Published in final edited form as:
Subcell Biochem. 2012 ; 61: . doi:10.1007/978-94-007-4525-4_10.

Basic Mechanisms in RNA Polymerase I Transcription of the Ribosomal RNA Genes

Sarah J. Goodfellow and **Joost C. B. M. Zomerdijk***
Wellcome Trust Centre for Gene Regulation and Expression, College of Life Sciences, University of Dundee , Dundee DD1 5EH , UK

Abstract

RNA Polymerase (Pol) I produces ribosomal (r)RNA, an essential component of the cellular protein synthetic machinery that drives cell growth, underlying many fundamental cellular processes. Extensive research into the mechanisms governing transcription by Pol I has revealed an intricate set of control mechanisms impinging upon rRNA production. Pol I-specific transcription factors guide Pol I to the rDNA promoter and contribute to multiple rounds of transcription initiation, promoter escape, elongation and termination. In addition, many accessory factors are now known to assist at each stage of this transcription cycle, some of which allow the integration of transcriptional activity with metabolic demands. The organisation and accessibility of rDNA chromatin also impinge upon Pol I output, and complex mechanisms ensure the appropriate maintenance of the epigenetic state of the nucleolar genome and its effective transcription by Pol I. The following review presents our current understanding of the components of the Pol I transcription machinery, their functions and regulation by associated factors, and the mechanisms operating to ensure the proper transcription of rDNA chromatin. The importance of such stringent control is demonstrated by the fact that deregulated Pol I transcription is a feature of cancer and other disorders characterised by abnormal translational capacity.

10.1 Introduction

In eukaryotic cells, the task of transcribing nuclear genes is shared by Pols I, II and III. Each of these polymerases is dedicated to the transcription of a different set of genes, known as class I, II or III genes, accordingly. Pol II produces messenger (m)RNAs, which code for cellular proteins, and many small nuclear (sn)RNAs, which are involved in mRNA processing. Pol III synthesises the transfer (t)RNAs, the 5S rRNA and a variety of other small, untranslated RNAs with essential roles in metabolism (White 2008). Unlike Pols II and III, which transcribe a variety of different genes, Pol I is dedicated to the synthesis of rRNA, and this accounts for up to 60% of transcriptional activity in a eukaryotic cell (Moss and Stefanovsky 2002). In mammals, the 47S pre-rRNA produced by Pol I is processed into mature 18S, 5.8S and 28S rRNAs, which are essential structural and catalytic components of ribosomes (Moore and Steitz 2002). Ribosomes constitute the core of the protein synthetic machinery; consequently, ribosome content is a critical determinant of protein accumulation and hence cell growth and division (Camacho et al. 1990; Siehl et al. 1985; Zetterberg and Killander 1965). The abundance of ribosomes within a cell depends upon the availability of rRNA (Liebhaber et al. 1978). Therefore, given the high metabolic burden of rRNA production and its direct influence on protein synthetic capacity, a tightly regulated

*j.zomerdijk@dundee.ac.uk.

Europe PMC Funders Author Manuscripts

transcription system has evolved, devoted to ensuring that rRNA synthesis is closely coupled with cellular growth demands.

10.2 The Ribosomal DNA

Ribosomal RNA synthesis by Pol I occurs in the nucleolus. This nuclear structure is also the site of ribosome assembly, which involves the incorporation of the rRNAs produced by Pol I along with Pol III-produced 5S rRNA and many ribosomal proteins (for reviews see Boisvert et al. 2007; Tschochner and Hurt 2003). The nucleolus forms around the nucleolar organiser regions (NORs) containing hundreds of rRNA genes, the majority (although not all) of which are organised head-to-tail into tandem arrays (Caburet et al. 2005; Nemeth and Langst 2011). Recent genome-wide mapping studies aimed at characterising the nucleolar genome have discovered that specific chromatin regions unrelated to the rDNA, and localised to distinct chromosomes, are also associated with nucleoli (Nemeth et al. 2010; van Koningsbruggen et al. 2010). These studies point towards a role for the nucleolus in the general organisation of chromosomes in the nucleus and imply a correlation between tethering to the nucleolar periphery and transcriptional silencing.

Approximately 150–200 rDNA repeats are found in the yeast genome, whereas human diploid cells have approximately 400 repeats (Birch and Zomerdijk 2008). However, only a subset of these genes (~50%) is transcribed at any given time. A recent study proposed that allelic inactivation of mammalian rRNA genes, occurring early in development, could account for this (Schlesinger et al. 2009). Active and inactive rDNA repeats are distinguished by distinct chromatin states, epigenetic marks, topological organisation and sub-nucleolar localisation (for reviews see Birch and Zomerdijk 2008; McStay and Grummt 2008; Nemeth and Langst 2011; Sanij and Hannan 2008). Transcriptionally inactive rDNA is found in a tightly packaged, heterochromatic state characterised by methylation of the DNA and repressive histone modifications, and is localised outwith the nucleolar fibrillar centre/fibrillar centre-dense fibrillar component border regions where rDNA transcription takes place. On the other hand, active rRNA genes are found in a more open chromatin state characterised by hypomethylated DNA, acetylated histones and, in mammals, an enrichment of the Pol I activator upstream binding factor (UBF). A related protein, Hmo1, specifically associates with active rDNA repeats in yeast (Merz et al. 2008). UBF binds DNA as a dimer through its high mobility group (HMG) domains, inducing substantial topological changes in DNA (Bazett-Jones et al. 1994; Jantzen et al. 1990). UBF binding is thought to be important for maintaining euchromatic rDNA, partly through its displacement of the repressive histone H1 (Kermekchiev et al. 1997; Nemeth and Langst 2011; Sanij et al. 2008) and is critical for nucleolar architecture, underpinning the structural organisation of rDNA into NORs (Mais et al. 2005). UBF has recently been shown to interact with the transcriptional regulator CTCF (CCCTC binding factor) (van de Nobelen et al. 2010), which has been implicated in the regional organisation of nucleolar rDNA (Guerrero and Maggert 2011). Extensive DNA looping is postulated to occur specifically in the active rDNA repeats, juxtaposing sites of transcription initiation and termination, mediated by Pol I-specific transcription factors and the proto-oncogene c-Myc (Denissov et al. 2011; Nemeth et al. 2008; Shiue et al. 2009). This higher order chromatin conformation of active rRNA genes further demonstrates the complexity of genome organisation within the nucleolus, the regulation of which is only just beginning to be understood.

In mammals, each rDNA repeat is approximately 43 kb and contains regulatory elements including promoters, repetitive enhancers and terminators within an intergenic spacer (IGS) of approximately 30 kb, and a single transcribed region of approximately 13 kb containing the 47S coding region (Fig. 10.1) (reviewed by McStay and Grummt 2008). In *S. cerevisiae*, each rDNA repeat is approximately 9.1 kb and contains a 6.9 kb 35S pre-rRNA coding

region and a comparatively short IGS (Albert et al. 2011; French et al. 2003). Although described as rDNA ‘repeats’, recent evidence indicates that the multiple rRNA genes are not simply identical copies of the same transcription unit but that, in fact, several rDNA variants exist and these can be differentially expressed and regulated (Santoro et al. 2010; Tseng et al. 2008).

Eukaryotic rRNA gene promoters contain two regulatory elements important for directing accurate and efficient transcription initiation: the core promoter and the upstream control element (UCE; functionally analogous to the yeast upstream promoter element (UPE)) (Fig. 10.1). The core promoter is sufficient for basal transcription by Pol I in most species (Paule and White 2000). The UCE lies further upstream (−156 to −107 relative to the transcription start site of human rRNA genes) and is important for stimulating transcription from the core promoter (Paule and White 2000; Russell and Zomerdijk 2005). Although the general layout of the rDNA promoter is conserved from yeast to humans, with the spacing and orientation of the core promoter and UPE/UCE being critical, there is little sequence similarity between elements and, as a result, Pol I transcription is highly species specific (for reviews see Grummt 2003; Heix and Grummt 1995). In addition to the main rRNA gene promoter, which directs pre-rRNA synthesis, related sequence elements known as spacer promoters have been identified within the IGS from several species (De Winter and Moss 1986; Grimaldi and Di Nocera 1988; Kuhn and Grummt 1987; Labhart and Reeder 1984) (Fig. 10.1). Studies using mouse cells suggest that transcripts produced by Pol I from these promoters (known as promoter RNA (pRNA)) are involved in transcriptional silencing of the rRNA genes (Mayer et al. 2006).

In plants, IGS transcripts of as yet undefined origin serve as precursors for the RNA-dependent RNA Polymerase 2 (RDR2). RDR2 works together with, among other enzymes, the plant-specific Pols IV and V in the production of siRNAs which also mediate class I gene silencing through epigenetic mechanisms (Lawrence et al. 2004; Pontes et al. 2006; Preuss et al. 2008).

10.3 Transcription by Pol I

Using the rDNA repeats as a template, Pol I catalyses the synthesis of rRNA. However, in order to do this accurately and efficiently, Pol I requires a number of accessory factors, which facilitate polymerase recruitment, initiation, promoter escape, elongation, termination and re-initiation, as discussed below.

10.3.1 Pre-initiation Complex (PIC) Assembly

RNA polymerases themselves have little affinity for promoter sequence elements and so rely upon specific transcription factors for accurate recruitment. Therefore, as with Pol II- and Pol III-driven transcription, the first stage of transcription by Pol I is the formation of a PIC at the gene promoter. A common feature of the basal transcription machinery used by Pols I, II and III is the requirement for a TBP-containing transcription factor complex. However, in each case, the combination of TBP-associated factors (TAFs) is polymerase-specific. Transcription by Pol I in mammalian cells is dependent upon selectivity factor 1 (SL1, termed TIF1B in mouse), which is a complex of TBP and at least four Pol I-specific TAFs: TAF_I110 (TAF1C), TAF_I63 (TAF1B), TAF_I48 (TAF1A) and TAF_I41 (TAF1D) (Comai et al. 1992, 1994; Eberhard et al. 1993; Gorski et al. 2007; Heix et al. 1997; Zomerdijk et al. 1994). An additional TAF, TAF12, which was originally described as a factor involved in the transcription of class II genes, has also been implicated as a component of the mammalian SL1 complex (Denissov et al. 2007).

SL1 is pivotal to PIC formation. It confers promoter specificity by recognising and binding the core promoter element in the rDNA repeat, it is essential for Pol I recruitment to the transcription start site, and it promotes a stable interaction between UBF and the rDNA promoter (Beckmann et al. 1995; Cavanaugh et al. 2002; Friedrich et al. 2005; Miller et al. 2001; Rudloff et al. 1994). Recently, SL1 has been shown to also have an essential post-polymerase recruitment role, operating through TAF1B (Naidu et al. 2011). TAF1B and the yeast orthologue Rrn7 are structurally and functionally related to TFIIB and the Brf proteins, which are involved in Pol II and Pol III transcription, respectively, thus extending and underscoring the parallels between the eukaryotic transcription machineries (Knutson and Hahn 2011 ; Naidu et al. 2011).

Furthermore, a role for SL1 in maintaining the promoters of active rRNA genes in a hypomethylated state has been proposed. This involves the TAF12-mediated recruitment of GADD45a (growth arrest and DNA damage inducible protein 45 alpha) and various components of the nucleotide excision repair (NER) machinery (Schmitz et al. 2009). In addition, SL1 is thought to contribute to the structural organisation of actively transcribed rRNA genes through interactions with promoter, upstream enhancer and terminator elements (Denissov et al. 2011). Transcription by Pol I in yeast also requires TBP and the core promoter binding complex core factor (CF), which is composed of three associated proteins, RRN6, 7 and 11 (Reeder 1999). RRN6, 7 and 11 are only distantly related to the mammalian SL1 subunits TAF_I110, TAF_I63 and TAF_I48, respectively, with limited sequence homologies (Boukhgalter et al. 2002; unpublished observations), highlighting the divergent nature of the rDNA promoter elements.

