Journal of Cellular Biochemistry Supplements 32/33:41–50 (1999)

Control of RNA Polymerase I-Directed Transcription: Recent Trends

Samson T. Jacob[1]* **and Asish K. Ghosh**[2]

[1]Department of Molecular and Cellular Biochemistry, Ohio State University College of Medicine, Columbus, Ohio 43210
[2]Section of Rheumatology, Department of Medicine, University of Illinois, Chicago, Illinois 60607

Eukaryotic ribosomal RNA gene (rRNA gene) is transcribed by RNA polymerase I (Pol I) in the nucleolus as a large precursor RNA (40S–47S). The rRNA precursor is then processed to mature rRNA species (28S, 18S, and 5.8S) by a series of specific endonucleolytic reactions that are mediated by U3 RNA, a small nucleolar RNA species [Sharma, and Tollervey, 1999]. The highly reiterated rRNA gene is arranged in a tandem array in clusters of head-to-tail repeats that can be visualized in electron micrographs as structures resembling Christmas trees. This unique structure representing the first reported eukaryotic transcriptional event seen under a microscope reveals the rRNA gene, functional RNA polymerase I molecules and growing RNA chains. rRNA accounts for as much as 80% of the total steady-state RNA molecules in a cell and its transcription can be either upregulated or downregulated dramatically in response to a variety of physiological stimuli and pathological conditions [for review, see Jacob, 1995; Paule, 1998; and Grummt, 1999]. A summary of the different *cis*-acting elements and the *trans*-acting factors involved in rDNA transcription has been presented elsewhere [Jacob, 1995]. The major *cis*-acting elements are composed of core and spacer promoters, enhancers, and terminators.

RNA polymerase I (Pol I) is a complex protein consisting of as much as 11 subunits in the mouse [Song et al., 1994] and 14 subunits in yeast [Carles and Riva, 1998]. The functional Pol I, designated Pol IB, also contains an associated factor called PAF 53. In adddition to Pol I, the rDNA transcription factors required for the basal expression include upstream binding factor (UBF) and selectivity factor (SL1), also called TIF-IB. SL1 is a complex consisting of TATA box-binding protein (TBP) and TBP-associated Pol I-specific factors (TAF_1). Human and mouse cells consist of three TAF1s ranging in size from 48 kDa to 95–110 kDa. The Pol I-associated factor PAF 53 interacts with UBF in vitro, is involved in specific initiation of Pol I transcription , and is concentrated in the nucleolus of growing cells [Hanada et al., 1996].

A unique characteristic of the Pol I transcription machinery is its species specificity. Although the transcription factors among the closely related species are compatible, the requirement of homologous species specific factor(s) is very rigorous with the rDNA promoter from the unrelated species. For example, rodent extracts can not transcribe insect rDNA whereas human and mouse extracts can transcribe the homologous promoters. Although fractions containing species-specific factor(s) have been described, the species-specific factor itself has not been identified. In vitro experiments suggest the potential role of the largest TAF_1 in the species-specificity of the mouse-human systems. The physiological relevance of this observation has not, however, been established. Further, the nucleotide sequences in the core promoter regions as well as the distance between the conserved regions that span from −45 to +20 bp in the animal cells appear to play a key role in the species-specific transcription.

Several recent review articles and a book [Jacob, 1995; Moss and Stefanovsky, 1995; Paule, 1998; Grummt, 1999] have discussed the *cis*-acting elements and *trans*activating factors for Pol I transcription. Therefore, the focus of

Grant sponsor: National Institutes of Health.

*Correspondence to: Samson T. Jacob, Department of Molecular and Cellular Biochemistry, Ohio State University College of Medicine, 333 Hamilton Hall, 1645 Neil Ave., Columbus, OH 43210

Received 6 October 1999; Accepted 7 October 1999

this review is Pol I holoenzyme, transcription of rRNA gene from the chromatin, roles of SV40 T antigen-associated protein kinase, DNA methylation, and repressors in Pol I-mediated transcription.

