REVIEW ARTICLE

Regulation of ribosomal gene transcription

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INTRODUCTION

In eukaryotic cells, ribosomal RNA (rRNA) gene is transcribed by RNA polymerase I (pol I) in the nucleolus as a large (40 S–47 S) precursor RNA (pre-rRNA) (for reviews, see refs. [1,2]). The mature rRNA species (28 S, 18 S and 5.8 S) are formed after a series of specific endonucleolytic cleavage reactions (for a review, see ref. [3]). The rRNA gene (rDNA) is highly reiterated and is arranged in a tandem array in clusters of head-to-tail repeats. Each transcription unit is separated from the next unit by an intergenic spacer that ranges in size from 2 kb to over 30 kb. The transcriptionally active rRNA genes can be visualized in electron micrographic spreads of nucleolar chromatin as structures resembling Christmas trees where the rRNA gene, functional RNA pol I molecules and growing RNA chains can be observed as discrete entities [4]. Ribosomal RNA accounts for as much as 80% of total steady-state cellular RNA. In general, rRNA gene is transcribed at the correct initiation site and with maximal efficiency only in the extracts from the corresponding, or very closely related, species [5]. Transcription of the rRNA gene is controlled by *cis*-acting sequences and *trans*-acting factors that can specifically interact with the DNA elements. Since recent review articles have dealt with the *cis*-acting and *trans*-acting elements [6,7], the present article will focus on the regulation of rRNA gene transcription. A brief summary of the various protein factors involved in rDNA transcription (see Table 1) and the *cis*-acting elements (Figure 1) is provided to facilitate discussion of the transcriptional control. A model for rDNA transcription initiation is presented in Figure 2.

CIS-ACTING ELEMENTS

The ribosomal gene consists of three important regions, promoters, enhancers and terminators. By systematically analysing the template capacity of a series of deletion mutants *in vivo* and *in vitro*, the core promoter regions of rRNAs from different species have been shown to reside approximately between +10 and −40 with respect to the initiation site [8]. The promoter sequences are markedly diverged (for a review, see ref. [9]), which may partly explain the species-specific transcription of rRNA genes. The 5′ end of the core promoter can be extended to around −150 under *in vivo* and stringent *in vitro* conditions [10–13]. This distal promoter element has been designated upstream control element (UCE) or upstream promoter element (UPE). Alteration of the spacing between the core promoter sequence and the UPE by insertion or deletion of about half a DNA duplex can adversely affect rRNA gene transcription in homologous systems. Paradoxically, if half of a helical turn (5 bp) is inserted in, or removed from, the region between the upstream and downstream domains of the frog promoter, the frog rDNA promoter becomes non-functional in the homologous system, but can function efficiently and correctly in the mouse extract [14]. This observation indicates that the spacing between the key promoter elements can play a significant role in conferring species-specific transcription by pol I.

The enhancer sequences constitute another *cis*-acting element. The first well-characterized enhancer elements were the 60 bp or 80 bp repetitive elements in the frog which occur in blocks of 6–12 units [15,16]. These elements can stimulate transcription from the promoter and generally function independent of orientation or distance. Subsequently, a 130 bp repeat (11.5 times) element upstream of the rat rDNA promoter was shown to augment rat pol I transcription *in vitro* [17,18]. Recently, the *in vivo* enhancer function and the pol I specificity [19] of this element was also established. An analogous 140 bp repetitive (13 times) enhancer element was characterized in the mouse system [20,21]. In addition to the repetitive enhancers, rat rDNA also

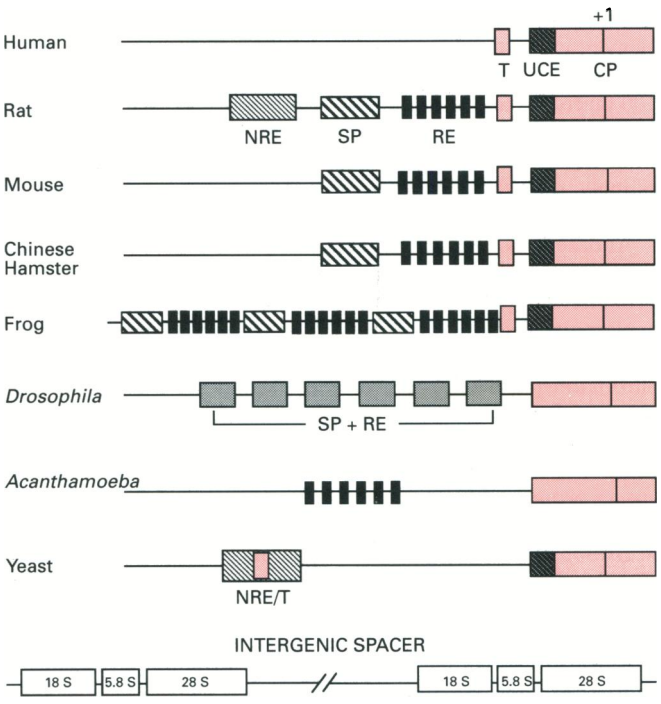

Figure 1 Schematic representation of eukaryotic rRNA gene promoters and other *cis*-acting regulatory elements in the intergenic spacer

Abbreviations: CP, rRNA gene promoter with transcription initiation site; UCE (UPE), upstream control element (upstream promoter element); T, terminator; RE, repeat enhancer; SP, spacer promoter; NRE, non-repeat enhancer; NRE/T, non-repeat enhancer and terminator.

Abbreviations used: CPBF, core promoter-binding factor; DNA-PK, DNA-activated protein kinase; E_1BF, enhancer 1-binding factor; E_1BF_c, control E_1BF from serum-enriched cells; E_1BF_s, E_1BF purified from serum-starved cells; GRE, glucocorticoid response element; HMG, high mobility group; PMA, phorbol 12-myristate 13-acetate; pol I, II and III, RNA polymerases I, II and III; SV40, simian virus 40; TAF, TBP-associated factor; TBP, TATA box-containing protein; UBF, upstream binding factor; UPE, upstream promoter element.