Core promoter selection and binding by SL1 is solely mediated by the TAFs, with TAF_I110, TAF_I63 and TAF_I48 being reported to make direct contacts with the DNA (Beckmann et al. 1995; Rudloff et al. 1994). SL1 TAFs are also crucial for the recruitment of Pol I. Pol I has 14 polypeptide subunits in yeast, homologues for 13 of which have been identified in mammals (Table 10.1). A catalytic core is formed by ten of these subunits, which are shared with or homologous to subunits found in Pols II and III (Kuhn et al. 2007; Werner et al. 2009). At the periphery, the A14 and A43 Pol I subunits associate as a heterodimer (Kuhn et al. 2007). Similar heterodimeric structures are also found in Pols II and III (see Table 10.1 for homologous subunits) (Werner et al. 2009). The remaining two Pol I-specific subunits (*S. cerevisiae* A49 and A34.5; mammalian PAF53 and CAST/PAF49) form a heterodimeric subcomplex that can dissociate from Pol I (Hanada et al. 1996; Huet et al. 1975; Kuhn et al. 2007; Yamamoto et al. 2004). Studies in yeast indicate that this subcomplex is structurally and functionally related to the TFIIE and TFIIF initiation factors used by Pol II (as indicated in Table 10.1), and can bind DNA and promote RNA cleavage (Geiger et al. 2010; Kuhn et al. 2007). These specific Pol I subunits function at multiple stages in the Pol I transcription cycle, playing important roles in polymerase recruitment, promoter escape and elongation (Albert et al. 2011; Beckouet et al. 2008; Kuhn et al. 2007; Panov et al. 2006a, b).

The multisubunit Pol I complex exists as at least two distinct subpopulations (Milkereit and Tschochner 1998; Miller et al. 2001), known as Pol Iα and Pol Iβ in mammalian cells (Miller et al. 2001). Both forms of Pol I are active and can catalyse the synthesis of RNA, but only Pol Iβ, which represents less than 10% of the total Pol I in a cell, can be incorporated into PICs and initiate accurate, promoter-specific transcription (Milkereit and Tschochner 1998; Miller et al. 2001). This is due, at least in part, to the association of Pol Iβ with RRN3 (Milkereit and Tschochner 1998; Miller et al. 2001) (murine TIF1A (Bodem et al. 2000)). RRN3 interacts directly with Pol I, through its A43 subunit (Cavanaugh et al. 2002; Peyroche et al. 2000). In addition, the Pol I-specific A49/A34.5 subcomplex is important for the association of RRN3 with Pol I, although it is unclear whether this is mediated by direct interactions between RRN3 and these subunits (Beckouet et al. 2008).

RRN3 also binds the CF subunit RRN6 in yeast, and SL1 subunits TAF_I110, TAF_I63 and TAF_I41 in mammals (Cavanaugh et al. 2002; Gorski et al. 2007; Miller et al. 2001; Peyroche et al. 2000). Therefore, RRN3 plays an essential, evolutionarily-conserved role in mediating specific transcription initiation at class I genes by connecting Pol I with an essential promoter-binding factor, and thus facilitating polymerase recruitment to the PIC at the rDNA promoter.

In addition to RRN3, various other proteins have been found specifically associated with Pol Iβ. For example, the serine/threonine kinase CK2 is present in Pol Iβ but not Pol Iα complexes and is found at the rDNA promoter in cells (Lin et al. 2006; Panova et al. 2006). Various roles have been proposed for this kinase in the regulation of transcription by Pol I (Bierhoff et al. 2008; Lin et al. 2006; Panova et al. 2006; Voit et al. 1992). Reports suggest that CK2 targets TAF_I110 and UBF and in this way regulates PIC assembly and stability, although the precise mechanistic details of this remain unclear (Lin et al. 2006; Panova et al. 2006). CK2 also phosphorylates the essential initiation factor TIF1A (the mouse counterpart of RRN3) (Bierhoff et al. 2008). However, rather than influencing PIC assembly, this modification seems important for the release of Pol I from promoter-bound initiation factors and thus elongation (Bierhoff et al. 2008). Another protein found specifically associated with the initiation-competent Pol Iβ complex is topoisomerase IIα (Panova et al. 2006). Interestingly, this topoisomerase IIα was found to be targeted by Pol Iβ-associated CK2. However, the significance of these observations to the regulation of transcription by Pol I have yet to be elucidated.

SL1 and Pol Iβ alone are sufficient to support basal levels of Pol I transcription *in vitro*. However, to achieve activated transcription, UBF must also be incorporated into the Pol I PIC. As discussed above, UBF binds throughout the rDNA in cells (O'Sullivan et al. 2002), playing critical roles as a nucleolar scaffold protein and in promoting decondensation of rDNA chromatin (Chen et al. 2004; Mais et al. 2005; Sanij et al. 2008). Crucially, UBF can also activate promoter-specific transcription by Pol I. UBF interacts cooperatively with SL1 at the rDNA promoter, with SL1 binding the highly acidic C-terminus of UBF through its TAF_I48 and TBP subunits (Beckmann et al. 1995; Bell et al. 1988; Hempel et al. 1996; Jantzen et al. 1992; Kihm et al. 1998; Tuan et al. 1999). This stabilises the association of UBF with the PIC (Friedrich et al. 2005), hence facilitating promoter-specific transcriptional activation. In addition to SL1, UBF also interacts with the PAF53 and PAF49/CAST subunits of Pol I (Hanada et al. 1996; Panov et al. 2006a, b; Seither et al. 1997; Whitehead et al. 1997). The HMG-box protein Hmo1 is involved in rDNA transcription in yeast and is, perhaps, the functional analogue of mammalian UBF (Gadal et al. 2002). Like UBF, Hmo1 binds throughout the rDNA repeat and acts synergistically with the Pol I-subunit A49 to activate transcription (Gadal et al. 2002; Hall et al. 2006; Kasahara et al. 2007). Interestingly, Albert et al. (2011) recently demonstrated the importance of the yeast A34.5-A49 subcomplex to nucleolar architecture. Given the fundamental role played by UBF as a nucleolar scaffold, it will be interesting to ascertain whether this property of these Pol I subunits is influenced by their interaction with Hmo1/UBF.

In summary, a series of cooperative protein-protein and protein-DNA interactions involving SL1, UBF (or their functional equivalents) and specific promoter elements are required for the recruitment of polymerase poised for the activated transcription of rRNA genes, as depicted in Fig. 10.2. Live cell imaging coupled with computational kinetic modelling has demonstrated a direct correlation between the efficiency of PIC assembly and transcriptional output in cells (Gorski et al. 2008).

10.3.2 Initiation and Promoter Escape

Following the assembly of a productive PIC at the rDNA promoter, promoter opening and transcription initiation by Pol I can commence, defined by the incorporation of the first ribonucleotides of the RNA chain. However, for productive RNA synthesis to ensue, Pol I must dissociate from the promoter-bound initiation factors in a process known as promoter escape (Panov et al. 2006a, b; Russell and Zomerdijk 2005). This post-PIC assembly event is rate-limiting for rRNA synthesis *in vitro* (Panov et al. 2001).

Promoter escape following transcription initiation coincides with the release of RRN3 from polymerase (Aprikian et al. 2001; Hirschler-Laszkiewicz et al. 2003; Milkereit and Tschochner 1998). In mouse cells, covalent attachment of RRN3 to the A43 Pol I subunit, with which RRN3 interacts, impairs rDNA transcription and cell cycle progression (Bierhoff et al. 2008). However, a similar approach pioneered in yeast strains lacking RRN3 and A43, but instead expressing a non-dissociable Pol I-RRN3 complex, did not detect any defects in rRNA synthesis or growth (Laferte et al. 2006). These studies suggest potential species-specific differences in the relative importance of RRN3 dissociation to the transcription cycle. The interaction between RRN3 and Pol I is controlled at least in part by phosphorylation. However, regulatory phosphorylation events also appear to vary from yeast to mammals: in yeast, Pol I phosphorylation apparently regulates this interaction, whereas in mammals, RRN3 phosphorylation seems important (Bierhoff et al. 2008; Cavanaugh et al. 2002; Fath et al. 2001). Bierhoff et al. (2008) looked specifically at the phosphorylation events regulating the dissociation of Pol I from RRN3 during promoter escape, and demonstrated that phosphorylation of two specific serine residues in mouse RRN3 (TIF1A) by CK2 promotes its release from polymerase.

The mammalian activator of Pol I transcription, UBF, also plays an important role in stimulating promoter escape (Panov et al. 2006a). UBF interacts with the Pol I-specific heterodimer PAF49/CAST-PAF53 (Hanada et al. 1996; Panov et al. 2006b), and this is important for transcriptional activation by UBF, which occurs subsequent to PIC assembly (Panov et al. 2006b). However, the mechanisms underlying this are unclear, although changes in DNA and/or polymerase conformation have been proposed (Panov et al. 2006b). In yeast, the homologues of these Pol I subunits (A34.5-A49) interact structurally and functionally with the probable yeast counterpart of UBF, Hmo1, and play an important role in promoter escape by promoting the release of RRN3 from elongating polymerase (Beckouet et al. 2008; Gadal et al. 2002; Schnapp et al. 1994). Conceivably, a network of interactions involving these factors could induce conformational changes in the PIC, triggering any post-translational modifications and the release of RRN3, converting initiation-competent Pol I into an elongating form.

10.3.3 Elongation

A mammalian cell requires approximately 8–10 million rRNA transcripts every 24 h to sustain adequate levels of ribosome biogenesis (Lewis and Tollervey 2000). Accordingly, transcription elongation by Pol I is highly efficient with, on average, 100 polymerases transcribing each active gene at a rate of approximately 95 nucleotides per second (Dundr et al. 2002). Similar elongation rates have also been observed for yeast Pol I (French et al. 2003). This impressive transcriptional output is achieved through the intrinsic processivity of Pol I and its cooperation with a multitude of other proteins.

Factors TFIIF and TFIIS are involved in elongation by Pol II (Saunders et al. 2006). Recent work using yeast has demonstrated that the A34.5-A49 subcomplex of Pol I is structurally and functionally analogous to TFIIF and is important for Pol I processivity (Geiger et al. 2010; Kuhn et al. 2007). Furthermore, the A12.2 subunit of Pol I stimulates the intrinsic

RNA cleavage activity of Pol I and shares functional and structural homology with TFIIS, which enhances the weak 3′-RNA cleavage activity of Pol II (Haag and Pikaard 2007; Kuhn et al. 2007). This cleavage activity might be required for RNA proofreading and to stimulate elongation by creating a new and correctly aligned 3′OH in the polymerase active site after stalling and backtracking of polymerase. A role for the A34.5-A49 subcomplex in permitting contact between adjacent Pol I molecules on the same rDNA template, which might contribute to efficient transcription elongation, has also been proposed (Albert et al. 2011).

Pol I-specific transcription factors are also thought to play a role in elongation. UBF is distributed throughout the rDNA repeats and has been reported to regulate Pol I elongation by phosphorylation-dependent remodelling of the rDNA chromatin (O'Sullivan et al. 2002; Stefanovsky et al. 2006). More recently, SL1 has been proposed to assist elongating Pol I via its role in anchoring the core promoter, upstream region and terminator, which provides a spatial arrangement favourable for productive rRNA synthesis (Denissov et al. 2011).

Furthermore, several additional factors are crucial for transcription elongation by Pol I in cells, allowing the polymerase to negotiate rDNA in the context of chromatin. For example, the histone chaperones nucleolin, nucleophosmin and FACT assist in rDNA transcription in mammalian cells (Birch et al. 2009; Murano et al. 2008; Rickards et al. 2007) and in yeast, Spt4/5 and Paf1C are important (Schneider et al. 2006, 2007; Zhang et al. 2009, 2010). Moreover, various chromatin remodelling and modifying activities have been shown to promote transcription by Pol I *in vivo* including Chd1p, Isw1p and Isw2p in yeast (Jones et al. 2007) and tip60, Williams syndrome transcription Factor (WSTF)-SNF2h and the histone methyltransferase G9a in mammalian cells (Halkidou et al. 2004; Percipalle et al. 2006; Yuan et al. 2007). Nuclear actin and myosin I drive transcription elongation by Pol I and this might, in part, be attributed to their interaction with the chromatin remodeler WSTF at the rDNA (Percipalle et al. 2006; Ye et al. 2008).

