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TRANSCRIPTION BY RNA POLYMERASE I

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1. ABSTRACT

The genes that code for 45S rRNA, the precursor of 18S, 5.8S and 28S rRNA, are transcribed by RNA polymerase I. In many eukaryotes the genes are arranged as tandem repeats in discrete chromosomal clusters. rDNA transcription and rRNA processing occur in the nucleolus.

In vertebrates, at least two factors, SL-1 and UBF, specific for transcription by RNA polymerase I cooperate in the formation of the initiation complex. Interestingly, there are proteins analogous to SL-1 in unicellular eukaryotes, but the requirement for a UBF-like factor appears to vary.

Recent advances in our understanding of the rDNA transcription system and its regulation have demonstrated overlap with the other nuclear transcription systems (RNA polymerase II and III). This is exemplified by the utilization of TBP as a component of SL-1 and the role of Rb in regulatory rDNA transcription.

2. INTRODUCTION

Protein synthesis is an essential process for all living cells. Cells must govern both the amounts of specific proteins synthesized as well as the total protein synthesized in response to environmental signals and internal programming (1,2,3,4). In cycling cells, this coordination insures successful cell division and daughter cell survival. Alternatively, during terminal differentiation or in response to environmental stress, a cell may withdraw from the cell cycle. In many cases this reduces the need for protein synthesis.

The protein synthetic capacity of a cell is dictated by a number of processes such as mRNA availability, efficiency of translation, availability of translation factors or the number of ribosomes. The evidence accumulated to date indicates that the protein synthetic capacity is primarily regulated by the steady state number of ribosomes. This in turn is dictated by the relative rates of ribosome synthesis and degradation (1,2,3,4,5). Ribosome synthesis or biogenesis, is a complex process dependent on the coordinated synthesis of approximately 85 ribosomal proteins, four ribosomal RNAs (rRNA), and their subsequent processing and assembly into mature ribosomes. In contrast, little is known about the process or regulation of ribosome degradation (1,2,3,4).

In the majority of cells, ribosomes are relatively stable thus their cellular content depends largely on the rate of ribosome biogenesis. Experimental evidence so far correlates regulation of ribosome biogenesis to altered rates of rRNA transcription rather than changes in rRNA processing or stability (2,3,4,6). Ribosomal DNA (rDNA) transcription is a major commitment for the cell since it accounts for approximately 40-60% of all cellular transcription and 80% of the steady-state cellular RNA content. The rate of rDNA transcription can vary over a wide range. For example, when *Acanthamoeba castellanii* encyst, rDNA transcription decreased from 75% to almost 0% of the total cellular transcription (3,7). Indeed rDNA transcription has been shown to be regulated in response to different stages of development or cell cycle, nutritional state and altered environmental or hormonal conditions (1,6,7,8,9,10,11,12,13,14,15,16,17). This illustrates that rDNA transcription, like the expression of cell-cycle specific genes, is a prime example of growth-regulated gene expression.

Present data suggest that cells can utilize a diverse array of mechanisms to coordinate the rate of rDNA transcription with altered cellular requirements for protein synthesis. The relative importance of the various mechanisms to a specific stimulus have not been thoroughly investigated in any single cell. However, the data suggests that these mechanisms tend to be both cell type and stimulus dependent. In many cases the exact molecular mechanism(s) and signaling pathways involved in regulating rDNA transcription are not well understood. Since the regulation of rDNA transcription is a critical component of cellular homeostasis, it is important for us to understand and characterize the process.

3. GENERAL BACKGROUND

3.1. The Nucleolus

The interphase nucleus contains varying numbers of nucleoli. In metazoans, the nucleolus is the site of 45S rRNA synthesis, *i.e.* transcription of the ribosomal genes (rDNA), rRNA processing and ribonucleoprotein (RNP) assembly (6,18). The only active genes in the metazoan nucleolus are the rRNA genes, and the only RNA polymerase is RNA polymerase I. Indeed the ribosomal genes are the central elements of the nucleolus and are localized at special chromosomal sites referred to as nucleolar organizer regions (NOR) (19). Surrounding the NOR is a fine network of filaments which forms a scaffolding distinguishable in both organization and composition from that of the "nuclear matrix". The scaffolding is thought to provide some "structural support" or organization to the arrangement of transcriptionally active rDNA and/or the assembly and transport of ribosomal subunits. This is supported by the observation that the nucleolar scaffolding is absent from cells which are inactive in rRNA synthesis such as nucleated erthyrocytes and spermatocytes (6,18).

Typically, mammalian nucleoli consist of three substructures which were named according to their appearance in transmission electron microscopy; i) Fibrillar Centers (FC); ii) Dense Fibrillar Component (DFC); and iii) Granular Component (GC) (6,20). The FC are pale staining regions in the center of the nucleoli consisting of a fine fibril (4-8 nm thick) network which is relatively opaque in the electron microscope (20). The rDNA, RNA polymerase I, and other components of the rDNA transcription system such as UBF, SL-1 and topoisomerase I have been localized to the periphery of this region (19,20,21,22,23). Thus, it is likely that the FC are the site where the primary rRNA transcript is generated. The DFC surrounds the FC and is characterized by densely packed fine fibrillae (3-5 nm thick), a high electron microscope contrast and a high content of a 34 kDa protein, fibrillarin (6,20). Fibrillarin is known to associate with proteins required in the early stages of rRNA processing, such as the U3, U8 and U13 snoRNP (small nucleolar RNP) (24). The GC is localized to the periphery of the nucleoli and consists of granular structures ranging in diameter from 10 to 15 nm, which are sometimes organized in short strings (20). The later stages of maturing ribosome precursor particles, before they are exported to the cytoplasm, have been localized to this region (20).

The boundaries of these substructures are not always discreet, in fact three different patterns of compartmentalization have been described and these are used to classify nucleoli. The type of nucleoli identified depends on the rate of ribosome production. Typically cells with a high rate of ribosome production, such as nerve and Leydig cells, have large and complex nucleoli described as compact or reticulate. Alternatively, cells with a lower rate of ribosome biogenesis, such as monocytes and lymphocytes, exhibit ring-shaped small nucleoli and a single FC (20).

A diploid human cell contains 10 NORs, thus it would be expected to have 10 nucleoli. However this is seldom the case. This discrepancy could be explained by two situations: i) not all NOR are active; or ii) more than one NOR can be included in a nucleolus. Both situations have been identified. For example, in some cells not all NOR's are active (19,20,21,22). In human Hep-2 cells the transcription factor UBF associates with only six-to-eight of the possible ten NOR and in PtK1 cells UBF is found in

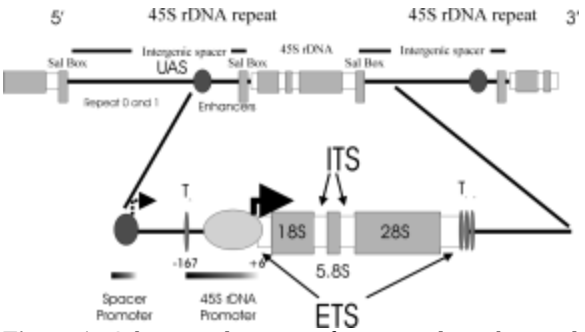

Figure 1. Schematic depiction of a mammalian ribosomal DNA repeat: The top portion of the cartoon depicts one and one-half ribosomal repeats in tandem, including the terminator Sal box, intergenic spacer, repetitive elements, enhancer region and the region transcribed to yield 45S rRNA. A section of the repeat is enlarged in the bottom portion of the cartoon. This section illustrates the placement of the spacer and 45S rDNA promoters, the proximal (To) and downstream promoter terminator elements (T1-7), the transcription initiation site (+6) and the external transcribed spacers (ETS).

50% of the NOR's (22). In each case, upon cell division there is an equal apportionment of UBF to the daughter cells. Alternatively, it has been shown that when lymphocytes are activated the previously inactive NOR fuse with the existing functional nucleoli (20).

During mitosis, as the cells enter prophase, the nuclei and nucleoli undergo rapid changes. For example the nuclear envelope disintegrates, chromosomes condense and subsequently the spindle apparatus form. In addition, the nucleoli disperse and disappear (20). At least a portion of some nucleolar components, such as RNA polymerase I, SL-1, UBF, and topoisomerase I, remain associated with the NOR (6,19,21,22,23), while others are released, such as NO38 (25) and the snoRNP (24,26). Nucleolar reformation usually begins during telophase with the daughter nucleoli forming at the NOR. Complete restoration of nucleolar morphology requires both ribosomal chromatin and active rDNA transcription (19). Thus, those NOR containing the RNA polymerase I transcription apparatus are more quickly able to initiate rDNA transcription and contribute to nucleolar regeneration (19,21,22,23).

In general, chromosomal DNA is organized in nucleosome structures. However, from electron microscopy, it has been suggested that the rRNA chromatin does not form a typical compact nucleosome structures. In fact, some reports suggest there are no nucleosomes on the transcribed rDNA (27,28). As one might expect, the nuclease digestion and psoralen cross-linking properties of the rDNA are atypical. This has also been examined using topoisomerase I digestion to examine the nucleoprotein structure of the rDNA. Topoisomerase I digestion sites were found to be spaced with a periodicity of 200 bp and concentrated in the regions encoding the 18S, 5.8S and 28S rRNA (28). This pattern was due to binding of nuclear proteins to the rDNA and not dependent on the DNA sequence itself (27,29). UV laser-induced histone-DNA cross-links studies demonstrated that the rDNA coding sequence, spacer enhancer and spacer promoter were associated with histones in both transcriptionally active and inactive cells (30,31). Interestingly, a recent study (32) suggested that the nucleosome structure may play a role in the regulation of initiation complex formation on the rDNA. That study demonstrated that histone octamers could compete with the transcription factors for the rDNA promoter, but only if the DNA was not first bound with an initiation complex (32). However, to date, a complete picture of the rDNA nucleosome structure and its function(s) is unclear.