RNA POLYMERASE I HOLOENZYME

Earlier preparations of RNA polymerase I purified from several sources, particularly from the animal cells, exhibited no more than 6 subunits. The most highly purified RNA polymerase I preparations obtained in recent years consisted of as much as 11–14 subunits, depending on the source. Even this multisubunit enzyme did not contain all the initiation-specific factors that are essential for efficient and accurate transcription from the correct site. Clearly, the core enzyme needed additional factors to achieve the Pol I-specifc transcription from the +1 site. An early report from our laboratory was the first indication that all the essential initiation-specific factors for rDNA transcription could be associated with Pol I [Kurl et al., 1984]. Although this study did not use affinity columns, the Pol I-associated fraction eluted from a linear heparin-Sepharose column contained all the essential factors that could initiate transcription of rDNA. More recent studies demonstrated the existence of a Pol I holoenzyme (approximately 10% of the population) completely devoid of the core Pol I, in plant [Saez-Vasquez and Pikaard, 1997], mouse [Seither et al., 1998], and rat [Hannan, 1998] cells. This observation was consistent with similar Pol II and Pol III holoenzyme preparations reported from other laboratories. The holoenzyme was obtained by multiple-column chromatographic fractionations that in some case included an affinity chromatography on a column constructed of antibodies against one of the larger subunits of Pol I. These preparations had a molecular mass of nearly 2,000 kDa and contained as many as 30 polypeptides in the case of plant. The holoenymes from the mouse and plant cells were associated with all the basal transcription factors and could accurately initiate rDNA transcription in vitro in the absence of any added factors, whereas the rat holoenzyme required the addition of partially purified SL-I. An interesting observation was the demonstration that the casein kinase II/nuclear kinase NII (a serine/threonine protein kinase implicated in the control of cell growth and proliferation), co-purified with Pol I and phosphorylated the largest subunit of Pol I [Hannan et al., 1998]. This key observation is in agreement with an observation made in our laboratory as early as 1981, in which we demonstrated the association of both subunits of this protein kinase with a highly purified enzyme preparation from a rapidly growing rat hepatoma [Rose et al., 1981]. Although the exact large subunit phosphorylated by the associated kinase is in dispute, these two studies performed independently almost 17 years apart seem to support the contention that casein kinase II may regulate Pol I activity and consequently rDNA transcription in vivo. It is crucial, however, to investigate the physiological significance of the holo RNA polymerases. In this context, it is noteworthy that more casein kinase II/nuclear kinase II becomes associated with purifed Pol I from rapidly growing/proliferating tissues/cells than from the less proliferative cells [Rose et al., 1981]. It is conceivable that the regulatory transcription factors become associated with Pol I only when the cells need maximal rRNA synthesis and ribosome production that are required for sustaining growth .

CHROMATIN AND POL I TRANSCRIPTION

Although considerable progress has been made to elucidate the regulatory *cis*-acting elements on the Pol I promoter and the factors that modulate Pol I transcription in reconstituted transcription system, the role of chromatin structure in this transcription process has not been adequately addressed. The lack of information concerning the transcription of ribosomal RNA genes in the chromatin structure has compromised complete understanding of the mechanism by which the transcription factors direct rDNA transcription in its natural setting. The transcriptional regulation of eukaryotic genes is achieved by alterations in the structure of chromatin. Nucleosomes mask the transcriptional regulatory elements such as the promoter and enhancer from the transcriptional machinery. The binding of the Pol I transcription complex to its promoter is known to alter positioning of downstream nucleosomes assembled in vitro [Georgel et al., 1993]. Studies in the yeast system suggest that the chromatin remodeling precedes the ribosomal gene promoter activation [Lucchini and Sogo, 1995]. It appears that the assembly of a functional transcriptional initiation complex occurs after a lo-

cal disruption of chromatin over the rRNA promoter. Recently, the role of the proximal transcription termination sequence (PT) located at approximately 200 bp upstream of the transcription initiation site in the remodeling of the repressive chromatin structure has been demonstrated [Langst et al., 1997]. The transcriptional termination by Pol I is mediated by the termination sequence in association with a specific transcription termination factor (TTF) and additional protein factors such as NURF or SW1/SNF [Langst et al., 1997; Coté et al., 1994; Peterson et al., 1994]. The interaction between the transcription termination factor and its target sequences are essential for nucleosome rearrangement as well as chromatin-specific activation of transcription. Although TTF-1 and its yeast homologue, Reb 1p, bind at the same regions of rDNA, these proteins appear to have definitive roles in both initiation and termination of rRNA chains.