Given the high loading density of Pol I on rRNA genes, it is important that any other physical impediments encountered by a transcribing polymerase, caused by topological changes in the rDNA or DNA damage, for example, are efficiently resolved. Consequently, mechanisms ensure that such constraints are minimised. For instance, topoisomerases promote transcriptional elongation in yeast by relieving the positive and negative supercoiling that occurs ahead of and behind transcribing Pol I, respectively (Brill et al. 1987; El Hage et al. 2010; French et al. 2011; Schultz et al. 1992). Topoisomerase IIα is a component of Pol Iβ in human cells, as discussed above (Panova et al. 2006). Furthermore, topoisomerase I was found associated with Pol I complexes in mouse cells (Hannan et al. 1999; Rose et al. 1988), and has been proposed to assist transcriptional elongation by Pol I in human cells (Zhang et al. 1988). Therefore, this function of topoisomerases in relieving torsional strain during transcriptional elongation by Pol I may be evolutionarily conserved.

Signalling pathways invoked by DNA damage lead to a transient repression of rRNA synthesis, partly through the ATM-mediated displacement of elongating polymerase (Kruhlak et al. 2007). Resumption of Pol I transcription is dependent upon functional DNA repair mechanisms (Kruhlak et al. 2007). Transcription-coupled DNA repair occurs at rDNA genes (Conconi et al. 2002) and various DNA repair proteins have been found in Pol I complexes, including TFIIH, Cockayne syndrome B protein (CSB), Werner's syndrome helicase (WRN), Ku70/80 and several components of the NER machinery (Bradsher et al. 2002; Hannan et al. 1999; Iben et al. 2002; Schmitz et al. 2009; Shiratori et al. 2002). In many cases, these interactions have been shown to promote transcription by Pol I. However, a direct role for these factors in the transcription-coupled repair of rDNA has yet to be demonstrated.

As elongation by Pol I proceeds, the nascent pre-rRNA associates with components of the processing machinery, allowing co-transcriptional maturation of the rRNA and assembly of ribosomal particles (reviewed by Granneman and Baserga 2005). As a result, pre-rRNA synthesis and processing are closely coordinated, such that defective transcription by Pol I impairs pre-rRNA processing and vice versa (Granneman and Baserga 2005; Schneider et al. 2006, 2007). Although the mechanisms responsible for this coupling are incompletely defined, factors implicated in yeast include Spt4 and Spt5, which interact both with elongating Pol I and components of the pre-rRNA processing machinery (Leporé and Lafontaine 2011; Schneider et al. 2006, 2007). Such rigorous coordination likely contributes to the highly efficient and tightly regulated production of ribosomes.

10.3.4 Termination

Transcription termination by Pol I is a multistep process involving specific DNA sequence elements and regulatory proteins. In mammals, transcription termination factor TTF-I binds terminator elements downstream of the rRNA gene (T_1-T_{10}; Fig. 10.1), causing polymerase pausing. Dissociation of the paused transcription complex is then mediated by Pol I and transcript release factor PTRF. A similar mechanism is thought to operate in yeast, involving the TTF-I homologue Reb1p (Jansa and Grummt 1999). However, recent studies using yeast have uncovered further complexity in the control of transcription termination by Pol I, by demonstrating the existence of a ‘torpedo’ mechanism, similar to that employed for the termination of transcription by Pol II. This process begins with cleavage of the nascent pre-rRNA by the endonuclease Rnt1, followed by the progressive digestion of the resulting Pol I-associated RNA cleavage product mediated by the cooperative actions of the 5′ to 3′ exonuclease Rat1 (mammalian Xrn2) and the RNA helicase Sen1 (Braglia et al. 2010, 2011; El Hage et al. 2008; Kawauchi et al. 2008). Recognition of the Rnt1-cleaved pre-rRNA by Rat1 is thought to be controlled by phosphorylation of the 5′end of the RNA by the polynucleotide kinase Grc3 (Braglia et al. 2010). Once Rat1 reaches elongating Pol I, the transcription complex becomes unstable and dissociates from DNA, thus resulting in transcription termination. In addition, the smallest Pol I subunit A12.2/RPA12 is critical for effective transcription termination, potentially mediated by its stimulation of the intrinsic 3′-end RNA cleavage activity of Pol I (Haag and Pikaard 2007; Kuhn et al. 2007; Prescott et al. 2004). The Pol II and III homologues of RPA12, RPB9 and RPC11, are also important for relief of polymerase pausing and termination. Furthermore, each of these polymerase subunits shares homology with the RNA cleavage enhancing factor TFIIS (Prescott et al. 2004). It is possible that several mechanisms co-exist to ensure accurate, efficient termination of transcription by Pol I and thus cell viability (Braglia et al. 2011).

10.3.5 Re-initiation

Correct termination of transcription and release of the nascent rRNA is required for re-initiation by Pol I. Once a gene is activated, the rate of re-initiation will contribute to the overall level of transcripts produced. In addition to terminator elements located downstream of the rRNA coding region, TTF-I binding sites are also found immediately upstream of the rDNA promoter (termed T_0) and downstream of the spacer promoter (T_{sp}) (Fig. 10.1) (Nemeth et al. 2008). Interactions between TTF-1 and its binding sites are thought to be important for epigenetic and topological regulation of the rDNA (for reviews see McStay and Grummt 2008; Nemeth and Langst 2011). In cells, efficient recycling and re-initiation by Pol I might be facilitated by TTF-1-mediated juxtaposition of the terminator and promoter elements, which results in the formation of DNA loops (Nemeth et al. 2008; Nemeth and Langst 2011; Shiue et al. 2009). In addition to TTF-I, SL1 and c-Myc have been implicated in the formation of such DNA loops (Denissov et al. 2011; Nemeth and Langst 2011; Shiue et al. 2009).

Another important event in transcription re-initiation by Pol I is the re-association of the essential initiation factor RRN3 with polymerase, allowing Pol I to re-assemble with SL1 and UBF, which remain promoter bound following escape of elongating polymerase (Lin et al. 2006; Panov et al. 2001). This reversible interaction between Pol I and RRN3 underpins critical transitions in the Pol I transcription cycle, and is evolutionarily conserved. However, the mechanisms underlying this are not fully elucidated. Such a dynamic partnership is likely controlled through reversible post-translational modifications (Bierhoff et al. 2008; Cavanaugh et al. 2002; Fath et al. 2001). In support of this, Bierhoff et al. (2008) demonstrated that dephosphorylation of the CK2 target sites (serines 170 and 172) in TIF-1A (mouse RRN3) by the protein phosphatase FCP1 is required for the re-association of TIF-1A with Pol I, and hence re-initiation. In contrast, these particular residues are not conserved in yeast RRN3 and phosphorylation of Pol I itself is thought to regulate Pol I-RRN3 complex formation (Bierhoff et al. 2008; Fath et al. 2001). FCP1 has been implicated in the regulation of Pol I transcription in yeast, but rather than functioning in re-initiation, this phosphatase appears to promote chain elongation (Fath et al. 2004). Whether additional kinases and/or phosphatases targeting RRN3 and/or Pol I are involved in a conserved mechanism for promoting multiple rounds of transcription remains to be determined.

10.4 Regulation of rDNA Transcription

Stringent regulatory mechanisms operate to ensure a precise balance between the requirement for and availability of rRNA, allowing cells to control their capacity for protein synthesis in response to changing metabolic needs. For example, transcription by Pol I is low when nutrients or mitogens are limiting, but upregulated when the availability of these growth stimuli increase. In addition, Pol I transcription is regulated in response to a range of cellular stresses and during many fundamental cellular processes, including cellular differentiation and throughout the cell cycle (for reviews see Drygin et al. 2010; Grummt 2003; Grummt and Voit 2010; Mayer and Grummt 2005; Moss 2004; Russell and Zomerdijk 2005). The level of cellular rRNA is determined by the rate at which active rRNA genes are transcribed, and also by the number of active genes. Some of the mechanisms influencing these different aspects of class I gene expression are discussed below.

10.4.1 Regulation of the Pol I Transcription Machinery

In yeast, growth-dependent changes in rRNA synthesis can be achieved both through altering the proportion of active genes, and the rate of transcription from already active loci (Grummt and Pikaard 2003; Russell and Zomerdijk 2005). However, in mammalian cells, changes in rRNA production in response to growth signals seem to be mediated mainly by the latter mechanism. A plethora of cellular control pathways have been shown to mediate such acute changes in rRNA synthesis by directly regulating the activity of the Pol I transcription machinery, with positive regulators of growth activating transcription and negative regulators of growth having repressive effects (Drygin et al. 2010; Grummt 2003; Moss 2004; Russell and Zomerdijk 2005). Some of these regulatory proteins, and their effects on the Pol I transcription machinery, are listed in Table 10.2.

10.4.2 Regulation of rDNA Chromatin

In addition to these mechanisms that modulate the activity of the Pol I transcription machinery, epigenetic regulation of the rDNA chromatin, which determines the number of active rRNA genes, can also influence the level of rRNA produced. Such epigenetic regulation is thought to be stably propagated throughout cell divisions to ensure that an appropriate proportion of active and inactive rDNA repeats is maintained. The importance of this to nucleolar integrity, genomic stability and the global regulation of gene expression has

been proposed (Espada et al. 2007; Guetg et al. 2010; Ide et al. 2010; McStay and Grummt 2008; Paredes and Maggert 2009).

One of the key factors in establishing the epigenetic state of rRNA genes in mammals is TTF-I, which, in addition to its role as a transcription terminator and potential regulator of rDNA topology, can define active or inactive rDNA conformations through its association with chromatin remodelling complexes (reviewed by McStay and Grummt 2008). Epigenetic silencing by TTF-I is mediated by its recruitment of the nucleolar remodelling complex (NoRC) to the promoter-proximal T_0 element. The NoRC subunit TIP5 interacts with TTF-I, and NoRC in turn recruits DNA methyltransferases DNMT1 and DNMT3, and the histone deacetylase-containing Sin3 complex, which mediate transcriptional repression (Santoro and Grummt 2005; Santoro et al. 2002; Zhou et al. 2002). Methylation of a single CpG dinucleotide at the mouse rDNA promoter seems particularly important for transcriptional silencing, as this diminishes binding of the activator UBF to the rDNA (Santoro and Grummt 2001, 2005). NoRC function is dependent on the association of TIP5 with pRNAs. These 150–300 nucleotide transcripts are derived from the IGS by Pol I-driven transcription from the spacer promoters of a subset of hypomethylated rRNA genes (Mayer et al. 2006, 2008; Santoro et al. 2010). Such transcripts are essential for epigenetic silencing of rDNA. Furthermore, a recent study has shown that pRNA can induce *de novo* methylation of rDNA and transcriptional silencing independently of TTF-I and NoRC, by interacting directly with the T_0 element forming a DNA-RNA triplex which is recognised by DNMT3b (Schmitz et al. 2010). In fact, binding of TTF-I and pRNA to T_0 are mutually exclusive (Schmitz et al. 2010). The levels of pRNA and its association with T_0 vary during S phase progression, suggesting a potential link between pRNA and the transmission of epigenetic rDNA silencing between cell divisions (Santoro et al. 2010; Schmitz et al. 2010). Studies of the molecular basis of nucleolar dominance in plant hybrids, whereby NORs from one parental species are dominant over the other, also suggest an involvement of non-coding RNAs derived from the rDNA IGS in determining a repressive pattern of DNA methylation and histone deacetylation (reviewed by Tucker et al. 2010). Therefore, the involvement of such RNAs in selecting the proportion of transcriptionally silenced rDNA repeats, through epigenetic mechanisms, appears to be evolutionarily conserved.