3.2. Synthesis and Assembly of Ribosomes

The synthesis of ribosomes requires the coordinate effort of all three DNA-dependent RNA polymerases (6,18). RNA polymerase I, in the nucleolus, transcribes the rRNA gene that encodes the 45S precursor of the 18S, 5.8S and 28S rRNAs (figure 1). The 45S precursor rRNA is neither capped nor polyadenylated and can account for 1/3 to 1/2 of all nuclear RNA synthesis. To a lesser extent RNA polymerase I transcribes another transcript which originates from the spacer promoter located in the intergenic spacer (figure 1). However, this second transcript is unstable and its function is yet to be established (4). RNA polymerase III, in the nucleus, transcribes the 5S RNA gene (6). RNA polymerase II, in the nucleoplasm, transcribes numerous genes encoding ribosome associated proteins (r-proteins). These mRNAs are transported to the cytoplasm, translated and the mature r-proteins returned to the nucleolus for assembly of the ribosome components (6).

Mammalian ribosomal subunits are assembled in discrete stages within the GC of the nucleolus. Initially the 45S precursor rRNA is processed via a complex series of specific exo- and endo-nucleolytic cleavages. The rRNA exons are not spliced together thus the 45S precursor generates the 18S, 5.8S and 28S rRNAs. rRNA processing is directed by snoRNPs such as nucleoli U3 snRNP. U3 snRNP has been implicated in several steps, including the earliest step in rRNA processing, the cleavage at -650 in the 5' external transcribed spacer (ETS) (figure 1) (6).

The 18S, 28S and 5.8S rRNAs associate with the 5S rRNA and r-proteins to form a complex referred to as the 80S preribosome. The 80S is further processed to generate the 40S and 60S ribosomal subunits. Studies have shown that the order of r-protein addition in this process is essential for successful assembly of the ribosomal subunits. For example, a decrease in the cellular content of the r-proteins L13 or L16 can result in a deficiency of the 60S ribosomal subunit (6). The 40S and 60S ribosomal subunits are transported to the cytoplasm, where they the final stages of maturation occur. This involves the association with additional proteins, such as initiation factors. They are then able to participate in translation (6).

The accumulation of mature ribosomes in the cell depends on the balance between the rate of subunit synthesis and the rate of degradation. Since mature ribosomes are fairly stable complexes, with half lives

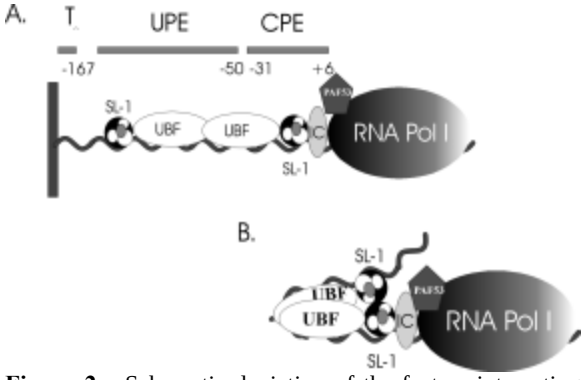

Figure 2. Schematic depiction of the factors interacting with the 45S rRNA promoter. The factors include SL-1, UBF, TFIC (IC), PAF53 and RNA polymerase I (RNA Pol I). A. Illustration of the DNA binding sites with which the factors interact. These include the upstream promoter element (UPE) and the core promoter element (CPE). B. Illustration of a model where UBF bends the rRNA promoter bringing two molecules of SL-1 in contact. Note that the stoichiometry of SL-1 to the promoter is not known.

ranging from 4.5 days in rat liver to more than 10 days in cultured L cells, ribosome degradation is not considered to contribute significantly to the regulation of ribosome content (2). To date little is known about the signaling mechanism(s) involved in ribosomal degradation, although a recent publication implicated a role for ubiquitin in this process (33). Ubiquitin may function by binding to the ribosome, thereby stabilizing or protecting it from degradation. Subsequent removal of ubiquitin would signal the cell to degrade that ribosome (6,33). Interestingly, in the majority of cells, it is the rate of ribosome synthesis, *i.e.* rDNA transcription, that is the primary determinant of ribosome content (2).

3.3. Proteins Associated with Ribosomes

Numerous proteins associate with ribosomes, some of which have obvious catalytic or structural functions, while for others it is unclear. For example, nucleolin (C23) is a 92-100 kDa phosphoprotein localized in the FC and DFC of the nucleolus, *i.e.* the sites for all stages of rDNA processing. Interestingly, nucleolin binds to the intergenic spacer region between the repeated rRNA genes. Moreover, one laboratory has demonstrated a correlation between nucleolin cellular content or activity, and the rate of rDNA transcription (34,35,36). However, the significance of this correlation is unclear. In addition, nucleolin has been implicated in the packaging and shuttling of the ribosome between the nucleus and cytoplasm. Also it has been suggested that nucleolins N-terminal HMG domain plays a role in the structure of the nucleus (20). While the extended conformation of the glycine rich C-terminal domain is suggestive of involvement in protein-protein interactions (6,18,20).

Like nucleolin, NO38 (B23, numatrin, or nucleophosmin) is an abundant 38 kDa nucleolar phosphoprotein (37) localized in the FC, which possibly plays a role in ribosome packaging and transportation. NO38 cooperatively binds, with high affinity, to single-stranded nuclei acids and exhibits an RNA helix destabilizing activity (6,18,20). Thus, NO38 may coordinate the attachment of r-proteins and other RNA-binding proteins to the rRNA (reviewed in 6,18,20).

4. rDNA TRANSCRIPTION

Essential components required for efficient rDNA transcription include the rRNA genes, RNA polymerase I, RNA polymerase I associated factors and a number of rDNA specific trans-acting factors such as SL-1, the homologue of TFIID, and UBF (17). In addition, other proteins have been reported to be components of the transcription initiation complex and may participate in the regulation of rDNA transcription (17). The specific contributions of these factors to the regulation of rDNA is poorly understood. However, they present interesting links between transcription by RNA polymerase II and RNA polymerase I.

4.1. The rRNA Genes

There are approximately 150-200 copies of mammalian rRNA genes (rDNA) present per haploid genome. In general, the genes are distributed among several chromosomes and arranged in tandem, head to tail arrays with the coding regions of the primary transcript being separated by nontranscribed or intergenic spacer regions (figure 1). The length of the transcript generated from the rDNA varies from ~8 kb (yeast, *Drosphila* and *Xenopus*) to ~13 kb (mammals), and this appears to be dependent on the length of the external and internal transcribed spacer regions (2,5,6). Although examination of the sequences of the rDNA promoters of different genera fails to demonstrate significant sequence identity, there is a high degree of conservation between the functional elements (3). In fact, the human, mouse, frog and rat rDNA promoters all share a similar molecular anatomy (4). In addition to the promoters, the vertebrate rDNA repeats contain terminator elements as well as additional transcription elements within the intergenic spacer. Surprisingly, the nontranscribed spacer of the yeast rDNA repeat also functions in termination and the anatomy of the yeast promoter is more similar to those of the vertebrate rRNA genes than the *Acanthamoeba* rDNA promoter is.

4.1.1. rDNA Promoter

Functionally, the vertebrate rDNA promoter consists of 2 domains, the core promoter element (CPE:~+6 to -31, with respect to the transcription initiation site), and the upstream promoter element (UPE) which extends from the CPE (-30) to ~ -167 (figure 2) (3,4,5,6,17). The CPE is necessary and sufficient for *in vitro* transcription, and is required but not sufficient for *in vivo* transcription. The UPE is not absolutely required for transcription initiation *in vitro*. However, it can stimulate transcription from the CPE under stringent conditions *in vitro* and is required for transcription *in vivo* (38). Transcription from the CPE occurs without the formation of a stable preinitiation complex, and the experimental evidence suggests that the UPE is essential for the formation of the stable preinitation complex *in vitro* (39,40). Studies using deletion, point, and

linker scanning mutants have demonstrated that the UPE is important for transcription but have, with one or two exceptions, failed to identify critical nucleotides within the CPE. Interestingly, the CPE has been demonstrated to consist of at least two functional domains, and individual nucleotides, such as the G's at -7 and -16, have been demonstrated to fulfill critical roles both *in vitro* and *in vivo* (2,41).

Experiments using distant-altering mutations demonstrated an interesting relationship between the UPE and CPE. For example, Pape *et al*. (42) demonstrated that altering the spacing between the UPE and the CPE of the *Xenopus* rDNA promoter allowed that promoter to be transcribed efficiently by mouse extracts (42). In addition, distant altering mutants of the rat rDNA promoter suggested that the distances between the UPE and CPE were critical for initiation. However, this response was not uniform across the entire UPE, suggesting that different segments of the UPE must have different functional roles, or are "neutral" with respect to their role in the structure of the preinitiation complex (43). To date, the results from published studies are consistent with a model in which the protein complexes that form on the UPE interact with and possibly stabilize those complexes bound to the CPE. This, might then enhance the rate of passage through the rate-limiting steps involved in the formation of an open initiation complex. At least two transcription factors have been shown to interact with the UPE and CPE, these are UBF and SL-1 (41,44,45,46). Interestingly, although the model by which this is accomplished on vertebrate promoters is physically different than that proposed for *Saccharomyces* promoter, it is biochemically similar (discussed below).

