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Review

A housekeeper with power of attorney: the rRNA genes in ribosome biogenesis

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Abstract. Ribosome biogenesis centres both physically and functionally on the activity of the ribosomal RNA (rRNA) genes. Ribosome assembly occurs co-transcriptionally on these genes, requires the coordinated expression and assembly of many hundreds of proteins and is finely tuned to cell and organism growth. This review presents contemporary understanding of the mode and the means of rRNA gene transcription and how growth factors, oncogenes and tumour suppressors regulate this transcription. It is argued that transcription elongation is a key mechanism regulating rRNA gene transcription. This unorthodox view provides a logical framework to explain the co-transcriptional phase of ribosome biogenesis.

Keywords. Ribosome biogenesis, growth regulation, gene regulation, RNA polymerase I, RPI, transcription, elongation, transcription-coupled ribosome assembly.

A senior professor once gave some stern advice: 'Keep well clear of muscle and ribosomes,' he said, 'they've been done to death.' Though this advice was followed assiduously during his postgraduate years, T. M. failed miserably as a postdoctoral fellow, becoming fascinated by the problem of how a few hundred ribosomal RNA (rRNA) genes are able to produce 80% of the total cellular RNA. Since that time, he has become ever more convinced that understanding the role these genes play in regulating cell growth is one of the most important challenges facing modern biology. Yet it is clearly also one of the most neglected challenges. Here, we attempt to review the knowledge of how, and indeed why, the rRNA genes are transcribed and summarise what is known of the mechanisms used to coordinate their output with cell growth.

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Why are the rRNA genes important?

The rRNA genes encode the major RNA components of the ribosome, the most ancient and most complex of all molecular machines. In eukaryotes, the synthetic activity of these genes generates the largest sub-nuclear structure, the nucleolus, and it is here that ribosomes are assembled. Given the overwhelming emphasis paid to protein-coding genes, it is a sobering thought that each organism must provide around 10 ribosomes for every messenger RNA it synthesizes. Ribosome biogenesis – the process of ribosome synthesis – therefore occupies a very large fraction of the metabolic effort of a cell. But does it also control cell growth, proliferation and perhaps differentiation, or is it just another 'housekeeping' function? Several recent studies give little alternative but to consider ribosome biogenesis as a defining element in the control of cellular and organism growth. Here we will review current understanding of the growth-related aspects of rRNA gene regul-

ation, emphasising their relevance to mammalian systems.

Assembling ribosomes

The ribosome, the factory of protein synthesis, is probably descended from a primitive catalytic RNA. The existence in modern organisms of ribozymes and the demonstration that peptide bond formation is catalysed predominantly by the rRNAs strongly suggest that when proteins finally arrived in the primaeval RNA world they were synthesized on an 'all-RNA' ribosome [see e.g. refs. 1, 2]. This central role in the development of life forms appears to have been carried over into modern organisms. In recent years we have come to understand that the ability to synthesise ribosomes determines growth and cell division rates and we now know that many oncoproteins and tumour suppressor proteins modulate ribosome biogenesis [3, 4]. More surprisingly, ribosome biogenesis appears in turn to be a regulator of several tumour suppressors, including p53 [5, 6].

The mammalian ribosome is a 4-MDa structure made up of two-thirds RNA and one-third protein and assembled into two distinct units referred to as the large, or 60S, and the small, or 40S, subunits. The large 60S subunit contains three RNA species, the 28S, 5.8S and 5S rRNAs, and ~49 ribosomal proteins (r-proteins), while the small 40S subunit contains a single RNA, the 18S rRNA, and ~33 r-proteins. However, several hundred other proteins have been implicated in the process of ribosome biogenesis [7–10]. The 18S, 5.8S and 28S rRNAs are synthesised, processed and assembled into ribosomes in the largest sub-nuclear structure, the nucleolus (Fig. 1). These three rRNAs are transcribed by a dedicated polymerase, RNA polymerase I (RPI, also called PolI), from a set of repeated genes, the rRNA genes or rDNA, as part of a single precursor, which in mammals is referred to as the 45 or 47S pre-rRNA (Fig. 2A). The short 5S rRNA is independently synthesised by RNA polymerase III (RPIII, also called PolIII), and since its regulation is beyond the scope of this review, the reader is referred to other review articles [11]. Initial assembly of the ribosome occurs co-transcriptionally with 47S pre-rRNA synthesis leading to a 90S precursor particle, a process elegantly visualized in the 'Miller spread' electron micrographs (Fig. 1A, B) [reviewed in ref. 12]. Structural studies of the ribosome suggest that this co-transcriptional assembly process is important in establishing the complex folding of the mature rRNAs and in positioning the r-proteins [13]. Hence, this co-transcriptional phase of assembly is probably a key factor in the fidelity of ribosome biogenesis [14]. Soon after its synthesis, the pre-rRNA is cleaved in a number of distinct steps, first to yield 40S and 60S subunit precursor complexes and finally the mature ribosomal subunits

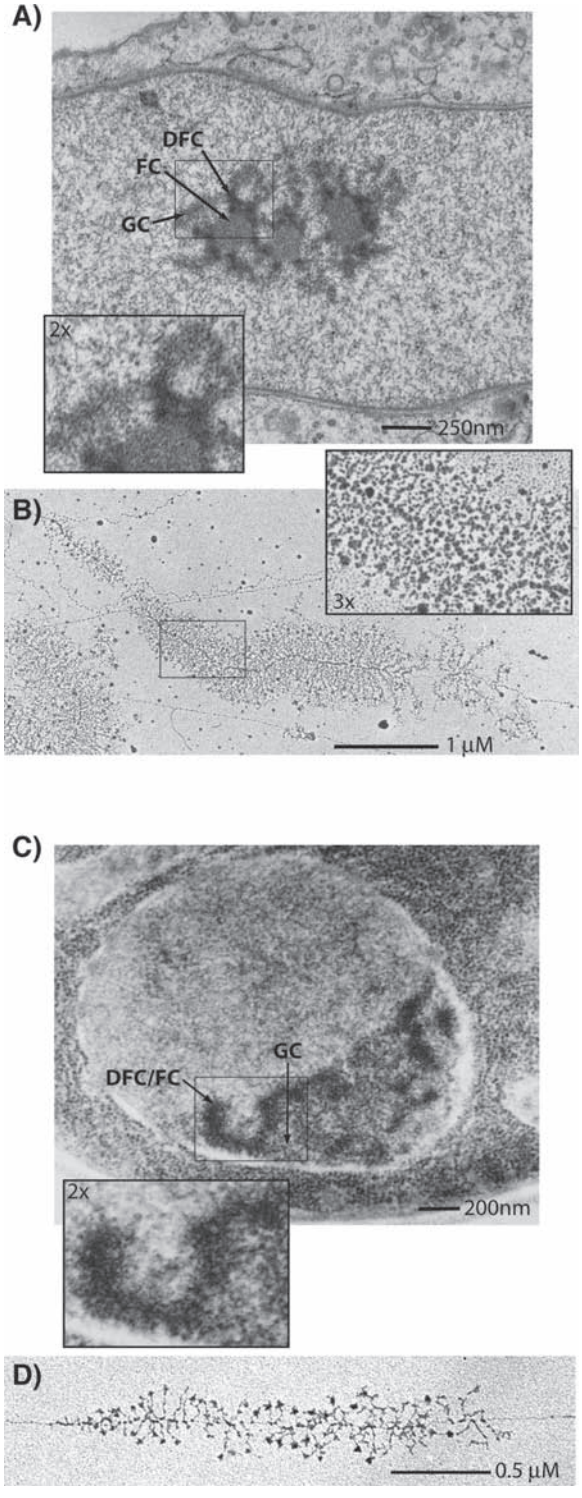

Figure 1. The cytological and low-resolution macromolecular structures of the nucleolus and active ribosomal genes. FC, fibrillar centre; DFC, dense fibrillar centre; GC, granular centre. (*A*) Electron micrograph of a thin section through the nucleus and nucleolus of a bovine endothelial cell. (*B*) A 'Miller spread' from a mouse Ltk$^-$ cell in culture showing closely packed transcribing polymerases. (*C*) Electron micrograph of a thin section through the nucleus and nucleolus of a *Saccharomyces cerevisiae* cell. (*D*) 'Miller spread' from an *S. cerevisiae* cell. *A* and *C* kindly provided by Dr N. Gas; *B* by Prof. U. Scheer; *D* by Dr Y. Osheim and Prof. A. Beyer.

[reviewed in refs. 15–17]. Not only is ribosome biogenesis the most complex undertaking of proliferating cells, it is also a major metabolic task. Ribosomes account for around 80% of total cellular RNA. In yeast, ribosome biogenesis accounts for >75% of all nuclear transcription, ~60% engaged in the production of the rRNAs themselves and ~15% in transcribing the 78 yeast ribosomal protein genes [18]. In proliferating mammalian cells, around 35% of nuclear transcription is dedicated to the production of the rRNAs, while a significant proportion of total mRNA gene transcription is required to produce the proteins needed for ribosome assembly. Interestingly, the rate of genome-wide transcription has been shown to be coordinated with ribosome biogenesis. Regardless of growth rate, yeast maintains a constant ratio of about 10 ribosomes per mRNA, though how this is accomplished remains a mystery [19].

