

At the Center of Eukaryotic Life

Minireview

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The ribosomal RNA genes encode the enzymatic scaffold of the ribosome and thereby perform perhaps the most basic of all housekeeping functions. However, recent data suggests that they might also control important aspects of cell behavior.

An actively cycling eukaryotic cell expends between 35% and 60% of its total nuclear transcription effort in making the 18S, 5.8S, and 28S ribosomal RNAs (rRNA) (Paule, 1998, and references therein). The 5S rRNA and the small nucleolar RNAs required for ribosome biogenesis, account for another 10% to 20%. Thus, the assembly of the translational machinery occupies around 80% of nuclear transcription in yeast, while in the proliferating mammalian cell as much as 50% is dedicated to this goal. Even relatively small changes in this commitment are likely to have extensive repercussions on the cell's economy, limiting proliferation rates and perhaps even cell fate. From the earliest steps in cell differentiation through to senescence and from rapid proliferation to contact- or nutrient-limited growth, cellular changes in model systems are accompanied by a regulation of ribosome biogenesis and in particular of rRNA synthesis (see Stefanovsky et al., 2001, and references therein). Though little is known of the changes that occur *in vivo*, one would suspect that, given the longevity of ribosomes and the highly variable proliferation rates of different somatic cell types, rRNA transcription rates must be regulated over a wide range if neither a ribosome deficit nor an overproduction is to occur.

The nucleolus, the site of rRNA gene transcription and ribosome assembly, has other far more diversified interests. Regulation of the cell cycle, of senescence, and of aspects of transport are among the other functions controlled by factors localized to the nucleolus (Olson et al., 2000). However, it is rRNA gene transcription that appears to lie at the heart of nucleolar events. Transcription of the rRNA genes is correlated with the size of the nucleolus (see Stefanovsky et al., 2001, and references therein), and the existence of the nucleolus depends on a functional transcription machinery (Nomura, 2002, and references therein). Given that the ribosome has a life of days to weeks, a recent demonstration that the rRNA genes constitute immediate response genes cannot be easily understood in terms of a rapid requirement for ribosome biogenesis (Stefanovsky et al., 2001). It might, however, make sense if the level of

rRNA transcription were a regulator of a broader range of nucleolar functions.

The Basic Players

Most eukaryotes contain a hundred or more chromosomal rRNA genes arranged in one or more tandem repeats. The rRNA gene promoter in mammals, amphibia, and yeasts consists of around 150 bp of DNA containing two sequence elements, the upstream control element (UCE or UE) and the core (Boukhgalter et al., 2002; Nomura, 2002; Paule, 1998, and references therein). In mammals, three basal transcription factors have been identified, the “selectivity” complex (SL1 or TIF-IB), the HMG1 box architectural upstream binding factor (UBF), and the dedicated DNA-dependent RNA polymerase I or A (Poll), as depicted in Figure 1B. Isolated SL1 contains four polypeptides, TATA box binding protein (TBP), and three Poll-specific TAFs, of 95/110, 68/63, and 48 kDa apparent molecular weight (Paule, 1998, chapters 6 and 11 and references therein). It is believed that UBF binds the promoter first, allowing the recruitment of SL1. Both SL1 and UBF contact the UCE and core promoter elements, and in the case of UBF, this is very probably due to the binding of two dimers. However, it is still unknown whether one or two SL1 complexes are recruited to the promoter (Figures 1B and 1C). The situation at present appears to be more complex in yeast, where distinct factors, the upstream activating factor (UAF) and the core factor (CF), have been found to bind the UE and the core (Figure 1A; Nomura, 2002; Paule, 1998, chapter 12 and references therein). The isolated UAF consists of Rrn5p, 9p, and 10p as well as Uaf30p and the histones H3 and H4, while the CF consists of Rrn6p, 7p, and 11p. Both UAF and CF can associate with TBP, but while TBP and the UAF are required for Poll transcription *in vivo*, they are not essential *in vitro* and neither is the upstream promoter element (UE). TBP, UAF, and the UE are, however, required *in vitro* for a high-level transcription. This is reminiscent of the mammalian data, where SL1 and the core promoter are often sufficient for transcription *in vitro* while *in vivo* the UCE plays an essential role (Paule, 1998, chapter 3 and references therein). Assembly of a preinitiation complex in yeast starts with the recruitment of the UAF to the UE. Subsequently, the CF and TBP and finally the polymerase are recruited. TBP is probably able to provide a bridge between the UAF and CF complexes, and it is tempting to suggest that this bridging might occur via a TBP dimerization. This could also be relevant to the interaction of mammalian SL1 with both the UCE and core promoter elements (see Figures 1A and 1C). CF is released from the promoter at each round of initiation, and mutations that inactivate Poll also prevent CF recruitment, suggesting that these factors may normally be recruited together (Bordi et al., 2001, and references therein). Thus, it is the UAF and the upstream promoter element that are required for stable promoter commitment in yeast.

