REVIEW

Life on a planet of its own: regulation of RNA polymerase I transcription in the nucleolus

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Mammalian cells contain 100 or more copies of tandemly repeated ribosomal RNA (rRNA) genes per haploid genome. These genes are transcribed with high efficiency to keep up with the cell's metabolic activity and demand for ribosomes. Alterations in cell proliferation are accompanied by profound changes in the transcription rate of rRNA genes. Thus, by responding to changes in the cellular environment, transcription by RNA polymerase I (Pol I) ultimately determines ribosome production and the potential for cell growth and proliferation. There are several comprehensive reviews that discuss regulation of rRNA synthesis in vertebrates and yeast (Grummt 1999; Reeder 1999; Warner 1999; Moss and Stefanovsky 2002). However, new data have been produced even since the latest of these reviews that uncover the mechanisms that link Pol I transcription to cellular physiology. In this review, I restrict the background information to the minimal level that is required for understanding initiation complex formation at the rDNA promoter before proceeding to review the regulatory pathways that adapt cellular rRNA synthesis to cell metabolism and growth.

Structural organization of the rRNA transcription unit

In higher vertebrates, a standard rDNA transcription unit encodes the precursor to 18S, 28S, and 5.8S rRNAs. Each unit also contains important sequence elements that regulate pre-rRNA transcription, such as the rDNA promoter, enhancers, spacer promoters, an origin of replication, transcription terminators, and a replication fork barrier that prevents replication forks from colliding with transcribing RNA polymerase I during S phase. The tandem arrangement of multiple rDNA genes may have been useful to increase gene dosage and to maintain the well-recognized rRNA sequence homology. With the exception of closely related species, eukaryotic rDNA promoter sequences have diverged significantly. Consistent

with this sequence disparity, rDNA transcription is generally specific to taxonomic orders, the promoter of one group not being recognized by the transcription machinery of others (for review, see Heix and Grummt 1995). With a few exceptions, rDNA promoters share a common modular organization, consisting of a start site proximal core promoter (CP) and an upstream control element (UCE). The stereospecific alignment of both sequence elements is crucial for efficient transcription initiation. Analysis of structural parameters of ribosomal gene promoters from human to lower plants revealed the conservation of specific structural features, rather than base sequence, that are fundamental for promoter function (Marilley and Pasero 1996; Marilley et al. 2002). Apparently, a structural code, in addition to primary sequence, directs specific DNA-protein interactions at the rDNA promoter and may play an important function in transcriptional control.

Basal Pol I transcription initiation factors

RNA polymerase I is unique in that in most eukaryotes its sole function is the transcription of genes encoding the large rRNAs. Like Pol II and Pol III, it requires auxiliary factors that mediate promoter recognition, promote transcription elongation, and facilitate transcription termination. Briefly, initiation of mammalian rDNA transcription is mediated by a specific multiprotein complex containing Pol I and at least four basal transcription initiation factors (Fig. 1). Preinitiation complex formation requires the synergistic action of the upstream binding factor (UBF; Jantzen et al. 1990) and the promoter selectivity factor, termed TIF-IB in mouse (Clos et al. 1986) and SL1 in humans (Learned et al. 1985). UBF contains several HMG boxes, a motif known to bend DNA. Like other HMG proteins, UBF interacts with the minor groove of DNA and binds to structured nucleic acids such as kinked DNA, cruciforms, or four-way junctions (Putnam et al. 1994). The tandem HMG boxes enable a single dimer of UBF to wrap the DNA in a right-handed direction, forming a loop of almost 360° once every 140 bp, thereby bringing the core and the UCE into close proximity (Bazett-Jones et al. 1994; Copenhagen et

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Article and publication are at <http://www.genesdev.org/cgi/doi/10.1101/gad.1098503R>.

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Figure 1. A diagram depicting the structural organization of the murine rDNA transcription unit and the basal factors required for faithful transcription initiation and termination. The green line marks sequences upstream of the transcription start site, including the rDNA promoter and the upstream terminator T_0 . The red line marks sequences downstream of the rRNA coding region, containing the terminator elements T_{1-10} . The cartoon below shows the basal transcription initiation factors (for details, see text), a schematic of the transcription initiation complex, and the two proteins, TTF-I (transcription termination factor) and PTRF (polymerase and transcript release factor) that are required for Pol I transcription termination.

al. 1994). This structure may provide the correct scaffolding for productive interactions between UBF and TIF-IB/SL1 bound to the two promoter elements and facilitate initiation complex formation. UBF is known to activate rDNA transcription by recruiting Pol I to the rDNA promoter, stabilizing binding of TIF-IB/SL1, and competing with nonspecific DNA-binding proteins, such as histone H1 (Kuhn and Grummt 1992; Kuhn et al. 1993). Moreover, UBF has been shown to bind to nucleosomes and displace histone H1 from the linker region on preassembled nucleosomes (Kermekchiev et al. 1997).