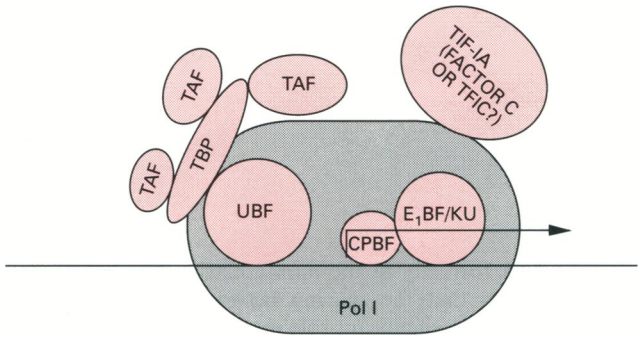

Figure 2 Model for ribosomal RNA gene transcription initiation in higher organisms

For details on the various *trans*-acting factors (coloured red), please refer to Table 1.

contains a non-repetitive enhancer element [17]. The 174 bp enhancer sequence located far upstream (approximately 2 kb from the initiation site) of the core promoter consists of at least two functional domains [22,23]. Unlike the repetitive enhancer, the non-repetitive enhancer can also stimulate transcription from the pol II promoters *in vivo* [19]. The latter effect may be due to the pol II regulatory elements associated with the 174 bp enhancer sequence [19]. A 190 bp enhancer element is required for maximal transcription of rDNA in *Saccharomyces cerevisiae* [24]. This element is located 2 kb upstream of the initiation site (similar to the position of rat non-repetitive enhancer element) and just 100 bp from the end of the rRNA precursor. A recent study [25] has shown that a 45 bp region at the 3′ end of the enhancer is both necessary and sufficient for full enhancer activity. Interestingly, the yeast enhancer also contains binding sites for three pol II transcription factors [26].

Another DNA element is the spacer promoter located upstream of the repetitive enhancer elements. The transcripts from these promoters appear to be relatively unstable [27], which could explain the absence of the transcripts within the spacer region in the electron micrographs of the functionally active nuclear chromatin [4]. The spacer promoters appear to enhance transcription from the adjacent core promoter in the rat [28] and frog [29]. The exact function of the spacer promoters has not been completely elucidated. Interestingly, transcription from the spacer promoters is active during development of *Drosophila* [30] and *Artemia* [31]. A recent study has shown that spacer promoters in *Drosophila* are important in regulating the initial expression of rDNA after fertilization (M. Pellegrini, personal communication). The function of the transcripts formed from the spacer promoter in the overall transcriptional regulation is not known.

A fourth *cis*-acting sequence is the terminator element. It consists of approximately 18 bp sequences which are called 'Sal boxes' in rodents and human (as each element contains a *Sal*I restriction site) or 'T3 boxes' in the frog (see ref. [7]). These short sequence motifs are recognized by a single termination factor, designated TTF I in mouse [32] or Rib 2 in frog [33, 33a]. In addition to its location at the 3′ end of the pre-rRNA coding region, the terminator is also found just upstream of the gene promoter. The termination reaction is pol I-specific [34] and pausing of pol I by the DNA-bound factor is followed by cleavage several bases upstream of the factor-binding site [35]. The mouse has at least eight *Sal*I boxes whereas the frog has only one terminator element at the end of the rRNA precursor. The latter terminator, however, contains a point mutation which prevents polymerase release after the 3′ end formation, allowing it to read through the intergenic spacer until the terminator just upstream of the core promoter stops further movement. The promoter proximal terminator could therefore protect the transcription complex on the adjacent gene promoter from the traversing polymerase molecules [36,37]. Several studies have shown that the promoter proximal terminator can also enhance transcription (see ref. [7]). This paradoxical observation is rather unique to the pol I transcription. In *Xenopus borealis*, the chromatin structures in the spacer region downstream of the rRNA genes are packaged in nucleosomes, whereas a heterogeneous structure is found in *Xenopus laevis*. This is consistent with the presence of a relatively weak terminator at the 3′ end of the latter frog species, which allows pol I to transcribe into the downstream spacer [38].

In *Saccharomyces cerevisae*, there are three termination sites, one within the enhancer distal to the promoter, and two located downstream between the enhancer and the 5 S gene. One of the terminators is located 108 bp downstream of the 3′ end of the 25 S rRNA and shares several characteristics with the vertebrate pol I terminator element [39].

RNA POLYMERASE I

RNA pol I is a multisubunit enzyme with a molecular mass of over 500 kDa (for review, see [6,40,41]). The exact composition of the functional enzyme is rather conjectural. Considerable advances have been made on the subunit gene structure of the yeast polymerase I [42,43]. These reports revealed considerable similarity among several subunits of pol I and those of pol II and pol III. RNA pol I, by itself, cannot direct transcription from the correct initiation site unless other *trans*-acting factors are present. Although pol I exclusively transcribes rRNA gene in yeast, plant, amoeba, insect, frog, rodents and humans, the parasitic protozoan *Trypanosoma brucei* is an exception to this rule. In this organism, certain protein-coding genes (proryclin and variant cell surface glycoprotein) are transcribed by pol I [44]. This phenomenon may be related to the novel mRNA maturation pathway in the protozoa, which involves *trans*-splicing of a 39-nucleotide RNA with a 5′ cap on to the pre-mRNA [45]. The presence of the cap structure of this RNA probably contributes to the efficient synthesis of the initial product by pol I and the production of a functional mRNA.