Synthesis of the rRNA precursor is the central focus of ribosome biogenesis. The nucleolus forms in the nucleus wherever the rRNA genes are transcribed [20]. Thus, the existence of the cellular ribosome factory is the consequence of rRNA gene activity. Consistent with this, the nucleolus shows a distinct sub-structure, the so-called fibrillar centres and associated dense fibrillar centres, which are the centres of pre-rRNA synthesis and co-transcriptional assembly, and the outer granular centres, the area where the large and small ribosomal subunits are independently matured (Fig. 1) [21–25]. The 5S rRNA is transcribed independently of the 18S, 5.8S and 28S rRNAs and is imported into the nucleolus, as are the r-proteins. The only known exception among eukaryotes is *Saccharomyces cerevisiae*, in which the 5S genes are linked to the rRNA genes and hence must necessarily be transcribed in the nucleolus (Fig. 2A). In mammals, however, transcription of the unlinked 5S and even tRNA

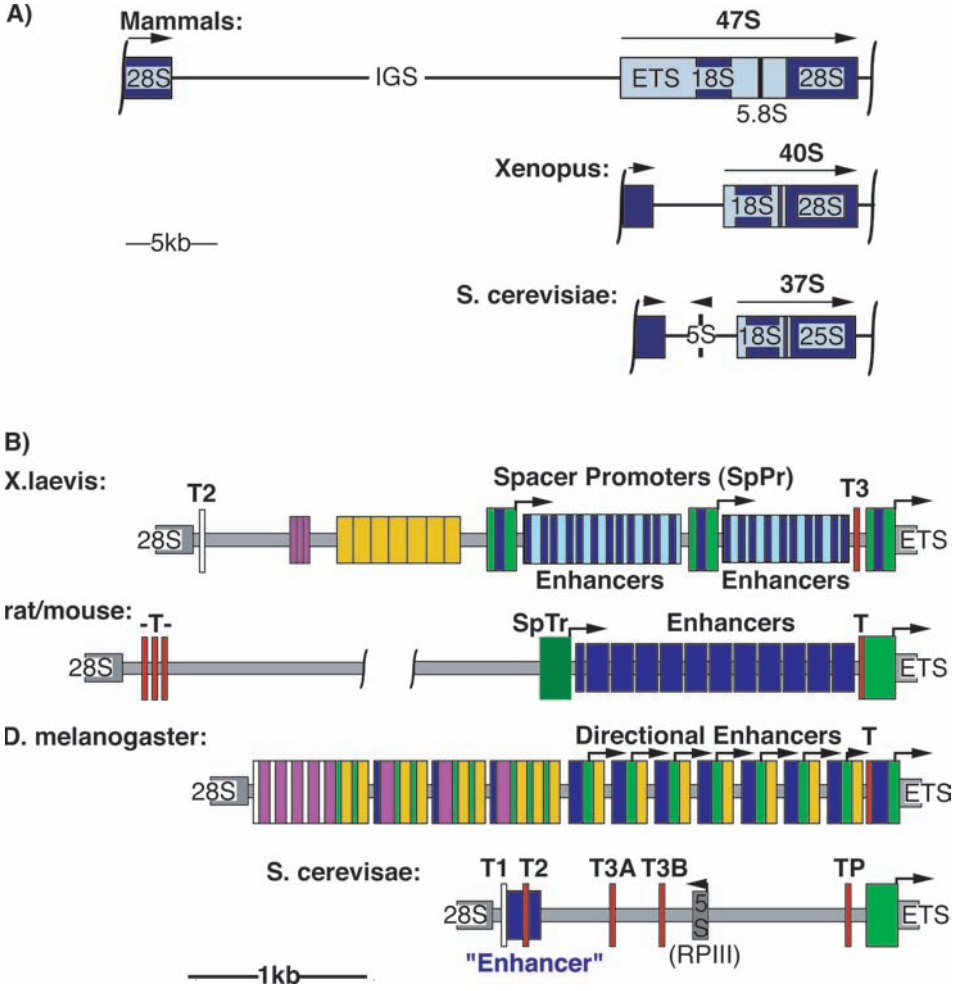

Figure 2. (*A*) Organisation of the rRNA genes in mammals, amphibia and yeast. (*B*) Organisation of the inter-genic spacer (IGS) in rat/mouse [221, 222] [36, 37, 60, 61], *Xenopus laevis*, *Drosophila melanogaster* and *S. cerevisiae* [reviewed in refs. 60, 61; reviews also available on request from the author]. Termination sites in *S. cerevisae* are taken from Van der Sande et al. [62], but some questions remain as to the functions of these sites *in vivo* [see e.g. refs. 223, 224].

genes occurs at the nucleolar periphery [26]. During the co-transcriptional phase of ribosome assembly, the rRNA is subjected to extensive, sequence specific modification. In vertebrates, around 115 residues of the rRNAs are 2′-O-methylated and about 95 uridines are converted to pseudo-uridine [27]. These modifications, which also occur on tRNAs and the 5S rRNAs, are dependent on several hundred complementary small nucleolar RNAs (snoRNAs) [27–30]. As can be seen, assembly and maturation of the ribosome subunits is a complex process and will not be discussed further here; the reader is referred to several specialised reviews [16, 17, 31]. For the purposes of the present review, it suffices to say that the transcriptional activity of the rRNA genes concentrates all these functions in the nucleolus.

The mode of rRNA gene transcription.

In trying to understand the mechanisms that underlie rRNA gene transcription and its regulation, we have generally made the assumption that, irrespective of the eukaryotic system studied, ribosome biogenesis and its regulation will be fundamentally the same. Given the primaeval origins of the ribosome and its conservation both structurally and functionally, this seems the most reasonable starting point, at least until solid evidence exists to the contrary. Thus, it is our contention that one should seek out the parallels between higher and lower eukaryotes rather than emphasise their apparent differences. As will be seen below, it could be argued from present incomplete knowledge that the promotion of rRNA gene transcription in yeast is quite different from that in mammals. But, I would suggest that this apparent difference is more than likely due to our present incomplete state of knowledge of the mammalian system rather than to a fundamental difference in molecular mechanism. When the existence of active promoters within the intergenic spacer of the rRNA genes was first demonstrated [32], this was seen as a peculiarity of the amphibian *Xenopus*. However, within a few months, *Drosophila* species were found to have such promoters [33–35], and a few years later mammals also underwent a rapid evolution [36–38]. Thus, until our knowledge of different systems is sufficient to demonstrate a clear lack of mechanistic conservation, it may be more profitable to seek out the commonalities rather than to stress the apparent differences. In this vein, let us first consider the fundamental mechanisms of rRNA gene transcription.

The 200 to 300 ribosomal genes per haploid mammalian genome exist as direct repeats at the secondary constrictions of acrocentric chromosomes, five in humans [39] and probably five in mouse [40]. Each of these sites has the potential to form a nucleolus and is hence referred to as a nucleolar organiser or *nor*, a term that predates knowledge of the genes these sites encompass [41, 42]. Each rRNA gene repeat is made up of an intergenic spacer (IGS), originally referred to as the non-transcribed spacer (NTS), but now known to be partly transcribed into non-coding, non-structural RNA. Early studies revealed that the DNA sequences surrounding the site coding the pre-rRNA 5′ terminus, and now known to promote its transcription, were also found repeated one or more times within the IGS (Fig. 2B) [32, 43–45]. These ‘spacer promoters’ were shown to direct transcription of the IGS and to be required for efficient pre-rRNA synthesis, though they could not direct this synthesis themselves [32, 46]. The ability of the spacer promoters to enhance pre-rRNA transcription was shown to require the adjacent short repeated sequences referred to as enhancers [32, 47, 48]. However, we showed that these sequences do not function to increase overall transcription but rather to greatly increase the likelihood of transcription from the linked gene [32, 48], and in this sense they are functionally similar to the so-called yeast enhancer [49, 50]. Later work has demonstrated the veracity of these findings [51–53]. Several explanations for IGS transcription have been suggested. The spacer promoters could represent a means of trapping polymerase and supplying it to the 45S pre-rRNA promoter [32, 54]. Alternatively, spacer transcription could represent a mechanism for activating or maintaining the active state of a gene, perhaps by maintaining chromatin of the IGS in some as yet undefined ‘open’ state or, as recently shown in yeast, for regulating recombination of the rRNA genes [55]. Most recently, it has been suggested that spacer transcription is required for rDNA silencing [56], a subject treated in depth elsewhere [4, 57]. Such a silencing function for the IGS transcripts is presently difficult to reconcile with the copious data showing an *in cis* positive selector function for the spacer promoters. However, it is consistent with the role of micro-RNAs in centromere silencing [58, 59] and almost certainly provides an important link in the complex chain of events leading to rRNA gene silencing.