Promoter specificity is conferred by TIF-IB/SL1, a protein complex containing the TATA-binding protein (TBP) and three Pol I-specific TBP-associated factors TAF₄₈, TAF₆₈, TAF_{95/110} (Comai et al. 1992; Eberhard et al. 1993; Heix et al. 1997). In contrast to TFIID, the factor that nucleates Pol II transcription initiation complexes, the TBP subunit of TIF-IB/SL1 does not bind to DNA, and promoter recognition is carried out by the associated TAFs. As is discussed below, the most important step in the assembly of a productive transcription initiation complex is the recruitment of Pol I to the rDNA promoter. This is achieved by interaction of UBF with PAF53, the mammalian homolog of the yeast Pol I subunit A49 (Hanada et al. 1996; Seither et al. 1997), and by interaction of TIF-IB/SL1 with TIF-IA. TIF-IA is the mammalian homolog of yeast Rrn3p (Bodem et al. 2000; Moorefield et al. 2000), a regulatory factor that is asso-

ciated with the initiation-competent subpopulation of Pol I (Miller et al. 2001; Yuan et al. 2002).

In *Saccharomyces cerevisiae*, rDNA transcription requires Pol I, the TATA-binding protein (TBP), Rrn3p, the core factor (CF), and the upstream activating factor (UAF). CF is composed of three stably associated proteins encoded by *RRN6*, *RRN7*, and *RRN11*. UAF is a complex of six polypeptides including Rrn5p, Rrn9p, Rrn10p, the two histones H3 and H4, and Uaf30p (see Fig. 2; for review, see Nomura 2001). Binding of UAF to the promoter is necessary to recruit CF and the Pol I-Rrn3p complex. Transcription experiments with immobilized templates revealed that CF and TBP, along with Pol I and Rrn3p, are released from the template upon transcription, whereas UAF remains associated with the upstream promoter element, presumably serving as a scaffold for reinitiation (Aprikan et al. 2001). These findings support a model in which essential components of the Pol I machinery cycle on and off the promoter with each round of transcription. In contrast, similar experiments with human Pol I and the respective factors demonstrated recycling of Pol I and TIF-IA/Rrn3p, but not of UBF and TIF-IB/SL1 (Panov et al. 2001).

The RNA polymerase I holoenzyme concept

The model of a stepwise preinitiation complex (PIC) formation at the rDNA promoter is now being reconsidered

Figure 2. A cartoon view of the basal components of the murine and yeast Pol I transcription initiation complexes. The green line marks rDNA promoter sequences, the bent arrow indicates the transcription initiation site, and the shaded objects represent proteins that are involved in preinitiation complex formation. TIF-IB is composed of four subunits; for example, TBP and three TBP-associated factors, TAF₉₅, TAF₆₈, and TAF₄₈ (yellow). UAF consists of Rm5p, Rm9p, Rm10p, histones H3 and H4, and Uaf30p (blue), whereas CF consists of Rm6p, Rm7p, and Rm11p (yellow). Rm3p is the yeast homolog of mammalian TIF-IA. PAF67 is a 67-kD Pol I-associated factor that, like TIF-IA, decorates the initiation-competent subpopulation of Pol I (Pol I β).

in view of reports of Pol I “holoenzymes.” In plants, mammals, and frogs, large Pol I-containing protein machineries have been identified that contain most, if not all, factors required for transcription initiation and combine several enzymatic entities (Saez-Vasquez and Pikaard 1997; Seither et al. 1998; Albert et al. 1999; Hannan et al. 1999). Although the composition of these large protein complexes is not well defined, they all contain Pol I and basal transcription factors to support rDNA transcription in vitro and additional polypeptides that play a role in protein phosphorylation (CKII), chromatin modification (PCAF), or DNA repair/replication (topoisomerase I, Ku70/80, and PCNA). Interestingly, TFIIF, the protein complex that plays an essential role in transcription of protein-coding genes and nucleotide excision repair, was also found to be an integral part of the Pol I holoenzyme (Iben et al. 2002). These findings are compatible with a mechanism by which Pol I is recruited to the rDNA promoter as a giant multiprotein complex that contains proteins required for rRNA synthesis and maturation, chromatin modification, and DNA repair. In support of this view, a novel ribonucleoprotein complex enriched in nucleolar proteins has been purified from yeast that contained Pol I, TBP, Rm3p, Rm5p, Rm7p, and Reb1p along with rRNA-processing factors like Nop1p, Cbf5p, Nhp2p, and Rrp5p as well as small nucleolar RNAs (Fath et al. 2000). The ribonucleoprotein complex supports accurate transcription, termination, and pseudouridylation of rRNA, suggesting that it represents a nucleolar subdomain that serves as a scaffold for coordinated rRNA synthesis and processing. However, it cannot be excluded that after breakage of the cells, such complexes are artificially generated by specific or non-specific macromolecular interactions. In support of this, recent FRAP experiments have demonstrated that Pol I subunits enter the nucleolus as distinct subunits rather than as a preassembled complex. These observations

suggest that assembly of Pol I and functional initiation complexes may proceed in a sequential manner via metastable intermediates, each with increasing stability as more subunits are added (Dundr et al. 2002).