GENE SILENCING AT THE rDNA REPEAT

Although rDNA is transcribed efficiently, and the transcript constitutes the majority of cellular RNA (60%), nearly one-half of the 100–200 copies of the rRNA genes are inactive at any given time [Straight et al., 1999]. Gene silencing in *Saccharomyces cerevisiae* occurs at three locations that include the silent-mating loci, telomeres, and the tandomly repeated rRNA genes [Bryk et al., 1997; Fritze et al., 1997]. Although considerable advance has been made in the understanding of gene silencing at the first two chromosomal regions, much less is known about the mechanism or the biological relevance of silencing at the rDNA repeat. Four unique silencing proteins, Sir 1, Sir 2, Sir 3, and Sir 4, are involved in silencing. In addition, a 175-kDa Sir 2-associated nucleolar protein, namely Net 1, has been identified recently [Straight et al., 1999], which plays a key role in rDNA silencing and maintaining the nucleolar integrity. Another unique observation by this same group has been the association of Net 1 with the Cdc 14 phosphatase. Net 1 could also regulate the mitotic exit function of Cdc 14 [Shou et al., 1999]. Interestingly, like Net 1 and Sir 2, Cdc 14 was preferentially associated with rDNA. These studies have demonstrated that the complex containing three proteins, Cdc 14, Sir 2 and Net 1, collectively called RENT (regulator of nucleolar silencing and tetophase exit) is involved in three unrelated cellular functions, namely rDNA silencing, nucleolar integrity, and the control of exit from mitosis. It would be of considerable interest to study the probable existence of similar protein complexes that are associated with rDNA from mammalian cells and mediate silencing at the rDNA repeat.

DNA METHYLATION IN THE CONTROL OF POL I TRANSCRIPTION

DNA methylation in the cytosines of the CpG dinucleotides occurs in mammals, and plays a critical role in X chromosome inactivation, epigenetic silencing, carcinogenesis, development, and certain human genetic diseases [Baylin, 1997]. As much as 80% of the CpG sequence is known to be methylated in the mouse cells. This DNA modification can lead to alteration in transcription of the gene [Kass et al., 1997]. In vertebrate and plant cells, some correlation exists between cytosine methylation and transcriptional activity of ribosomal RNA gene. For example, hypomethylated rDNA is preferentially transcribed, whereas the inactive genes and their flanking sequences are generally hypermethylated. Recently, the nucleolar dominance in somatic cell hybrid cell lines has been attributed to silencing of the inactive ribosomal gene in the hybrid by hypermethylation [Chen and Pikaard, 1997]. Earlier studies have suggested that the absence of a species-specific transcription factor was responsible for the nucleolar dominance in mouse-human somatic cell hybrid lines. In other words, the inactivation of genes that encode human or mouse species specific factor (SL1 complex) in a mouse-human somatic cell hybrid may determine which species-specific ribosomal gene expression will dominate in the somatic cell hybrid. This hypothesis was challenged by Reeder [1985], who argued against the possibility of the species-specific factor in determining the nucleolar dominance in closely related species. Indeed, Pikaard and colleagues have directly demonstrated that the rRNA gene promoters of the closely related *Brassica* species are functional in either species in transient transfection assays [Chen and Pikaard, 1997]. Further, the ribosomal gene promoter of *Arabidopsis thaliana*, a related species, is functional in vitro in a *B. oleracea* (broccoli) cell extract, which suggests utilization of the same factors for Pol I transcription in similar species. Subsequent studies by this group demonstrated that de-

methylation of DNA by 5-Aza-deoxycytidine, an inhibitor of DNA methyltransferase reactivated the silent ribosomal gene in *Brassica* as well as *Arabidopsis* polyploids.