Earlier studies showed that pol I from higher eukaryotes consists of 5–8 subunits, whereas that of yeast contains several additional subunits. A recent purification of mouse RNA pol I using a novel procedure has, however, revealed additional subunits for this enzyme as well [46]. This study showed that the smaller subunit RPA 40 is a homologue of yeast RPC40. It also exhibited significant sequence identity to the α-subunit of *Escherichia coli* RNA polymerase, yeast RPB3 and human RPB33 RNA pol II subunits [46]. The overall structural conservation of this subunit suggests an important role for this subunit in the transcriptional process.

TRANS-ACTING FACTORS

Upstream binding factor (UBF)

The UBF is the first well-characterized RNA pol I *trans*-acting factor. This factor interacts with UPE and core promoter as well as the repetitive enhancer element. Although it has been identified in several lower and higher eukaryotes, extensive purification of the protein has been achieved only from the human, frog and rodent cells (see Table 1). The purified protein from these higher

Table 1 RNA polymerase I transcription factors

Abbreviation: n.d., not determined.

Transcription factor	Species	Molecular mass (kDa)	rDNA binding element(s)	Probable function(s)
UBF 1 and 2	Human	97 and 94	Core promoter, UCE (UPE), enhancer element (repetitive).	Enhances stable initiation complex formation and activates transcription. UBF1 is the functional species.
	Mouse	97 and 94		Also involved in the growth-dependent regulation of rRNA gene transcription.
	Rat	97 and 94		
	Frog	85 and 82		
TBP–TAFs complex				
SLI	Human, rat	100, 68 and 48 TAFs in human.	Core promoter. Human SLI binding to the core promoter requires UBF	Required for basal transcription and species-specific formation of transcription initiation complex.
TIF-IB/TFID	Mouse	95, 68 and 48 TAFs		
Rib I	Frog	n.d.		
TIF I	*Acanthamoeba*	145, 99, 95 and 91 TAFs		
E_1BF/Ku	Rat	85 and 72	Core promoter. Enhancer elements (repetitive and non-repetitive).	Involved in the pre-initiation complex formation and stimulation of transcription.
				Post-translationally modified form produced during serum starvation represses rDNA transcription by inhibiting the initiation reaction.
CPBF	Rat	44 and 39	Core promoter.	Involved in transcription initiation complex formation and activates basal transcription.
	Human	44		
TIF-IA	Mouse	75	No direct interaction.	Increases the rate of reinitiation (recycles). Involved in growth dependent regulation of rRNA gene transcription.
Factor C	Mouse	n.d.		Growth regulated; responsible for down-regulation of rRNA gene transcription due to growth arrest of cells in stationary phase or by protein synthesis inhibition. Activity rather than the amount is altered. Consumed during transcription.
TFI C	Mouse	55, 50 and 42		Involved in the down-regulation of rRNA gene transcription in glucocorticoid-treated mouse lymphosarcoma (P1798) cells. Activity is altered.
Reb 1p (REB 1)	Yeast	125	Promoter, enhancer.	Stimulates rRNA gene transcription. Also exhibits stimulatory effects on some pol II genes. Can also induce termination and pol I release.
TIF-IC	Mouse	65		Participates in the initiation and elongation reactions.
TTFI	Mouse, human,	100, 90, 80 and 65	Terminator element GGGTCGACCAG (human) AGGTCGACCAGT/AA/TNTCCA (mouse)	Termination of pol I-mediated transcription by binding to terminator elements.
Rib 2	*Xenopus*	n.d.	Terminator element GACTTGCNC	Termination of pol I-mediated transcription.

eukaryotes exhibits a molecular mass ranging from 85 kDa to 97 kDa. It exists in two forms which arise from alternative splicing of the primary transcript [47]. The larger and smaller forms are designated UBF1 and UBF2 respectively. In the rodents, UBF1 is at least one order of magnitude more transcriptionally active than UBF2 [48,49]. It is a highly conserved protein which shows only a single amino acid change between primates and rodents [50]. A unique feature of the protein is the presence of DNA-binding domains (four in mammals and three in the frog) that are homologous to the high mobility group (HMG) proteins 1 and 2. These are referred to as the HMG boxes consisting of reiterated 80-amino-acid domains [51]. The absence of the second HMG box motif in UBF2 appears to be responsible for the reduced *trans*-activating potential of this isoform. Since the discovery of the HMG boxes in UBF, other proteins such as sex determination factor, tissue-specific regulatory factor and yeast mitochondrial non-histone protein NHP6 have been added to the list. UBF has been shown to dimerize in solution, which requires 80–100 amino acids in the N-terminus region. Removal of this sequence results in complete inactivation of the protein. Similarly the *trans*-activation in rodents requires a hyperacidic C-terminus [52,53]. This domain is essential for the transport of UBF to the nucleolus (the site of its function) both in the mouse [54] and the frog [55]. Interestingly, this sequence is susceptible to phosphorylation which appears to activate transcription [56]. Unlike other HMG box-containing proteins, UBF requires more than two HMG boxes for specific DNA binding. Although UBF is essential for the basal transcription in the human systems, it may not be essential for the basal transcription in other systems (for a review, see ref. [8]). Transcriptionally competent fractions from rat cells do not contain measurable levels of UBF when assayed with monospecific antibodies that can detect as little as 10 fmol of rat UBF [48]. In the human system, cooperation of UPE and the core promoter (the two elements with which UBF interacts) is essential for the formation of a stable initiation complex.

On the contrary, the rodent UBF appears to augment the binding of another factor (SL1/TIF-IB/TF ID/Factor D/Rib I) to the core promoter (see the next section) and the subsequent transcription initiation. Recent studies [57,58] have demonstrated the sequence-tolerant nature of UBF–nucleic acid interactions and have further revealed the ability of UBF to bend and wrap DNA [58a], which explains its interaction with both the upstream and downstream sequences. Furthermore, this study [58] has proposed a mechanism by which the frog UBF recruits the TATA box-binding protein complex. Considerable evidence, accumulated in recent years, also indicates potential regulatory roles for this unique protein.