4.1.2. Intergenic Spacer

The intergenic spacer lies between the transcribed regions and is bound at both ends by transcription termination signals (2,4,5,17). In *Xenopus laevis* the intergenic spacer is punctuated by 2-7 spacer promoters and in turn these are separated by six to twelve 60 and 81 bp directly repeating elements (47). The spacer promoter is almost a perfect duplication of the rRNA promoter with as high as $\underline{\sim}$ 90% homology in the regions -145 to +4, and in the imperfect copy of a 42 bp sequence (active core), that localizes to the -72 to -114 region of the gene promoter (48). However, in rat and mouse the spacer and 45S promoters contain only one conserved block of 12-13 bp which includes the G's at -7 and -16 (3,48). The spacer promoter is transcribed by RNA polymerase I producing a transcript which terminates just upstream of the rRNA promoter, ~ -167 bp. In *Xenopus*, the spacer promoter may also enhance transcription from the gene promoter, possibly by delivering RNA polymerase I to the gene promoter (49). However, other studies contradict this observation (48).

The intergenic spacer of *Xenopus* contains additional repetitive elements. The most notable of these are the 60 and 81 bp repetitive elements which are homologous to a portion of the 45S promoter. The 81 bp elements are identical to the 60 bp elements except they contain an additional 21 bp of unique sequence (47). The 3' end of the intergenic spacer (-2300 to -3950), i.e. the region near the 3' end of the 45S rRNA transcript, is a region that shows little homology with either the spacer promoter or the 60/81 bp elements. It consists of at least two repetitive elements (repeat 0 and 1) and some non-repetitive elements (figure 1) (48).

The intergenic spacers of *Xenopus*, yeast, Drosophila, mouse and rat, contain elements which enhance transcription from their "major" promoters (3,4,5). In *Xenopus*, the cis-acting 60 or 81 bp repeat elements, enhance transcription from both the 40S preribosomal RNA and the spacer promoters. In this case the rate of transcription has been shown to be directly proportional to the number of repeat elements and independent of their orientation or distance from the promoter (4,5). Such characteristics are typical of enhancers described in RNA polymerase II transcription. Other enhancer elements have been reported in the rat and mouse intergenic spacers, including the 130 bp element which comprises the variable region of the rDNA repeat (41) and the 37 bp enhancer motif localized in the rat 174 bp non-repetitive region which is able to enhance both RNA polymerase I and RNA polymerase II transcription (3). Additional 140 bp and 200 bp element have also been identified in rat and yeast, respectively (3,50). To date the mechanism by which these elements enhance rDNA transcription is not clear. The repeated elements in the mammalian intergenic spacers and the 60/81 bp repeats of the *Xenopus* spacers have been shown to bind UBF and to act across species. However, it is not clear if UBF is the only factor that binds to the repeated elements and if UBF is solely responsible for enhancer activity.

4.1.3. Terminators

At the 3' end of the primary transcript of mammalian 45S rRNA genes lie several copies of a 17 bp motif, referred to as the Sal box (figure 1). The Sal box functions as orientation dependent terminators of transcription (2,4,17,18,51,52). The 13 bp promoter proximal terminator (T_0) located ~ -167 bp +1 is a Sal box (figure 1). In both cases, the terminator elements act as binding sites for the 105 kDa RNA polymerase I transcription termination factor, TTF-1 (51,53). TTF-1 binds to DNA in a polymerase specific but not a species specific manner. This suggests that TTF-1 once bound to the terminator site acts by interacting with one of the unique subunits of RNA polymerase I (53). Interestingly, recent studies indicate that TTF-1 can associate with RNA polymerase I in the absence of DNA (R. Hannan and L. Rothblum, unpublished observation).

In vertebrates, the process of transcription termination requires two steps: i) RNA polymerase I pausing and its subsequent release; and ii) release and processing of the 3' end of the pre-rRNA (4,18). Mammalian transcription termination requires TTF-1 for the pausing of RNA polymerase I ~11 bp upstream of the Sal box. Interestingly, the second step in termination requires a T-rich element upstream of the TTF-1 binding site and a releasing factor (51,54). In *Xenopus* a terminator

factor (54) that binds to the T3 box in the intergenic spacer has been identified, Rib2 (54,55).

Yeast rDNA repeats contain unique termination elements, which are comprised of two domains. The first domain consists of an 11 bp element, sometimes referred to as a REB1 element, which serves as the binding site for Reb1p (2,56,57). Transcription termination for yeast RNA polymerase I also requires about 46 bp of T-rich 5' flanking sequence. It has been suggested that the Reb1p-DNA complex comprises a pause element, while the 5' flanking sequence contains a release element. In contrast to the TTF-1-DNA complex, the Reb1p-DNA complex is not specific for polymerase I (57,58,59).

The terminators may also serve secondary functions. The promoter proximal terminator element (T_0) not only serves to terminate transcripts originating from the spacer promoter, but it may also activate transcription. It has been suggested that T_0 may function to prevent promoter occlusion. Occlusion is a phenomemon by which transcription through a promoter disrupts the semistable preinitiation complex (3). Thus, in the absence of TTF-1, the transcription factors SL-1 and/or UBF would be displaced from the rDNA by a polymerase that was transcribing the promoter. Recent studies suggest that TTF-1 dependent transcription activation is dependent on chromatin and involves repositioning nucleosomes in an ATP dependent fashion (29,60). However, the exact mechanism by which TTF-1 catalyzes these functions is unknown. It has also been proposed that TTF-1 may function in DNA replication, as it results in the arrest of replication fork movement and thus directs DNA replication in the same direction as transcription (61).

4.2. Proteins Involved in rDNA Transcription

4.2.1. RNA polymerase I

The core mammalian RNA polymerase I is a large, complex enzyme with a total approximate Mr of 500-600,000 and the subunit composition is yet to be confirmed. Varying reports suggest a subunit composition ranging from 11 subunits and 2-3 associated factors to only 2 large and 3-4 smaller subunits depending on the purification method implemented (62,63,64,65,66). Two recent studies, employing different purification schemes, report that mammalian RNA polymerase I is composed of at least 12 subunits with 3 associated factors (PAFs) (67, 68). In contrast, yeast RNA polymerase I has been subject to a detailed series of studies, and fourteen subunits have been identified and cloned (62,64,69).

To date only four of the mammalian RNA polymerase I subunits have been cloned, including the two largest subunits of 190 kDa and 127 kDa, which are analogous to the archaebacterial β' and β subunits, respectively (62,67). The other cloned subunits, AC40 and AC19 (64,69), are common to both RNA polymerase I and III. The yeast subunits are classified into three groups: i) four core subunits: β'-like (A190), β-like (A135) and two which are similar to the bacterial α subunits (AC40 and AC19); ii) five subunits common to all three RNA polymerases: ABC27, 23, 14.5, 10α, 10β; and iii) five specific subunits: A49, 43, 34.5, 14, 12.2 (62). There is a large degree of sequence conservation between the homologous mammalian and yeast homologous RNA polymerase I subunits and also between the RNA polymerases I, II and III subunits themselves (62). Interestingly, the β and β' subunit of yeast RNA polymerase I are more identical to the β and β' subunit of rat RNA polymerase I than they are to the β and β' subunit of yeast RNA polymerase II.

The majority of the yeast RNA polymerase I subunits are essential for growth especially the five ABC and two AC subunits (62). However, the A34.5 and A49 subunits are not strictly essential for cell growth. For example, mutations of A49 generate slow growing colonies with only reduced RNA polymerase I activity illustrating that it is important, but not essential for cell viability (62). Identification of the specific functions of the RNA polymerase I subunits has been limited and restricted mainly to the yeast system. Experimental evidence to date demonstrated that the A190 and A135 subunits cross-link to nascent chain RNA and contain putative Zn^{2+} fingers. Other subunits containing putative zinc binding domains include A12.2, ABC10α and ABC10β. Independent studies have suggested that Zn^{2+} binding may be essential for activity and/or the structural integrity of RNA polymerase I (62).

In order for functional RNA polymerase I to initiate transcription it must recognize and bind the transcription initiation site. The A190, A135, A125 and ABC23 subunits have been implicated in this process. However, the domain(s) involved in this process are yet to be defined (62). Furthermore, the A135 subunit contains a putative nucleotide binding domain suggestive of a role in elongation (62,70). In addition, studies suggest that A190 may also play a role in elongation since resistance to α-amanitin, a drug which interferes with chain elongation, maps to the β' subunit of RNA polymerase II (62,71).

In order for RNA polymerase I to mediate transcription initiation and elongation, it needs to interact with other proteins. To date a number of proteins termed RNA polymerase I associated factors, such as TFIC, Factor C*, TIF-IA, TIF-IC and PAF53 (68,72,73,74) have been shown to closely interact with RNA polymerase I. In addition, there is evidence suggesting that RNA polymerase I itself may interact with the transcription factor UBF. One study has demonstrated that UBF interacts with a 62kDa subunit of murine RNA polymerase I *in vitro* (75). However, RNA polymerase I purified by another group did not contain a 62 kDa subunit, and that laboratory reported an interaction between UBF and the 180, 114 and 44 kDa subunits of mouse RNA polymerase I, as well as with PAF53 *in vitro* (64,68). The association of RNA polymerase I with PAF53 has been confirmed (67,76). However, the interaction between RNA polymerase I and UBF has proven more problematic (67,77). The reasons for these disparities are unclear and further investigation is required.

4.2.2. RNA Polymerase I Associated Factors

4.2.2.1. TFIC, TIF-IA and Factor C*

TFIC (15), TIF-IA (78) and Factor C* (72,79) are factors closely associated with RNA polymerase I and

thought to be intimately involved in regulating its activity under certain growth conditions. The majority of studies examining these factors have been carried out in systems where rDNA transcription is virtually shut off. For example, depriving tumor cells of essential nutrients and growth factors, or treating hormone sensitive lymphosarcoma cells with glucocorticosteroids reduces both rDNA transcription and RNA polymerase I ability to initiate specific transcription (70,80,81). In each case the identified RNA polymerase I associated factor has been shown to restore the ability of RNA polymerase I to initiate specific transcription (15,72,78). Moreover, studies indicate that while not critical for the formation of a stable pre-initiation complex, all three factors are required for the formation of the first phosphodiester bond of nascent pre-rRNA (78,80). Interestingly, a factor, with properties similar to the mammalian polymerase associated factors, has been identified in yeast. This protein, Rrnp3, has been shown to interact directly with RNA polymerase I, independent of the DNA. It has been suggested that Rrnp3 stimulates the recruitment of the polymerase to the stable complex containing the rDNA promoter, and the Rrn6/7/11 and the Rrn5/9/10 complexes (82).