In most systems, though perhaps not all, IGS transcription is terminated immediately preceding the true pre-rRNA promoter (Fig. 2B) [32, 60, 61]. A similar termination site also occurs in yeast, despite the apparent absence of any IGS transcription directed towards it [62]. These promoter-proximal terminators have been ascribed the functions of (i) preventing promoter occlusion, that is the inactivation of the promoter by displacement of essential factors [63] or (ii) recycling polymerase molecules delivered there by the spacer promoters or by ‘read-through’ from the upstream gene [64, 65]. More recently, the promoter proximal terminator in mammals has been suggested to mediate gene silencing [57], though again this is difficult to reconcile with the transcription enhancing functions of the terminator.

The means of rRNA gene transcription

A specialised set of proteins has evolved uniquely to transcribe the rRNA genes. With rare exceptions [66], the α-amanitin-resistant DNA-dependent RNA RPI is dedicated solely to the transcription of the rRNA genes, synthesising both the non-coding spacer transcripts and the productive pre-rRNA transcripts. In yeast, RPI is an enzyme of 14 subunits, half of which are shared with one or both of the other two eukaryotic RNA polymerases, RPII and RPIII (Table I). In vertebrates and yeasts, the RPI promoter is a sequence of 140–160 bp encompassing at least the first four nucleotides downstream of the mapped pre-rRNA initiation site (Fig. 3). The very poor sequence conservation of RPI promoters is consistent with the extreme species specificity of the RPI transcription system and the rapid evolution of the rRNA gene spacer. Despite this, functional studies have shown that most RPI promoters contain two distinct sequence elements, a core promoter (Core) sequence and an upstream promoter element (UPE or UE), originally called the upstream control sequence (UCE) in humans. The spacing of these promoter elements is crucial to *in vivo* function, but often the Core promoter element is sufficient to specify correct transcription initiation *in vitro* (Fig. 3). Short promoter sequences, similar in length to these Core promoters appear to be the norm in plants and some single-cell organisms (Fig. 3) [67–69].

There is general agreement that the formation of an RPI pre-initiation complex requires the TATA box-binding protein (TBP) and a group of RPI-specific TBP-associated factors (TAF_Is) that form one or two complexes able to recognize the promoter (Table 2). But here the similarities appear to end. In human and mouse, formation of the

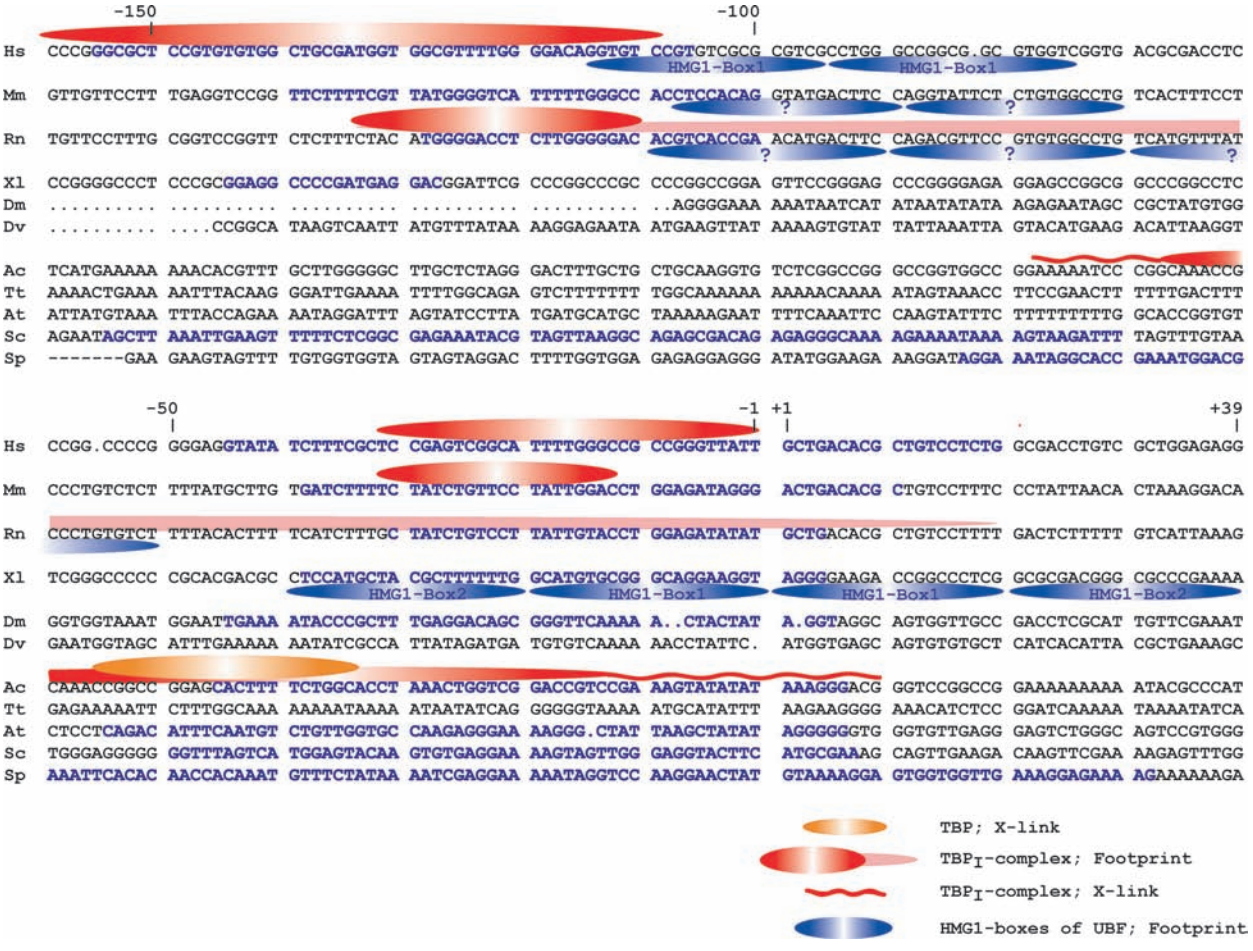

Figure 3. Structure of RPI promoters. Promoter sequences are shown aligned to the mapped initiation site (+1) with some small adjustments of alignment to emphasise the limited homologies. Mapped functional sequence elements are shown in blue and the regions demonstrated by footprinting or crosslinking to be physically contacted by the TBP_I complex (SL-1, TIF-IB), by UBF or by TBP are indicated graphically. [The data for human (Hs) were taken from refs. 74, 80, 225–228, for mouse (Mm) from refs. 229, 230, for rat (Rn) from refs. 231–233, for *Xenopus laevis* (Xl) from refs. 81, 234–238, for *Drosophila melanogaster* (Dm) from ref. 239; the *Drosophila* virilis (Dv) promoter sequence was deduced from comparisons of repetitive IGS sequences in ref. 35; the data for *Acanthamoeba castallanii* (Ac) was taken from refs. 118, 119, 240, for Arabidopsis thaliana (At) from ref. 241, for *Saccharomyces cerevisiae* (Sc) from refs. 101, 242 and for *Schizosaccharomyces pombe* from ref. 69.]

pre-initiation complex requires SL1 [for selectivity factor 1, referred to in mouse as transcription initiation factor IB (TIF-IB)], which has been shown to contain TBP and three TBP-associated factors, TAF_I48, 63 and 94 (Table 2). A second non-specific DNA-binding protein, upstream binding factor (UBF), has been shown to enhance RPI transcription and will be discussed in greater detail below. On the other hand, in the yeast *S. cerevisiae*, two factors are required for pre-initiation complex formation, the upstream activating factor (UAF) and the core factor (CF). The isolation of factors from *Schizosaccharomyces pombe* has suggested that parallels exist between the CF components of the yeast and those of mammals (Table 2) [69]. However, these parallels still remain tentative and no mammalian equivalents to the yeast UAF are known.

Table 1. List of RPI subunits and associated factors in yeast and mammals.

Yeast RPI subunits and associated proteins	Yeast gene	Yeast null mutant	Human and mouse
A190	RPA190	lethal	RPA194
A135	RPA135	lethal	RPA135
A49	RPA49	conditional	PAF53/PAF51?
N/I			PAF49/Ase-1/CAST
A43	RPA43	lethal	RPA43 = TWISTNB
AC40	RPC40	lethal	RPA40
A34.5	RPA34	viable	not identified
ABC27	RPB5	lethal	RPB25
ABC23	RPB6	lethal	RPB14.4/RPB6
AC19	RPC19	lethal	RPA16
ABC14.5	RPB8	lethal	RPB17
A14	RPA14	viable	not identified
A12.2	RPA12	conditional	RPA12
ABC10α	RPC10	lethal	RPB10α
ABC10β	RPB10	lethal	RPB10β
Rrn3p	RRN3	lethal	Rrn3(TIF-IA)

A, B and C refer to the three RNA polymerase forms RPI, RPII, and RPIII, e.g. in yeast 'ABC' indicates a common subunit. In mammals, the shared subunits were named after the polymerase with which they were first associated. The data are mainly taken from Carles and Riva [219] combined with searches of the BioBase Proteome Library and the recent identification of PAF49 [220].