As yet, the cloned components of SL1 have proven insufficient for *in vitro* transcription (see Paule, 1998,

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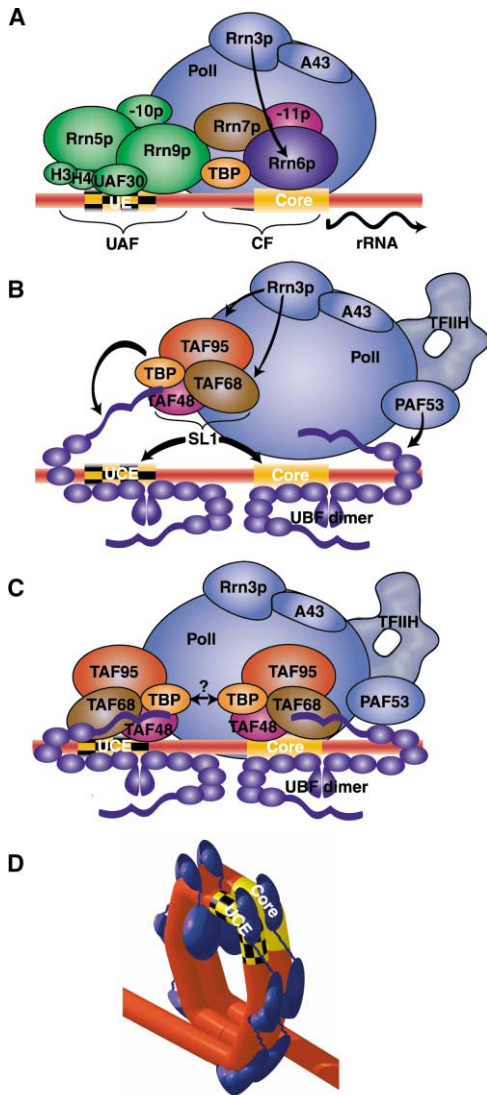


Figure 1. Assembly of the Poll Initiation Complex
(A) In yeast and (B–D) in mammals; see text for details. DNA is shown in red and promoter elements in checkered yellow. We adapted (A) from an illustration in Paule (1998, chapter 12).

chapter 11 and references therein). However, mammalian TAF68 and TAF48 do show homology, respectively, to Rrn7p and Rrn11p of yeast and other fungi (Boukhgalter et al., 2002) and like the CF, SL1 can be recruited with Poll, since it forms part of an initiation-competent holo-Poll (see Iben et al., 2002, and references therein). Thus, either a mammalian UAF simply does not exist or it remains to be discovered. UBF was first discovered as a binding factor for the UCE of the human Poll promoter and has often been equated with the yeast UAF (see Paule, 1998, chapters 6 and 7 and references therein). Consistent with this, promoter commitment in mammals is generally believed to depend on the ability of UBF to interact with the promoter and to catalyze the recruitment of SL1. However, UBF is a single polypeptide with no structural homology to the yeast UAF, and its binding is certainly not restricted to the upstream promoter ele-