Mechanisms also exist to maintain a proportion of rDNA repeats in an active chromatin conformation. For instance, binding of TTF-I to the T_0 elements of certain rDNA repeats in mouse cells induces chromatin remodelling and transcriptional activation (Langst et al. 1997). This is mediated by an interaction between TTF-I and the chromatin remodeler CSB (Yuan et al. 2007). Transcriptional activation by CSB is dependent on its intrinsic ATPase activity, and also its association with the histone methyltransferase G9a (Yuan et al. 2007). Therefore, TTF-I is integral in determining whether rDNA repeats adopt an active or an inactive epigenetic conformation. However, it is unclear how TTF-I interacts differentially with these positive and negative regulators of transcription to achieve a precise balance between these alternative chromatin states.

Other factors proposed to maintain rDNA in a euchromatic state include the methyl-CpG binding domain protein MBD3, TAF12-recruited GADD45a and the putative chromatin remodeler CHD7 (chromodomain helicase DNA-binding protein 7). These proteins prevent repressive methylation of the rDNA and/or promote the active demethylation of this region (Brown and Szyf 2007; Schmitz et al. 2009; Zentner et al. 2010). In addition to DNA methylation, the methylation state of the rDNA-associated histones also correlates with transcriptional activity: di- and tri-methylation of Lys4 and mono- and di-methylation of Lys36 of histone H3 mark active repeats, whereas di-methylation of Lys9 of histone H3 is associated with silenced rDNA chromatin. The JmjC domain-containing lysine

demethylases JHDM1B, KDM2A and PHF8 associate with rDNA and influence this histone methylation pattern (Feng et al. 2010; Frescas et al. 2007; Tanaka et al. 2010).

UBF is also involved in determining the number of active rDNA repeats (Sanij et al. 2008). However, this does not appear to involve epigenetic modifications of the chromatin, but instead occurs through the ability of UBF to displace histone H1, thus preventing H1-induced chromatin condensation (Sanij et al. 2008). In yeast, the UBF-related protein Hmo1 localises to active rDNA repeats, and a recent study has demonstrated the importance of this to the maintenance of a transcriptionally-competent chromatin state, established following DNA replication through the Pol I transcription-dependent (and potentially histone chaperone-dependent) displacement of nucleosomes (Wittner et al. 2011).

Although mechanisms directly influencing the activity of the Pol I transcription machinery are important for the modulation of rRNA production in response to growth signals, alterations in the rDNA chromatin are also likely to contribute. For instance, Murayama et al. (2008) have described a complex known as eNoSC (energy-dependent nucleolar silencing complex), which mediates the epigenetic repression of rRNA genes in response to energy deprivation. Furthermore, the lysine demethylase KDM2A targets mono- and di-methylated Lys36 of histone H3 and in this way represses transcription by Pol I in response to starvation (Tanaka et al. 2010). In addition to these dynamic alterations in rDNA chromatin in response to changing metabolic conditions, reducing the number of active rDNA repeats has been proposed to contribute to the down-regulation of Pol I transcription that accompanies differentiation (Sanij and Hannan 2008). Moreover, the proportion of active rDNA repeats varies depending on the developmental stage and cell type, indicating that lineage-specific regulation of the number of actively transcribed rRNA genes could be important for vertebrate development (Haaf et al. 1991; Schlesinger et al. 2009).

10.4.3 Deregulated Transcription by Pol I and Disease

The regulation of Pol I transcription is clearly a crucial feature of normal cellular growth and proliferation. The importance of such stringent control is highlighted by the fact that transcription by Pol I is deregulated in various disease states. Most notably, pre-rRNA levels are elevated in a wide range of tumour types and this is thought to be a general feature of human cancers (reviewed by Ruggero and Pandolfi 2003; White 2008). Inactivation of tumour suppressors, aberrant activation of oncogenes (many of which target the Pol I transcription machinery directly as outlined in Table 10.2) and loss of rDNA methylation, are all thought to play a role in this abnormal activation of rDNA expression, contributing to the uncontrolled cell growth and division that is characteristic of tumour cells. In addition, elevated transcription by Pol I underlies the hypertrophic growth of cardiomyocytes, which is a characteristic feature of various cardiovascular disorders (Brandenburger et al. 2001). In contrast, decreased rRNA production, as a result of rDNA promoter hypermethylation in the cerebral cortex, has been described as a feature of the neurodegeneration that accompanies Alzheimer's disease (Pietrzak et al. 2011). Furthermore, the demethylase PHF8 might link dynamic histone methylation at rDNA to mental retardation with cleft lip and palate (Feng et al. 2010).

The consequences of deregulated Pol I transcription during development are highlighted by recent findings regarding the genetic basis of Treacher Collins Syndrome, which can be caused by mutations in Pol I/III subunits or the UBF-interacting protein Treacle (Dauwerse et al. 2011; Valdez et al. 2004). This craniofacial autosomal-dominant disorder is characterised by a deficiency in neural crest cells, resulting from inadequate ribosome production during development. Abnormalities in ribosome biogenesis give rise to a variety of other congenital disorders, emphasizing the importance of understanding the mechanisms impinging upon ribosome production (Narla and Ebert 2010).

10.5 Conclusions

Transcription by Pol I underlies fundamental cellular functions. Our understanding of this process has grown in recent years, revealing unanticipated complexity. An increasing number of functions are being attributed to the Pol I-specific transcription factors, unravelling the means by which they facilitate rRNA synthesis. Furthermore, many additional factors are now known to bind and regulate this machinery. Recent insights into the intricacies of the structural and topological organisation of rDNA further demonstrate the multifaceted cellular control mechanisms which impinge upon rRNA production. Such elaborate regulation highlights the importance for precise control of transcription by Pol I, a point further emphasized by the apparently universal deregulation of rRNA expression in human tumours. Despite the ever-expanding list of factors and epigenetic control mechanisms that influence transcription by Pol I, the full implications of many of these discoveries have yet to be established. Therefore, further research directed towards resolving the many unanswered questions regarding the complex interplay between regulatory mechanisms targeting the rDNA chromatin and the Pol I transcription machinery is essential, not only to enhance our understanding of cellular growth controls, but also to enable the development of prognostic tools and therapeutic strategies for disease.

Acknowledgments

We thank Dr Jackie Russell for critical reading and helpful comments. We thank the Wellcome Trust for supporting our research through a Wellcome Trust Programme Grant (085441/Z/08/Z) awarded to JCBMZ.

References

Albert B, Leger-Silvestre I, Normand C, Ostermaier MK, Perez-Fernandez J, Panov KI, Zomerdijk JC, Schultz P, Gadal O. RNA polymerase I-specific subunits promote polymerase clustering to enhance the rRNA gene transcription cycle. J Cell Biol. 2011; 192:277–293. [PubMed: 21263028]

Aprikian P, Moorefield B, Reeder RH. New model for the yeast RNA polymerase I transcription cycle. Mol Cell Biol. 2001; 21:4847–4855. [PubMed: 11438642]

Arabi A, Wu S, Ridderstrale K, Bierhoff H, Shiue C, Fatyol K, Fahlen S, Hydbring P, Soderberg O, Grummt I, Larsson LG, Wright AP. c-Myc associates with ribosomal DNA and activates RNA polymerase I transcription. Nat Cell Biol. 2005; 7:303–310. [PubMed: 15723053]

Ayrault O, Andrique L, Fauvin D, Eymin B, Gazzeri S, Seite P. Human tumor suppressor p14ARF negatively regulates rRNA transcription and inhibits UBF1 transcription factor phosphorylation. Oncogene. 2006; 25:7577–7586. [PubMed: 16924243]

Bazett-Jones DP, Leblanc B, Herfort M, Moss T. Short-range DNA looping by the Xenopus HMG-box transcription factor, xUBF. Science. 1994; 264:1134–1137. [PubMed: 8178172]

Beckmann H, Chen JL, O'Brien T, Tjian R. Coactivator and promoter-selective properties of RNA polymerase I TAFs. Science. 1995; 270:1506–1509. [PubMed: 7491500]

Beckouet F, Labarre-Mariotte S, Albert B, Imazawa Y, Werner M, Gadal O, Nogi Y, Thuriaux P. Two RNA polymerase I subunits control the binding and release of Rrn3 during transcription. Mol Cell Biol. 2008; 28:1596–1605. [PubMed: 18086878]

Bell SP, Learned RM, Jantzen HM, Tjian R. Functional cooperativity between transcription factors UBF1 and SL1 mediates human ribosomal RNA synthesis. Science. 1988; 241:1192–1197. [PubMed: 3413483]

Bierhoff H, Dundr M, Michels AA, Grummt I. Phosphorylation by casein kinase 2 facilitates rRNA gene transcription by promoting dissociation of TIF-IA from elongating RNA polymerase I. Mol Cell Biol. 2008; 28:4988–4998. [PubMed: 18559419]

Birch JL, Zomerdijk JC. Structure and function of ribosomal RNA gene chromatin. Biochem Soc Trans. 2008; 36:619–624. [PubMed: 18631128]

Europe PMC Funders Author Manuscripts

Birch JL, Tan BC, Panov KI, Panova TB, Andersen JS, Owen-Hughes TA, Russell J, Lee SC, Zomerdijk JC. FACT facilitates chromatin transcription by RNA polymerases I and III. EMBO J. 2009; 28:854–865. [PubMed: 19214185]

Bodem J, Dobreva G, Hoffmann-Rohrer U, Iben S, Zentgraf H, Delius H, Vingron M, Grummt I. TIF-IA, the factor mediating growth-dependent control of ribosomal RNA synthesis, is the mammalian homolog of yeast Rrn3p. EMBO Rep. 2000; 1:171–175. [PubMed: 11265758]

Boisvert FM, van Koningsbruggen S, Navascues J, Lamond AI. The multifunctional nucleolus. Nat Rev Mol Cell Biol. 2007; 8:574–585. [PubMed: 17519961]

Boukhgalter B, Liu M, Guo A, Tripp M, Tran K, Huynh C, Pape L. Characterization of a fission yeast subunit of an RNA polymerase I essential transcription initiation factor, SpRrn7h/TAF(I)68, that bridges yeast and mammals: association with SpRrn11h and the core ribosomal RNA gene promoter. Gene. 2002; 291:187–201. [PubMed: 12095692]

Bradsher J, Auriol J, Proietti de Santis L, Iben S, Vonesch JL, Grummt I, Egly JM. CSB is a component of RNA pol I transcription. Mol Cell. 2002; 10:819–829. [PubMed: 12419226]

Braglia P, Heindl K, Schleiffer A, Martinez J, Proudfoot NJ. Role of the RNA/DNA kinase Grc3 in transcription termination by RNA polymerase I. EMBO Rep. 2010; 11:758–764. [PubMed: 20814424]

Braglia P, Kawauchi J, Proudfoot NJ. Co-transcriptional RNA cleavage provides a failsafe termination mechanism for yeast RNA polymerase I. Nucleic Acids Res. 2011; 39:1439–1448. [PubMed: 20972219]

Brandenburger Y, Jenkins A, Autelitano DJ, Hannan RD. Increased expression of UBF is a critical determinant for rRNA synthesis and hypertrophic growth of cardiac myocytes. FASEB J. 2001; 15:2051–2053. [PubMed: 11511532]

Brill SJ, DiNardo S, Voelkel-Meiman K, Sternglanz R. Need for DNA topoisomerase activity as a swivel for DNA replication for transcription of ribosomal RNA. Nature. 1987; 326:414–416. [PubMed: 2436053]

Brown SE, Szyf M. Epigenetic programming of the rRNA promoter by MBD3. Mol Cell Biol. 2007; 27:4938–4952. [PubMed: 17452452]

Caburet S, Conti C, Schurra C, Lebofsky R, Edelstein SJ, Bensimon A. Human ribosomal RNA gene arrays display a broad range of palindromic structures. Genome Res. 2005; 15:1079–1085. [PubMed: 16024823]

Camacho JA, Peterson CJ, White GJ, Morgan HE. Accelerated ribosome formation and growth in neonatal pig hearts. Am J Physiol. 1990; 258:C86–91. [PubMed: 1689115]

Cavanaugh AH, Hempel WM, Taylor LJ, Rogalsky V, Todorov G, Rothblum LI. Activity of RNA polymerase I transcription factor UBF blocked by Rb gene product. Nature. 1995; 374:177–180. [PubMed: 7877691]