Table 2. List of yeast core factor (CF) and upstream activating factor (UAF) and mammalian SL1 (TIF-IB) subunits and their possible correspondences.

Yeast CF	Yeast UAF	Yeast null mutant	Mammalian SL1 (TIF-IB)
Rrn6p >? (102 kDa)		lethal	$TAF_I110/95$ (95/92 kDa)
Rrn7p (60 kDa)		lethal	$TAF_I63/68$ (68 kDa)
Rrn11p (59 kDa)		lethal	TAF_I48 (53 kDa)
TBP = >	< = TBP	lethal	TBP
	Rrn5p	viable	
	Rrn9p	viable	
	Rrn10p	viable	
	UAF30	viable	
	H3		
	H4		

In the cases of the TAF_Is, the original protein names are indicated for human and mouse, respectively, followed by the calculated molecular weight(s) rounded to the nearest kDa. See text for the origins of the data.

Initiation complex formation

The mechanisms of initiation of RPI transcription have been studied in yeast, *Acanthamoeba castellani*, human, rat and mouse. In each case, reconstruction of the pre-initiation and initiation complexes has been studied in solution using cell-free extracts and purified factors. A summary of the known interactions of the various factors with their cognate promoters is given in Figure 3.

1. Mammals

The earliest data came from studies of the human and mouse factors and established a paradigm that is only

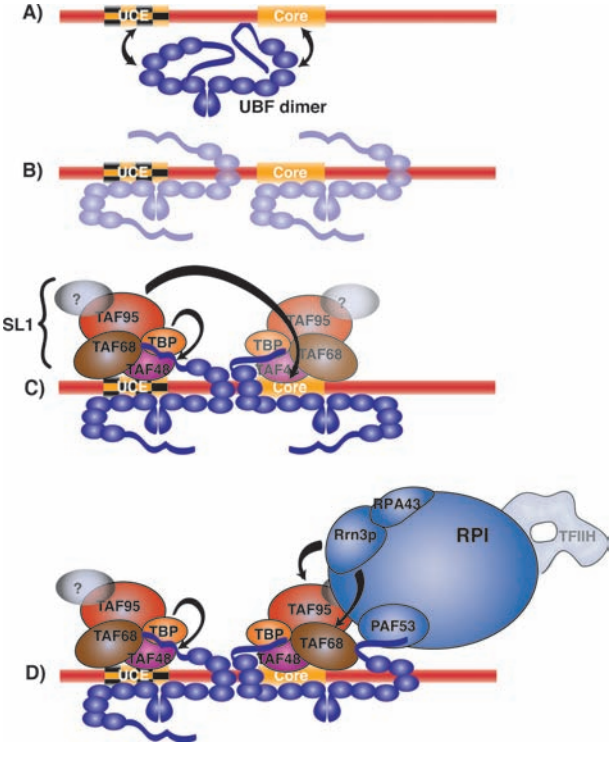

Figure 4. Assembly of the pre-initiation complex and the initiation cycle in mammals. DNA is shown in red and promoter elements in yellow and chequered yellow. The possibility of further SL1 subunits (?) and the possible implication of TFIIH are indicated. Probable correlations between SL1 subunits and yeast CF subunits is indicated by colouring (compare with Fig. 6).

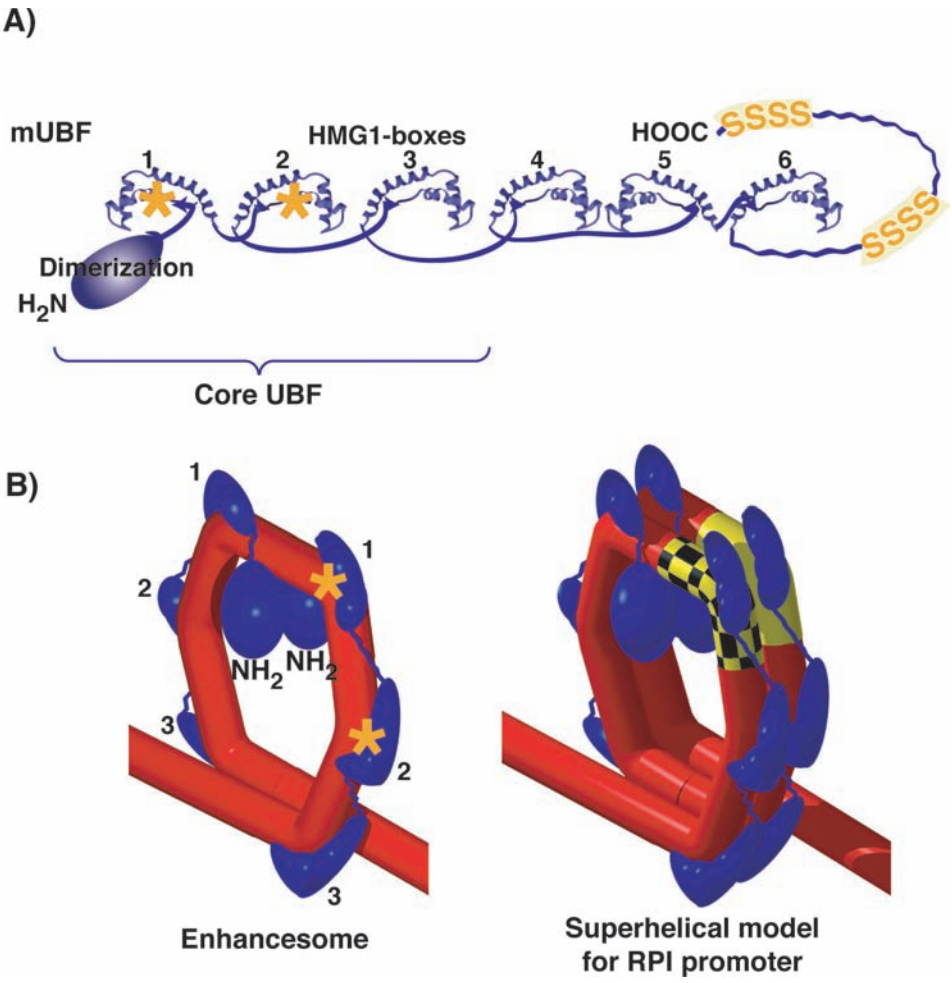

Figure 5. (*A*) The domain structure of mammalian UBF. Each HMG box is indicated by the homologous fold of HMG-D [243] and inter-box peptides as arrows of approximately correct length. The acidic residues of the C-terminal tail are indicated by a wavy line and the approximate positions of the blocks of serine residues are in yellow. (*B*) Low-resolution structure of the enhancesome. Left, the low-resolution structure of a single enhancesome as determined by electron spectroscopic imaging [83, 84, 186]; right, possible folding of the RPI promoter by two adjacent enhancesomes induced by UBF binding. Promoter sequences are indicated as in Figure 4. Only the Core UBF region is shown in *B* and inter-HMG1 box linkers are shown generically. UBF is in blue and DNA in red, and ∗ indicates sites of phosphorylation by ERK.