Because many fully methylated genes can be transcribed at nearly normal rates in the absence of methyl-CpG binding proteins [Nan et al., 1997], it is unlikely that the CpG methylation by itself renders these sites inaccessible to the basal transcriptional machinery or prevent interaction of the transcription factors with the promoters. It is important to elucidate the molecular mechanisms by which DNA methylation brings about repression of Pol I transcription. Recent studies by Bird and colleagues identified two major repressor proteins, MeCP1 and MeCP2, that bind specifically to methyl-CpG without apparent sequence specificity [Nan et al., 1997]. MeCP2 is an abundant nuclear protein that is essential for mouse embryogenesis. This is the only MeCP that has been well characterized with respect to its function and other properties. It contains a transcription repression domain (TBD) and binds tightly to the gene in a methylation-dependent manner. TBD interacts with a corepressor complex containing the transcriptional repressor Sin3A and histone deacetylase (HDAC) [Nan et al., 1998]. The restoration of transcription from the methylated DNA by trichostatin A, a potent inhibitor of HDAC [Yoshida et al., 1995], indicates the role of histone deacetylation, DNA methylation and MeCP2 in the suppression of gene expression. Further, methylated DNA assembled into chromatin binds the transcriptional repressor MeCP2 which cofractionates with Sin 3A and histone deacetylase [Jones et al., 1998]. Unlike MeCP2, MeCP1 is less abundant, loosely bound to the methylated sequences and requires multiple methyl-CpGs for tight binding to DNA. MeCP1 is a large protein complex (400–800 kDa), and its activity is not altered by trichostatin A, which suggests a mechanism of action distinct from that of MeCP2. It is not known whether any of these MeCPs or other related repressors play a role in the suppression of Pol I transcription, and whether any one of these MeCPs is localized in the nucleolus. Interestingly, maize histone deacetylase HD2, an acidic phosphoprotein, is localized in the nucleolus, which may have a role in the regulation of ribosomal chromatin structure and function [Lusser et al., 1997]. Pikaard and colleagues treated *Brassica* and *Arabidopsis* tetraploids with sodium butyrate that is known to inhibit histone deacetylase activity and enhance acetylation of histones [Chen and Pikaard, 1997]. This treatment also resulted in reactivation of the silent ribosomal RNA genes in maize [Lusser et al., 1997]. These studies have suggested that DNA methylation in concert with histone deacetylation plays a key role in the suppression of ribosomal RNA gene that is responsible for nucleolar dominance in closely related species. It is not evident from these data whether cytosine methylation of ribosomal RNA genes is the cause or effect of nucleolar dominance. Clearly, genetic experiments using strains defective in DNA methylation and additional biochemical studies will be required to address this issue.

REPRESSORS OF POL I TRANSCRIPTION

Although considerable effort has been expended to purify and characterize several factors that *trans*activate rRNA gene promoter, the probable existence of the repressors of Pol I transcription has received much less attention. Three proteins that appear to function as repressors have been described. Two of these proteins represent the tumor suppressor gene products, namely Rb and p53, and the third is the DNA-activated protein kinase, that is associated with Ku in a large complex. In addition, the transcription factor CPBF/USF could function as a repressor in vivo in its homodimeric form.

Ku or Ku-associated protein(s)

The autoantigen Ku is a heterodimeric DNA-binding protein that plays an important role in several cellular processes in eukaryotes [for a review, see Ghosh, 1997]. We have shown that anti-Ku antibodies raised against a synthetic peptide corresponding to a small segment of Ku protein inhibited Pol I transcription from rat or mouse rRNA promoter, and that addition of exogenous Ku restored the transcriptional activity [Hoff et al., 1994]. Because anti-Ku antibodies were highly specific for Ku, and exogenous Ku was essentially a homogeneous preparation, it is highly unlikely that the effect of Ku was mediated by a contaminating protein. At higher protein-to-DNA ratios, Ku suppressed transcription in both rat [Hoff et al., 1994] and mouse [Kuhn et al., 1993] systems, which was probably caused by a nonspecific effect of Ku. Although Ku exerted a positive effect under normal physiological conditions, it was associ-

ated with a repressor during growth restriction by serum depletion and inhibited transcription from rRNA promoter in vitro [Niu and Jacob, 1994]. The addition of purified Ku from the control cells relieved the repression caused by Ku-associated inhibitor found under growth-deprived conditions. Ku may be susceptible to post-translational modification during serum deprivation and the modified Ku functioned as a repressor. Alternatively, post-translational modification of Ku may result in enhanced affinity of this protein for a repressor and that Ku-repressor complex functions as an active repressor. Our study (unpublished data) has ruled out the possibility of modification of Ku by phosphorylation or dephosphorylation during growth restriction. The general characteristics of Ku from the control cells and growth-restricted cells were identical. The precise nature of the Ku-associated repressor or the molecular mechanism of the Ku-mediated repression of Pol I transcription after serum deprivation has not been completely elucidated.