ment. UBF is able, through three of its HMG1 boxes, to induce a chromatin-like structure, the *enhancesome*, in which about 140 bp of DNA is looped into a single turn (Paule, 1998, chapter 7; Stefanovsky et al., 2001, and references therein). Two such adjacent structures may occur at the Poll promoter, possibly explaining the cooperative recruitment of SL1 to the UCE and core (Figure 1D). UBF may also recruit Poll, perhaps via the polymerase-associated factor PAF53 (RPA53 in yeast) (see Paule, 1998, chapter 8; Voit and Grummt, 2001, and references therein). It can also displace histone H1 and thus compete with the repressive effects of chromatin. Thus, UBF may function both at the level of gene activation, by displacing repressive chromatin, and during the formation of the preinitiation complex. Considering the role of UBF in SL1 and Poll recruitment, it was very surprising to realize that UBF binds indiscriminately throughout the rRNA gene locus (O'Sullivan et al., 2002, and references therein). The precise positioning of UBF on the Poll promoter is probably the result of a preferential phasing with respect to the underlying DNA structure, similar to that seen for the nucleosome of chromatin. These properties of UBF clearly make it unable to target SL1 and Poll to the promoter in the absence of other parameters such as a specific interaction of SL1 and perhaps Poll with the promoter DNA. Although UBF has been found in mammals, amphibia, and fish, no convincing evidence exists for a UBF homolog in other eukaryotes, and genetic screens in yeast have not identified any HMG1 box proteins implicated in ribosomal RNA transcription. However, given the chromatin-like role of UBF, i.e., its high abundance, low sequence selectivity, and ubiquitous presence throughout the rRNA genes, it remains possible that this function is replaced by one of the eight smaller HMG1 box proteins encoded in the yeast genome.

Silent rRNA genes display the classic nucleosomal chromatin structure. Active ribosomal genes do not display this structure but do remain associated with the core histones H3, H4, H2A, and H2B (Paule, 1998, chapter 20 and references therein). The UBF *enhancesome* structure observed *in vitro* is clearly incompatible with nucleosomal chromatin and hence its formation would require nucleosome disruption (Stefanovsky et al., 2001, and references therein). The recruitment of a chromatin remodeling complex to the promoter-proximal transcription termination site in mouse via the Reb1 p-related termination factor TTF-I could provide such a function (Strohner et al., 2001). The association of histones H3 and H4 with the yeast UAF is intriguing since it also suggests a relationship with chromatin remodeling.

Recruitment of Poll to the preinitiation complex in both yeast and mammals requires the conserved factor Rrn3p (or TIF-IA), which is found associated with yeast CF and with the yeast and mammalian Poll. There is good agreement in both yeast and mammalian systems that Rrn3p forms a bridge between Poll and the factor CF or SL1 (see Figures 1 and 2 and Fath et al., 2001, and references therein). This again underlines the strong functional and structural homologies which can sometimes be drawn between the two systems.

Digital or Analog Regulation?

Since eukaryotic cells contain several hundreds of copies of the ribosomal RNA genes, an on-off gene switch

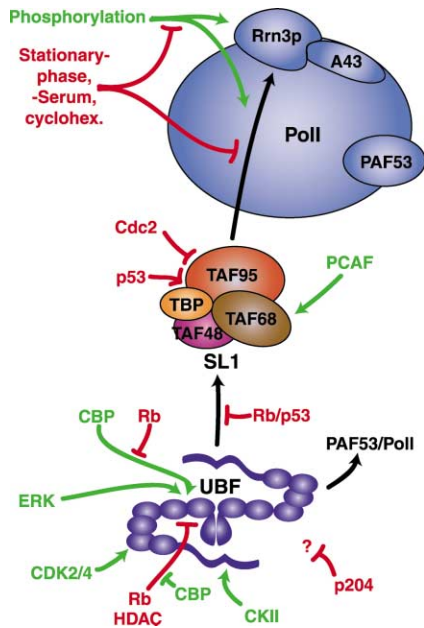


Figure 2. Regulation of the Pol II Basal Factors
Black arrows indicate interactions, green indicate activating and red indicate repressive interactions or posttranslational modifications; see text for details.

could provide a finely graduated gene dosage control. However, though in yeast the number of active ribosomal genes has been observed to diminish as growth is severely limited, such regulation has to date not been observed in other eukaryotes (Paule, 1998, and references therein, chapter 20). Further, even at maximum rRNA output, only around 50% of the genes are transcribed. Thus, though rRNA gene dosage is a critical parameter of organism development and survival, the full transcriptional potential of the genes is apparently never utilized (see below).