Cavanaugh AH, Hirschler-Laszkiewicz I, Hu Q, Dundr M, Smink T, Misteli T, Rothblum LI. Rrn3 phosphorylation is a regulatory checkpoint for ribosome biogenesis. J Biol Chem. 2002; 277:27423–27432. [PubMed: 12015311]

Chen D, Belmont AS, Huang S. Upstream binding factor association induces large-scale chromatin decondensation. Proc Natl Acad Sci USA. 2004; 101:15106–15111. [PubMed: 15477594]

Claypool JA, French SL, Johzuka K, Eliason K, Vu L, Dodd JA, Beyer AL, Nomura M. Tor pathway regulates Rrn3p-dependent recruitment of yeast RNA polymerase I to the promoter but does not participate in alteration of the number of active genes. Mol Biol Cell. 2004; 15:946–956. [PubMed: 14595104]

Comai L, Tanese N, Tjian R. The TATA-binding protein and associated factors are integral components of the RNA polymerase I transcription factor, SL1. Cell. 1992; 68:965–976. [PubMed: 1547496]

Comai L, Zomerdijk JC, Beckmann H, Zhou S, Admon A, Tjian R. Reconstitution of transcription factor SL1: exclusive binding of TBP by SL1 or TFIID subunits. Science. 1994; 266:1966–1972. [PubMed: 7801123]

Conconi A, Bespalov VA, Smerdon MJ. Transcription-coupled repair in RNA polymerase I-transcribed genes of yeast. Proc Natl Acad Sci USA. 2002; 99:649–654. [PubMed: 11782531]

Europe PMC Funders Author Manuscripts

Dauwerse JG, Dixon J, Seland S, Ruivenkamp CA, van Haeringen A, Hoefsloot LH, Peters DJ, Boers AC, Daumer-Haas C, Maiwald R, Zweier C, Kerr B, Cobo AM, Toral JF, Hoogeboom AJ, Lohmann DR, Hehr U, Dixon MJ, Breuning MH, Wieczorek D. Mutations in genes encoding subunits of RNA polymerases I and III cause Treacher Collins syndrome. Nat Genet. 2011; 43:20–22. [PubMed: 21131976]

De Winter RF, Moss T. Spacer promoters are essential for efficient enhancement of *X. laevis* ribosomal transcription. Cell. 1986; 44:313–318. [PubMed: 3943126]

Denissov S, van Driel M, Voit R, Hekkelman M, Hulsen T, Hernandez N, Grummt I, Wehrens R, Stunnenberg H. Identification of novel functional TBP-binding sites and general factor repertoires. EMBO J. 2007; 26:944–954. [PubMed: 17268553]

Denissov S, Lessard F, Mayer C, Stefanovsky V, van Driel M, Grummt I, Moss T, Stunnenberg HG. A model for the topology of active ribosomal RNA genes. EMBO Rep. 2011; 12:231–237. [PubMed: 21331097]

Drygin D, Rice WG, Grummt I. The RNA polymerase I transcription machinery: an emerging target for the treatment of cancer. Annu Rev Pharmacol Toxicol. 2010; 50:131–156. [PubMed: 20055700]

Dundr M, Hoffmann-Rohrer U, Hu Q, Grummt I, Rothblum LI, Phair RD, Misteli T. A kinetic framework for a mammalian RNA polymerase in vivo. Science. 2002; 298:1623–1626. [PubMed: 12446911]

Eberhard D, Tora L, Egly JM, Grummt I. A TBP-containing multiprotein complex (TIF-IB) mediates transcription specificity of murine RNA polymerase I. Nucleic Acids Res. 1993; 21:4180–4186. [PubMed: 8414971]

El Hage A, Koper M, Kufel J, Tollervey D. Efficient termination of transcription by RNA polymerase I requires the 5′ exonuclease Rat1 in yeast. Genes Dev. 2008; 22:1069–1081. [PubMed: 18413717]

El Hage A, French SL, Beyer AL, Tollervey D. Loss of Topoisomerase I leads to R-loop-mediated transcriptional blocks during ribosomal RNA synthesis. Genes Dev. 2010; 24:1546–1558. [PubMed: 20634320]

Espada J, Ballestar E, Santoro R, Fraga MF, Villar-Garea A, Nemeth A, Lopez-Serra L, Ropero S, Aranda A, Orozco H, Moreno V, Juarranz A, Stockert JC, Langst G, Grummt I, Bickmore W, Esteller M. Epigenetic disruption of ribosomal RNA genes and nucleolar architecture in DNA methyltransferase 1 (Dnmt1) deficient cells. Nucleic Acids Res. 2007; 35:2191–2198. [PubMed: 17355984]

Fath S, Milkereit P, Peyroche G, Riva M, Carles C, Tschochner H. Differential roles of phosphorylation in the formation of transcriptional active RNA polymerase I. Proc Natl Acad Sci USA. 2001; 98:14334–14339. [PubMed: 11717393]

Fath S, Kobor MS, Philippi A, Greenblatt J, Tschochner H. Dephosphorylation of RNA polymerase I by Fcp1p is required for efficient rRNA synthesis. J Biol Chem. 2004; 279:25251–25259. [PubMed: 15073185]

Feng W, Yonezawa M, Ye J, Jenuwein T, Grummt I. PHF8 activates transcription of rRNA genes through H3K4me3 binding and H3K9me1/2 demethylation. Nat Struct Mol Biol. 2010; 17(4):445–450. [PubMed: 20208542]

French SL, Osheim YN, Cioci F, Nomura M, Beyer AL. In exponentially growing *Saccharomyces cerevisiae* cells, rRNA synthesis is determined by the summed RNA polymerase I loading rate rather than by the number of active genes. Mol Cell Biol. 2003; 23:1558–1568. [PubMed: 12588976]

French SL, Sikes ML, Hontz RD, Osheim YN, Lambert TE, El Hage A, Smith MM, Tollervey D, Smith JS, Beyer AL. Distinguishing the roles of Topoisomerases I and II in relief of transcription-induced torsional stress in yeast rRNA genes. Mol Cell Biol. 2011; 31:482–494. [PubMed: 21098118]

Frescas D, Guardavaccaro D, Bassermann F, Koyama-Nasu R, Pagano M. JHDM1B/FBXL10 is a nucleolar protein that represses transcription of ribosomal RNA genes. Nature. 2007; 450:309–313. [PubMed: 17994099]

Europe PMC Funders Author Manuscripts

Friedrich JK, Panov KI, Cabart P, Russell J, Zomerdijk JC. TBP-TAF complex SL1 directs RNA polymerase I pre-initiation complex formation and stabilizes upstream binding factor at the rDNA promoter. J Biol Chem. 2005; 280:29551–29558. [PubMed: 15970593]

Gadal O, Labarre S, Boschiero C, Thuriaux P. Hmo1, an HMG-box protein, belongs to the yeast ribosomal DNA transcription system. EMBO J. 2002; 21:5498–5507. [PubMed: 12374750]

Geiger SR, Lorenzen K, Schreieck A, Hanecker P, Kostrewa D, Heck AJ, Cramer P. RNA polymerase I contains a TFIIF-related DNA-binding subcomplex. Mol Cell. 2010; 39:583–594. [PubMed: 20797630]

Gorski JJ, Pathak S, Panov K, Kasciukovic T, Panova T, Russell J, Zomerdijk JC. A novel TBP-associated factor of SL1 functions in RNA polymerase I transcription. EMBO J. 2007; 26:1560–1568. [PubMed: 17318177]

Gorski SA, Snyder SK, John S, Grummt I, Misteli T. Modulation of RNA polymerase assembly dynamics in transcriptional regulation. Mol Cell. 2008; 30:486–497. [PubMed: 18498750]

Grandori C, Gomez-Roman N, Felton-Edkins ZA, Ngouenet C, Galloway DA, Eisenman RN, White RJ. c-Myc binds to human ribosomal DNA and stimulates transcription of rRNA genes by RNA polymerase I. Nat Cell Biol. 2005; 7:311–318. [PubMed: 15723054]

Granneman S, Baserga SJ. Crosstalk in gene expression: coupling and co-regulation of rDNA transcription, pre-ribosome assembly and pre-rRNA processing. Curr Opin Cell Biol. 2005; 17:281–286. [PubMed: 15901498]

Grimaldi G, Di Nocera PP. Multiple repeated units in *Drosophila melanogaster* ribosomal DNA spacer stimulate rRNA precursor transcription. Proc Natl Acad Sci USA. 1988; 85:5502–5506. [PubMed: 2840664]

Grummt I. Life on a planet of its own: regulation of RNA polymerase I transcription in the nucleolus. Genes Dev. 2003; 17:1691–1702. [PubMed: 12865296]

Grummt I, Pikaard CS. Epigenetic silencing of RNA polymerase I transcription. Nat Rev Mol Cell Biol. 2003; 4:641–649. [PubMed: 12923526]

Grummt I, Voit R. Linking rDNA transcription to the cellular energy supply. Cell Cycle. 2010; 9:225–226. [PubMed: 20023389]

Guerrero PA, Maggert KA. The CCCTC-binding factor (CTCF) of Drosophila contributes to the regulation of the ribosomal DNA and nucleolar stability. PLoS One. 2011; 6:e16401. [PubMed: 21283722]

Guetg C, Lienemann P, Sirri V, Grummt I, Hernandez-Verdun D, Hottiger MO, Fussenegger M, Santoro R. The NoRC complex mediates the heterochromatin formation and stability of silent rRNA genes and centromeric repeats. EMBO J. 2010; 29:2135–2146. [PubMed: 20168299]

Haaf T, Hayman DL, Schmid M. Quantitative determination of rDNA transcription units in vertebrate cells. Exp Cell Res. 1991; 193:78–86. [PubMed: 1995304]

Haag JR, Pikaard CS. RNA polymerase I: a multifunctional molecular machine. Cell. 2007; 131:1224–1225. [PubMed: 18160031]

Halkidou K, Logan IR, Cook S, Neal DE, Robson CN. Putative involvement of the histone acetyltransferase Tip60 in ribosomal gene transcription. Nucleic Acids Res. 2004; 32:1654–1665. [PubMed: 15016909]

Hall DB, Wade JT, Struhl K. An HMG protein, Hmo1, associates with promoters of many ribosomal protein genes and throughout the rRNA gene locus in *Saccharomyces cerevisiae*. Mol Cell Biol. 2006; 26:3672–3679. [PubMed: 16612005]

Hanada K, Song CZ, Yamamoto K, Yano K, Maeda Y, Yamaguchi K, Muramatsu M. RNA polymerase I associated factor 53 binds to the nucleolar transcription factor UBF and functions in specific rDNA transcription. EMBO J. 1996; 15:2217–2226. [PubMed: 8641287]

Hannan RD, Cavanaugh A, Hempel WM, Moss T, Rothblum L. Identification of a mammalian RNA polymerase I holoenzyme containing components of the DNA repair/replication system. Nucleic Acids Res. 1999; 27:3720–3727. [PubMed: 10471742]

Hannan KM, Hannan RD, Smith SD, Jefferson LS, Lun M, Rothblum LI. Rb and p130 regulate RNA polymerase I transcription: Rb disrupts the interaction between UBF and SL-1. Oncogene. 2000; 19:4988–4999. [PubMed: 11042686]

Hannan KM, Brandenburger Y, Jenkins A, Sharkey K, Cavanaugh A, Rothblum L, Moss T, Poortinga G, McArthur GA, Pearson RB, Hannan RD. mTOR-dependent regulation of ribosomal gene transcription requires S6K1 and is mediated by phosphorylation of the carboxy-terminal activation domain of the nucleolar transcription factor UBF. Mol Cell Biol. 2003; 23:8862–8877. [PubMed: 14612424]

Heix J, Grummt I. Species specificity of transcription by RNA polymerase I. Curr Opin Genet Dev. 1995; 5:652–656. [PubMed: 8664554]

Heix J, Zomerdijk JC, Ravanpay A, Tjian R, Grummt I. Cloning of murine RNA polymerase I-specific TAF factors: conserved interactions between the subunits of the species-specific transcription initiation factor TIF-IB/SL1. Proc Natl Acad Sci USA. 1997; 94:1733–1738. [PubMed: 9050847]