Transcription initiation factor

The first pol I transcription factor involved in the initiation of rDNA transcription was initially identified in the mouse system [59] and subsequently purified from human cells [60]. It has been called SL1 in human and rat, TIF-IB, Factor D or SL1 in the mouse, and Rib I in the frog (for a review, see [8]). Human SL1 or frog Rib-I does not bind the respective core promoters unless UBF is present, whereas the rodent factor can independently interact with the core promoter. It is not evident why there is a different requirement for the promoter binding of similar factors from different organisms. Although the function of SL1 from different organisms is identical, it exhibits selective affinity for the homologous promoter. This observation suggested that SL1 has an intrinsic activity that confers the species-specific transcription of rDNA. Until 1992, the exact nature of this factor eluded the investigators. Recently, Tjian and his co-workers [61] demonstrated that SL1 consists of the TATA box-containing factor TBP and pol I-specific TBP-associated proteins (TAFs). Subsequent studies in other laboratories confirmed this key observation and extended it to pol II and pol III transcription machinery (for reviews, see refs. [62–65]). TBP is, therefore, an integral component of the transcription initiation factor for all three classes of RNA polymerase. X-ray crystallography of TBP has shown that it resembles a molecular 'saddle' with a curved DNA-binding surface and, upon binding to DNA, the convex surface would be available for interaction with the transcription initiation and regulatory factors [66]. The TAFs associated with TBP provide the polymerase specificity of transcription. The pol I-specific TAFs from humans contain three TAFs of 110, 68 and 48 kDa [61] whereas the mouse TAFs contain three TAFs of 95, 68 and 48 kDa [67]. The largest polypeptide appears to confer the species-specific transcription in humans and the mouse [67]. Interestingly, the *Acanthamoeba* TAFs [68] consist of four polypeptides of relatively larger size (145, 99, 95 and 91 kDa). It is not certain which polypeptide(s) provides the species specificity in this organism.

Although the N-terminal domains of human and mouse TBP are structurally different, this difference does not seem to contribute to the species-specific pol I transcription [67]. The lack of species specificity for TBP in rDNA transcription has been further confirmed in another study which showed that yeast TBP can function with human TAFs and direct human rDNA transcription [68a]. Although rodent SL1 can interact with the core promoter independent of UBF, UBF can enhance the promoter binding, as demonstrated by extended DNAase I footprinting [69]. UBF probably recruits SL1 and facilitates stable initiation complex formation which leads to stimulation of transcription.

Additional factors (see below) may be involved in the overall transcription of rDNA. It is likely that these factors are closely associated with pol I, depending upon the purification strategies used in different laboratories, and once dissociated from pol I their requirement in the initiation complex formation becomes evident. This would explain why there is some discrepancy as to the requirement of certain factors in the initiation or regulation of rDNA transcription in the same system. Since at least seven distinct factors (TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH and TFIIJ) are required for accurate initiation of pol II promoters (for a review, see ref. [70]), it is conceivable that initiation of transcription by pol I also requires several factors in addition to UBF and TBF/TAFs complexes. Improvements in the purification protocols are likely to dissociate these factors from pol I. Indeed, recent studies in Grummt's and our laboratory have resulted in the characterization of additional factors that are crucial for the initiation and/or elongation reactions (see the following sections).

ENHANCER BINDING FACTORS

E_1BF/Ku

A factor that is involved in the basal level pol I transcription was purified in our laboratory from rat mammary adenocarcinoma ascites cells. Since this protein was purified using an affinity column constructed of a 37 bp oligonucleotide corresponding to the non-repetitive rat enhancer motif, it was designated E_1BF, enhancer 1-binding factor [71]. It consists of a heterodimer of 85 and 72 kDa subunits. It interacts with the core promoter and the enhancer (both repetitive and non-repetitive) elements [18,71], and modulates rDNA transcription *in vitro* [18]. Subsequently, the same factor was purified from a rat hepatoma and was shown

to resemble the human Ku autoantigen with respect to the immunological and DNA-binding characteristics, and overall size of the two polypeptides [72]. Specific polyclonal and monoclonal antibodies raised against the human Ku protein cross-react with the rat E_1BF in a Western-blot assay. The smaller polypeptide is the major DNA-binding subunit [72,73]. Dissociation of the two subunits with the anti-Ku antibodies results in inhibition of rDNA transcription, suggesting that both polypeptides, probably in a heterodimeric form, are required for rDNA transcription [74]. Inhibition of rat or human rDNA transcription by the anti-Ku antibodies can be overcome by the addition of purified rat E_1BF to the transcription reaction. The inhibitory effect of the antibodies is most predominant when added prior to preinitiation complex formation and is reduced significantly when added after establishment of the initiation complex. The effect of E_1BF in rDNA transcription was observed irrespective of the source of the cell extract (rat, mouse or human) or nature of the template (linear or circular) [74]. These results suggest that E_1BF is not a species-specific factor, and that unlike the classical Ku protein [75], E_1BF can also interact with the internal DNA sequences.

In contrast to pol I transcription, pol II transcription from the adenovirus major late promoter or from simian virus 40 (SV40) and cytomegalovirus (CMV) promoters, and pol III transcription of 5S and U6 RNA genes are either unaffected or minimally altered by the anti-Ku antibodies [73]. Interestingly, a Ku-related protein, PSEl, which interacts with the PSE element of U1 RNA gene, can modulate transcription of the latter gene [76,77]. Further study is needed to explore the effect of the Ku-related protein(s) in the transcription of selected genes.