now being questioned. Essentially, this paradigm is described in the cartoons depicting the steps of human initiation complex assembly in Figure 4 [see refs. 4, 70–73 for reviews]. The non-specific DNA-binding HMG1-box protein UBF was shown to bind the upstream and/or core promoter regions, creating a situation propitious for the SL1 complex to bind and form a 'stable' pre-initiation complex [74]. This complex is able to recruit RPI and, in the presence of nucleotide triphosphates, initiate transcription. How UBF is able to aid in RPI initiation remains somewhat of a mystery. If this is truly its function, several observations suggest how it might occur. The C-terminal domain of UBF is made up almost exclusively of blocks of aspartic and glutamic acid residues, each terminating in blocks of serine residues (Fig. 5A). This domain can bind and recruit SL1, binding being enhanced by phosphorylation of the serine motifs, probably by casein kinase II [75–78]. However, early studies also suggested that UBF could more weakly enhance initiation complex assembly even in the absence of this C-terminal domain [79, 80]. When it was discovered that the N-terminal half of UBF (core UBF) could form the 'enhancesome', a nucleoprotein structure that somewhat resembles the nucleosome (Fig. 5B), the finding immediately led to a speculative explanation for the cooperativity between UBF and SL1 and the bimodal organisation of RPI promoters [81–84]. It was suggested that by binding two regions of the promoter, UBF would juxtapose key promoter sequences and thus present the SL1-binding sites on the same surface of a DNA superhelix (Fig. 5B). These interpretations were based on the original observations in the human cell-free system showing that UBF was required for SL1 recruit-

ment and RPI promotion. However, it is clear that UBF is not essential for promotion in the mouse and rat cell-free systems and, indeed, it is often difficult to find conditions under which it has any positive effect at all on RPI transcription initiation. *In vitro*, UBF has been found variously to activate RPI transcription at pre-initiation [79, 80] or promoter release [73, 85], to relieve H1 repression [86] or simply to be unnecessary [87]. These conflicting observations may be the result of the polarised basic-acidic nature of UBF, allowing it to compete non-specifically for inhibitory DNA-binding activities in *in vitro* assays. But they may also be related to the low DNA-binding constant of UBF combined with its ability to interact non-specifically with almost all DNA sequences [81, 83, 88]. Most recently, it has been argued that human SL1 is able to functionally bind the promoter in the absence of UBF, something already known for rodent SL1, and that UBF binding is dependent on SL1 rather than the converse [89]. However, given the rapid off-rate of UBF [90, 91], it may be difficult to determine the true order of binding of these factors to the DNA. SL1 binds to both the promoter DNA and to UBF. SL1 would then naturally reduce the off-rate of UBF by its cooperative interaction with both components of the UBF-DNA complex. Hence, we do not believe that the data to date provide definitive information on the order of promoter association of UBF and SL1, and may, rather, reflect the changes in DNA-protein complex stabilities. The observation that UBF is not restricted to the RPI promoter but is also found to bind throughout the rDNA, further complicates the issue of whether or not UBF has a specific function in transcription initiation [92]. Rather, UBF appears to form a nucleolar or rRNA gene chromatin and this may regulate multiple aspects of transcription, including rRNA gene accessibility, much as nuclear histone chromatin does for the rest of the genome. Clearly, this rRNA gene chromatin is able to recruit SL1 and RPI regardless of the underlying DNA [93–95]. Consistent with the role of UBF in the formation of an rRNA gene chromatin, the most recent data show that growth factor-dependent remodelling of this chromatin controls rRNA synthesis by regulating RPI elongation [96] (see below).

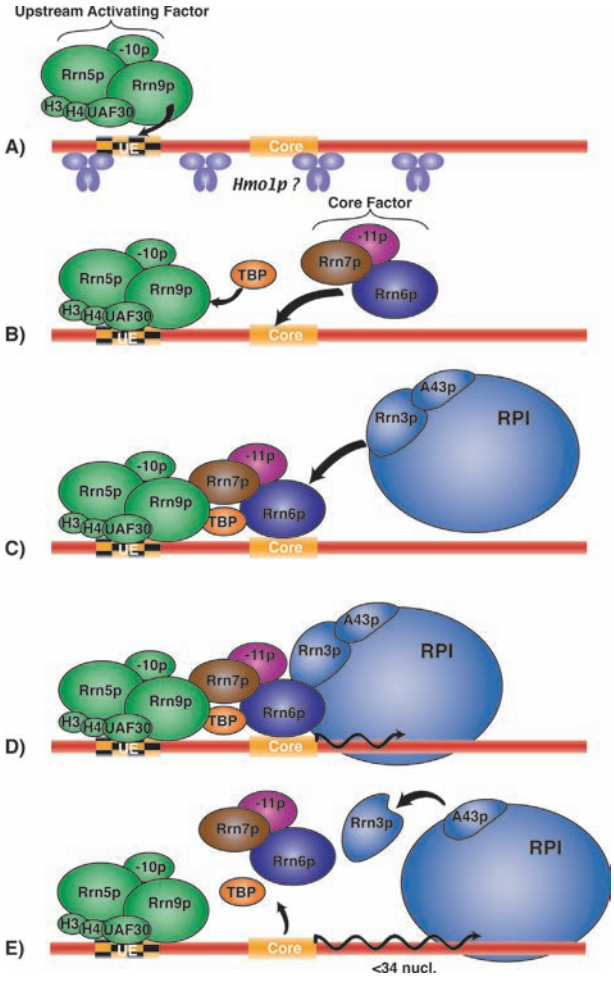

Figure 6. Cartoon of the assembly of a pre-initiation complex and the initiation cycle in the yeast *S. cerevisae*, based on Nomura [20] and Aprikian et al. [97]. DNA is shown in red and promoter elements in yellow. Colouring of the different factors indicates probable equivalence with the mammalian factors shown in Figure 4.

2. Yeast

RPI initiation complex formation in yeast is in broad terms similar to that in mammals. However, the number of factors directly involved appears to be significantly greater, suggesting that much may still need to be learnt about the mammalian situation. The steps of *in vitro* assembly of a yeast initiation complex in yeast are shown in the cartoons of Figure 6 [20, 97]. After establishment of the UAF-UE complex, TBP is either already present or is recruited along with the CF [98–100]. Efficient promotion requires the UAF complex, though low-level specific transcription initiation *in vitro* does not require this complex, nor does it require TBP or the UE of the promoter [99, 101, 102]. This is reminiscent of the mammalian, plant and single-cell organism data, where in general only a core promoter and a single TBP complex are absolutely required *in vitro* (Fig. 3). A significant increase in the TBP concentration in the absence of functional UAF enhances RPI transcription from the yeast core promoter *in vitro*, but *in vivo*, over-expression of TBP is unable to rescue the loss of UAF [103, 104]. UAF subunits are not strictly required for yeast survival. However, viability then depends on polymerase switching, a phenomenon in which yeast uses RPII to produce functional levels of rRNA and which requires an increase in rDNA copy number [105]. Thus, UAF is in fact essential for the functional synthesis of rRNA by RPI.

Once the yeast pre-initiation complex has been assembled, recruitment of the polymerase permits transcription

initiation and the complex is released into the elongation phase (Fig. 6). The CF complex may be disrupted and reformed at each new round of initiation [97], though it is unclear whether or not this occurs *in vivo* and it is certainly not obligatory for re-initiation [106]. HmoIp, a UBF-like protein, is not essential for yeast viability, but its loss induces a slow-growth phenotype and is lethal in combination with inactivation of non-essential RPI subunits [107]. Recent work has shown that like UBF, Hmo1p is found bound throughout the rDNA and hence may also define an rDNA chromatin [108]. However, Hmo1p may also be required for r-protein gene transcription [108].

3. Protozoa

In vitro RPI promotion in cell-free extracts from *A. castellanii* resembles basal *in vitro* transcription in rodent and yeast extracts. A single TBP complex, TIF-IB, is required for *in vitro* RPI transcription from a short, core-like, promoter (Fig. 3). TIF-IB is purified as a five-subunit complex that includes TBP and its high-affinity binding within the promoter region has been mapped by both footprinting and protein-DNA crosslinking (Fig. 3). The data from *Acanthamoeba* is probably the best in terms of the details of the early steps in initiation [109–117]. The system has shown that the exact site of RPI initiation is determined by the positioning of the TBP complex and is relatively sequence insensitive [110]. *Acanthamoeba* TBP was shown to contact the DNA at around –45 bp, and RPI in the pre-initiation complexes sits across the initiation site, protecting the DNA from the downstream edge of the TBP complex to around +20 (Fig. 3) [118, 119].

Transcription initiation and the transition to elongation

There is general agreement that unlike RPII, but like bacterial polymerases, RPI initiation does not require triphosphate hydrolysis. This was initially demonstrated in cell-free rat extracts [120], but has also been shown in the mouse and *Acanthamoeba* systems [121, 122]. Initiation in yeast and mammals requires the RPI-associated factor Rrn3. Rrn3p was initially identified in yeast as an essential factor for RPI transcription (Fig. 6) [123]. It was shown to associate with a fraction of the RPI, and to be essential for functional recruitment of the polymerase to the pre-initiation complex [101, 124]. Though RPI can be recruited to the yeast promoter in the absence of Rrn3p, initiation does not occur [97]. Rrn3p is normally found associated with a small fraction of RPI, and in yeast this association requires RPI phosphorylation [125]. Rrn3p is also phosphorylated, but in yeast this does not appear to be required for initiation [125]. Soon after the polymerase initiates transcription, it releases Rrn3p, somewhat in the vein of the release of bacterial σ factor and the RPII factor TFIIF [124, 126]. This release is not obligatory for normal elongation, as fusion of Rrn3p to the RPI subunit A43 has no effect on viability or growth rate in yeast but does prevent normal down-regulation of RPI transcription [106]. In this context, it is worth noting that σ factor is often maintained throughout elongation of the bacterial rRNA genes, its stochastic release being only necessary to allow a more rapid reprogramming of transcription levels [127, 128]. This said, dephosphorylation of RPI by Fcp1p does appear to enhance the early phase of RPI elongation *in vitro* [129]. The mammalian Rrn3 homologue was first identified in human [130], where it has been shown to be required for RPI recruitment [131, 132]. Recycling of mammalian Rrn3 (TIF-IA) requires a post-translational modification that is lost during initiation [133] and in contrast to yeast Rrn3p, phosphorylation does indeed appear to play a role in its activity [134].