Hempel WM, Cavanaugh AH, Hannan RD, Taylor L, Rothblum LI. The species-specific RNA polymerase I transcription factor SL-1 binds to upstream binding factor. Mol Cell Biol. 1996; 16:557–563. [PubMed: 8552083]

Hirschler-Laszkiewicz I, Cavanaugh AH, Mirza A, Lun M, Hu Q, Smink T, Rothblum LI. Rrn3 becomes inactivated in the process of ribosomal DNA transcription. J Biol Chem. 2003; 278:18953–18959. [PubMed: 12646563]

Hoppe S, Bierhoff H, Cado I, Weber A, Tiebe M, Grummt I, Voit R. AMP-activated protein kinase adapts rRNA synthesis to cellular energy supply. Proc Natl Acad Sci USA. 2009; 106:17781–17786. [PubMed: 19815529]

Huet J, Buhler JM, Sentenac A, Fromageot P. Dissociation of two polypeptide chains from yeast RNA polymerase A. Proc Natl Acad Sci USA. 1975; 72:3034–3038. [PubMed: 1103135]

Iben S, Tschochner H, Bier M, Hoogstraten D, Hozak P, Egly JM, Grummt I. TFIIH plays an essential role in RNA polymerase I transcription. Cell. 2002; 109:297–306. [PubMed: 12015980]

Ide S, Miyazaki T, Maki H, Kobayashi T. Abundance of ribosomal RNA gene copies maintains genome integrity. Science. 2010; 327:693–696. [PubMed: 20133573]

Jansa P, Grummt I. Mechanism of transcription termination: PTRF interacts with the largest subunit of RNA polymerase I and dissociates paused transcription complexes from yeast and mouse. Mol Gen Genet. 1999; 262:508–514. [PubMed: 10589839]

Jantzen HM, Admon A, Bell SP, Tjian R. Nucleolar transcription factor hUBF contains a DNA-binding motif with homology to HMG proteins. Nature. 1990; 344:830–836. [PubMed: 2330041]

Jantzen HM, Chow AM, King DS, Tjian R. Multiple domains of the RNA polymerase I activator hUBF interact with the TATA-binding protein complex hSL1 to mediate transcription. Genes Dev. 1992; 6:1950–1963. [PubMed: 1398072]

Jones HS, Kawauchi J, Braglia P, Alen CM, Kent NA, Proudfoot NJ. RNA polymerase I in yeast transcribes dynamic nucleosomal rDNA. Nat Struct Mol Biol. 2007; 14:123–130. [PubMed: 17259992]

Kasahara K, Ohtsuki K, Ki S, Aoyama K, Takahashi H, Kobayashi T, Shirahige K, Kokubo T. Assembly of regulatory factors on rRNA and ribosomal protein genes in *Saccharomyces cerevisiae*. Mol Cell Biol. 2007; 27:6686–6705. [PubMed: 17646381]

Kawauchi J, Mischo H, Braglia P, Rondon A, Proudfoot NJ. Budding yeast RNA polymerases I and II employ parallel mechanisms of transcriptional termination. Genes Dev. 2008; 22:1082–1092. [PubMed: 18413718]

Kermekchiev M, Workman JL, Pikaard CS. Nucleosome binding by the polymerase I trans-activator upstream binding factor displaces linker histone H1. Mol Cell Biol. 1997; 17:5833–5842. [PubMed: 9315641]

Kihm AJ, Hershey JC, Haystead TA, Madsen CS, Owens GK. Phosphorylation of the rRNA transcription factor upstream binding factor promotes its association with TATA binding protein. Proc Natl Acad Sci USA. 1998; 95:14816–14820. [PubMed: 9843972]

Knutson BA, Hahn S. Yeast Rrn7 and human TAF1B are TFIIB-related RNA polymerase I general transcription factors. Science. 2011; 333:1637–1640. [PubMed: 21921198]

Kruhlak M, Crouch EE, Orlov M, Montano C, Gorski SA, Nussenzweig A, Misteli T, Phair RD, Casellas R. The ATM repair pathway inhibits RNA polymerase I transcription in response to chromosome breaks. Nature. 2007; 447:730–734. [PubMed: 17554310]

Europe PMC Funders Author Manuscripts

Kuhn A, Grummt I. A novel promoter in the mouse rDNA spacer is active in vivo and in vitro. EMBO J. 1987; 6:3487–3492. [PubMed: 3428263]

Kuhn CD, Geiger SR, Baumli S, Gartmann M, Gerber J, Jennebach S, Mielke T, Tschochner H, Beckmann R, Cramer P. Functional architecture of RNA polymerase I. Cell. 2007; 131:1260–1272. [PubMed: 18160037]

Labhart P, Reeder RH. Enhancer-like properties of the 60/81 bp elements in the ribosomal gene spacer of *Xenopus laevis*. Cell. 1984; 37:285–289. [PubMed: 6722873]

Laferte A, Favry E, Sentenac A, Riva M, Carles C, Chedin S. The transcriptional activity of RNA polymerase I is a key determinant for the level of all ribosome components. Genes Dev. 2006; 20:2030–2040. [PubMed: 16882981]

Langst G, Blank TA, Becker PB, Grummt I. RNA polymerase I transcription on nucleosomal templates: the transcription termination factor TTF-I induces chromatin remodeling and relieves transcriptional repression. EMBO J. 1997; 16:760–768. [PubMed: 9049305]

Lawrence RJ, Earley K, Pontes O, Silva M, Chen ZJ, Neves N, Viegas W, Pikaard CS. A concerted DNA methylation/histone methylation switch regulates rRNA gene dosage control and nucleolar dominance. Mol Cell. 2004; 13:599–609. [PubMed: 14992728]

Leporé N, Lafontaine DLJ. A functional interface at the rDNA connects rRNA synthesis, pre-rRNA processing and nucleolar surveillance in budding yeast. PLoS One. 2011; 6:e24962. [PubMed: 21949810]

Lessard F, Morin F, Ivanchuk S, Langlois F, Stefanovsky V, Rutka J, Moss T. The ARF tumor suppressor controls ribosome biogenesis by regulating the RNA polymerase I transcription factor TTF-I. Mol Cell. 2010; 38:539–550. [PubMed: 20513429]

Lewis JD, Tollervey D. Like attracts like: getting RNA processing together in the nucleus. Science. 2000; 288:1385–1389. [PubMed: 10827942]

Liebhaber SA, Wolf S, Schlessinger D. Differences in rRNA metabolism of primary and SV40-transformed human fibroblasts. Cell. 1978; 13:121–127. [PubMed: 202397]

Lin CY, Navarro S, Reddy S, Comai L. CK2-mediated stimulation of Pol I transcription by stabilization of UBF-SL1 interaction. Nucleic Acids Res. 2006; 34:4752–4766. [PubMed: 16971462]

Mais C, Wright JE, Prieto JL, Raggett SL, McStay B. UBF-binding site arrays form pseudo-NORs and sequester the RNA polymerase I transcription machinery. Genes Dev. 2005; 19:50–64. [PubMed: 15598984]

Mayer C, Grummt I. Cellular stress and nucleolar function. Cell Cycle. 2005; 4:1036–1038. [PubMed: 16205120]

Mayer C, Zhao J, Yuan X, Grummt I. mTOR-dependent activation of the transcription factor TIF-IA links rRNA synthesis to nutrient availability. Genes Dev. 2004; 18:423–434. [PubMed: 15004009]

Mayer C, Bierhoff H, Grummt I. The nucleolus as a stress sensor: JNK2 inactivates the transcription factor TIF-IA and down-regulates rRNA synthesis. Genes Dev. 2005; 19:933–941. [PubMed: 15805466]

Mayer C, Schmitz KM, Li J, Grummt I, Santoro R. Intergenic transcripts regulate the epigenetic state of rRNA genes. Mol Cell. 2006; 22:351–361. [PubMed: 16678107]

Mayer C, Neubert M, Grummt I. The structure of NoRC-associated RNA is crucial for targeting the chromatin remodelling complex NoRC to the nucleolus. EMBO Rep. 2008; 9:774–780. [PubMed: 18600236]

McStay B, Grummt I. The epigenetics of rRNA genes: from molecular to chromosome biology. Annu Rev Cell Dev Biol. 2008; 24:131–157. [PubMed: 18616426]

Merz K, Hondele M, Goetze H, Gmelch K, Stoeckl U, Griesenbeck J. Actively transcribed rRNA genes in *S. cerevisiae* are organized in a specialized chromatin associated with the high-mobility group protein Hmo1 and are largely devoid of histone molecules. Genes Dev. 2008; 22:1190–1204. [PubMed: 18451108]

Milkereit P, Tschochner H. A specialized form of RNA polymerase I, essential for initiation and growth-dependent regulation of rRNA synthesis, is disrupted during transcription. EMBO J. 1998; 17:3692–3703. [PubMed: 9649439]

Europe PMC Funders Author Manuscripts

Miller G, Panov KI, Friedrich JK, Trinkle-Mulcahy L, Lamond AI, Zomerdijk JC. hRRN3 is essential in the SL1-mediated recruitment of RNA polymerase I to rRNA gene promoters. EMBO J. 2001; 20:1373–1382. [PubMed: 11250903]

Moore PB, Steitz TA. The involvement of RNA in ribosome function. Nature. 2002; 418:229–235. [PubMed: 12110899]

Moss T. At the crossroads of growth control; making ribosomal RNA. Curr Opin Genet Dev. 2004; 14:210–217. [PubMed: 15196469]

Moss T, Stefanovsky VY. At the center of eukaryotic life. Cell. 2002; 109:545–548. [PubMed: 12062097]

Murano K, Okuwaki M, Hisaoka M, Nagata K. Transcription regulation of the rRNA gene by a multifunctional nucleolar protein, B23/nucleophosmin, through its histone chaperone activity. Mol Cell Biol. 2008; 28:3114–3126. [PubMed: 18332108]

Murayama A, Ohmori K, Fujimura A, Minami H, Yasuzawa-Tanaka K, Kuroda T, Oie S, Daitoku H, Okuwaki M, Nagata K, Fukamizu A, Kimura K, Shimizu T, Yanagisawa J. Epigenetic control of rDNA loci in response to intracellular energy status. Cell. 2008; 133:627–639. [PubMed: 18485871]

Muth V, Nadaud S, Grummt I, Voit R. Acetylation of TAF(I)68, a subunit of TIF-IB/SL1, activates RNA polymerase I transcription. EMBO J. 2001; 20:1353–1362. [PubMed: 11250901]

Naidu S, Friedrich JK, Russell J, Zomerdijk JC. TAF1B is a TFIIB-like component of the basal transcription machinery for RNA polymerase I. Science. 2011; 333:1640–1642. [PubMed: 21921199]

Narla A, Ebert BL. Ribosomopathies: human disorders of ribosome dysfunction. Blood. 2010; 115:3196–3205. [PubMed: 20194897]

Nemeth A, Langst G. Genome organization in and around the nucleolus. Trends Genet. 2011; 27:149–156. [PubMed: 21295884]

Nemeth A, Guibert S, Tiwari VK, Ohlsson R, Langst G. Epigenetic regulation of TTF-I-mediated promoter-terminator interactions of rRNA genes. EMBO J. 2008; 27:1255–1265. [PubMed: 18354495]

Nemeth A, Conesa A, Santoyo-Lopez J, Medina I, Montaner D, Peterfia B, Solovei I, Cremer T, Dopazo J, Langst G. Initial genomics of the human nucleolus. PLoS Genet. 2010; 6:e1000889. [PubMed: 20361057]

O'Sullivan AC, Sullivan GJ, McStay B. UBF binding in vivo is not restricted to regulatory sequences within the vertebrate ribosomal DNA repeat. Mol Cell Biol. 2002; 22:657–668. [PubMed: 11756560]

Panov KI, Friedrich JK, Zomerdijk JC. A step subsequent to preinitiation complex assembly at the ribosomal RNA gene promoter is rate limiting for human RNA polymerase I-dependent transcription. Mol Cell Biol. 2001; 21:2641–2649. [PubMed: 11283244]