Dynamics of the Pol I transcription apparatus

The classical view of transcription initiation complex formation is that of an ordered stepwise assembly of multiple proteins on the promoter via specific protein-protein interactions or, alternatively, binding of a preassembled ready-to-use multiprotein complex, often termed a “factory.” Indeed, the nucleolus can be regarded as the archetype of such a transcription factory because it represents a stable, architectural framework within which most steps of ribosome biosynthesis, that is, rRNA transcription, processing, maturation, and ribosome assembly, take place in a spatial and temporal order. The view of the nucleolus as a highly organized environment has been eclipsed by recent findings that monitored the movement of individual subunits of Pol I and basal factors, such as UBF, TAF₄₈, and TIF-IA, in mammalian nucleoli (Dundr et al. 2002). Using green fluorescent protein (GFP) tags that permit the observation of proteins in living cells by the FRAP (fluorescent recovery after photobleaching) technique, the kinetics of assembly and elongation of mammalian Pol I has been analyzed. The data revealed that the Pol I transcription machinery is a highly dynamic complex that assembles in a stochastic fashion from freely diffusible subunits. Each of the components is steadily and rapidly exchanged between the nucleoplasm and the nucleolus. Real-time evaluation of promoter-associated multiprotein complexes revealed occupancy of only a few seconds, indicating that the association of transcription factors with their binding sites is transient. A hit-and-run mechanism was proposed, in which transcriptional fac-

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tors quickly exchange between individual rDNA promoters. Pol I subunits have been found to enter the nucleolus as distinct subunits rather than as a preassembled holoenzyme. Surprisingly, Pol I appears to break apart after transcription termination and needs to reassemble before transcription initiation. Calculations of the FRAP data indicate that transcription initiation at a ribosomal promoter occurs every ~1.4 sec, Pol I subunits reside in the pool for ~9 to ~37 sec, and the residence time of elongating Pol I is 2–3 min. With the use of computational modeling of imaging data, the *in vivo* elongation time of Pol I has been determined as ~140 sec, corresponding to an elongation rate of 95 nt/sec for a human rDNA gene of 13.3 kb. Although one can question whether imaging and mathematical models can provide such an unambiguous picture of assembly, the estimated numbers are approximately the same as those obtained by French et al. (2003), who calculated the elongation rate of yeast Pol I directly from the number of Pol I molecules per rRNA gene and the rate of rRNA synthesis.

Mechanisms regulating Pol I transcription

Transcription of rRNA genes is highly regulated to be responsive to both general metabolism and specific environmental challenges (for review, see Grummt 1999). Changes in Pol I transcription regulate ribosome production and thus determine the potential for cell proliferation. rDNA genes are present in multiple copies, and therefore, rRNA synthesis could be modulated by varying the transcription rate per gene or by varying the number of active genes. Although there are several reports demonstrating that in yeast both of these mechanisms may operate under certain conditions (for review, see Reeder 1999), a recent electron microscopy study revealed that the overall initiation rate, and not the number of active genes, determines the rate of rDNA transcription during exponential growth in yeast (French et al. 2003). Similarly, in vertebrates the level of cellular rRNA is regulated by changing the rate of transcription initiation at active rDNA genes rather than by activating silent transcription units. *In vivo* psoralen-cross-linking studies that can distinguish between transcriptionally active and inactive genes have revealed that even in exponentially growing mammalian cells that synthesize high levels of pre-rRNA, only half of the rDNA genes are transcriptionally active and maintained in an “open” chromatin conformation. The other half that corresponds to inactive gene copies resides in a compact, nucleosomal structure. The ratio of active and inactive rRNA genes is stably propagated through the cell cycle and is independent of the cellular rRNA synthetic activity (Conconi et al. 1989). The present view is that growth-dependent modulation of Pol I transcription occurs at transcriptionally competent gene copies, and “opening” or “closing” of ribosomal genes is not involved in short-term Pol I transcription regulation. Epigenetic mechanisms that mediate rDNA silencing will

be discussed in another review (Grummt and Pikaard 2003).

Early studies in mice and rats have demonstrated up- or down-regulation of rRNA synthesis after partial hepatectomy, hormone administration, cycloheximide treatment, or nutrient starvation (for reviews, see Jacob 1995; Grummt 1999). Deprivation of a single amino acid from the culture medium has been demonstrated to cause a rapid shut-off of nucleolar transcription (Grummt et al. 1976). Meanwhile, several studies have been published that address the mechanisms underlying cell-cycle- and growth-factor-dependent fluctuations of Pol I activity. Indeed, almost any perturbation that slows down cell growth or protein synthesis decreases rDNA transcription. Evidence accumulated to date indicates that almost any of the proteins required for Pol I transcription can serve as a target for regulatory pathways. For example, changes in the phosphorylation pattern of UBF play a key role in modulating rDNA activity during cell cycle progression. UBF is phosphorylated at multiple sites, and phosphorylation of the C terminus by casein kinase II facilitates the interaction between UBF and TIF-IB/SL1 (Tuan et al. 1999). In quiescent cells, UBF is hypophosphorylated and transcriptionally inactive (O'Mahony et al. 1992; Voit et al. 1992, 1995). Moreover, interactions with pRb, p130, and p53 have been shown to impair UBF functions, such as DNA binding or the interaction with TIF-IB/SL1 (Cavanaugh et al. 1995; Voit et al. 1997; Budde and Grummt 1999; Zhai and Comai 2000; Ciarmatori et al. 2001). Finally, acetylation of UBF by the histone acetyltransferase CBP has been reported to enhance UBF activity *in vitro*, and overexpression of both CBP and p300 enhances Pol I activity *in vivo* (Hirschler-Lankiewicz et al. 2001). Besides UBF, the TAF₆₈ subunit of TIF-IB/SL1 is acetylated by PCAF. Acetylation enhances binding of TAF₆₈ to rDNA and augments Pol I transcription. Conversely, deacetylation of TAF₆₈ by the NAD⁺-dependent histone deacetylase mSir2a represses Pol I transcription (Muth et al. 2001). As is discussed below, there is evidence for changes in the phosphorylation pattern of SL1 (Heix et al. 1998), UBF (Klein and Grummt 1999; Voit et al. 1999), and TTF-I (Sirri et al. 2000, 2002) that correlate with cell-cycle-specific fluctuations of rDNA transcription. Thus, reversible acetylation and phosphorylation of basal components of the Pol I transcription machinery may be an effective means to regulate rDNA transcription.