Despite repeated attempts, we failed to obtain a convincing peptide sequence of either the 72 kDa or the 85 kDa polypeptide. Preliminary indications are that E_1BF is not identical to Ku; rather, E_1BF and Ku belong to the same family of proteins. Recent studies have demonstrated direct interaction between E_1BF and core promoter-binding factor, CPBF [78] (see the next section for the characteristics of this factor). The overall conclusions from these studies are that E_1BF/Ku is a basal pol I transcription factor, that it interacts with CPBF both physically and functionally to promote rDNA transcription, and that it is not a general pol II or pol III transcription factor.

REB-I (Reb 1p)

An enhancer-binding protein called REB1 or Reb 1p with an apparent molecular mass of 125 kDa has been extensively purified [79]. Subsequently, the same investigators cloned this protein and demonstrated that it is essential for cell growth, shares several features with the general DNA-binding proteins of *Saccharomyces cerevisiae* and exhibits some sequence similarity to the oncogene *myb* [80]. Reb 1p binds to a recognition sequence which is an essential component of the terminator element. Recently, this protein has been shown to induce complete termination and pol I release of the transcript correctly initiated on a 3′-tailed template by purified pol I [80a].

OTHER FACTORS

TIF-IA, TFIC, Factor C

Three factors, designated TIF-IA, TFIC or Factor C by different laboratories, have been extensively purified. These factors are closely associated with RNA pol I and are involved in the growth-regulated transcription of ribosomal genes. For a detailed discussion of these factors, see the section on Regulation and Table 1.

TIF-IC

In addition to TIF-IA and TIF-IB (TBP/TAFs), a factor designated TIF-IC is required for the initiation of rDNA transcription in the mouse [81]. Other investigators failed to identify this factor in a reconstituted transcription system from mouse [2], human [69], rat [82] and frog [83]. As indicated earlier (see section on Transcription initiation factor), the close association of this and other related factors with pol I may have prevented their identification in these laboratories. Recently, this protein has been further characterized and shown to participate in the initiation and elongation of rDNA transcription [84]. It resembles functionally the pol II transcription factor TFIIF [85–87]. Both factors interact with the respective RNA polymerase, suppress non-specific initiation, and facilitate stable association of the polymerase with the promoter and formation of functional pre-initiation complexes.

CPBF

A factor called CPBF (core promoter-binding factor) that specifically interacts with the rRNA gene core promoter was purified from rat mammary adenocarcinoma ascites cells by fractionation on a series of columns including a final oligodeoxynucleotide affinity column [88]. The purified factor consists of two polypeptides of 44 kDa and 39 kDa. A similar factor has been purified from HeLa cells (Z. Liu and S. Jacob, unpublished work). The rat and human factor can bind and trans-activate heterologous promoters, which suggests that CPBF is not a species-specific factor. No significant pol I transcription was noted in a reconstituted transcription system that is devoid of CPBF, and addition of relatively low concentrations of the purified factor to the depleted system results in a marked increase in the initiation of transcription. CPBF is not related to the pol I transcription factors identified to date. It does not appear to be one of the TAFs, as immunoprecipitation with anti-TBP antibodies does not precipitate CPBF. The lack of detection of this factor by other investigators is probably due to its association with pol I or other factors. The relatively small quantity of this factor present in the cell (approximately 5000 molecules/cell) [88] may have escaped detection in other studies. The major function of this protein appears to be interaction with the basal transcription factor E_1BF/Ku (see previous section) and to promote initiation of rDNA transcription.

DNA topoisomerase I

DNA topoisomerase I is another factor that is involved in rDNA transcription. We have shown that this enzyme is required for the transcription of supercoiled rDNA *in vitro* [89]. Inhibition of the topoisomerase by the specific drug camptothecin markedly reduced transcription of supercoiled rDNA, but not that of linear template. This observation is in agreement with the requirement of the topoisomerase activity for rRNA synthesis in yeast [90,91]. Further studies have demonstrated that the topoisomerase activity is required for the elongation of rRNA chains [92]. However, transcription of ribosomal minigenes on extrachromosomal plasmids *in vivo* [92] or the nuclear run-on transcription near the 5′ end of the human rDNA was stimulated following inhibition of topoisomerase I [92a]. It appears that DNA topoisomerase I plays a dual role in rDNA transcription depending upon the accumulation of positive superhelical structure or negative superhelical form during transcription.

REGULATION OF rRNA GENE TRANSCRIPTION

The rate of rRNA gene transcription is dramatically altered in response to a need for increased production of ribosomes [2]. The pol I transcription can be either up-regulated or down-regulated depending upon the conditions. It is up-regulated by glucocorticoid in non-lymphoidal cells/tissues, SV40-induced infection, in response to cell proliferation and growth, or down-regulated by glucocorticoid treatment of lymphosarcoma cells, nutrient deprivation, polio virus infection and in response to differentiation, heat shock or drug-induced inhibition of protein synthesis.

Hormonal regulation

The first key observation of hormone-induced alteration in ribosomal RNA synthesis was made a quarter of a century ago [93]. This study showed that administration of hydrocortisone to adrenelectomized rats caused a marked increase in pre-rRNA (45 S) synthesis and processing in rat liver *in vivo*. A corresponding increase in RNA pol I activity in the isolated nucleoli and in the solubilized enzyme was also observed in the liver following glucocorticoid treatment [94,95] and in the kidney from the mouse treated with androgen or progestin [96,97]. Subsequently, we achieved the hormone-mediated stimulation of specific rDNA transcription using an *in vitro* transcription system which mimics the *in vivo* effects [98]. Contrary to the up-regulation of rDNA transcription by the glucocorticoid in liver cells, treatment of mouse lymphosarcoma cells P1798 with the hormone results in the down-regulation of pol I transcription [99]. This inhibition of transcription was attributed to the decreased activity of a transcription initiation factor, designated TFIC [100]. Inactivation of this factor prevents formation of the initiation complexes, which could be restored by addition of the purified TFIC [101]. This study has suggested that TFIC–pol I complex may be the transcriptionally competent form of the polymerase. Purified TFIC consists of three polypeptides of 55, 50 and 42 kDa. Because of the relative instability of TFIC, the protein reconsitituted with the three polypeptides has not been functionally active.

