect and mammalian parasites, utilize pol I to express a family of coat proteins (variable surface glycoprotein, VSG, in the bloodstream and procyclin, PARP, in the insect). The primary transcripts are initially uncapped, but are made competent for translation by receiving a cap by a trans-splicing mechanism. Van der Ploeg (Rahway, Merck) and Clayton (Heidelberg, ZMBH) presented compelling evidence that both VSG and PARP genes are transcribed by pol I. This is the first demonstration of mRNA transcription by pol I. Presumably it only works because of the trans-splicing mechanism allowing capping, a process usually cotranscriptional with pol II. A PARP promoter/*neo* fusion integrated at an αβ-tubulin locus was localized by in situ hybridization to the nucleolus, suggesting that the pol I promoter, or pol I itself, serves an important role in nucleolar organization.

Regulation of rDNA transcription

There is general agreement that the primary mechanism of regulation of rDNA transcription involves modification of pol I or of a transcription factor tightly associated with it. Grummt first showed (1981) that proliferation rate-dependent regulation of rDNA transcription could be reproduced in vitro using mouse extracts. Paule then demonstrated that it was the pol I fraction that lost activity when Acanthamoeba entered stationary phase (cysts). This activity loss could be reproduced with homogeneous pol I, and the enzymes purified from growing cells and cysts had different heat stabilities (Paule et al., 1984). This led Paule to conclude that pol I itself was modified. Modification prevents the inactive polymerase from binding to the promoter. He reported that recent structural studies have demonstrated changes in the electrophoretic mobility of the 39 kDa subunit that correlate with both down and up regulation of rDNA transcription. This subunit is the homologue of the yeast AC40 subunit and of the E. coli α subunit. The latter has recently been implicated in making direct contact with several regulatory proteins, leading to activation of the bacterial polymerase (reviewed in Russo and Silhavy, 1992). Paule also reported that a monoclonal antibody directed against the native form of Acanthamoeba AC39 inhibits specific rDNA transcription, but a similar monoclonal antibody which reacts only with the denatured form of the AC39 has no effect. Neither antibody inhibits nonspecific transcription or elongation, suggesting a specific effect upon the interaction of polymerase with transcription factor(s) bound at the promoter.

Grummt has a different view of how regulation is accomplished (Schnapp et al., 1990). Her group has been able to separate the mouse polymerase fraction into three components: pol I proper and two factors, TIF-IA and TIF-IC. The first factor has been implicated in regulation, since its addition to inactive extracts can reactivate them. The relationship between this finding and those in the Acanthamoeba system is not clear, but distinguishing between a modified polymerase subunit and a distinct factor will have to await the cloning of the regulated component. One important difference between the modes of regulation is the stage that seems to be blocked by the inactive component. In Acanthamoeba, polymerase will not bind to the promoter when in its inactive state. In mouse, polymerase will bind, but it cannot initiate without the subsequent binding of TIF-IA. Sollner-Webb and Poretta (Baltimore) reported that a TIF-IA-like factor is consumed during the earliest stages of rDNA transcription in mouse. Loss of TIF-IA activity requires active transcription, with loss occurring after approximately 40 residues have been polymerized into RNA.

Pellegrini (Los Angeles, USC) reported on the regulation of rDNA transcription in Drosophila. Drugs which alter intracellular calcium (TPA, calcium ionophores) or affect calmodulin activity—but not cyclic nucleotides—alter rDNA transcription. This implicates the protein kinase C pathway. Extracts from Schneider's line-11 mimic serum regulation. Regulation requires

core and UCE promoter sequences only. As above, initial fractionation of the extracts reveals correspondence of the regulated component with the polymerase fraction. It is unlikely, however, that PKC directly participates in polymerase or factor modification. Except for phosphorylation of UBF, extensive investigation has revealed no other component of the pol I system that requires phosphorylation for activity.

Processing

The primary processing site for rRNA occurs a few hundred nucleotides from the 5′ end (mouse, 651; human, 414; Xenopus, 105). Sollner-Webb (Baltimore) reported that only the 25 nucleotides surrounding the site are required, though efficiency is low with this minimal sequence. Assembly of the processing complex is cotranscriptional. Sollner-Webb presented compelling evidence that the terminal knobs observed in electron micrographs of rRNA transcription complexes are the processing complex. She showed that this complex has as its core U3 snRNA. However, U3 has no complementarity to the processing site.

Termination

Transcription termination by pol I is signaled by a short sequence—the Sal box in mouse (Kuhn et al., 1988), T3 in Xenopus (Labhart and Reeder, 1987). Labhart (San Diego, Scripps) created mismatch mutants in the Xenopus terminator using synthetic DNA. Any sequence alteration in the double-stranded DNA eliminated termination, even if the sequence of the resulting RNA transcript was normal. This agrees with the notion that a DNA-binding factor, TTF-I, not the RNA sequence, causes termination. However, based upon carefully controlled oligonucleotide competition experiments, the amount of TTF-I in extracts seems impossibly large unless it binds to the template by a mechanism coupled to active transcription. Planta (Amsterdam) commented that in Saccharomyces the pol I preparation used affects termination efficiency. This may be related to Labhart's finding. Perhaps TTF-I or a TTF-I loading factor moves along the DNA with pol I until the termination sequence is encountered. Schultz (Seattle) reported that in Saccharomyces only the REB1 binding site and a few surrounding nucleotides are needed for termination in vitro, and competing REB1-binding oligonucleotide inhibits it. However, inhibition could not be overcome by added recombinant REB1 from Warner (Albert Einstein), and extracts containing temperature-sensitive REB1 still terminate. Thus, it is still not certain that REB1 is both an enhancer and a termination factor.

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