If Ku itself functions as a repressor, overexpression of Ku in cells should result in inhibition of Pol I transcription. On the contrary, Rat 1 cells that overexpress Ku heterodimer exhibit higher level of rRNA synthesis in vivo, and disruption of individual subunits of Ku do not lead to augmented rRNA synthesis (K. Ghoshal and S. Jacob, unpublished data). Interestingly, the Ku-overexpressing cells are protected from the cellular damage caused by heavy metals or by oxidative stress (K. Ghoshal and S. Jacob, unpublished data). This observation is of interest, as agents that cause cellular damage usually inhibit rRNA gene transcription. None of these studies that use a molecular genetics approach supports the notion that Ku by itself can function as Pol I transcriptional repressor. Indeed, Grummt and colleagues now report that DNA-activated protein kinase (DNA-PK) is a much more potent repressor of Pol I transcription than Ku itself [Kuhn et al., 1995]. DNA-PK is composed of a large catalytic subunit with an approximate molecular mass of 460 kDa (DNA-PK_{cs}) and Ku, the DNA-binding component. Recruitment of DNA-PK_{cs} by Ku results in activation of the kinase. Their investigation was an extension of the study by Labhart [1995], who showed that DNA-PK can specifically repress initiation of transcription by Pol I. Both reports demonstrated that DNA-PK inhibits the initiation rather than elongation of transcription. One of the functions of Ku is to recruit DNA-PK to DNA [Dvir et al., 1993; Gottlieb and Jackson, 1993]. Grummt's group hypothesizes that after recruitment to rRNA gene, DNA-PK phosphorylates a specific component of the transcription complex. The identity of this transcriptional factor is, however, not known. Although DNA-PK_{cs} can phosphorylate several peptides in vitro, the in vivo targets of this unique protein kinase are largely unknown. It has not been established whether DNA-PK can repress Pol I transcription in vivo. Mutant cells that are defective in DNA-PK are available to test directly the role of this enzyme in the suppression of rRNA gene transcription. Unfortunately, a molecular genetics approach using these cells has not been undertaken. In the absence of these studies, the physiological relevance of the repressor effect of DNA-PK on Pol I transcription is rather conjectural.

CPBF/USF

The Pol I transcription factor (core promoter binding factor (CPBF) characterized in our laboratory [Liu and Jacob, 1994] was later shown to be related to the Pol II transcription factor USF, a helix-loop-helix-zipper protein [Datta et al., 1995]. CPBF/USF, a heterodimeric protein could interact with Ku and stimulate transcription of rRNA gene in vitro. DNA transfection studies showed that the USF heterodimer could stimulate rDNA transcription in vivo as well. By contrast, USF homodimers suppressed Pol I transcription in vivo [Ghosh et al., 1997]. The USF homodimer-mediated repression of rDNA transcription is consistent with the inhibition of cellular proliferation by USF homodimers [Luo and Sawadgo, 1996]. Because ribosome biogenesis is essential for cellular proliferation, the inhibition of Pol I transcription by USF homodimers may be one of the ways to regulate this process. Post-translational modification of USF is known to occur during transition of cells from resting to growing state. It would be of considerable interest to investigate whether such USF modification or the ratio of USF homodimer to heterodimer could alter rRNA synthesis under a variety of physiological, pathological and nutritional conditions. It is also important to study the mechanism by which the USF homodimers mediate the suppression of rDNA transcription.