Panov KI, Friedrich JK, Russell J, Zomerdijk JC. UBF activates RNA polymerase I transcription by stimulating promoter escape. EMBO J. 2006a; 25:3310–3322. [PubMed: 16858408]

Panov KI, Panova TB, Gadal O, Nishiyama K, Saito T, Russell J, Zomerdijk JC. RNA polymerase I-specific subunit CAST/hPAF49 has a role in the activation of transcription by upstream binding factor. Mol Cell Biol. 2006b; 26:5436–5448. [PubMed: 16809778]

Panova TB, Panov KI, Russell J, Zomerdijk JC. Casein kinase 2 associates with initiation-competent RNA polymerase I and has multiple roles in ribosomal DNA transcription. Mol Cell Biol. 2006; 26:5957–5968. [PubMed: 16880508]

Paredes S, Maggert KA. Ribosomal DNA contributes to global chromatin regulation. Proc Natl Acad Sci USA. 2009; 106:17829–17834. [PubMed: 19822756]

Paule MR, White RJ. Survey and summary: transcription by RNA polymerases I and III. Nucleic Acids Res. 2000; 28:1283–1298. [PubMed: 10684922]

Pelletier G, Stefanovsky VY, Faubladier M, Hirschler-Laszkiewicz I, Savard J, Rothblum LI, Cote J, Moss T. Competitive recruitment of CBP and Rb-HDAC regulates UBF acetylation and ribosomal transcription. Mol Cell. 2000; 6:1059–1066. [PubMed: 11106745]

Percipalle P, Fomproix N, Cavellan E, Voit R, Reimer G, Kruger T, Thyberg J, Scheer U, Grummt I, Farrants AK. The chromatin remodelling complex WSTF-SNF2h interacts with nuclear myosin 1

and has a role in RNA polymerase I transcription. EMBO Rep. 2006; 7:525–530. [PubMed: 16514417]

Peyroche G, Milkereit P, Bischler N, Tschochner H, Schultz P, Sentenac A, Carles C, Riva M. The recruitment of RNA polymerase I on rDNA is mediated by the interaction of the A43 subunit with Rrn3. EMBO J. 2000; 19:5473–5482. [PubMed: 11032814]

Pietrzak M, Rempala G, Nelson PT, Zheng JJ, Hetman M. Epigenetic silencing of nucleolar rRNA genes in Alzheimer's disease. PLoS One. 2011; 6:e22585. [PubMed: 21799908]

Pistoni M, Verrecchia A, Doni M, Guccione E, Amati B. Chromatin association and regulation of rDNA transcription by the Ras-family protein RasL11a. EMBO J. 2010; 29:1215–1224. [PubMed: 20168301]

Pontes O, Li CF, Costa Nunes P, Haag J, Ream T, Vitins A, Jacobsen SE, Pikaard CS. The Arabidopsis chromatin-modifying nuclear siRNA pathway involves a nucleolar RNA processing center. Cell. 2006; 126:79–92. [PubMed: 16839878]

Prescott EM, Osheim YN, Jones HS, Alen CM, Roan JG, Reeder RH, Beyer AL, Proudfoot NJ. Transcriptional termination by RNA polymerase I requires the small subunit Rpa12p. Proc Natl Acad Sci USA. 2004; 101:6068–6073. [PubMed: 15073335]

Preuss SB, Costa-Nunes P, Tucker S, Pontes O, Lawrence RJ, Mosher R, Kasschau KD, Carrington JC, Baulcombe DC, Viegas W, Pikaard CS. Multimegabase silencing in nucleolar dominance involves siRNA-directed DNA methylation and specific methylcytosine-binding proteins. Mol Cell. 2008; 32:673–684. [PubMed: 19061642]

Reeder RH. Regulation of RNA polymerase I transcription in yeast and vertebrates. Prog Nucleic Acid Res Mol Biol. 1999; 62:293–327. [PubMed: 9932458]

Rickards B, Flint SJ, Cole MD, LeRoy G. Nucleolin is required for RNA polymerase I transcription in vivo. Mol Cell Biol. 2007; 27:937–948. [PubMed: 17130237]

Rose KM, Szopa J, Han FS, Cheng YC, Richter A, Scheer U. Association of DNA topoisomerase I and RNA polymerase I: a possible role for topoisomerase I in ribosomal gene transcription. Chromosoma. 1988; 96:411–416. [PubMed: 2851418]

Rudloff U, Eberhard D, Tora L, Stunnenberg H, Grummt I. TBP-associated factors interact with DNA and govern species specificity of RNA polymerase I transcription. EMBO J. 1994; 13:2611–2616. [PubMed: 8013460]

Ruggero D, Pandolfi PP. Does the ribosome translate cancer? Nat Rev Cancer. 2003; 3:179–192. [PubMed: 12612653]

Russell J, Zomerdijk JC. RNA-polymerase-I-directed rDNA transcription, life and works. Trends Biochem Sci. 2005; 30:87–96. [PubMed: 15691654]

Sanij E, Hannan RD. Chromatin organization and expression. Genome Biol. 2008; 9:305. [PubMed: 18423066]

Sanij E, Poortinga G, Sharkey K, Hung S, Holloway TP, Quin J, Robb E, Wong LH, Thomas WG, Stefanovsky V, Moss T, Rothblum L, Hannan KM, McArthur GA, Pearson RB, Hannan RD. UBF levels determine the number of active ribosomal RNA genes in mammals. J Cell Biol. 2008; 183:1259–1274. [PubMed: 19103806]

Santoro R, Grummt I. Molecular mechanisms mediating methylation-dependent silencing of ribosomal gene transcription. Mol Cell. 2001; 8:719–725. [PubMed: 11583633]

Santoro R, Grummt I. Epigenetic mechanism of rRNA gene silencing: temporal order of NoRC-mediated histone modification, chromatin remodeling, and DNA methylation. Mol Cell Biol. 2005; 25:2539–2546. [PubMed: 15767661]

Santoro R, Li J, Grummt I. The nucleolar remodeling complex NoRC mediates heterochromatin formation and silencing of ribosomal gene transcription. Nat Genet. 2002; 32:393–396. [PubMed: 12368916]

Santoro R, Schmitz KM, Sandoval J, Grummt I. Intergenic transcripts originating from a subclass of ribosomal DNA repeats silence ribosomal RNA genes in trans. EMBO Rep. 2010; 11:52–58. [PubMed: 20010804]

Saunders A, Core LJ, Lis JT. Breaking barriers to transcription elongation. Nat Rev Mol Cell Biol. 2006; 7:557–567. [PubMed: 16936696]

Schlesinger S, Selig S, Bergman Y, Cedar H. Allelic inactivation of rDNA loci. Genes Dev. 2009; 23:2437–2447. [PubMed: 19833769]

Schmitz KM, Schmitt N, Hoffmann-Rohrer U, Schafer A, Grummt I, Mayer C. TAF12 recruits Gadd45a and the nucleotide excision repair complex to the promoter of rRNA genes leading to active DNA demethylation. Mol Cell. 2009; 33:344–353. [PubMed: 19217408]

Schmitz KM, Mayer C, Postepska A, Grummt I. Interaction of noncoding RNA with the rDNA promoter mediates recruitment of DNMT3b and silencing of rRNA genes. Genes Dev. 2010; 24:2264–2269. [PubMed: 20952535]

Schnapp G, Santori F, Carles C, Riva M, Grummt I. The HMG box-containing nucleolar transcription factor UBF interacts with a specific subunit of RNA polymerase I. EMBO J. 1994; 13:190–199. [PubMed: 8306961]

Schneider DA, French SL, Osheim YN, Bailey AO, Vu L, Dodd J, Yates JR, Beyer AL, Nomura M. RNA polymerase II elongation factors Spt4p and Spt5p play roles in transcription elongation by RNA polymerase I and rRNA processing. Proc Natl Acad Sci USA. 2006; 103:12707–12712. [PubMed: 16908835]

Schneider DA, Michel A, Sikes ML, Vu L, Dodd JA, Salgia S, Osheim YN, Beyer AL, Nomura M. Transcription elongation by RNA polymerase I is linked to efficient rRNA processing and ribosome assembly. Mol Cell. 2007; 26:217–229. [PubMed: 17466624]

Schultz MC, Brill SJ, Ju Q, Sternglanz R, Reeder RH. Topoisomerases and yeast rRNA transcription: negative supercoiling stimulates initiation and topoisomerase activity is required for elongation. Genes Dev. 1992; 6:1332–1341. [PubMed: 1321070]

Seither P, Zatsepina O, Hoffmann M, Grummt I. Constitutive and strong association of PAF53 with RNA polymerase I. Chromosoma. 1997; 106:216–225. [PubMed: 9254723]

Shiratori M, Suzuki T, Itoh C, Goto M, Furuichi Y, Matsumoto T. WRN helicase accelerates the transcription of ribosomal RNA as a component of an RNA polymerase I-associated complex. Oncogene. 2002; 21:2447–2454. [PubMed: 11971179]

Shiue CN, Berkson RG, Wright AP. c-Myc induces changes in higher order rDNA structure on stimulation of quiescent cells. Oncogene. 2009; 28:1833–1842. [PubMed: 19270725]

Siehl D, Chua BH, Lautensack-Belser N, Morgan HE. Faster protein and ribosome synthesis in thyroxine-induced hypertrophy of rat heart. Am J Physiol. 1985; 248:C309–319. [PubMed: 3156508]

Stefanovsky VY, Pelletier G, Hannan R, Gagnon-Kugler T, Rothblum LI, Moss T. An immediate response of ribosomal transcription to growth factor stimulation in mammals is mediated by ERK phosphorylation of UBF. Mol Cell. 2001; 8:1063–1073. [PubMed: 11741541]

Stefanovsky V, Langlois F, Gagnon-Kugler T, Rothblum LI, Moss T. Growth factor signaling regulates elongation of RNA polymerase I transcription in mammals via UBF phosphorylation and r-chromatin remodeling. Mol Cell. 2006; 21:629–639. [PubMed: 16507361]

Tanaka Y, Okamoto K, Teye K, Umata T, Yamagiwa N, Suto Y, Zhang Y, Tsuneoka M. JmjC enzyme KDM2A is a regulator of rRNA transcription in response to starvation. EMBO J. 2010; 29:1510–1522. [PubMed: 20379134]

Tschochner H, Hurt E. Pre-ribosomes on the road from the nucleolus to the cytoplasm. Trends Cell Biol. 2003; 13:255–263. [PubMed: 12742169]

Tseng H, Chou W, Wang J, Zhang X, Zhang S, Schultz RM. Mouse ribosomal RNA genes contain multiple differentially regulated variants. PLoS One. 2008; 3:e1843. [PubMed: 18365001]

Tuan JC, Zhai W, Comai L. Recruitment of TATA-binding protein-TAFI complex SL1 to the human ribosomal DNA promoter is mediated by the carboxy-terminal activation domain of upstream binding factor (UBF) and is regulated by UBF phosphorylation. Mol Cell Biol. 1999; 19:2872–2879. [PubMed: 10082553]

Tucker S, Vitins A, Pikaard CS. Nucleolar dominance and ribosomal RNA gene silencing. Curr Opin Cell Biol. 2010; 22:351–356. [PubMed: 20392622]

Valdez BC, Henning D, So RB, Dixon J, Dixon MJ. The Treacher Collins syndrome (TCOF1) gene product is involved in ribosomal DNA gene transcription by interacting with upstream binding factor. Proc Natl Acad Sci USA. 2004; 101:10709–10714. [PubMed: 15249688]

van de Nobelen S, Rosa-Garrido M, Leers J, Heath H, Soochit W, Joosen L, Jonkers I, Demmers J, van der Reijden M, Torrano V, Grosveld F, Delgado MD, Renkawitz R, Galjart N, Sleutels F. CTCF regulates the local epigenetic state of ribosomal DNA repeats. Epigenetics Chromatin. 2010; 3:19. [PubMed: 21059229]

van Koningsbruggen S, Gierlinski M, Schofield P, Martin D, Barton GJ, Ariyurek Y, den Dunnen JT, Lamond AI. High-resolution whole-genome sequencing reveals that specific chromatin domains from most human chromosomes associate with nucleoli. Mol Biol Cell. 2010; 21:3735–3748. [PubMed: 20826608]