It has been suggested that TFIC, TIF-IA and Factor C may well represent the same biological component. However, there are no antibodies to these factors and none have been cloned. Thus, this conclusion remains to be verified. Accordingly, our understanding of the specific role these factors play in rDNA transcription is limited.

Arguments against the possibility that these activities represent the same factor include differences in the subunit composition of the purified factors. For example, mouse TFIC activity co-purifies with three polypetides present in a stoichiometric ratio of 1:1:1, with approximate molecular mass of 55, 50 and 42 kDa (81). In contrast, TIF-IA activity purifies with one 75 kDa polypeptide (78). In addition, TIF-IA has been found to be far less abundant in cells compared to TFIC. Moreover, TIF-IA can be liberated from the initiating complex and recycled to facilitate transcription from other templates (78). In contrast, Factor C* or TFIC functions stoichiometrically *in vitro* (79,83), *i.e.* it can activate one round of transcription and is then "used up." Interestingly, if elongation is halted within a critical distance (54 bp) Factor C* remains active (79).

It is not possible to compare all of the properties reported for each factor, as different laboratories have carried out different characterizations. For example, mouse TFIC activity was reported to be heat stable (15), and mouse TIF-IA has not been found to be species specific.

4.2.2.2. TIF-IC

TIF-IC has been identified as a 65kDa factor associated with RNA polymerase and is required for the assembly of the initiation complex, formation of first internucleotide bond and chain elongation (84). TIF-IC contributes to the chain elongation by stimulating elongation and suppressing RNA polymerase I pausing. TIF-IC also inhibits nonspecific initiation and supports the synthesis of full-length, run-off transcripts (84). However, as with the above RNA polymerase I associated activities, there are no antibodies to TIF-IC and this factor has not been cloned. This precludes a more complete analysis of its contribution to rDNA transcription.

4.2.2.3. PAFs

Recently, three polymerase associated factors have been isolated and cloned from mouse cells, PAF53, PAF51 and PAF49 (68). All three factors are tightly associated with RNA polymerase I but dissociable under certain purification conditions, indicating that they are probably not core subunits of this enzyme. PAF53 and PAF51 are structurally related proteins since they are both recognized by anti-PAF53 antibodies. It is as yet unknown if PAF51 is a degradative product, an alternatively spliced isoform, or a post translationally modified form of PAF53. PAF49 however, is not detected by anti-PAF53 antibodies thus it appears to be a distinctively different protein (68).

PAF53 is associated with RNA polymerase I purified from exponentially growing 3T3 cells but not with RNA polymerase from quiescent NIH3T3 cells. In addition, antibodies to PAF53 block specific, but not random, transcription from the rDNA promoter. These observations suggest that PAF53 is not involved in template binding, nucleotide incorporation, polymerization activity or elongation. Instead they suggest that PAF53 is required for initiation of specific transcription from the rDNA promoter (68). *In vitro* studies indicate that PAF53 has the potential to interact with the transcription factor, UBF (68), suggesting a role for this factor in the recruitment of RNA polymerase I to the initiation complex. However, the mechanism by which PAF53 contributes to the regulation of rDNA transcription remains to be elucidated.

4.2.3. rDNA Trans-acting Factors

There are at least two trans-acting factors required for efficient transcription of rDNA by RNA polymerase I. In mammals, they are referred to as SL-1 (selectivity factor 1) and UBF (upstream binding factor). Studies in *Acanthamoeba*, described below, have unambiguously identified a multimeric complex, TIF-IB, with properties and a functional role similar to SL-1. Interestingly, studies on transcription by yeast RNA polymerase I, have identified two complexes, described below, with properties similar to what might be considered to be a combination of SL-1 and UBF. Briefly, SL-1 is absolutely required for rDNA transcription *in vitro* (85,86). In contrast, UBF is not absolutely required for specific initiation on the rDNA promoter *in vitro*, although its addition to UBF-depleted extracts increases the efficiency of *in vitro* transcription in a dose dependent manner (87,88,89). In addition, overexpression of UBF1 in cell lines or primary cultures of cardiomyocytes is sufficient to directly increase transcription of a reporter for rDNA transcription (90), as well as the endogenous rRNA genes (R. Hannan and L. Rothblum, unpublished observation).

4.2.3.1. Factors which bind to the core promoter element

There is evidence that several of the rDNA transcription factors may interact with the core promoter element. However, the experimental evidence accumulated

from studies on the mechanism of rDNA transcription in *Saccharomyces* and *Acanthamoeba* suggests that SL-1, or rather its paralogues in those systems, must be considered the primary factor that interacts with the CPE.

Saccharomyces cerevisiae core factor: Genetic studies in yeast demonstrated that TATA-binding protein (TBP), the highly studied component of TFIID, was involved in transcription by RNA polymerase I and III, before biochemical studies had demonstrated that it was a component of any of the factors which interacted with the core promoter elements of the rDNA (91,92,93,94,95). Subsequent biochemical and genetic experiments confirmed that TBP was a component of the human, mouse, yeast, and *Acanthamoeba* rDNA transcription systems. Biochemical and genetic studies in yeast provided evidence for two transcription factors, referred to as UAF (upstream activation factor) and CF (core factor). UAF (discussed below) is a multiprotein transcription factor which consists of at least five proteins. Both biochemical and genetic analyses confirm that CF is also a multiprotein transcription factor, and it consists of at least three proteins, Rrn6p, Rrn7p and Rrn11p (96,97). CF interacts with the core promoter element, but does not by itself form a stable DNA-protein complex. However, in the presence of UAF, which forms a stable complex with the upstream element (98). CF becomes committed to the template and directs the initiation of transcription. It is not clear, at this time, whether TBP is a component of CF as suggested by Lin *et al*. (99) or if TBP is a "bridge" between CF and UAF (100).

Acanthamoeba TIF-IB: Studies on rDNA transcription have demonstrated that one protein, TIF-IB, is the TBP-containing transcription factor that binds the rDNA promoter to form the committed complex (86,101,102). While, TIF-IB has not been cloned, it has been purified to homogeneity and its interactions with the rDNA promoter have been studied extensively. TIF-IB consists of TAF_Is of 145, 99, 96, and 91 kDa as well as TATA-binding protein. Site-specific cross-linking experiments demonstrated that the TIF-IB contacts mapped from -19 to -66 (86,102,103). Interestingly, TBP, as part of TIF-IB, only made contact with promoters derivatized between -38 and -43. This site is 22 bp upstream of the bend in the promoter induced by contact with TIF-IB, and consistent with the hypothesis that the DNA binding region of TBP may not be as involved in DNA-binding by TIF-IB as it is in TIF-ID (86).

Subsequent studies on the the interaction of TIF-IB and RNA polymerase I with the *A. castellanii* promoter demonstrated that TIF-IB could direct transcription from a core promoter terminated at -6. Additional cross-linking experiments demonstrated that, when assayed in combination, both TAF_I96 and the 133 kDa subunit of RNA polymerase I interacted with the region between -1 and -7. This region contains a conserved sequence which is present in a large number of rRNA promoters: n(g/r)(g/r)**G**t(**T**/A)a**T**n**TA**g**GG**(a/g)g**A**n (**A**=+1). This leads to the hypothesis that the CPE of RNA polymerase I promoters contains both an upstream site that interacts with TIF-IB and an Inr-like element that strengthens the interaction between TIF-IB and the promoter (104). Interestingly, these observations, using state-of- the-art techniques and highly purified reagents are consistent with conclusions drawn in earlier studies using various promoter mutants (105 and references therein).

SL-1: The mammalian homologue of TIF-IB is referred to as SL-1. Like TIF-IB, SL-1 is a "basal" rDNA transcription initiation factor capable of directing multiple rounds of RNA polymerase I recruitment to the rDNA promoter. SL-1 was first identified and its subunits cloned in humans (106). Subsequently, homologous proteins have been identified in rat (SL-1) (107), mouse (TIF-IB, factor D) (101,108,109,110), and frog (Rib1) (111). SL-1 exists as a complex containing the TATA-binding protein (TBP) and at least three RNA polymerase I specific TBP associated factors (TAF_Is) (106, 109).

As mentioned, TBP is a the subunit common to the fundamental transcription factors for all three nuclear transcription systems. In every case, the functional regions of TBP are localized to the highly conserved C-terminal domain, which consists of two copies of an imperfect repeat of 61-62 amino acids. This region is sufficient for the correct assembly of SL-1 and is necessary for transcriptional activity (112).

In contrast to TBP, the RNA polymerase I TAFs exhibit no homology to the TAFs involved in transcription by RNA polymerase II or III (86,106). In addition, the molecular masses of the RNA polymerase I TAFs differ between species for example, the human TAF_Is are 110, 63, and 48 kDa (106), and the mouse TAFs 95, 68 and 48 kDa (108,110). TAF_I48 exhibits the highest degree of conservation among species and contains two stretches near the N-terminus which are imperfectly repeated at the C-terminal (106,110). The largest TAF_Is, mouse TAF_I95 and human TAF_1110, are the least conserved, demonstrating only 66% identity at the amino acid level. The second largest TAFs also differ. Human TAF_I63 contains an unique 40 amino acid N-terminal extension and mouse TAF_I68 has 66 unique amino acids in its C-terminal region. Both proteins contain two putative Zn^{2+} fingers, although $mTAF_1 68$ may have a third Zn finger (106,110). To date, the 5' end of the cDNA for human TAF_I68 has not been cloned (106).