The control of the *per gene* transcription rate can be achieved at two levels, initiation and elongation. In turn, initiation can be regulated at preinitiation complex formation, polymerase recruitment, or promoter clearance. Regulation at each of these levels has been described for the Pol II promoter. In several systems, two distinct forms of Pol II could be isolated, both capable of RNA synthesis but only one of which was able to specifically initiate transcription *in vitro*. Loss of the initiation-competent form was correlated with encystment in *Acanthamoeba*, stationary growth phase in yeast, and prolonged serum starvation, glucocorticoid induced arrest, and cycloheximide block in mammalian cell culture (see Paule, 1998, and references therein, chapters 11, 15, 16, and 20). Subsequently it was found that yeast Rrn3p and the serum- and cycloheximide-regulated factor TIF-IA were interchangeable and that both were associated with active Pol II. Phosphorylation of Pol II has been found necessary for its interaction with Rrn3p (Figure 2), suggesting that rRNA transcription can be regulated by the availability of a Pol II-Rrn3p complex (Fath et al., 2001, and references therein).

Mitotic silencing causes a temporary but complete

cessation of rRNA transcription and is associated with modifications in two distinct factors. SL1 is inactivated by a Cdc2-dependent phosphorylation, while two CDK2/4-catalyzed activating phosphorylations on UBF are lost during mitosis (see Voit and Grummt, 2001, and references therein). Surprisingly, the majority of other growth-related regulatory mechanisms target UBF (Figure 2). The C-terminal acidic region of UBF is phosphorylated by a casein kinase II-like activity, and this has been correlated with an enhanced binding to SL1. A rapid activation of endogenous rRNA transcription by epidermal growth factor was shown to be mediated by direct MAP-kinase phosphorylation of UBF (see Stefanovsky et al., 2001, and references therein). Further, despite UBF being relatively abundant, it is in fact limiting for rRNA gene transcription, and variations in its concentration correlate with growth rate changes (Paule, 1998, chapter 17 and references therein). Acetylation of UBF by CBP and of TAF68 by PCAF were reported to enhance their activities *in vitro*, and both CBP and p300 were shown to enhance Pol II transcription *in vivo*, probably via acetylation of both UBF and the core histones (Hirschler-Laszkiwicz et al., 2001, and references therein). The retinoblastoma tumor suppressor protein Rb represses Pol II transcription, partly by preventing an interaction between UBF and SL1 (Hannan et al., 2000). Rb also competes with CBP for a common binding site on UBF and probably catalyzes UBF deacetylation (Pelletier et al., 2000). The interferon-inducible p204 nucleolar protein also binds UBF and inhibits rRNA transcription by an unknown mechanism (Liu et al., 1999). Similarly, the tumor suppressor p53 represses Pol II transcription by preventing an interaction of UBF with SL1, but unlike Rb it does so by binding to SL1 (Zhai and Comai, 2000). The possibility that tissue-specific factors can also regulate rRNA gene expression was raised when it was found that basonuclin plays a role in activating Pol II transcription during mouse oogenesis (Tian et al., 2001, and references therein). The possible existence of tissue-specific factors opens the rRNA genes to a whole new level of regulation perhaps required for cell differentiation.

Given that UBF has little or no sequence selectivity and is bound throughout the ribosomal genes (O'Sullivan et al., 2002, and references therein), it is difficult to understand how the plethora of regulatory interactions it supports can specifically target the assembly of the preinitiation complex at the Pol II promoter. CBP and Rb could regulate global access to the rRNA gene chromatin via UBF and histone acetylation-deacetylation cycles. However, how could Rb and perhaps p204 be targeted to prevent SL1 interactions specifically at the Pol II promoter? Also, would not the large excess of UBF over SL1 tend to sequester this factor away from the promoter and "squench" its activity? In fact, such squelching may have been the basis for *in trans* transcription suppression by the enhancers of the *Xenopus* rRNA genes (Paule, 1998, and references therein, chapter 18). Perhaps mechanisms of regulation via UBF do not directly affect preinitiation complex assembly at all but rather the ability of UBF to attract SL1 and Pol II to sites throughout the rRNA gene locus from whence they may be handed over to the promoter. In this scenario UBF would, like chromatin, play several roles (i.e., gene activation, initiation complex formation, and transcription

enhancement) depending on its position on the rRNA genes. Alternatively, the UBF molecules bound at the promoter could be differentially phosphorylated or acetylated and thus target SL1 and Poll to the promoter.