Vincent T, Kukalev A, Andang M, Pettersson R, Percipalle P. The glycogen synthase kinase (GSK) 3beta represses RNA polymerase I transcription. Oncogene. 2008; 27:5254–5259. [PubMed: 18490923]

Voit R, Grummt I. Phosphorylation of UBF at serine 388 is required for interaction with RNA polymerase I and activation of rDNA transcription. Proc Natl Acad Sci USA. 2001; 98:13631–13636. [PubMed: 11698641]

Voit R, Schnapp A, Kuhn A, Rosenbauer H, Hirschmann P, Stunnenberg HG, Grummt I. The nucleolar transcription factor mUBF is phosphorylated by casein kinase II in the C-terminal hyperacidic tail which is essential for transactivation. EMBO J. 1992; 11:2211–2218. [PubMed: 1600946]

Voit R, Schafer K, Grummt I. Mechanism of repression of RNA polymerase I transcription by the retinoblastoma protein. Mol Cell Biol. 1997; 17:4230–4237. [PubMed: 9234680]

Voit R, Hoffmann M, Grummt I. Phosphorylation by G1-specific cdk-cyclin complexes activates the nucleolar transcription factor UBF. EMBO J. 1999; 18:1891–1899. [PubMed: 10202152]

Werner M, Thuriaux P, Soutourina J. Structure-function analysis of RNA polymerases I and III. Curr Opin Struct Biol. 2009; 19:740–745. [PubMed: 19896367]

White RJ. RNA polymerases I and III, non-coding RNAs and cancer. Trends Genet. 2008; 24:622–629. [PubMed: 18980784]

Whitehead CM, Winkfein RJ, Fritzler MJ, Rattner JB. ASE-1: a novel protein of the fibrillar centres of the nucleolus and nucleolus organizer region of mitotic chromosomes. Chromosoma. 1997; 106:493–502. [PubMed: 9426281]

Wittner M, Hamperl S, Stockl U, Seufert W, Tschochner H, Milkereit P, Griesenbeck J. Establishment and maintenance of alternative chromatin states at a multicopy gene locus. Cell. 2011; 145:543–554. [PubMed: 21565613]

Yamamoto K, Yamamoto M, Hanada K, Nogi Y, Matsuyama T, Muramatsu M. Multiple protein-protein interactions by RNA polymerase I-associated factor PAF49 and role of PAF49 in rRNA transcription. Mol Cell Biol. 2004; 24:6338–6349. [PubMed: 15226435]

Ye J, Zhao J, Hoffmann-Rohrer U, Grummt I. Nuclear myosin I acts in concert with polymeric actin to drive RNA polymerase I transcription. Genes Dev. 2008; 22:322–330. [PubMed: 18230700]

Yuan X, Feng W, Imhof A, Grummt I, Zhou Y. Activation of RNA polymerase I transcription by cockayne syndrome group B protein and histone methyltransferase G9a. Mol Cell. 2007; 27:585–595. [PubMed: 17707230]

Zentner GE, Hurd EA, Schnetz MP, Handoko L, Wang C, Wang Z, Wei C, Tesar PJ, Hatzoglou M, Martin DM, Scacheri PC. CHD7 functions in the nucleolus as a positive regulator of ribosomal RNA biogenesis. Hum Mol Genet. 2010; 19:3491–3501. [PubMed: 20591827]

Zetterberg A, Killander D. Quantitative cytophotometric and autoradiographic studies on the rate of protein synthesis during interphase in mouse fibroblasts in vitro. Exp Cell Res. 1965; 40:1–11. [PubMed: 5838948]

Zhai W, Comai L. Repression of RNA polymerase I transcription by the tumor suppressor p53. Mol Cell Biol. 2000; 20:5930–5938. [PubMed: 10913176]

Zhang H, Wang JC, Liu LF. Involvement of DNA topoisomerase I in transcription of human ribosomal RNA genes. Proc Natl Acad Sci USA. 1988; 85:1060–1064. [PubMed: 2829214]

Zhang C, Comai L, Johnson DL. PTEN represses RNA Polymerase I transcription by disrupting the SL1 complex. Mol Cell Biol. 2005; 25:6899–6911. [PubMed: 16055704]

Zhang Y, Sikes ML, Beyer AL, Schneider DA. The Paf1 complex is required for efficient transcription elongation by RNA polymerase I. Proc Natl Acad Sci USA. 2009; 106:2153–2158. [PubMed: 19164765]

Zhang Y, Smith AD, Renfrow MB, Schneider DA. The RNA polymerase-associated factor 1 complex (Paf1C) directly increases the elongation rate of RNA polymerase I and is required for efficient regulation of rRNA synthesis. J Biol Chem. 2010; 285:14152–14159. [PubMed: 20299458]

Zhao J, Yuan X, Frodin M, Grummt I. ERK-dependent phosphorylation of the transcription initiation factor TIF-IA is required for RNA polymerase I transcription and cell growth. Mol Cell. 2003; 11:405–413. [PubMed: 12620228]

Zhou Y, Santoro R, Grummt I. The chromatin remodeling complex NoRC targets HDAC1 to the ribosomal gene promoter and represses RNA polymerase I transcription. EMBO J. 2002; 21:4632–4640. [PubMed: 12198165]

Zomerdijk JC, Beckmann H, Comai L, Tjian R. Assembly of transcriptionally active RNA polymerase I initiation factor SL1 from recombinant subunits. Science. 1994; 266:2015–2018. [PubMed: 7801130]

Europe PMC Funders Author Manuscripts

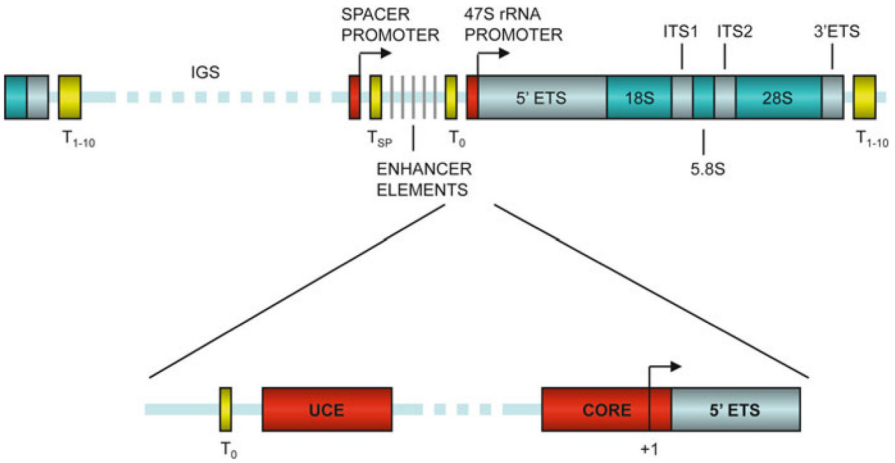

Fig. 10.1. Mammalian rDNA repeat and 47S rRNA promoter

The *top* panel illustrates key elements and the general organisation of a mammalian rDNA repeat. The IGS includes the spacer and 47S rRNA promoters, enhancer repeats and the TTF-I binding sites T_0 and T_{sp}. *Arrows* indicate start sites and direction of transcription. The coding region contains 5′ and 3′ external transcribed spacer (ETS) and two internal transcribed spacer (ITS) regions, along with regions encoding 18S, 5.8S and 28S rRNAs. Terminator elements (T_{1-10}) downstream of the 47S rRNA gene are also indicated. The *lower* panel illustrates the layout of the 47S rRNA promoter, which directs the assembly of the Pol I PIC and consists of an upstream control element, and a core promoter element overlapping the transcription start site.

Europe PMC Funders Author Manuscripts

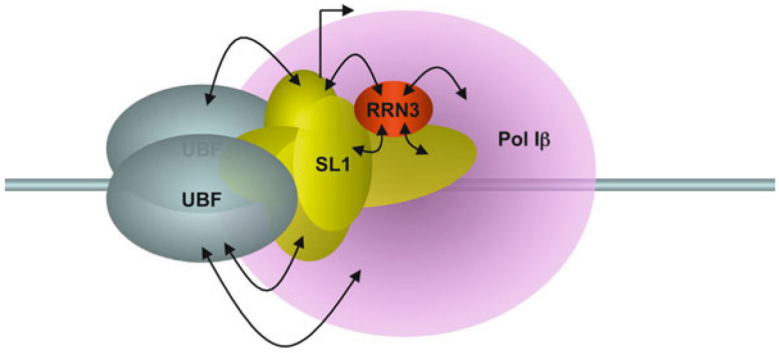

Fig. 10.2. The mammalian Pol I pre-initiation complex

Activated transcription by Pol I requires the assembly of Pol I-specific transcription factors SL1 and UBF at the rRNA promoter. In addition to contacts made between these transcription factors and the rDNA, several protein-protein interactions are also known to facilitate PIC assembly, as indicated by double-headed *arrows* (described in the text). A multitude of other factors cooperate with this transcription machinery to enhance PIC assembly and promote efficient rRNA synthesis by Pol I *in vivo*.

Table 10.1

Eukaryotic RNA polymerase subunits

***S. cerevisiae* Pol I subunits**	**Human Pol I subunits**	**Homologues in Pols II/III [or associated factors]**
Shared subunits		
RPB5 (ABC27, POLR2E)	hRPB5	shared
RPB6 (ABC23, POLR2F)	hRPB6	shared
RPB8 (ABC14.5, POLR2H)	hRPB8	shared
RPB10 (ABC10β, POLR2L)	hRPB10	shared
RPB12 (ABC10α, POLR2K)	hRPB12	shared
RPA40 (AC40, POLR1C)	hRPA40	RPB3/shared
RPA19 (AC19, POLR1D)	hRPA19	RPB11/shared
Homologous subunits		
RPA190 (A190, POLR1A)	hRPA190	RPB1/RPC160
RPA135 (A135, POLR1B)	hRPA135	RPB2/RPC128
RPA43 (A43, POLR1F)	hRPA43	RPB7/RPC25
RPA14 (A14)	*	RPB4/RPC17
RPA12 (A12.2, POLR1H)	hRPA12	RPB9/RPC11
RPA49 (A49, POLR1E)	PAF53	[TFIIF (Rap74 subunit) & TFIIE-β/RPC37 & RPC34]
RPA34.5 (A34.5, POLR1G)	CAST (PAF49)	[TFIIF (Rap30 subunit)/ RPC53]

* Human counterpart not yet identified

Table 10.2
Positive and negative regulators of cell growth target the Pol I transcription machinery

Regulatory factor	Targets in Pol I transcription machinery	References
Activators		
G1-specific cyclin/CDKs	UBF	Voit et al. (1999) and Voit and Grummt (2001)
ERK	RRN3, UBF	Stefanovsky et al. (2001) and Zhao et al. (2003)
RSK	RRN3	Zhao et al. (2003)
CK2	UBF, RRN3	Lin et al. (2006), Panova et al. (2006), and Bierhoff et al. (2008)
mTOR	RRN3, UBF	Hannan et al. (2003), Claypool et al. (2004), and Mayer et al. (2004)
CBP	UBF	Pelletier et al. (2000)
PCAF	SL1	Muth et al. (2001)
TIP60	UBF	Halkidou et al. (2004)
c-Myc	SL1	Arabi et al. (2005) and Grandori et al. (2005)
RasL11a	UBF	Pistoni et al. (2010)
Repressors		
p53	SL1	Zhai and Comai (2000)
RB/p130	UBF	Cavanaugh et al. (1995), Voit et al. (1997), and Hannan et al. (2000)
CK2	SL1	Panova et al. (2006)
PTEN	SL1	Zhang et al. (2005)
p14ARF	UBF, TTF-I	Ayrault et al. (2006) and Lessard et al. (2010)
GSK3β	SL1	Vincent et al. (2008)
AMPK	RRN3	Hoppe et al. (2009)
JNK2	RRN3	Mayer et al. (2005)