An attractive hypothesis is that the up- and down-regulation of rDNA transcription by glucocorticoid in different cell types is caused by the activation or inactivation of the same factor. No definitive experiments to test this idea have been performed. It is noteworthy that the rRNA genes from the mouse, rat, rabbit, rhesus monkey, chimpanzee, and human contain a consensus sequence that exhibits striking sequence similarity to the glucocorticoid response element (GRE) [102]. This element is known to bind the glucocorticoid receptor, although the binding affinity is considerably weaker than the canonical GRE. Although one investigation [102] has indirectly ruled out the involvement of the hormone receptor, additional data are required to determine unequivocally the potential role of the glucocorticoid receptor on rDNA transcription.

Regulation mediated by cell growth

Changes in cell growth parallel changes in the rate of ribosomal RNA synthesis. Several investigators have attempted to identify the factor(s) involved in the growth-regulated rDNA transcription and to elucidate its molecular mechanism of action. A factor, designated TIF-IA [103,104] is inactivated in the cells grown to stationary phase. Although this factor is tightly associated with pol I, it is separable as a separate entity under certain chromatographic conditions [104]. Recently, this laboratory [105] has extensively purified this factor, and has shown that it is an authentic transcription initiation factor, with a molecular mass of 75 kDa. It is present in relatively small amounts (approximately 500–1000 molecules/cell) and its association renders the abundant free pol I molecules in the cell transcriptionally competent. It does not exhibit species specificity, a general characteristic of pol I transcription, which suggests that the mechanisms for the growth-mediated regulation of ribosome synthesis are well conserved. TIF-IA resembles the bacterial sigma factor and the RNA pol II transcription factor TFIIF (RAP 30/74) with regard to their requirements in the transcription initiation and their interaction with the respective RNA polymerases.

Down-regulation of rDNA transcription by drugs or arrest of cells in the stationary phase

Contrary to the growth-regulated factor (TIF-IA) that is devoid of pol I, deficiency in an activated subform of RNA pol I called factor C is responsible for the down-regulation of pol I transcription in the cells arrested at the stationary phase or those whose protein synthesis is inhibited by specific drugs [106]. Although factor C, TIF-IA and TFIC (the factor inactivated by glucocorticoid) were initially considered to be identical proteins characterized in three laboratories under different conditions, Sollner-Webb and co-workers suggest a contrary view (see ref. [107] for detailed discussion). Unlike TIF-IA, factor C exhibits different chromatographic elution characteristics, and is consumed during transcription. Although Sollner-Webb's laboratory has now been able to separate this factor from the bulk of RNA pol I using an additional chromatographic fractionation, there is no evidence for the existence of a free factor C in the cell [107]. It appears to be a post-translationally modified subunit of RNA pol I, which can be immunoprecipitated by the anti-pol I antibodies. The molecular size of this postulated regulatory pol I subunit is not known.

The down-regulation of rDNA transcription in encysting cells of the soil amoeba *Acanthamoeba castellanii* is also mediated by the inactivation of the functional form of RNA pol I [108]. This modification in the pol I activity appears to be due to a structurally altered 39 kDa subunit of the enzyme, which is homologous to the yeast AC 40 subunit and the *E. coli* α subunit [8].

Despite several attempts, none of the laboratories has identified the exact nature of the pol I modification in the growth-arrested cells. Our laboratory was the first to demonstrate that pol I can be phosphorylated by nuclear kinase II [109,110] and that the pol I activity is augmented by this modification [111]. It does not, however, seem likely that general phosphorylation/dephosphorylation of pol I subunit(s) is responsible for the growth-mediated regulation of rDNA transcription. Analogous to factor C, TFIC, the factor involved in the down-regulation of rDNA transcription by glucocorticoid, exhibits strong affinity for RNA pol I and is rapidly inactivated immediately after initiation of transcription [100]. However, it has not been determined whether this factor is completely inactivated during transcription and whether it can restore transcription in the cells grown to the stationary phase or in the protein synthesis-inhibited cells. The relatively large amounts of TFIC present relative to factor C cast further doubt as to the structural or functional relationship of these factors despite several similarities. At present, there is also no direct evidence for a potential relationship among any of these factors and the short-lived polypeptide that has been implicated in the regulation of rRNA synthesis *in vivo* [112–116].

Recently, the potent anti-cancer drug cisplatin has been shown to bind human UBF with high affinity and specificity [117]. These

investigators have proposed that cisplatin–DNA adducts act as molecular decoys for hUBF, which leads to disruption of rRNA synthesis. Since elevated rRNA synthesis is a hallmark of rapidly proliferating cells, removal of a functional pol I transcription factor should be an effective means for arresting the growth of tumour cells. It is worthwhile to explore other potential agents with more potency and less toxicity, which could block rDNA transcription by this novel mechanism.