Growth-dependent transcription regulation by TIF-IA

Conditions that harm cellular metabolism, that is, nutrient starvation, toxic lesion, aging, cancer, and viral infections, down-regulate rDNA transcription. Conversely, rDNA transcription is up-regulated upon reversal of such conditions and by agents that stimulate growth. This growth-dependent regulation of rRNA synthesis is evolutionarily conserved and has been observed in bacteria, yeast, plants, and vertebrates. The key player in growth-dependent regulation of rDNA transcription is the transcription initiation factor TIF-IA, the mamma-

lian homolog of yeast Rrn3p (Buttgereit et al. 1985; Schnapp et al. 1993; Bodem et al. 2000; Moorefield et al. 2000). A preinitiation complex containing Pol I can be formed in the absence of TIF-IA/Rrn3p; however, formation of the first phosphodiester bond requires the presence of TIF-IA/Rrn3p (Schnapp and Grummt 1991; Schnapp et al. 1993). Following initiation, TIF-IA is released from the ternary complex and can associate with another preinitiation complex. The activity of TIF-IA/Rrn3p is regulated by diverse extracellular signals, suggesting that this factor adapts Pol I transcription to cell growth. In both mammals and yeast, a large fraction of Pol I, the “bulk” enzyme, termed Pol I α , is unable to support specific initiation, despite its ability to synthesize RNA from nonspecific templates (Tower and Sollner-Webb 1987; Schnapp et al. 1990; Miller et al. 2001). Only the fraction of Pol I (Pol I β) that is associated with TIF-IA/Rrn3p is capable of assembling into a productive initiation complex (Yamamoto et al. 1996; Milkereit and Tschochner 1998), suggesting that TIF-IA/Rrn3p bridges Pol I to the preinitiation complex. Importantly, the amount of TIF-IA/Rrn3p associated with Pol I, but not the overall level of TIF-IA/Rrn3p, is decreased in growth-arrested cells, indicating that transcriptional shut-off is caused by dissociation of the Pol I/Rrn3p complex (Milkereit and Tschochner 1998; Cavanaugh et al. 2002; Yuan et al. 2002). These and other experiments demonstrate that most, if not all, growth-dependent control of rDNA transcription may be exerted by the formation and recruitment of TIF-IA/Rrn3p–Pol I complexes to the rDNA promoter. This suggests a regulatory cycle in which TIF-IA/Rrn3p dissociates from Pol I during initiation or after promoter escape, is inactivated after release, and must be reactivated before association with another polymerase and assembly into a new preinitiation complex (Aprikian et al. 2001).

The role of TIF-IA/Rrn3p as a bridge between Pol I and TIF-IB/SL1 or CF, respectively, has been supported by genetic and biochemical experiments in *S. cerevisiae* and mammals demonstrating that TIF-IA/Rrn3p interacts with RPA43, a unique subunit of Pol I (Peyroche et al. 2000; Fath et al. 2001; Cavanaugh et al. 2002; Yuan et al. 2002). In addition, TIF-IA has been found to interact with PAF67, a 67-kD Pol I-associated factor that decorates the initiation-competent form of Pol I (Seither et al. 2001). This suggests that by interacting with PAF67, TIF-IA may target a functional subset of Pol I molecules into a productive transcription initiation complex. TIF-IA also interacts with two TAF_I subunits of TIF-IB/SL1 (Miller et al. 2001; Yuan et al. 2002) and the Rrn6p subunit of CF (Peyroche et al. 2000). Thus, by associating with both Pol I and the promoter selectivity factor, TIF-IA may link both protein complexes.