The mechanism determining the association of TBP with the TAF_Is rather than other TAFs to form TFIID, TFIIIB and SNAPc is not known. *In vitro* experiments demonstrated that when TBP is bound to any of the TAF_{II}s, it will no longer bind the TAF_Is, and *vice versa* (106). These studies suggest a mutually exclusive binding and that this binding specificity will direct the formation of the promoter- or polymerase-selective TBP-TAF complexes (106). SL-1 activity could be reconstituted from the three human TAF_Is (106). However, functional mouse SL-1 could not be reconstituted from recombinant mouse TBP and TAF_Is, although they did form a high molecular weight complex (109), and could complex with the human TAF_Is. This observations suggests that SL-1 may contain additional components, or that additional factors may be

required to mediate the interaction between SL-1 and RNA polymerase I.

Formation of a stable SL-1 complex involves multivalent contacts between TBP and the TAFs as well as between the individual TAFs (106,109). These contacts appear to be conserved, as the interactions between the mouse TAFs and human TBP appears to be the same as that observed with the human TAFs (106). How the SL-1 complex interacts with the rDNA promoter and thus mediates rDNA transcription is currently under investigation.

The original studies on human SL-1 suggested that, by itself, SL-1 was not a DNA-binding protein (113). Human SL-1 did not footprint the human rDNA promoter. However, the addition of SL-1 to a UBF footprinting assay resulted in a 5' extension of the UBF footprint (113,114,115). Rat SL-1 was found to be sufficient to drive transcription from a promoter that extended from -37 to +164 (44), but footprinted over the UPE of the rat rDNA promoter (44). It was noted that the position of that footprint was similar to the "extension" of the human UBF footprint by human SL-1 (44). Interestingly, mouse SL-1 yielded a "disperse" footprint, but that footprint included the CPE (113,116). This binding was abolished by a mutation at -16 with respect to +1. The same mutation results in a decrease in rDNA transcription, confirming that mouse SL-1 binding is required for promoter recognition and transcription initiation (116). Studies on the purified, recombinant hTAFIs suggest that hSL-1 is a DNA-binding protein. One study reported that both hTAFI110 and hTAFI63 bound to the rDNA promoter (117), while a second paper demonstrated that human TAFI48 and TAFI63 (or mouse TAFI68) can bind to DNA (106,118).

Although it has not been tested, experiments examining the interactions between the core promoter binding factors suggest an ordered strength of DNA-binding, mSL-1>rSL-1>hSL-1. This may explain the relative importance of UBF in these various transcription systems. In this regard it should be noted that *A. castellanii* TIF-IB has an very strong affinity for its promoter (kDa of 30x10-9), and it is a matter of discussion if there is a UBF-like activity in that organism (119).

The interaction between SL-1 and UBF appears to be critical for UBF-dependent activation of transcription (115). It has been suggested that basal rDNA transcription requires SL-1 and the CPE, while elevated levels of transcription also require UBF and the distal promoter elements (44). Coimmunoprecipitation studies demonstrated that SL-1 can bind to UBF in the absence of DNA (77,120). UBF antibody depleted extracts of SL-1 activity but not TFIIIB activity, demonstrating that this is a specific interaction (77, 120). *In vitro* studies suggest that this interaction may be mediated by the SL-1 components, TBP and TAFI48 (117). However, the domains of the proteins involved are as yet undetermined. These studies suggest that SL-1 serves to communicate between UBF and RNA polymerase I.

The interaction between SL-1 and the rDNA promoter is species specific, *e.g.* human SL-1 is required for transcription from the human rDNA promoter (3,4,121,122). In contrast, UBF and RNA polymerase I are, at least to some degree, interchangeable between species (116,123). For example, extracts prepared from primate cells that actively transcribe the human rRNA promoter fail to initiate transcription from a rodent rRNA promoter, but will do so when supplemented with either mouse or rat SL-1 (4,44,116,124). However, this only extends so far, a similar study showed that frog and human extracts could not be "reprogrammed" to accurately transcribe one anothers genes (125). [Interestingly, mouse extracts would initiate transcription on the *Xenopus* promoter but at +4 (3,126). Another publication reported that rat SL-1 can utilize primate RNA polymerase I transcription machinery (127)] The subunit of SL-1 responsible for reprogramming has not been identified. The two largest TAFs are the least conserved thus, are the most likely candidates for conferring species specificity. However, this needs to be established.

4.2.3.2. UBF

UBF has been cloned from humans (84), mice (128), rats (123,129) and *Xenopus* (130,131). In *Acanthamoeba* a 125kDa protein has also been identified which has some functional characteristics similar to UBF (3). UBF is a highly conserved protein. Human and rat UBF1 are 97% identical, and there is only one, nonconservative amino acid change between the two (128). Even between mammals and *Xenopus* there is a 73% conservation of the amino acids overall. This conservation becomes 90% when the N-terminal domains are compared (2).

Purified UBF consists of two polypeptides, UBF1 and UBF2, the sizes of which vary depending upon the species (128,129). The human and rodent UBF isoforms are 97 kDa (UBF1) and 94 kDa (UBF2), whereas in *Xenopus laevies* they are 85 and 83 kDa (18,129). The mouse UBF gene consists of 21 exons extending over 13 kb (128). Transcription of this gene generates a single transcript which results in the mRNA for UBF1 (764 amino acids), or, due to alternative splicing at exon 8, UBF2. The result of the splicing event is that UBF2 mRNA contains an in frame deletion of 37 amino acids in HMG box 2 (128,129). In contrast, *Xenopus* UBF1 and UBF2 are generated by transcription from two different genes (130), and there is evidence for additional UBF genes or pseudogenes in the *Xenopus* genome (131). The xUBF1 gene encodes a protein which has 93% identity to xUBF2 and contains an insert of 22 unique amino acids between HMG box 3 and 4 (3,130,131).

Both isoforms of UBF can bind to the rDNA promoter, form homo or hetero dimers in solution and bind to synthetic DNA cruciforms with a similar affinity (46,85,132). However, UBF1 has been shown to be a more potent activator of transcription *in vitro*. UBF2 is 1/3 to 1/10 as active as UBF1 (87,132). This suggests that the activity difference is due to the alteration of HMG box 2 as found in UBF2. One study suggests that UBF2 may have a unique function in the formation of loops between the enhancers of the gene promoter (133). However, when

COS cells overexpress $p21^{h\text{-}ras}$ they only express UBF1 and these cells are viable (132). This suggests that, at least in COS cells, UBF2 is not essential for cell viability (132). One report found that the ratio of UBF1/UBF2 in a cell reflected the growth state of the cell, i.e. the ratio of UBF2/UBF1 was approximately two in stationary 3T3 or MH134 cells (128) and the ratio approached 1 upon nutritional upshift. It has also been reported that the ratio of UBF1 to UBF2 changes with development. For example, during differentiation of F9 cells or mouse embryogenesis the ratio of UBF1 to UBF2 decreases both at the mRNA and protein level (132). Cumulatively, these data raise the possibility that UBF2 may have an as yet unrecognized function(s).

The mechanism(s) by which UBF expression is regulated is unknown. It is clear that the gene is subject to both positive and negative regulation (9,11,134). However, further analysis is required in order to determine the direct mechanisms by which UBF expression is regulated.

UBF Structure: The dominant structural elements of UBF are the HMG boxes which are similar to the DNA binding domain of the chromosomal high mobility group proteins 1 and 2 (HMG-1, HMG-2) (89). Other proteins belonging to this family include T-lymphocyte receptor α-enhancer factor, sex determining region Y protein, mitochondrial transcription factor and the yeast mitochondrial nonhistone protein NHP6 (3).

HMG boxes are usually 80 amino acids long (4). The number "found" in UBF depends upon the stringency of homology to the consensus sequence used for classification. Thus, there are reports of four to six HMG boxes in UBF (75,89,128). For example, when the definition of a HMG box is applied stringently, mammalian UBF and *Xenopus* UBF have four and three HMG boxes, respectively (4,75,135,136). However, many papers cite six and five boxes respectively. Interestingly, each HMG box appears to play a specific role (131,135). An HMG box cannot be replaced with another box from the same protein. However, they can be replaced with the same HMG box from a distantly related species. UBF requires the correct number and order of HMG boxes for it to function in transcript (135). The finding that Xenopus UBF failed to activate transcription in an extract from human cells may be explained by the observation that *Xenopus* UBF lacks HMG box3 as found in human UBF (131).

Functions other than DNA-binding have been assigned to the HMG boxes. Part of the UBF nucleolar localization signal is found in the NH_2-terminal region, which includes HMG box 1, (137,138). In addition, nuclear transport, requires a short 24 amino acid sequence near HMG box 5 as well as the CO_2H-terminus (137). Similar to other HMG-like proteins UBF contains a highly acidic CO_2H-terminal domain and an NH_2-terminal dimerization domain. The acidic CO_2H-terminal domain of UBF consists of a stretch of 89 amino acids of which 68% are Glu or Asp, 25% are serines, and 7% glycine (89). The acidic regions are interrupted by conserved serine-rich blocks (3). The NH_2-terminal dimerization domain contains two short regions that are hypothesized to form amphipathic helices similar to a helix-loop-helix motif. The dimerization domain is also required for optimal DNA binding along with at least one HMG box (43,136), and additional HMG boxes appear to stabilize DNA-binding (43,139).