Depending on the promoter context, bacterial RNA polymerases and eukaryotic RPII may pass through a phase called promoter escape, during which the polymerase repeatedly aborts synthesis and re-initiates, producing many short transcripts of 10–20 bases. Eventually, the polymerase escapes the influence of the promoter and makes the transition to a highly processive elongation phase [see e.g. ref. 135]. Despite the potential importance of promoter escape as a means of regulating transcription, little is known about RPI in this respect, and no published data on abortive initiation exist. Studies have inferred from the measurement of *in vitro* transcription kinetics that RPI passes through a rate-limiting post-initiation step, consistent with promoter escape [85, 136]. However, while studying the transition of RPI from initiation to elongation, we were unable to detect the production of abortive transcripts, despite a highly sensitive single-round initiation assay [ref. 96 and unpublished data]. These findings are consistent with the data for the rRNA gene promoters of *Escherichia coli*, which do not generate abortive transcripts and are not limited by promoter escape [137]. Thus, if promoter release of RPI is rate limiting, the underlying enzymatic mechanism must be distinctly different from that of either *E. coli* RNA polymerase or RPII. Clearly, the role of promoter escape in RPI initiation warrants more detailed investigation.

Once RPI has made the transition from initiation to processive elongation it may encounter various impediments to continued rRNA synthesis, such as chromatin. As mentioned above, UBF is a major component of the rRNA gene chromatin. Surprisingly, we found that rather than aiding RPI elongation, in its unmodified state, UBF very effectively blocks elongation. Hence, UBF is a potential modulator of RPI elongation and, as will be seen below, this property is growth regulated. These data may also help to explain the very varied properties that have been

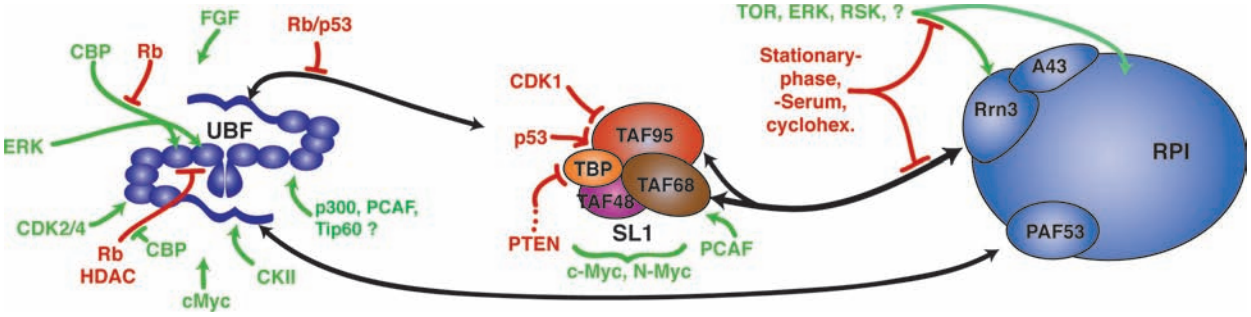

Figure 7. Summary of the regulatory targets of growth signalling, oncogenes and tumour suppressors within the RPI transcription machinery.

attributed to UBF in the past. Given the complexities of RPII elongation that have been revealed over the last few years [see e.g. ref. 138], it is highly likely that yet more regulators of RPI elongation will be discovered.

In vivo regulation of rRNA gene expression

Without new ribosomes, a cell cannot make protein and hence cannot grow and proliferate. Thus, an increased rate of ribosome biogenesis is a fundamental factor in hypertrophic disease. But is ribosome biogenesis a controlling factor or simply a housekeeping function? The most probable answer is: a bit of both. The inability to make ribosomes quickly enough will, without doubt, limit cell growth and slow proliferation [3, 19, 139, 140]. The many signalling pathways, tumour suppressors and oncogenes that impinge on mammalian ribosome biogenesis would suggest that it is the cell and its environment that control ribosome biogenesis (see Fig. 7). However, the rate at which ribosomes are made in turn determines whether a cell will enter S phase and commit to cell growth and division [3, 140]. Thus, we must consider ribosome biogenesis as one component of a communication network controlling growth and proliferation.

In bacteria, growth is associated with a high level of ribosome synthesis, while severe nutrient deprivation is associated with a rapid shutdown of this synthesis. In eubacteria, the shutdown is known as the 'stringent response' and is related to the production of (p)ppGpp by the idling ribosome [137, 141]. The key elements in this form of growth regulation were shown to be the proximal promoters of the rRNA genes. These rRNA gene promoters are regulated by nutrient availability while the weaker distal promoters and the r-protein gene promoters are not. R-protein expression is believed to be kept in check by an autoregulatory loop, free r-protein levels negatively regulating their corresponding genes. Thus, in bacteria, ribosome biogenesis appears to be regulated at the level of rRNA synthesis, this in turn regulating r-protein concentrations and hence their synthesis rates by driving ribosome assembly [142–144].

In eukaryotes the situation seems to be more complex, both r-protein and rRNA genes being growth regulated. For example, yeast mRNA and r-protein levels are both directly regulated in response to nutrient availability [145] and even in the absence of rRNA synthesis, HeLa cells continue to make r-proteins [146]. Thus, we must assume that the mechanisms coordinating ribosome biogenesis do not simply rely on rRNA levels controlling r-protein synthesis via an autoregulatory loop as occurs in bacteria. However, a very recent study in yeast in which the factor Rrn3p was fused to the A43 subunit of RPI (Table 1, Fig. 6) demonstrated clearly that expression of the r-protein genes depends on the level of rRNA synthesis [106]. Thus, as in bacteria, transcription of the yeast rRNA genes appears to determine r-protein expression levels. The regulation of ribosome biogenesis in eukaryotes, then, probably involves specific signalling and feedback networks to coordinate r-protein and rRNA synthesis precisely. At least some of these signals may rely on detecting functional 60S ribosome subunit levels, since inhibition of large subunit nuclear export leads to a coordinate down-regulation of rRNA and r-protein synthesis [147]. Strangely, this is not the case for the small ribosomal subunit.

Despite the differences between pro- and eukaryotes, a stringent-like response has not only been identified in eukaryotic micro-organisms such as yeast [148], but also much more recently in mammalian cells [149]. In the late 1960s, ribosome production in mammalian cell cultures was shown to be down-regulated in conditions of amino acid starvation [150, 151], and a few years later this effect was shown to be due at least in part to a down-regulation of rRNA gene transcription [152]. Glucocorticoid was also shown to down-regulate the mammalian rRNA genes, as was the global arrest of translation by cycloheximide [153–156] and serum withdrawal [157]. Encystment of *Acanthamoeba* was shown to cause a complete shutdown of rRNA transcription but also of all other nuclear transcription [158]. However, the responses of mammalian

cells to nutrient withdrawal, hormones and drugs were generally considered to be slow; for example, a 16-h treatment was used to observe repression of transcription with glucocorticoid [155], and cycloheximide required 2 h to reach maximal effect [153], though histidine withdrawal gave a relatively rapid response, transcriptional activity dropping by 50% in 60 min [152, 159]. Not until much later did studies of the response of mammalian cells to growth factor and MAP kinase activation reveal an immediate effect on rRNA gene transcription *in vivo* [149]. Indeed, these studies showed that a reversible response to growth factor (EGF) stimulation or direct MAP-kinase (ERK) activation occurred within 10 min, identifying a stringent-like response in mammals. Subsequent studies have confirmed these observations and extended them to include serum, fibroblast growth factor (FGF), insulin and insulin-like growth factor (IGF) responses [134, 160, 161]. However, before considering the mechanisms underlying these regulatory responses, we should first consider the potential cellular responses leading to rRNA gene regulation.

Growth response or stress response?

To come to terms with various, often conflicting data it may be necessary to consider at least two distinct rRNA gene regulatory modes. The first is a true growth regulation, in which rRNA synthesis and ribosome biogenesis are modulated to meet specific growth rate requirements. The second is a 'stress' response, in which the cell establishes a protective mode as an antecedent to the resumption of growth or to cell death. UV or other DNA damage and possibly cycloheximide or long-term withdrawal of nutrients may result in a stress response in which most if not all ribosome biogenesis is shut down. On the other hand, a short-term reduction in nutrients, changes in growth factors, or cell differentiation would be expected to lead to the adaptation of ribosome biogenesis to the new growth conditions, more akin to letting the engine 'tick over' rather than stalling it. Unfortunately, it is not always possible to determine which experiments fall into which of these categories, and this should be borne in mind when attempting to reconcile conflicting data.