Response to nutrients

rDNA transcription can respond dramatically when the cells are grown in serum- or amino-acid-deficient medium [103,118]. Shifting *Drosophila* from serum-deficient (2%) medium to serum-enriched (15%) medium resulted in as much as a 15-fold increase in rRNA synthesis in a nuclear run-on assay [119]. The increase in rRNA synthesis in response to serum could be correlated with the growth rate of the cells. A similar increase in rRNA synthesis independent of the growth state of the cells was also observed following exposure of the *Drosophila* cells to the tumour promoter phorbol 12-myristate 13-acetate (PMA), which may involve the action of protein kinase C. Although the core promoter region is sufficient for the serum response, maximal stimulation with either serum or PMA occurred when the recombinant plasmid contained sequences extending to −150 (with respect to the +1 site). Recent study in our laboratory has shown that serum deprivation of rat Nl-Sl cells for just 4 h led to virtual cessation of rDNA transcription [78]. Extensive purification of the nuclear extract from the serum-enriched and serum-starved cells resulted in the identification of a repressor protein in the growth-restricted cells (see section on Repressors of rDNA transcription for detailed discussion). In *Saccharomyces cerevisiae*, a 45 bp element at the 3′ end of the enhancer is essential for the stimulation of rDNA transcription that occurs when cells are shifted from a poor carbon source to a good carbon source such as glucose [25].

Ribosomal gene transcription during differentiation, heat shock and virus infection

The rates of ribosome production and rDNA transcription are markedly reduced during differentiation of rat L6 myoblasts to myotubes [120,121]. The reduction in pol I transcription was accompanied by corresponding decreases in the levels of the transcription factor UBF [122]. Similar reduction in the rate of rRNA synthesis was also observed in the isolated nuclei from the mouse teratocarcinoma cells that are undergoing retinoic acid-induced differentiation (P. K. Datta, R. Reichel and S. T. Jacob, unpublished work). The decline in rDNA transcription occurred in parallel with a diminished level of E_1BF/Ku and of mRNA for the smaller polypeptide of this protein, whereas the level of laminin B1 mRNA, a marker for differentiation, actually increases linearly at least for 6 h during the differentiation process. The activity of CPBF was not altered under this condition. These studies have collectively shown that at least two key factors involved in rDNA transcription are altered during the differentiation of different cell types. It would be of interest to know whether the TBP–TAFs complex is also modified during the differentiation process.

Ribosomal RNA synthesis is also suppressed following infection of cells with adenovirus 2, poxvirus and poliovirus [123]. A cell-free RNA pol I transcription system from HeLa cells (see ref. [123] and references therein) was used to demonstrate that human rDNA is transcribed at a significantly reduced level in the extracts from the infected cells, which is generally consistent with the *in vivo* results. Mixing the extracts from the infected and uninfected cells did not reveal the presence of specific virus-encoded repressors of rDNA transcription in the infected cells. It therefore seems likely that the virus infection leads to either depletion or specific inactivation of host rDNA transcription factor. Inhibition of human rRNA synthesis *in vivo* by poliovirus infection has been mimicked in the cell extracts prepared from the infected cells [124]. This study showed that an initiation-specific transcriptional activity containing RNA pol I and the associated factor C [106] is significantly reduced in the virus-infected cells and that a fraction containing a similar activity from the mock-infected cells can restore rDNA transcription. Although this study has partially revealed the nature of the factor(s) involved in the down-regulation of rDNA transcription in the poliovirus-infected cells, the true identity of the factor(s) has not been elucidated. Furthermore, it is not evident whether the same factor plays a similar role in the inhibition of pol I transcription in the cells infected with other viruses.

Contrary to the inhibition of rDNA transcription by certain viruses, a marked stimulation of pol I transcription has been observed following infection of host cells with SV40 or polyoma virus [125,126]. The activation of rDNA transcription in the SV40-infected cells is probably due to expression of the viral A gene, large T antigen [127]; as specific mutation in the viral A gene can render the virus incapable of activating rDNA transcription. Furthermore, addition of purified SV40 large T antigen markedly stimulated human rDNA transcription in a cell-free system [123].

rDNA transcription can also respond to heat shock. Early studies showed that raising the temperature by a few degrees above the physiological temperature primarily blocks rRNA processing in *Drosophila* [60] and frog [128]. Hyperthermia can, however, inhibit rRNA synthesis in HeLa cells [129,130]. The discrepancy among these studies is probably due to the different cell types used for heat treatment and the shorter time intervals for maintaining the cells at higher temperatures (1–4 h versus 13 h). Prolonged heat treatment may have resulted in some degree of thermotolerance, allowing at least partial recovery of rDNA transcription [130]. The most dramatic effect of heat shock is exerted at the level of termination, which decreased to the same extent as initiation [130]. The coupling of initiation and termination observed in response to heat shock is consistent with the fundamental observation made in other laboratories that termination can enhance initiation (see ref. [7]).

A recent study in our laboratory (K. Ghoshal and S. Jacob, unpublished work) has demonstrated that heat shock of mouse lymphosarcoma P1798 cells, by raising the temperature from 37 °C to 42 °C for 4 h, resulted in complete inhibition of rat rDNA transcription. Under this condition, pol II transcription from the adenovirus major late promoter and pol III transcription of 5 S RNA gene remained unaffected. The 72 kDa subunit of E_1BF/Ku was not detectable after heat shock whereas the amount of p86 subunit was reduced by 50% of control. Concurrently, the DNA binding of E_1BF/Ku was abolished. The amount and DNA-binding activity of UBF, as well as the promoter-binding activity of CPBF, were not altered following heat shock. This study has concluded that a decrease in the expression of initiation-specific factor E_1BF/Ku is one of the primary mechanisms for the down-regulation of rDNA transcription by elevated temperature.

REPRESSORS OF rDNA TRANSCRIPTION

Transcription of genes can be modulated either by specific activators or repressors [131–134]. The transcription reaction

appears to involve two independent, but interrelated, steps [134–136]. The initial step, called anti-derepression [137], removes inhibitors of transcription, whereas the second step, the gene activation, results in higher levels of the gene product.