Given the essential role for TIF-IA/Rrn3p in targeting Pol I to promoter-bound TIF-IB/SL1, the interactions with TAF_Is, RPA43, and PAF67 are expected to be major targets of regulatory pathways that control the assembly of Pol I preinitiation complexes. Indeed, interactions between TIF-IA/Rrn3p with Pol I are affected by diverse regulatory pathways that link the cell's biosynthetic ac-

tivities to environmental conditions. Nutrient starvation, density arrest, and protein synthesis inhibitors lead to inactivation of TIF-IA (Cavanaugh et al. 2002; Yuan et al. 2002). TIF-IA is phosphorylated at multiple sites, and signals that affect cell metabolism alter the phosphorylation pattern of TIF-IA (Zhao et al. 2003). In density-arrested, cycloheximide-treated, and amino-acid-starved cells, TIF-IA is hypophosphorylated and incapable of binding to Pol I (Yuan et al. 2002). Thus, cellular signaling cascades directly target TIF-IA, and reversible phosphorylation regulates the association of TIF-IA with Pol I and hence transcription initiation complex formation.

TOR and MAP kinase signaling pathways target TIF-IA

We are just beginning to understand some of the complex pathways the cell uses to gauge external growth signals and coordinate regulatory inputs to modulate Pol I activity. It is well established that starvation or lack of nutrients impairs ribosome and protein synthesis, the major energy-consuming processes of the cell. An important finding is that the target of rapamycin (TOR)/p70 S6 kinase (p70^{S6k}) pathway senses the availability of nutrients and regulates both rRNA and protein synthesis (for review, see Proud 2002). The TOR/p70^{S6k} pathway controls translation, ribosome biogenesis, and many growth-related processes in response to nutrients and environmental conditions. Nutrient deprivation inhibits mTOR kinase activity and blocks cell growth. The mechanism by which TOR senses nutrient availability is unknown, as is its involvement in regulation of rDNA transcription. Extracts from cells treated with the mTOR inhibitor rapamycin are transcriptionally inactive (Mahajan 1994; Zaragoza et al. 1998), and treatment of yeast cells with rapamycin leads to inhibition of rRNA synthesis (Powers and Walter 1999). Interestingly, transcriptional activity of rapamycin-treated cell extracts can be restored by mTOR, p70^{S6k}, or recombinant TIF-IA (I. Grummt, unpubl.). This suggests that phosphorylation by mTOR, p70^{S6k}, or downstream kinase(s) is required for TIF-IA activity. In support of this, the assembly of TIF-IA/Pol I complexes has been found to be impaired in rapamycin-treated cells. Thus, TOR/p70^{S6k} signaling regulates rDNA transcription by modulating the activity of TIF-IA.

A different pathway, but similar scenario, mediates transcriptional activation by growth factors. After mitogenic stimulation of quiescent cells, a transient 10-fold increase in pre-rRNA synthesis was observed (Zhao et al. 2003). The rapid response of rDNA transcription to growth factors was blocked by PD98059, an inhibitor of MEK1/2, indicating that Pol I transcription is up-regulated by activation of the Ras–ERK pathway. Transcriptional activation correlates with phosphorylation of TIF-IA at two specific serine residues (S633 and S649) by ERK and RSK kinases. Phosphorylation at these serine residues activates TIF-IA and increases cellular pre-rRNA synthesis. Replacement of Ser 649 by alanine, on the other hand, abolishes TIF-IA activity, impairs Pol I transcription in vivo and in vitro, and retards cell growth.

Thus, growth factors regulate rRNA synthesis and nucleolar activity by ERK/RSK-mediated phosphorylation of TIF-IA (Fig. 3). These results underscore the molecular cross-talk between the p70^{S6k} and ERK signaling pathways (Wang et al. 2001) and demonstrate that TIF-IA is a common final target for growth factor-dependent activation of ribosome biogenesis.

One additional point is worth mentioning. TIF-IA contains a conserved sequence motif, known as the Walker type A or P-loop motif. The P-loop is a flexible glycine-rich sequence that is embedded in a well-defined tertiary structure and has been implicated in ATP and GTP binding (Walker et al. 1982). The presence of a potential ATP- and GTP-binding site in TIF-IA is interesting, because rRNA synthesis in mouse cells has been shown to be regulated by the intracellular pool sizes of ATP and GTP (Grummt and Grummt 1976). Moreover, recent studies in *Escherichia coli* have demonstrated that the concentration of initiating nucleoside triphosphates, that is, ATP or GTP, regulate rRNA transcription in a growth-rate-dependent manner (Gaal et al. 1997). This suggests that NTP-sensing by *rnm* P1 promoters links cellular rRNA synthesis to the level of translation and the available energy resources (Schneider et al. 2002). It is tempting to speculate that eukaryotes use a similar ATP/GTP-sensing mechanism to integrate extracellular signals into growth-rate-dependent regulation of rRNA synthesis. Alternatively, the pool sizes of ATP and GTP could

regulate TIF-IA activity indirectly as a consequence of an effect on mTOR signaling. mTOR itself has been shown to function as an ATP sensor, and mTOR signaling is controlled by intracellular ATP concentrations (Dennis et al. 2001). This finding, together with the role of mTOR signaling in the regulation of TIF-IA activity, may provide a link between nutrient availability, cellular ATP levels, and regulation of rRNA synthesis.