Mechanisms of growth regulation

Unlike protein-coding genes, only four distinct possibilities exist to regulate the rRNA genes: gene activation, productive transcription initiation, transcription elongation and rRNA degradation. Since the last of these does not appear to be a significant factor, it will not be considered further.

1. rRNA gene activation

Since the ribosomal genes are present in several hundred copies and only a proportion appear to be transcribed, modulating the number of actively transcribed genes could in principle regulate rRNA synthesis. Growth of yeast into stationary phase leads to a reduction in the numbers of active ribosomal genes [162, 163]. However, growth stimulation of mammalian cell cultures does not detectably change the active gene number, despite a several-fold increase in rRNA synthesis rates [164]. This said, we have recently found that artificial changes in the heterochromatic state of the rRNA genes can lead to an increase in the active gene number in mammalian cells, though this does not induce a corresponding increase in transcription [T. Gagnon-Kugler and T. Moss unpublished data]. Furthermore, the number of active rRNA genes has not to our knowledge been determined under conditions of stress in mammals. Hence, though gene activation does not explain the rapid growth factor-mediated regulation of rRNA synthesis in mammalian cells in culture, its importance *in vivo* cannot be excluded.

2. Regulating initiation of rRNA transcription

Situations in which RPI transcription is very strongly repressed, for example long-term serum withdrawal, cycloheximide treatment and encystment of *Acanthamoeba*, have been used as the basis for attempts to identify mechanisms of rRNA gene regulation. Factors from active and inactive cells have been isolated and their abilities to support specific RPI transcription initiation *in vitro* investigated. Such studies led to the identification of active and inactive RPI fractions. These fractions of polymerase are equally capable of DNA-templated nucleotide polymerisation, but only one form retains the capacity to specifically initiate transcription from the RPI promoter [156, 165]. The ability to initiate transcription was associated with polymerase phosphorylation [124, 165] and/or with soluble factors TIF-IA, TIFIC or factor C [156, 157, 166–169]. More recent data show that TIF-IA and probably factor C correspond to the yeast and human Rrn3 (Figs. 4, 6) [131], but that TIFIC may be a distinct activity [133]. Later work demonstrated that mammalian Rrn3 (TIF-IA) phosphorylation changes with cell treatment and correlates with *in vivo* rRNA gene transcription levels [133, 134]. Phosphorylation of mammalian Rrn3 at several sites has been demonstrated to be due to a combination of RSK and ERK activities, and mutation of these sites in mouse Rrn3 suppresses transcription in transfected mouse and human cells (Fig. 7) [134]. Mouse Rrn3 was also shown to be regulated via the mTOR nutrient-sensing pathway and most recently via Jun N-terminal kinase (JNK) during a true stress response [170, 171]. A model emerged from these data of rRNA gene regulation occurring exclusively at the level of transcription initia-

tion via activation and inactivation of Rrn3/TIF-IA [71]. This model is consistent with stationary phase regulation and TOR inhibition in yeast [163], however, as will be seen below, it is not consistent with our knowledge of growth regulation in mammalian systems.

Despite intense study of RPI and Rrn3, the first molecular pathway from growth signalling to rRNA gene transcription actually came from a study of UBF [149]. Response to stimulation of human and mouse cells by EGF and by direct activation of the Raf-MEK-ERK pathway was shown to require phosphorylation of UBF. The two N-terminal HMG boxes of UBF display on their DNA-binding surfaces consensus ERK phosphorylation sites. Phosphorylation of these sites was shown to be required for stimulation of rRNA gene transcription. Recent data have shown that this phosphorylation regulates RPI elongation rates (see below). UBF has been implicated in the largest number of rRNA gene regulatory responses (Fig. 7), and it is even a direct target of the FGF2 growth factor [172, 173].

SL1 function (Figs. 4, 6), is regulated by PCAF acetylation of its TAF_I68 subunit [174]. SL1 is also inactivated by CDK1 phosphorylation [175]. Furthermore, the SL1 complex is disrupted in cells overexpressing the phosphatase PTEN [176]. At first sight, this would suggest that gene activation should be regulated, leading to a change in the number of active rRNA genes. However, as we have seen, this does not usually occur [164]. Rather, the data suggest that the level of active SL1 does not define the number of active genes. Such an interpretation is consistent with observations in yeast showing that CF, the SL1 homologue, is released at each new round of initiation and hence forms part of a catalytic cycle [97]. It may, then, be wrong to consider the formation of an SL1-promoter complex as a gene activation step, and it should rather be perceived of as a catalytic event much like initiation itself. The observation of 'holo-polymerase' complexes that include SL1 further supports this contention [177–181]. We may then find that mammalian SL1 performs a growth-regulated function that is unrelated to rRNA gene activation.

3. Regulating the rRNA transcription elongation rate

Despite the emphasis that until recently was placed on initiation as a means of regulating rRNA gene transcription, several early studies suggested that the capacity of RPI to initiate does not explain growth regulation *in vivo* [154, 182–184]. Most telling, however, are the Miller spread observations of tightly packed transcription complexes along the coding regions of the rRNA genes (Fig. 1). The dense packing of polymerases that these pictures reveal clearly excludes any large increase in polymerase loading. Yet, similar observations of tight polymerase packing have been made in animal cells exhibiting a wide range of rRNA synthesis rates [see ref. 60 for a discussion], suggesting that *in vivo* elongation must be regulated. A notable exception to this occurs in yeast, where the stationary phase shutdown of transcription correlates with a reduction in the density of transcribing polymerases [162, 163, 185]. However, even in this extreme case of regulation, yeast makes an attempt to maintain a high density of transcription by also reducing the number of active rRNA gene repeats.

The obvious inconsistency between mammalian models that invoke regulation solely at the level of initiation [see e.g. ref. 71], and the observations of near saturating levels of transcription complexes in Miller spreads, led us to ask whether changes in the RPI loading do in fact occur during growth factor induction of the rRNA genes [96, 164]. What we found was fully consistent with the electron microscope data. Using both nuclear run-on and ChIP approaches, we established that growth factor and MAP kinase activation of rRNA synthesis in human and mouse cells does not correlate with a significant increase in the total number of RPI transcription complexes required by models of regulation at initiation. The obvious explanation was that RPI transcription elongation rates were also modulated. This we demonstrated to be the case *in vivo* by directly measuring RPI elongation under different conditions of growth stimulation [96].

To achieve near constant RPI loadings over a wide range of rRNA synthesis rates, either (i) elongation and initiation must be coordinately regulated or (ii) elongation must limit the rate of initiation of new transcripts. The latter would appear much the simpler explanation mechanistically, since limiting elongation rates would naturally limit initiation. Several observations suggested that regulation of elongation was dominant over initiation [96]. More recent work has also shown that the capacity of nuclear extracts to specifically initiate RPI transcription *in vitro* does not change when cells are stimulated by growth factor (Fig. 8A). Thus, fivefold or greater changes in rRNA synthesis can occur in the absence of changes in the competence of SL1, RPI and Rrn3 to initiate new transcripts. We have further shown that the growth rates of human colon cancer cells are also quantitatively explained by changes in the RPI elongation rate [T. Gagnon-Kugler, unpublished data], suggesting that regulation of elongation is a general phenomenon in mammalian cells.

How is RPI elongation regulated? As mentioned above, some years ago we demonstrated that the ability of UBF to enhanced RPI transcription *in vivo* depends on a reversible phosphorylation of its two N-terminal HMG1 boxes by ERK1/2, and this phosphorylation was necessary for EGF to stimulate rRNA gene transcription (Fig. 5A) [149]. Each of the three most N-terminal HMG boxes of UBF is able to bend DNA and a dimer of UBF induces a 360° loop in the DNA template [83, 84, 186]. The resulting enhancesome structure resembles the nucleosome of