Elongation, a Key Factor in Regulation?

Almost without exception, the well-known "Miller" spreads of active rRNA genes show a close packing of transcription complexes. This suggests that it is in fact transcription elongation and not initiation that may be rate limiting. Three recent articles lend support to this view. Panov et al. (2001) presented evidence that UBF was required during promoter clearance or early elongation. Stefanovsky et al. (2001) argued that since growth factor stimulation of rRNA gene transcription by MAP-kinase phosphorylation of UBF prevented rather than enhanced its interaction with DNA, a cyclic phosphorylation of UBF may be required for elongation *in vivo*. Further, mitotic silencing mediated in part by UBF dephosphorylation does not displace Poll from the rRNA genes and therefore logically must arrest transcription elongation. Most recently, the Poll elongation and transcription-coupled repair factor TFIH was shown to be required for a post-initiation step in rRNA gene transcription (Iben et al., 2002). Contrary to its activity in Poll promoter opening, neither CDK7 activity nor ATP β - γ hydrolysis were required for TFIH enhancement of Poll transcription, suggesting a postinitiation role for TFIH (Iben et al., 2002, and references therein). Thus, understanding elongation could be a key step in resolving some of the mysteries of rRNA gene regulation.

Gene Silencing: A Regulation or a Protection?

Let us return to another mystery, that of rRNA gene silencing and why 50% of these genes are apparently never activated (Paule, 1998, and references therein, chapter 20). DNA methylation is a well-documented means of gene silencing, and methylation of a single CpG in the mouse UCE was recently shown to inactivate the Poll promoter and prevent UBF binding to rRNA chromatin *in vitro* (Santoro and Grummt, 2001). In yeast, silencing of Poll genes inserted into the rRNA gene locus requires the RENT complex of proteins, named for regulator of nucleolar silencing and telophase exit. RENT consists of Net1p, the NAD-dependent deacetylase Sir2p, and the phosphatase Cdc14p (Shou et al., 2001, and references therein). However, disruption of RENT not only leads to mitotic arrest but also severely limits rRNA gene transcription. One member of RENT, Net1, has been directly implicated in Poll transcription (Shou et al., 2001). This protein, which is the core of RENT, is required for the nucleolar localization of Sir2p and Cdc14p and several other proteins including Poll, to which it binds directly. Thus, one function of Net1p may be to retain Poll concentrated within the nucleolus. The UAF components, Rrn5p, Rrn9p, Rrn10p, and Uaf30p (Figure 1A), have all been shown to be required to silence Poll transcription of the yeast rRNA genes (Nomura, 2002, and references therein). The combined efforts of RENT and UAF to include Poll and exclude Poll from the nucleolus are probably related to the evolutionary advantage a distinct Poll machinery must afford the eukaryotic cell (Nomura, 2002). But the nature of this advantage is still unclear and does not appear to be related to regulation of rRNA gene transcription.

So why so much effort to silence the rRNA genes if

it is not to regulate Poll transcription? Transcribed genes are generally more accessible and are therefore probably subject to considerably more damage than inactive ones. During the life of an organism, the rRNA genes are probably subject to the most intense transcription of all genes. Randomly silenced genes might, then, provide a source of pristine or perhaps just good "partly used" genes that could replace irretrievably damaged ones, essentially providing a somatic rRNA gene germline of randomly selected master genes. Alternatively, the pool of silenced genes might be necessary as landing sites for factors involved in the diverse functions of the nucleolus.

In Conclusion

Recent work argues that the rRNA genes are not simply bystanders in the decisions on cell fate. Understanding the regulatory network surrounding the rRNA genes is then an essential part of understanding cell growth regulation. It may even turn out that the housekeeper is in fact keeping the house.

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