TFIIF and CSB link Pol I transcription to DNA repair

A dedicated network of DNA repair mechanisms and cell cycle checkpoints safeguards DNA integrity to prevent the deleterious consequences of mutations that lead to cancer and aging. One of these DNA repair pathways, nucleotide excision repair (NER), removes a broad range of helix-distorting injuries, for example, UV-light-induced pyrimidine dimers and bulky chemical adducts. The multistep NER process requires the coordinated action of at least 25–30 polypeptides. It is becoming increasingly evident that NER and transcription are tightly linked by the basal Pol II transcription factor TFIIF. Mutations within the XPB and XPD helicase subunits of TFIIF give rise to genetic disorders such as xeroderma pigmentosum, Cockayne's syndrome, and trichothiodystrophy (Lehmann 2001).

The most striking feature of TFIIF is its multifunctionality. TFIIF is engaged in promoter opening and

Figure 3. A model for activation of Pol I transcription by MAPK signaling pathways. The response of cells to growth factors and other mitogens is mediated by specific receptors including protein tyrosine kinase- and G protein-coupled receptors (R). In response to stimulation, the receptors are activated and initiate signaling events leading to various cellular responses. One such pathway activates the core unit of the MAPK cascade, composed of Raf, MEK1/2, and ERK1/2. Once activated, ERK1/2 phosphorylates and activates 90-kD ribosomal S6 kinase (RSK). After translocation to the nucleus, RSK phosphorylates TIF-IA at Ser 649. Subsequently, ERK1/2 phosphorylates TIF-IA at Ser 633. Phosphorylation at these serine residues is required for interaction with Pol I and transcription complex formation.

phosphorylation of the C-terminal domain of Pol II in the context of mRNA transcription and DNA opening in the setting of nucleotide excision and transcription-coupled repair. In addition, TFIIF serves an essential role in Pol I transcription. GFP-tagged TFIIF is homogeneously distributed through the nucleoplasm, with discrete clusters colocalizing with Pol I in nucleoli (Hoogstraten et al. 2002). Electron microscopy and immunogold labeling have shown enrichment of TFIIF at the dense fibrillar component of nucleoli, that is, sites of active rDNA transcription (Iben et al. 2002). Microinjection of antibodies against subunits of TFIIF induced a strong, rapid reduction of rRNA synthesis, demonstrating the requirement of TFIIF in rDNA transcription. In yeast strains carrying temperature-sensitive mutations in Tfb1 and Kin28, the homologs of mammalian p62 and Cdk7, pre-rRNA synthesis declines at a similar rate as in Pol I mutants upon shift to the restrictive temperature. Moreover, biochemical studies have revealed that TFIIF is associated with a subpopulation of TIF-IB/SL1 as well as with the initiation-competent form of Pol I (Pol I_β). Reconstituted transcription systems lacking TFIIF are transcriptionally inactive, and transcriptional activity can be restored by purified TFIIF (Iben et al. 2002). TFIIF is required for productive but not abortive rDNA transcription, implying a role in transcription elongation. These findings suggest that errors in the DNA template encountered during transcription might be corrected by TFIIF-mediated processes. Noteworthy, recent *in vivo* photobleaching studies have revealed that TFIIF moves freely and is capable of rapid switching between Pol I, Pol II, and NER complexes with an average residence time of ~25 sec, ~6 sec, and ~4 min, respectively (Hoogstraten et al. 2002). Thus, a stochastic exchange of TFIIF occurs between different multiprotein complexes involved in different DNA transactions.

The close interrelationship between DNA repair and rRNA synthesis has further been documented by the finding that CSB, a protein that is defective in Cockayne's syndrome (CS), is required for Pol I transcription. CSB is localized at sites of rDNA transcription and restores rRNA synthesis when transfected in CSB-deficient cells. CSB is part of a megadalton-size protein complex that contains Pol I, TFIIF, and basal Pol I transcription initiation factors and promotes efficient rRNA synthesis *in vitro* (Bradsher et al. 2002). Mutations in *CSB*, as well as *XPB* and *XPD* genes, all of which confer Cockayne's syndrome, disturb the Pol I/TFIIF/CSB complex and reduce rRNA synthesis *in vivo*. The fragility of this complex could be the molecular basis for some of the clinical features that are associated with the CS phenotype.

Regulation of Pol I transcription during the cell cycle

Coordination between cell growth and division is a requisite feature of cell proliferation, and unexpected links between nucleolar proteins and the machinery that regulates the cell cycle have been uncovered. In yeast, no significant cell cycle-dependent fluctuations of Pol I

transcription have been observed (Elliot and McLaughlin 1979). In mammalian cells, on the other hand, rDNA transcription strongly oscillates during cell cycle progression. Transcription is maximal in the S and G₂ phases, shuts down in mitosis, and slowly recovers in G₁. Mitotic silencing of human Pol I transcription is caused by phosphorylation of the TAF₁₁₀ subunit of SL1 by cdk1/cyclin B at Thr 852 (Heix et al. 1998; Kuhn et al. 1998). As a consequence of this specific phosphorylation, the capability of TIF-IB/SL1 to interact with UBF is impaired, and Pol I transcription is repressed. Moreover, UBF is inactivated during mitosis, presumably both by loss of essential phosphorylations and mitosis-specific inhibitory phosphorylation(s) (I. Grummt, unpubl.). Thus, reversible phosphorylation of TIF-IB/SL1 and UBF is used as a molecular switch to shut down rDNA transcription during mitosis. The mechanisms that trigger reactivation of transcription at the end of mitosis are unknown. Conceivably, cellular phosphatases have to reverse cdk1/cyclin B-mediated phosphorylations to recover TIF-IB/SL1 activity during telophase. A candidate enzyme for reactivation of the Pol I transcription machinery could be Cdc14B, a phosphatase that is sequestered in an inactive state in the nucleolus for most of the cell cycle and is released into the nucleus and cytoplasm at the exit from mitosis (Mailand et al. 2002).