*Mechanism of UBF Action: T*he action of UBF depends on the formation of homo- and/or hetero-dimers (3,136,148) and its binding to DNA, via the minor groove (140). Various manuscripts have reported that UBF binds to the CPE, UPE, spacer promoters and the enhancer repeats in the intergenic spacer (44,46,47,89,123). In addition, *Xenopus* UBF can bind on each side of the promoter proximal terminator (3). Interestingly, footprinting analysis has demonstrated that the DNase footprint obtained with UBF depends on the rDNA promoter being footprinted, and is independent of the origin of the UBF used in the assay (18,46,89,123). As discussed above, this would suggest that rDNA promoters share underlying structural similarities despite their sequence differences. In general, UBF footprints the rDNA promoter in the UPE, from ~ -50 to ~ -130 (89). However, UBF can also protect the CPE, from ~ -45 to ~ +20 (43, 89,123). As discussed previously, a mutation at either the guanine at -16 or -7 eliminates promoter activity (41,44,89), but did not affect UBF binding to the DNA (41). As mentioned, the interaction of UBF with SL-1 results in an extension of the UBF footprint (44,113,114,115,141). This is believed to be part of the mechanism by which UBF facilitates the generation of the pre-initiation complex on the promoter.

Initially it was reported that UBF bound predominantly to a GC rich consensus sequence (4). However, recent studies suggest that UBF may recognize a specific DNA structure, such as synthetic DNA cruciforms, four way junctions and tRNA, rather than a sequence (2,132,137,139). The domains of UBF required for DNA binding and the DNA binding sequence recognized are controversial. UBF with every HMG box deleted, except HMG box 1, is able to bind DNA (as long as the dimerization domain is present). The addition of HMG boxes increases the strength of DNA binding (4,89,136). These results may be explained if UBF binding to DNA is the result of a summation of multiple HMG box-DNA contacts. This would also lessen the requirement that any single sequence (DNA recognition site) be stringently maintained.

UBF binds to DNA and by inducing folding and bending shortens the DNA contour by ~190 bp (141,142). This generates a disk-like UBF-DNA complex which has been referred to as an enhancersome (2,43,141). The enhancersome contains a low-density protein core around which the DNA loops, probably by in-phase bending. UBF can force the DNA to generate a 360^0 loop with a diameter of 19 nm (141). In this structure the HMG boxes would interact with the promoter in a colinear manner. This model is consistent with those reported by Xie *et al.* (41). In that study spacing changes of half a helical turn significantly decreased rDNA promoter activity, while a full turn only

mildly affected promoter activity (41). Thus it would appear that UBF binds to the DNA and bends it. It is not clear if UBF binds cruciform DNA and then bends the remaining DNA to form an enhancersome or if UBF binds to the DNA, bends it facilitating the formation of a cruciform. In either case, bending the rDNA promoter would make it possible for the two bound SL-1's to interact generating the pre-initiation complex model illustrated in figure 2.

*Regulation of UBF Specific Activity***:** Cells can modify the specific activity of UBF by at least two different mechanisms: i) phosphorylation; and ii) sequestration. UBF is a phosphoprotein (13), especially the CO_2H-terminal tail which can be extensively phosphorylated (88). The ability of UBF to trans-activate the rDNA promoter is reduced when UBF is treated with phosphatase (13) or when the phosphorylated CO_2H-terminal tail is deleted (88). This suggests that phosphorylated UBF is the more active form of the transcription factor. UBF contains numerous consensus motifs for characterized kinases including CKII and MAPK. Interestingly, treatment of UBF with CKII *in vitro* increases UBF phosphorylation (13,88,139) and enhances transcriptional activity (88). However, it is not known whether or not UBF is an endogenous substrate of CKII *in vivo***.** In many models of growth the phosphorylation status of UBF has been demonstrated to correlate positively with the rate of rDNA transcription (13,88,143). These experiments are discussed in more detail in section 5.4.2.

The activity of UBF can also be down-regulated by the interaction of UBF with the product of the retinoblastoma susceptibility gene, Rb^{110} (10,144). Studies indicate that the ability of UBF to transactivate the rDNA promoter is severely compromised when UBF is sequestered, either directly or indirectly, by Rb^{110}. Moreover, the physiological relevance of this mechanism in the regulation of rDNA transcription has been demonstrated *in vivo* (10). These experiments are discussed in greater detail in section 5.4.2.

Cellular Distribution: In some cell types the localization of UBF can change during the cell cycle. For example, during early S phase there is an increased association of UBF, RNA polymerase I and SL-1 within the nucleolus (21). The increase in UBF association with nucleoli may be due to an increase in the ability of UBF to compete with the histones for binding to the rDNA. Typically, UBF localizes to the FC and DFC of the nucleolus where it forms small bead like structures in a folded filament pattern (22). This distribution is sustained during the G2 phase when the cells are actively transcribing the rDNA (23). At the end of G2, when rDNA transcription is "shut off", UBF, RNA polymerase I and SL-1 accumulate in the mitotic NORs forming a few intensive spots on the chromosomes (21).

4.2.3.3. Ku/E_1BF

Ku/E_1BF was originally detected as an human autoantigen reacting with antibodies from patients with rheumatic disorders and has now been widely identified in a number of species (3). Ku/E_1BF exists as a heterodimer of two polypeptides, 70 and 86 kDa polypeptide (145,146,147). Ku/E_1BF tends to bind DNA in a non-specific manner, while recent studies have shown that it binds the rDNA promoter with high specificity (145,148,149). Interestingly, when Ku/E_1BF is added to cell-free transcription assays it can affect the rate of rDNA transcription (to be discussed below).

Ku/E_1BF has been shown to be the DNA-binding component of the DNA-dependent protein kinase (DNA-dependent PK) (150,151). DNA-dependent PK is a nuclear, serine/threonine protein kinase consisting of a 350 kDa catalytic subunit and Ku/E_1BF. The enzyme is most active when bound to DNA, a process dependent on Ku/E_1BF. To date DNA-dependent PK has been shown to be important in various cellular processes such as, cell signaling, DNA replication, RNA polymerase II transcription activation and DNA repair.

4.2.3.4. CPBF

Interestingly Ku/E_1BF has been shown to interact with another potential rDNA transcription factor, CPBF (core promoter-binding factor) (3). CPBF is a rDNA binding protein, which has been isolated from both rat mammary adenocarcinoma ascites and HeLa cells. CPBF purifies as two polypeptides of 44 and 39 kDa (152). The 44 kDa peptide binds to Ku/E_1BF (146). Both CPBF peptides specifically interact with the rDNA core promoter sequence resulting in trans-activation of the rDNA promoter *in vitro* (152). Moreover, CPBF and Ku/E_1BF function synergistically to enhance RNA polymerase I transcription (146).

CPBF has been found to be the rat homologue of human USF which also consists of two peptides, 44 and 43 kDa (153). USF is a basic helix-loop-helix zipper, DNA binding protein which specifically binds E-boxes in genes transcribed by RNA polymerase II. Interestingly, USF and CPBF bind to the same E-box in the rat rRNA promoter suggesting a possible mechanism for their action on rDNA transcription. Oligonucleotides to the E-box sequence inhibit rDNA transcription possibly by preventing USF/CPBF binding to the DNA (154).

4.2.3.5. Topoisomerases

Topoisomerases are enzymes which modulate DNA topology by catalyzing cleavage-rejoining reactions of the phosphodiester bonds. There are two classes of topoisomerases, I and II. Topoisomerase II includes both α (170 kDa) and β (180 kDa) isoforms (27,155). Topoisomerase may act as a swivel, relieving torsonal stress generated during transcription. This would allow for a rotation of the transcribed DNA segments without having to turn any other part of the DNA or the transcription ensemble (155,156).

Both topoisomerase I and II are nuclear enzymes. Topoisomerase I and II α are found in both the nucleoplasm and nucleolus. Topoisomerase II β is exclusively localized in the nucleolus (20,157,158,159,160). Topoisomerase I preferentially associates with actively transcribed regions of chromatin, and has also been implicated in the regulation of rDNA transcription (159,160). Topoisomerase I has been

demonstrated to be required for rDNA transcription and replication in yeast (157,161).

4.2.3.6. p16

p16 is an HMG-like, DNA-binding protein isolated from Novikoff hepatoma ascites and Hela cells (162). p16 binds to the oligo d(A).d(T) tracts found both within the UPE (-620 to -417) and external transcribed spacer (+352 to +525) of the rat rRNA gene. p16 was demonstrated to stimulate rDNA transcription in a dose dependent and saturable fashion when either of those sites was *in cis* with the target promoter. To date, this factor has not been cloned, thus, further details on the nature and mechanism of the interaction between p16 and the rDNA transcription apparatus are not available.

4.3. Formation of Preinitiation Complexes

The transcription cycle involves four distinct steps: i) initiation; ii) promoter clearance; iii) elongation; and iv) termination (4). Initiation involves the assembly of the pre-initiation complex on the rDNA promoter, isomerization of the closed pre-initiation complex to form an initiation competent open complex and, finally, generation of the first phosphodiester bond (84). Once the first bond is formed, and the promoter is cleared, an alteration occurs in RNA polymerase I conformation which commits the enzyme to undergo RNA chain elongation (163). Elongation involves catalyzing the processive addition of ribonucleotides to the 3' end of the growing RNA chain until specific attenuation or termination signals are encountered. Lastly, transcription is terminated and the product of the polymerase released from the template (84).

The preponderance of evidence is consistent with the model that regulation of rDNA transcription occurs at the level of formation of the initiation complex (163,164). Once formed, the initiation complex is quite stable and may remain in place on the promoter, even as initiation rates vary widely, suggesting that the number of complexes is determined by limiting the amounts of a transcription factor (164). Once these complexes form, the actual rate of transcription will depend upon the ability of RNA polymerase I to recognize the complex and initiate transcription. The steps which may be involved in this regulation are dependent on the system being investigated.