Most investigations on the identification and characterization of the negative transcription factors or repressors have been confined to transcription of the protein-coding genes. Recently, Brou et al. [138] have shown that DNA topoisomerase II can repress both pol I and pol II transcription *in vitro*. Interestingly, the topoisomerase-induced inhibition of rDNA transcription could be alleviated by the pol I transactivator UBF. This observation is consistent with an increase in rDNA transcription in yeast following inactivation of toposiomerase II [92]. On the contrary, injection of VM-26 (teniposide), the specific topoisomerase II inhibitor, into *Xenopus* oocytes did not inhibit rDNA transcription [139]. Since VM-26 inhibits the toposiomerase activity by more than 90%, this DNA-binding protein does not seem to play a direct role in pol I transcription. In another study, VM-26-induced inhibition of topoisomerase II blocked rRNA synthesis in a human tumour cell line [140]. There is no direct explanation for the contradictory results obtained in different laboratories on the potential relationship between DNA topoisomerase II and rDNA transcription. The *in vitro* studies have, however, utilized a well-characterized cell-free transcription system and have in addition eliminated the use of drugs which might have other undesirable effects. Further study is needed to resolve these issues.

Recently, we have shown that the enhancer 1 binding factor, E_1BF, the Ku-related protein, purified from the serum-starved rat cells (E_1BF_S) can completely inhibit rDNA transcription *in vitro* [78]. The purified protein exhibited only two silver-stained bands corresponding to the 85 and 72 kDa polypeptides of E_1BF. The inhibition of rDNA transcription could be overcome by the addition of control E_1BF (E_1BF_C) from the cells grown in serum-enriched medium. Immunodepletion of purified E_1BF_S followed by addition of the supernatant to the transcription reaction relieved the inhibition significantly, whereas the control mouse IgG had no effect. It did not inhibit the non-specific pol I transcription in a filter binding assay or pol II transcription from the adenovirus major late promoter. It is, therefore, unlikely that E_1BF is a non-specific inhibitor of pol I transcription. The inhibition occurs at the level of initiation with minimal effect on the elongation reaction. The amount or the promoter-binding activity of E_1BF was not altered in the serum-starved cells, implying that the DNA-binding domain of the protein remains unaltered after serum starvation. This study suggests that E_1BF_C is post-translationally modified following serum deprivation to a form with altered *trans*-activating domain, which functions as a negative transcription factor in rDNA transcription. The proportion of the two forms of E_1BF may determine the rate of rDNA transcription. It is not known whether this factor is also involved in the down-regulation of rDNA transcription by other stimuli, and whether it interacts with the positive growth-regulated factors that control pol I transcription. Other factors involved in the growth-regulated transcription of rRNA gene act as positive factors (see previous sections). E_1BF_S may counteract the activation of transcription by forming an inactive protein complex with the positive factor(s). It is likely that the inhibitor activity identified in the unfractionated extracts from the growth-arrested cells [141] may be related to E_1BF_S.

E_1BF_C or the Ku protein is known to inhibit rDNA transcription *in vitro* when used at relatively high concentrations (low template to high factor ratio) [74,142], which does not appear to be the physiological function of the factor. The striking observation is that even at a very low protein concentration relative to that of the template, E_1BF_S could inhibit rDNA transcription completely [78]. There is ample evidence for the dual function of the same protein in the transcription process. Analogous to E_1BF_C [71,72] or the Ku protein [142], the *Drosophila* Kruppel proteins can activate or repress transcription depending upon their concentrations. The tumour suppressor protein p53 consists of domains that can activate or repress promoters [143]. Similarly, the pol III transcription factor TF IIIB can be activated or repressed, depending upon its phosphorylation state [144]. Phosphorylation of this factor by the mitosis-specific protein kinases results in inhibition of 5 S gene transcription, which may explain the reduced 5 S RNA synthesis during mitosis. Although E_1BF is a phosphoprotein, the overall phosphorylation of the protein was not altered following serum deprivation (K. Ghoshal and H. Niu, unpublished work).

A recent study has shown that DNA-activated protein kinase (DNA-PK), which consists of a catalytic subunit and the Ku autoantigen, is a potent inhibitor of pol I transcription *in vitro* [144a]. This study has also shown that the inhibition requires ATP hydrolysis and that the inhibition is confined to the formation of the first phosphodiester bond formation. Since transcription repression by DNA-PK requires DNA ends and does not occur with circular templates, it is not evident whether this repression also occurs *in vivo*.

Conclusions and perspectives

In this review article, I have attempted to summarize the role of various factors in the regulation of ribosomal RNA gene transcription and the molecular mechanism of this important process. Although there is general agreement that TBF-pol I-specific TAFs and RNA pol I can initiate transcription correctly from the core promoter of most organisms, other factors are required for stable initiation complex formation and maximal rDNA transcription. Improvements in the purification protocols are likely to reveal additional regulatory factors. There is enough evidence to suggest that these factors are closely associated with some pol I preparations, but may be separable as distinct functional entities under rigorous fractionation conditions. A crucial area that has received some attention in recent months is in regard to the repression of rDNA transcription. More efforts should be made to understand the interplay between the negative and positive factors and how it affects the overall rate of rDNA transcription. It is important to identify potential silencer elements in the ribosomal gene, which may be the recognition sequence(s) for the repressor molecules. We can anticipate significant progress in understanding the gene structure of different TAFs and other key factors and their potential relationship to other key regulatory proteins and the molecular mechanism by which species-specific transcription of the rRNA gene is achieved. Finally, rDNA transcription in the native chromatin should be investigated. The role of chromatin structure in the regulation of gene activity must be evaluated to assess the physiological significance of the studies carried out in the cell-free systems. The availability of functionally active chromatin preparations will be an asset to achieving this goal.

Note added in proof (received 16 January 1995)

A major development has been the cloning of RNA polymerase I promoter-specific TAFs and the reconstitution of the transcriptionally active initiation factor SLI from the recombinant subunits [145,146].

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