In early G₁ phase, rDNA transcription remains low although the activity of TIF-IB/SL1 has been fully restored. The key player for activation of Pol I transcription during G₁ progression is UBF. To achieve high levels of rRNA synthetic activity, UBF has to be phosphorylated at two serine residues by G₁-specific protein kinases. Cdk4/cyclin D1 targets Ser 484 (Voit et al. 1999), and cdk 2/cyclin E&A phosphorylates Ser 388 (Voit and Grummt 2001). Mutations that prevent phosphorylation of Ser 388 impair the ability of UBF to associate with Pol I and abrogate transcription. The finding that specific cdk/cyclin complexes modulate the activity of TIF-IB/SL1 and UBF in a cell-cycle-dependent manner links the control of cell cycle progression to regulation of Pol I transcription (Fig. 4).

Another link between cell cycle regulation and rDNA transcription has recently been uncovered by the finding that TAF₁, the largest subunit of the Pol II-specific TFIID complex, binds to UBF (Lin et al. 2002). TAF₁ (also known as CCG1) has been implicated in the regulation of G₁-to-S-phase progression (Hisatake et al. 1993; Ruppert et al. 1993). Interaction of TAF₁ with UBF stimulates human rDNA transcription *in vivo* and *in vitro*. The results suggest that TAF₁ may be specifically engaged in the regulation of genes, including the ribosomal genes, that play a critical role in the coordinate control of cell growth and division.

The nucleolar RENT complex

Recent studies in yeast point to an important role of the nucleolus in the senescence of eukaryotic cells. Ribosomal RNA genes are located at the AGE locus and some nucleolar functions are impaired in old yeast cells (Gotta

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Figure 4. Regulation of Pol I transcription during the cell cycle. (A) Cell-cycle-dependent fluctuations of pre-rRNA synthesis. FT210 cells, a murine mammary tumor cell line carrying a temperature-sensitive mutant of *cdc2* (Yasuda et al. 1991), were synchronized in G2 by culturing at 39°C and released from the G2 block by shifting to the permissive temperature (33°C). At the indicated times, cells were subjected to FACS analysis and nascent RNA was extracted, dotted onto a membrane, and hybridized to a labeled rDNA probe (Klein and Grummt 1999). The rDNA transcription levels differ by about two orders of magnitude in G2- and M-phase cells. (B) Regulation of TIF-IB/SL1 and UBF activity during M and G1 phases. The activity of both TIF-IB/SL1 and UBF is regulated, at least in part, by phosphorylation. At the entry of mitosis, phosphorylation by *cdk1/cyclin B* inactivates TIF-IB/SL1 and UBF. After mitotic exit, transcriptional activity remains low despite the fact that TIF-IB/SL1 activity has recovered. UBF is activated during G1 progression by phosphorylation of S484 by *cdk4/cyclin D* and S388 by *cdk2/cyclin E* and A.

et al. 1997). Transcription of RNA polymerase II genes integrated within the rDNA array is repressed, and this repression is dependent on both UAF (Vu et al. 1999) and Sir2 (silent information regulator #2), a protein that is conserved from archaea to metazoa. The repressive chromatin structure associated with rDNA silencing also functions in suppressing recombination among rDNA repeats, increasing rDNA stability and extending the yeast life span (Gottlieb and Esposito 1989). Mutations that inactivate Sir2 shorten the yeast life span, and overexpression of Sir2 extends it (Kaeberlein et al. 1999). Sir2 has been shown to be an NAD⁺-dependent histone deacetylase (Imai et al. 2000), implying that Sir2-induced transcriptional silencing is brought about by deacetylation of either histones or components of the transcription apparatus.

In budding yeast, Sir2 executes functions in rDNA transcription as a component of a nucleolar complex designated RENT (regulator of nucleolar silencing and telophase exit), consisting of at least three proteins, Sir2,

Net1, and Cdc14. Net1, the core subunit of the RENT complex, localizes to rDNA and is required for silencing (for review, see Guarente 2000). Net1 physically interacts with Pol I *in vitro* and stimulates rRNA synthesis (Shou et al. 2001). Net1 and Sir2 cross-link throughout individual rDNA repeats, and recent data demonstrate that the Net1/Sir2 complex spreads unidirectionally downstream of an active rDNA transcription unit (Buck et al. 2002). Silencing requires transcription by Pol I, and the direction of spreading is controlled by the direction of Pol I transcription. To reconcile these findings, a model has been proposed in which the interaction of Net1 with Pol I recruits Sir2 to active rDNA repeats for histone deacetylation, and the unidirectional spreading of RENT/Sir2 is mediated by its association with Pol I.