A working model for initiation can be based on studies from *Acanthamoeba castellani.* In this system, TIF (SL-1) binds to the promoter, in the absence of either UBF (or a UBF-like factor) or RNA polymerase I, and causes a distinctive DNAse I footprint (163,165). TIF then recruits RNA polymerase I by protein-protein interactions. RNA polymerase I binding results in an extension of the TIF footprint to include the +1 site (164). Upon the addition of nucleotide triphosphates, elongation occurs and the RNA polymerase I footprint moves down the template leaving the original TIF footprint behind (163).

In mammalian and *Xenopus* transcription systems, initiation is also believed to be a multistaged process. In mammals, initiation involves SL-1 binding to the core promoter a process which is facilitated by UBF, and possibly TIF-IC, to form a stable pre-initiation complex (166). This complex is stable for a number of rounds of transcription and able to recruit RNA polymerase I and the RNA polymerase I associated factors, such as TIF-IC, TIF-IA and PAF53, to form the second pre-initiation complex. The result of these steps is a complex which, with the addition of ATP/CTP (mouse; GTP/CTP in human) and further NTPs, becomes an initiation competent complex (166,167). The complex is now ready for elongation during which time RNA polymerase I moves past the initiation complex and leaves the pre-initiation complex intact.

5. REGULATION OF rDNA TRANSCRIPTION

Potential sites for regulation of ribosome synthesis include transcription of the ribosomal precursor genes (45S and 5S), pre-ribosomal splicing, and assembly of the ribosomal subunits, and transport from the nucleus to the cytoplasm. However, in the majority of cases ribosome synthesis has been shown to be regulated largely at the level of transcription of the ribosomal genes (rDNA). Theoretically, regulation of rDNA transcription can involve: i) changes in chromatin structure; ii) alterations in the amount, localization, or activity of RNA polymerase I; and/or iii) similar alterations in the associated transcription factors. Moreover, recent studies suggest that the rDNA transcription apparatus can assemble (or colocalize) on the rDNA, but not be actively transcribing, suggesting that there may also be mechanisms for inhibiting transcription (23,168,169,170).

5.1. Chromatin

Chromatin may regulate gene activity by limiting the access of transcription factors to their DNA binding sites on the promoter. However, this may not be the case for all transcription factors since UBF can associate with either naked or nucleosome associated rDNA. Like other genes, the ribosomal genes upon activation require a chromatin modification. For example, inactive *Xenopus* rRNA genes have nucleosomes occupying the complete 40S transcribed region and most of the intergenic spacer, including the repetitive enhancers. Transcribed genes do not appear to contain nucleosomes. Both UBF and TTF-1 may play roles in altering the structure of the chromatin. The association of UBF with chromatin *in vitro* results in the displacement of the linker histone H1, without affecting the core histones. The spacer promoter may also play a role in opening the enhancer chromatin to activating factors and thus be involved in an early stages of gene activation (2). To date, limited experimental techniques are available to fully examine and understand chromatin's role in the regulation of rDNA transcription.

5.2. RNA Polymerase I

Efficent rDNA transcription requires active RNA polymerase I. Only a fraction of the total amount of RNA polymerase I purified from cells is capable of participating in transcription *in vitro*. As stated above *Acanthamoeba castellanii* encyst when starved, and their rate of rDNA transcription decreases concomitant with an increase in the content of a modified form of RNA polymerase I (PolA) and a decrease in PolB. PolA does not support specific

transcription *in vitro* (66,72), while the other form, PolB, can initiate both specific and non-specific transcription (7,66,72,171). The difference between these two forms is unclear and has been be ascribed to a modification of one of the subunits of the enzyme (*A. castellanii*), and/or a change in its association with other RNA polymerase I associated factors.

Interestingly, several yeast RNA polymerase I subunits, including A190, A43, A34.5, ABC23 and AC19 and possibly C53 (62), as well as the A194 subunit of mammalian RNA polymerase I (67) are modified by phosphorylation. However, the role of phosphorylation in RNA polymerase I activity has yet to be established. Alternatively, as mentioned above, the difference between PolA and PolB in mammals may be due to changes in either their association with RNA polymerase I-associated factor(s) or to a change in that factor. These activities have been referred to as TFIC, Factor C*, TIF-IA, TIF-IC or PAF53 (15,78,80). Specific examples of such modifications are discussed below.

5.3. RNA Polymerase I Associated Factors

5.3.1. TFIC, TIF-IA and Factor C*

In a number of studies the rate of rDNA transcription was shown to correlate with the activity of TFIC, TIF-IA or Factor C*. For example, reduced rDNA transcription observed with cyclohexamide or glucocorticoid treatment of mouse P1798 lymphosarcoma cells was attributed to a decrease in either the amount and/or activity of TFIC (15,80). Interestingly, serum starvation of the same cell line did not alter TFIC activity even though rDNA transcription was reduced (74). Thus, regulation of RNA polymerase I activity by TFIC, in these cells, appears to be stimulus-dependent. rDNA transcription is also reduced in response to treatment with cyclohexamide or in post confluent suspension cultures of L 1210 and Ehrlich ascites cells. In these cells, this correlated with reduced Factor C* activity (72). Similarly, post confluent suspension cultures of Ehrlich ascites cells demonstrated reduced rates of rDNA transcription and reduced levels of TIF-IA activity (73,74,171). Paradoxically, if cells are arrested in mitosis by nocododazole they exhibit a high level of TIF-IA activity but a reduced rate of rDNA transcription (78).

In general the amounts of the activities referred to as TFIC, TIF-IA, Factor C* and TIF-IC correlated with rDNA transcription and thus appear to be critical in regulating the ability of RNA polymerase I to initiate specific transcription. However, further knowledge of the exact functions of these factors and their relationship with RNA polymerase I or other transcription factors, will remain limited until they have been studied in great detail.

5.3.2. PAF53

As mentioned above, PAF53 is one of a group of recently purified proteins that associate with RNA polymerase I. To date two lines of evidence support a role for PAF53 in the regulation of rDNA transcription. 1) There is a positive correlation between the accumulation of PAF53 in the nucleoli of 3T3 cells and the rate of rDNA transcription; and 2) PAF53 is isolated in a complex with PolB (the transcriptionally active form of RNA polymerase I), but not with PolA (68). It has been suggested that PAF53 mediates an interaction between RNA polymerase I and UBF (68). However, these results were obtained in vitro and await additional corroboration.

5.4. rDNA Trans-acting Factors:

5.4.1. SL-1

There has been only one published report of a physiologically relevant alteration in the amount or activity of SL-1. A priori one might predict that SL-1 would be a primary target for regulation in that: i) it is the RNA polymerase I paralogue of TFIID; and ii) it is absolutely required for rDNA transcription. Zhai et al. (173) have reported that SV40 large T antigen can bind to SL-1 and activate rDNA transcription (173). In contrast, SL-1 activity has been examined in extracts from growing and non-growing Ehrlich ascites cells and found to be unchanged (114). However, since the SL-1 subunits has only recently been cloned, studies on its regulation are still in their infancy. It is likely, as with the core RNA polymerase II transcription factors, that it will prove to be regulated at either the post-translational level (e.g., phosphorylation, acetylation) or by changes in its specific interaction with other positive or negative regulators of rDNA transcription.

5.4.2. UBF

The hypothesis that regulating UBF activity in the cell might have an effect on rDNA transcription is controversial. For instance, there are believed to be 10 000-100, 000 copies of UBF in the cell (22). This number, is in vast excess when compared to both the number of active ribosomal genes and to the estimated number of SL-1 and RNA polymerase I complexes (1,5). This would suggest that UBF is not a rate-limiting component of the rDNA transcription apparatus. However, these estimates are based on the amount of UBF present in rapidly dividing, immortal cell lines. The cellular content of UBF in differentiated cells such as, adult hepatocytes, neonatal and adult cardiomyocytes is significantly lower than that observed in immortal cell lines (D. O'Mahony, R. Hannan, and L. Rothblum, unpublished observation). Moreover, the transfection and overexpression of UBF1 in neonatal cardiomyocytes is sufficient to stimulate transcription from a reporter construct for rDNA transcription in a dose dependent manner (90). Such observations have led groups to examine if UBF is a potential target for regulation during altered growth conditions.

In theory, the cellular activity of UBF can be regulated by either altering the amount of UBF available to transactivate the rDNA promoter or by changing the activity of an individual molecule by postranslational modifications such as phophorylation. In fact, both have of these mechanisms have been demonstrated to occur. Moreover, while these two mechanisms are not mutually exclusive, they appear to be dependent on both the cell type and stimulus being examined.

Regulation of UBF Content: Numerous studies have demonstrated a correlation between the cellular content of UBF and rDNA transcription. For example, the differentiation of L6 myoblasts into myotubes correlates with a decrease in UBF mRNA which precedes the decrease in UBF content and rDNA transcription (11). At the same time, myosin heavy chain protein accumulates, the mRNA level of myogenin increases, and transcription of the tubulin, r-protein L32, and 5S rRNA genes do not change (11). Thus, the observed decrease in UBF content during differentiation is not due to a general decrease in gene expression or translation (11).

Serum starvation of cells, such as 3T6 cells, reduces rDNA transcription due to a decrease in the availability of the mitogenic factors found in serum that these cells require for growth. This decrease correlated with a decrease in the cellular content of UBF. Refeeding serum-starved 3T6 cells with serum restored UBF content, which preceded the elevation of rDNA transcription to levels observed in control cells. Accumulation of UBF protein was found to result from regulation at the level of transcription of the UBF gene, in a manner similar to that of c-*myc* (12).

The regulation of rDNA transcription has been studied in LNCAP cells, an androgen dependent cell line. Nuclear run-on data demonstrated that DHT treatment of these cells increases rDNA transcription which correlated with an increase in UBF cellular content (174). In addition, extracts of prostate cells from orchiectomized rats showed a decrease in rDNA transcription and UBF protein. However, if the rats were treated with testosterone these levels did not decrease (174). Thus, androgens appear to, at least in part, stimulate rRNA synthesis by regulating the quantities UBF.