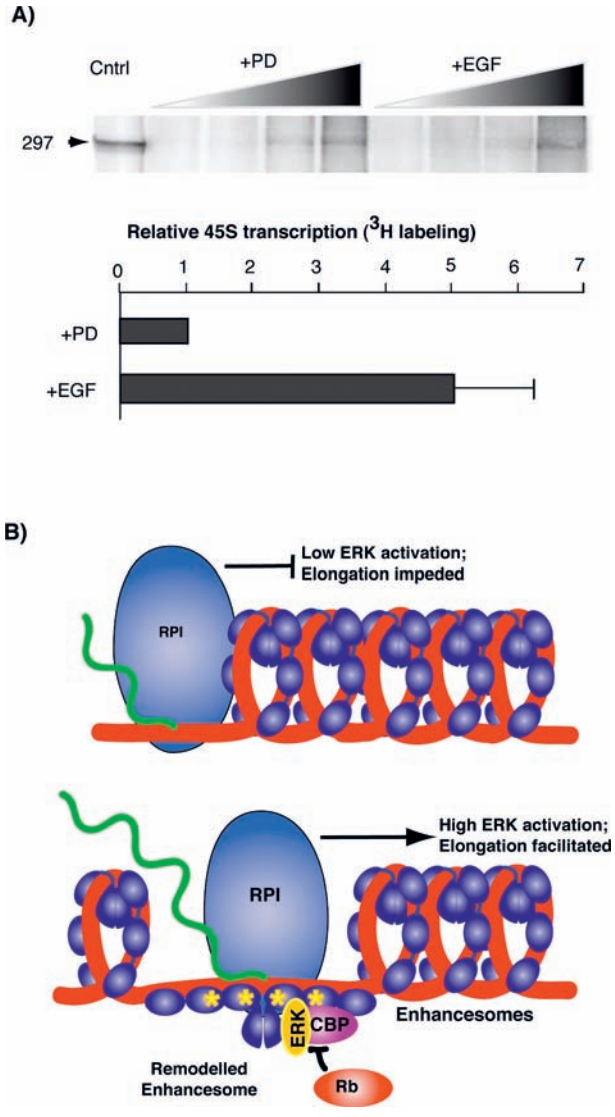

Figure 8. (*A*) The rapid changes of *in vivo* rRNA synthesis rates upon growth factor stimulation are not reflected in a change in the competence of RPI to initiate. Upper panel, RPI-specific *in vitro* transcription in nuclear extracts from NIH3T3 cells treated for 30 min with the MEK inhibitor PD98059 (PD) or with EGF [96, 149]. Exactly equal but increasing amounts of nuclear proteins were used in otherwise identical *in vitro* transcription reactions performed in parallel. Cntrl, *in vitro* transcription of the RPI template by a highly active nuclear extract from mouse L1210 cells. Lower panel, parallel measurement of *in vivo* 45S rRNA transcription rates determined in a 30-min [^{3}H]-uridine pulse labelling [96, 149]. (*B*) Regulation of RPI elongation by nucleolar chromatin remodelling. The rRNA genes are shown folded into a series of enhancesomes by the binding of consecutive UBF dimers. Only the Core of UBF consisting of the dimerisation domains and HMG boxes 1–3 are indicated (Fig. 5). ERK interaction with the first two HMG boxes and their subsequent phosphorylation leads to an unfolding of the enhancesome, permitting passage of the RPI elongation complex. The interaction of CBP with the same region of UBF may lead to a cooperative effect on transcription and may also be required *in vivo*. Rb is able to compete for CBP and perhaps ERK binding and hence would inhibit elongation. As in Figures 4 and 5, UBF is shown in dark blue and the DNA in red.

chromatin in protein-DNA composition but contains only a single loop of DNA (Fig. 5B). We showed that ERK phosphorylation of UBF remodels the enhancesome and in this way determines the rate of elongation of the RPI transcription complexes through nucleolar chromatin (Fig. 8B) [96, 187]. This reaction is probably mediated by a direct interaction between ERK and the HMG boxes it phosphorylates [149].

The demonstration that the RPI elongation rate is a key growth regulator of rRNA synthesis also suggests a means to coordinate this synthesis with pre-ribosome assembly. During the co-transcriptional phase of pre-ribosome assembly, processing factors and r-proteins must be assembled in the correct order on the nascent rRNA. A feedback mechanism allowing the regulation of RPI elongation dependent on correct ribonucleoprotein (RNP) assembly could provide an important means of 'proof-reading' pre-ribosome assembly. Indeed, recent studies of the processome, the earliest visible co-transcriptional RNP structure, suggest that its assembly on the pre-rRNA regulates the rate of rRNA synthesis in yeast [188].

The role of oncogenes and tumour suppressors

Tumour suppressors Rb and p53 have been implicated in limiting rRNA synthesis and affect the interaction between UBF and SL1 (Fig. 7) [189–192]. The acetyltransferase CBP has been shown to enhance rRNA gene transcription by competing for the Rb-binding site on UBF. CBP binding to HMG boxes 1 and 2 causes UBF acetylation, while Rb displaces CBP from its binding site and recruits HDAC1 to catalyse the deacetylation of UBF. A tantalizing correlation exists between this Rb-CBP competition and ERK phosphorylation of UBF. All three proteins bind to the same or adjacent sites on UBF [149, 193]. Furthermore, CBP is known to be bound and activated by ERK [194, 195]. Thus, ERK and CBP may act cooperatively in growth factor activation of the rRNA genes by targeting the same site on UBF. In support of this, we have shown that activation of the rRNA genes with the histone deacetylase (HDAC) inhibitor trichostatin A (TSA) depends on a functional ERK signalling pathway [164, 193]. UBF has also been reported to be acetylated by p300, PCAF and the isolated Tip60 acetyltransferase subunit [196, 197]. However, the functional significance of UBF acetylation is still poorly understood.

The ARF tumour suppressor was shown to regulate rRNA processing independently of p53 by catalysing the degradation of B23/nucleophosmin, a key protein in ribosome assembly [198–200]. However, it has also been shown to inhibit rRNA synthesis by an unknown mechanism [199].

Most recently, c-Myc was shown to enhance ribosome biogenesis, though the mechanism for its action is still

far from clear [201–204]. A search for c-Myc and N-Myc gene targets has consistently identified many genes that are implicated directly in ribosome biogenesis, including many r-protein genes and processing factors such as B23/nucleophosmin as well as genes that are indirectly implicated via cell cycle switches such as $p27^{cip}$, CDK4 and cyclin D2 [205–214]. Since it is evident that a coupling exists between r-protein and rRNA synthesis, it was not surprising to find that c-Myc levels modulated rRNA synthesis. The first study to demonstrate this directly showed that in a human B cell line, rRNA processing was more efficient when c-Myc expression was induced [215]. However, this study detected no change in rRNA synthesis rates. A second study investigated c-Myc/MAD function in mouse granulocytes and found that these proteins had reciprocal effects on rRNA synthesis rates [204]. It was argued that this was due to direct regulation of the UBF gene, enhanced UBF levels having previously been shown to drive rRNA synthesis [216–218]. Consistent with these data, a more recent study in *Drosophila* demonstrated that c-Myc drives rRNA synthesis indirectly by activating the genes encoding the RPI machinery and the great majority of genes required for ribosome assembly [201]. However, two further studies have shown that c-Myc and its co-factor TRRAP can also be found in the nucleolus and are able to interact directly with the rRNA genes [202, 203]. In one study, it was argued that nucleolar localisation and rRNA gene interaction of c-Myc was enhanced by inhibition of its proteasomal degradation, while in the other study, proteasome inhibition was not found to be a factor. In contrast, both studies showed that when c-Myc levels were manipulated by small interfering RNA and inducible expression, these levels correlated positively with rRNA gene activity and with the level of histone H3 and H4 acetylation on these genes. However, the mechanism of direct regulation of the rRNA genes by c-Myc remains a mystery, even more so since nucleolar c-Myc levels appear to be exceedingly low.

It should be noted that a common factor of many oncogenes and tumour suppressors is that they do not specifically target the promoter of the rRNA gene. Rather, they act through proteins such as UBF or directly interact with DNA sites spread widely throughout the genes. This suggests that they could be important for controlling the rate of transcription elongation or even pre-ribosome assembly.

In summary

In the last few years, several reappraisals of the basic parameters of rRNA gene regulation have succeeded in pinpointing the levels at which regulation can and does occur. We can be certain that rRNA gene transcription is co-regulated with growth and, as we should expect, changes in rRNA synthesis precede detectable changes in growth rate. To increase the rate of ribosome biogenesis, it is necessary to up-regulate the production of several hundred proteins in addition to the rRNAs. Who is driving whom – rRNA or r-protein expression – and the role they play in growth regulation are still open questions. In contrast to prokaryotes, the answer for eukaryotes may be that neither rRNA nor r-protein is dominant and that a complex feedback network exists to coordinate their synthesis. Clearly, isolated mechanisms of controlling rRNA initiation rates via RPI activity cannot explain this coordination, though they likely play their part. Regulation at the level of transcription elongation provides a more satisfactory explanation since it allows for feedback mechanisms that could coordinate rRNA synthesis with pre-ribosome assembly. The challenge now is to find ways of identifying and piecing together the components of the network regulating ribosome biogenesis.

Acknowledgement. We have attempted to review work of direct relevance to the growth regulation of rRNA gene transcription. For this reason and due to constraints on space, much important work had to be omitted and we wish to apologise to those whose work has apparently been overlooked. We thank Drs N. Gas, A. Beyer, Y. Osheim and U. Scheer for kindly providing electron micrographs. We would also like to thank those who provided updates of their most recent work and J. Smith and M. Nomura who took the time to discuss various mechanistic scenarios. Thanks finally to B. Muntwyler for critical reading and the editorial staff of *CMLS* for their patience. This work was supported by an operating grant from the Canadian Institutes of Health Research (CIHR) and a CIHR scholarship to T. G.-K. The University Laval Cancer Research Centre is supported by the Fonds de Recherche sur la Santé du Québec (FRSQ).

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