Besides its role in rDNA silencing, Net1 regulates both the exit from mitosis and the activity of Cdc14, the third component of RENT (Shou et al. 1999). The Cdc14 phosphatase is sequestered in an inactive state in the nucle-

lus and is released from the RENT complex at the end of anaphase. Thus, destabilization of the RENT complex appears to be a critical step in provoking exit from mitosis and triggering cell cycle progression. It remains to be investigated whether higher eukaryotes use a complex similar to RENT that links nuclear integrity, transcriptional silencing, and cell cycle control.

Repression of rDNA transcription by the tumor suppressor proteins pRb and p53

Tumor suppressor proteins are common targets for genetic alteration in human cancers and have been implicated as key mediators for suppression of cell transformation (for review, see Levine 1997). The related "pocket" proteins pRb, p107, and p130 restrict cellular proliferation and have been implicated in cell cycle regulation. The view that pRb restrains cell proliferation by inactivating factors that are needed for the transcription of genes required for DNA synthesis and cell proliferation was challenged by the discovery that pRb also represses Pol I transcription. UBF is the target for pRb-induced repression of Pol I transcription. pRb accumulates in the nucleoli of differentiated or cell-cycle-arrested cells and has been shown to repress rDNA transcription in vitro and in vivo (Cavanaugh et al. 1995; Voit et al. 1997; Hannan et al. 2000). Transcriptional repression is brought about by interaction of the C-terminal part of pRb with HMG boxes 1 and 2 of UBF. Thus, inactivation of UBF appears to be a most effective way for pRb to shut down rRNA synthesis and inhibit cell growth. The acetyltransferase CBP that activates Pol I transcription by acetylating UBF competes with pRb for binding to UBF, suggesting that the competitive recruitment of CBP and pRb regulates UBF acetylation and rDNA transcription (Pelletier et al. 2000). Interestingly, rRNA synthesis was unaffected in Rb^{-/-} cells, whereas Pol I transcription was elevated in cells lacking either all three pocket proteins or pRb and p130. This suggests overlapping functions of the pRb family members in the regulation of rRNA synthesis. Consistent with such functional redundancy, the pocket protein p130 shares with pRb the ability to interact with UBF and repress Pol I transcription in vivo and in vitro (Ciarmatori et al. 2001).

Similar to pRb and p130, the tumor suppressor p53 has also been shown to repress Pol I transcription in vivo and in vitro. Wild-type, but not mutant, p53 can suppress Pol I transcription in cotransfection experiments, and p53-deficient cells display increased pre-rRNA levels (Budde and Grummt 1999; Zhai and Comai 2000). p53 interacts with two subunits of SL1, TBP and TAF₁₁₀, which, in turn, impairs initiation complex formation. These results reveal a novel mechanism by which the tumor suppressors pRb and p53 inhibit cell proliferation, namely, by direct inhibition of cellular rRNA synthesis. Given that many tumor cells harbor mutations that affect both pRb and p53, the combined effect of both mutations may have an added impact on Pol I activity and tumor progression.

Conclusions and perspectives

In this review, I have summarized our present knowledge of the highly coordinated networks that regulate rRNA synthesis, and hence ribosome production, in response to external signals. Although the emerging picture of transcriptional regulation is one of unanticipated variety and complexity, we are beginning to understand the function of individual components of the Pol I transcription apparatus, the pathways that link rDNA transcription to cell growth, and the role of epigenetic mechanisms that establish the active and inactive state of ribosomal RNA genes. We shall soon be able to unravel the multiple pathways the cell uses to transfer extracellular signals into the nucleolus and modulate the activity of the Pol I transcription machinery. We need to understand the functional consequences of modifications of individual transcription factors as well as some more perplexing aspects of rDNA transcriptional regulation, such as the dynamics of assembly of different proteins into regulatory complexes. Knowing this is also of clinical importance, as the regulation of rRNA synthesis is abrogated in cancer cells, and will be essential for the discovery of novel therapeutic agents that target rDNA transcription regulators. Inactivating mutations in tumor suppressors and up-regulation of protein kinases that control rDNA transcription correlate with elevated pre-rRNA levels in transformed cells. Overexpression of rRNA, in turn, could lead to excess protein synthesis and thus could be an initiating step in tumorigenesis. The comparative application of candidate-gene and proteomics approaches should uncover key pathways that are deregulated in cancer cells. Although there are many questions yet to be answered, the elucidation of the signaling pathways that transmit information on the growth state of a cell population to the Pol I transcription apparatus represent challenging and rewarding subjects for future studies.

Acknowledgments

I apologize to those colleagues whose work I did not cite because of restriction of space. I acknowledge the numerous students and postdoctoral fellows who have contributed over the years to the results of my group. My special thanks go to one of the referees, who provided exceptionally thorough and insightful suggestions on the manuscript. Our work has been supported by the Deutsche Forschungsgemeinschaft, the European Union, the Fonds der Chemischen Industrie, and the BMBF.

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Genes Dev. 2003, **17**:

Access the most recent version at doi:[10.1101/gad.1098503R](https://doi.org/10.1101/gad.1098503R)

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