A correlation between UBF content and the regulation of rDNA transcription has been extensively studied in primary cultures of neonatal cardiomyocytes. When neonatal cardiomyocytes are treated with various growth promoting stimuli such as adrenergic agents, they undergo hypertrophy. This is associated with an elevated protein synthetic capacity due to increased ribosome biogenesis, which is achieved by increasing rDNA transcription (8,143). A good correlation is observed between the degree to which cells grow (hypertrophy) in response to a growth stimulus and the degree to which rDNA transcription is increased. Phenylephrine affected neither the content of RNA polymerase I nor UBF phosphorylation. However, there were significant increases in the cellular contents of UBF mRNA and protein which correlated, both temporally and quantitatively, with changes in rDNA transcription (8). This correlation was confirmed by the observation that overexpressing UBF1, in the absence of hypertrophic stimuli, increased the activity of a cotransfected reporter for rDNA transcription (90).

Regulation of UBF Phosphorylation: Stimulation of neonatal cardiomyocytes with two other hypertrophic agents, phorbol 12-myristate 13-acetate (PMA) or endothelin-1 (ET-1) does not change the cellular content of UBF. Instead a significant increase in UBF phosphorylation was observed. This increase in UBF phosphorylation correlated, both temporally and quantitatively, with elevated rDNA transcription. The affects were not seem until 6-12 h after the onset of PMA or ET-1 treatment (143), suggesting that they did not result from the activation of protein kinase C. These findings emphasize that even within one cell type the mechanisms utilized to regulate rDNA transcription and UBF activity are stimuli specific.

A correlation between the phosphorylation status of UBF and rDNA transcription has been observed in other cell culture systems. For example, the decreased rate of rDNA transcription that accompanies serum starvation of CHO cells correlates with a slow decrease in UBF phosphorylation, in the absence of changes in cellular content (13,88). The addition of serum restores both rDNA transcription and the degree of UBF phosphorylation. Pulse-chase experiments demonstrated that the decrease in UBF phosphorylation was due to a reduction in phosphorylation, and not the result of "active" dephosphorylation. Similarly, treatment of vascular smooth muscle cells (VSMC) with Angiotensin II (AII), a hypertrophic stimulus, rapidly (within 30 min) increases both rDNA transcription and UBF phosphorylation in the absence of changes in UBF content (175). The activity of CKII, an enzyme which phosphorylates UBF *in vitro*, is not altered in those cells, suggesting that either a serine kinase other than CKII is responsible for AII stimulation of UBF phosphorylation or the ability of CKII to specifically phosphorylate UBF was being regulated.

None of the above studies have established if there are qualitative changes in the specific serine residues phosphorylated. In addition, while *in vitro* experiments have demonstrated that phosphorylated UBF is more transcriptionally active than dephosphorylated UBF, it remains to be determined whether alterations in the phosphorylation state are necessary or sufficient to effect changes in rDNA transcription rates *in vivo*. Future studies will have to define the sites phosphorylated and identify the enzymes responsible for phosphorylating UBF *in vivo*.

Sequestration of UBF: Recent studies indicate that a direct measurement of the total cellular content of UBF or its degree of phosphorylation may not necessarily correlate with the amount of UBF available to transactivate the rDNA promoter or the specific activity of UBF. This conclusion stems from the observation that UBF can be sequestered into an inactive complex with Rb^{110}. Rb^{110} functions as a tumor suppresser and is a negative regulator of growth (1,144,176), acting at the G_1 checkpoint. It is the underphosphorylated (hypophosphorylated) form of Rb^{110} which is the most active. This form predominates in quiescent cells, while the hyperphosphorylated form is prevalent in actively growing cells (1,144,176). The hypophosphoryated form of Rb predominates in Go and G1 phase cells. Hyperphosphorylated Rb predominates in the G2, M and S phases of the cell cycle (176).

The initial observations of an interaction between UBF and Rb^{110} came from studies on the regulation of

rDNA transcription in differentiating U937 cells (10). It was noted that when U937 cells differentiate, Rb^{110} accumulates in the nucleolus and rDNA transcription decreases in the absence of changes in UBF content. This study also demonstrated, using cell-free transcription assays, that the addition of Rb^{110} to an extract containing limiting amounts of Rb resulted in inhibition of UBF-dependent rDNA transcription *in vitro* (10). Coimmunopreciptiation experiments demonstrated an increase in the association between Rb^{110} and UBF with differentiation (10). Moreover, affinity chromatography experiments demonstrated that this interaction was specific and required the A/B pocket of Rb. This was deduced since Rb^{110}209, a biologically inactive form of Rb^{110} containing a cysteine to phenylalanine mutation at amino acid 706 (in the A/B pocket), did not interact with UBF and did not inhibit rDNA transcription *in vitro*. In addition, the UBF-Rb interaction could be inhibited by a synthetic peptide that has been shown to interact with the A/B pocket and block the interaction of other proteins with the pocket (10,144).

A second study, by a different group, confirmed that that Rb could inhibit rDNA transcription and that this was due to an interaction between Rb and UBF (177). They also found that Rb inhibited UBF binding to the rDNA promoter, but did not affect the ability of UBF to interact with SL-1 or RNA polymerase I (177). Interestingly, in these experiments Rb209 was just as affective as Rb, and the authors concluded that the CO_2H-terminal domain of Rb, and not the A/B pocket, was required for the interaction between Rb and UBF (177). This finding contradicts the results of the initial study (10), and the observation that Rb209 (as found in H209 cells) cannot be coimmunoprecipitated with UBF (K. Hannan, L. Jefferson, and L. Rothblum, manuscript in preparation). These discrepancies suggest that further experiments are required in order to determine the exact mechanism involved in Rb^{110} regulation of rDNA transcription.

5.5. Other Factors:

5.5.1. Ku/E_1BF and CPBF

In some reports Ku/E_1BF markedly inhibited rDNA transcription (146,178,179), while in others it stimulated transcription (145,148). The first finding corroborates *in vivo* studies which demonstrated that the expression of Ku/E_1BF correlates negatively with the proliferation state of the cell (3,180). However, the question remains as to how Ku/E_1BF acts as both a positive and negative regulator of rDNA transcription. It has been suggested that low concentrations of Ku/E_1BF have a positive effect on rDNA transcription whereas higher concentrations repress transcription (178,179,181).

Anti-Ku antibodies can precipitate a repressor activity from HeLa cells, and stimulate rDNA transcription. The addition of UBF can also overcome Ku/E_1BF repression (147), thus suggesting that one mechanism by which UBF may enhance rDNA transcription is by releasing Ku/E_1BF repression rather than by directly stimulating transcription (168). Since Ku/E_1BF interacts with the UBF and SL-1 binding sites on the rDNA promoter it may compete with them for their DNA-binding sites on the rDNA promoter (147). In this model, a high concentration of Ku/E_1BF would titrate the UBF and/or SL-1 binding sites and reduce rDNA transcription. One other study, which examined Ku/E_1BF in serum-starved rat NISI cells, suggested that there are probably two forms of Ku/E_1BF; one which enhances (Ku/E_1BF_c*) and one which inhibits transcription (Ku/E_1BF_s: isolated from serum starved cells) (179). The difference between these two forms is unclear and may involve alternative splicing or a post translational modification of Ku/E_1BF (179). It is likely that, in this case, the effects of Ku/E_1BF on rDNA transcription reflect the ratio of active (Ku/E_1BF_c*) to repressive (Ku/E_1BF_s) forms in the cell. Ghoshal and Jacob have reported that heat shock (42^{o}C, 3h) repressed rDNA transcription and leads to a reduction (90%) in E_1BF, demonstrating a correlation between the regulation of E_1BF and rDNA transcription (182).

Interestingly, when Ku/E_1BF is complexed with DNA-dependent PK the complex represses rDNA transcription to a greater extent than Ku/E_1BF alone (150,151). It is possible that the enhanced repression may be due to DNA-dependent PK phosphorylation of certain components of the RNA polymerase I transcription complex.

Cell extracts lacking CPBF are rDNA transcriptionally inactive and the subsequent addition of CPBF restores transcription in an dose dependent fashion (3). Interestingly, when the human homologue of CPBF, USF1 is overexpressed as a homodimer in CHO cells it represses rDNA transcription. However, when USF1 forms heterodimers with USF2, rDNA transcription is stimulated. It is possible that the form of dimer may affect the ability of USF to bind the E boxes of the rDNA promoter and thus alter transcription (3,153).

5.5.2. Topoisomerases

The effect of topoisomerase on rDNA transcription have been examined in a number of systems. Treatment of HeLa cells with topoisomerase I-specific inhibitors, such as camptothecin, rapidly inhibits 45S rRNA synthesis which is reversible with drug removal (183). Interestingly, topoisomerase I coprecipitates with TBP (184), a subunit of the transcription factor SL-1, and has been reported to copurify with RNA polymerase I (185,186). These results suggest that it may be a component of an RNA polymerase I holoenzyme involved in the formation of the pre-initiation complex and thus rDNA transcription. While that model would suggest that topoisomerase I plays a positive role in rDNA transcription, there is additional evidence that it may negatively regulate transcription. Topoisomerase II has been found to bind to the CPE of the rDNA promoter and inhibit transcription by preventing pre-initiation complex formation (158). This process is counteracted by UBF. UBF may be competing with topoisomerase for the same DNA binding sites, thus if UBF is bound to the promoter Topo II is unable to repress transcription (158). The inhibition of topoisomerase I was also shown to generate a graded decrease (5' to 3') in the number of RNA polymerase I molecules associated with

the transcription unit. This has been interpreted as evidence that the inhibition of topoisomerase I results in the inhibit elongation (183). In addition, mutagenesis studies have demonstrated that both topoisomerase I and II are important for rRNA synthesis in *S. cerivisiae* (156).

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