RB, THE RETINOBLASTOMA SUSCEPTIBILITY GENE PRODUCT

Recent studies have shown that Rb, the 110 kDa protein product of the retinoblastoma susceptibility gene is a repressor of Pol I transcription in vivo and in vitro [Cavanaugh et al., 1995; Voit et al., 1997]. The original study of Rothblum and colleagues stemmed from the reports that after growth arrest or differentiation of the human monocyte-like cells U-2 or U 937, Rb is translocated to the nucleoli [Rogalsky et al., 1993] , and that UBF binds to RB in a dual hybrid screen [Shan et al., 1992]. Rb has also been shown to inhibit Pol II [Weinberg, 1995] and Pol III [White, 1997] transcription. Inhibition of rRNA gene transcription is consistent with the Rb-mediated negative regulation of cellular proliferation by preventing the progression of the cells from G1 to S phase. Further study demonstrated a highly specific molecular mechanism of Pol I repression by Rb. The Rb-mediated suppression of Pol I transcription was relieved by excess of the Pol I specific transcription factor UBF, which suggests sequestration of UBF by Rb. Such a contention was supported by the observation that affinity chromatography of nuclear extracts resulted in the association of UBF with the biologically active form of Rb (GST-Rb), but not with the biologically inactive form of the tumor suppressor gene product [Huang et al., 1991]. The A/B pocket domain of RB was involved in the interaction of Rb with UBF, as E7 peptide that binds to the A/B pocket could inhibit co-immunoprecipitation of Rb with UBF [Cavannaugh et al., 1995]. Further, co-immunoprecipitation of these two proteins occurred only from the extracts of cell cycle-arrested cells, but not from the extracts of exponentially growing cells [Hannan et al., 1998]. Although Grummt and colleagues have confirmed the Rb-mediated inhibition of rRNA gene transcription, and the role of UBF in this process [Voit et al., 1997], they proposed a mechanism of repression that does not involve the A/B pocket of Rb or UBF-SL1 complex formation. Further study is required to resolve the discrepancies concerning the detailed mechanism of Rb-mediated suppression of Pol I transcription.

What is the molecular mechanism that facilitates association of Rb with UBF and their dissociation. Dephosphorylation of Rb can lead to its association with a specific transcription factor, particularly with E2F, preventing Pol II-directed transcription of E2F-responsive genes [for review, see Hollingsworth et al., 1993; Weinberg 1995]. On the contrary, phosphorylation by cyclin-dependent kinases releases Rb from the associated transcription factors such as E2F which then become available for transcription of genes needed for the entry of cells into S phase. It is conceivable that phosphorylation by these kinases could also dissociate Rb from UBF, resulting in the UBF-mediated transcription of rRNA gene as the cells recover from the growth-arrested phase. One cannot rule out the possibility that phosphorylation/dephosphorylation of UBF, a phosphoprotein, also determines its association with or dissociation from Rb, and that the protein kinases distinct from cyclin-dependent kinases may also be involved in this process. Finally, additional mechanism(s) other than sequestration of UBF by Rb may also be operative in the cells, which could result in suppression of Pol I transcription by Rb. In this context, it is noteworthy that Rb can inhibit the intrinsic kinase activity of TATA-binding protein-associated factor TAF_{II} 250 [Siegert and Robbins, 1999]. The inability of this kinase to phosphorylate itself as well as the RAP74 subunit of the Pol II transcription factor TF IIH, and possibly other unknown targets could be an alternate mechanism for the Rb-mediated suppression of Pol II transcription. Similarly, Rb could inhibit other related kinases that may be involved in the inactivation of Pol I-specific transcription factors. Further study will address these issues. Nevertheless, the original observation [Cavanaugh et al., 1995] that Rb can indeed downregulate Pol I transcription and that this is achieved by interaction between Rb and UBF remains an important means to control rRNA gene expression.

The recent demonstration from several laboratories that Rb interacts with histone deacetylase (HDAC) has provided new insight into the general mechanism of Rb-induced repression of gene expression [Luo et al., 1998; Brehm et al., 1998; Magnaghi-Jaulin et al., 1998]. These observations are consistent with the known association of several transcriptional repressors with HDACs that are presumed to promote nucleosome formation by deacetylating histones. This mechanism of Rb-mediated suppression of certain promoters is distinct from the direct interaction of Rb with specific transcription factors.

It now appears that HDAC activity is required for the efficient inhibition of E2F by Rb. The probable recruitment of HDAC by Rb to inactivate specific Pol I transcription factors under certain conditions has not been explored. In this context, it is noteworthy that Pol I holoenzyme from the frog cells copurified with histone acetyltransferase (HAT) [Albert et al., 1999]. Because Pol I holoenzyme is considered the functional Pol I, its association with HAT supports the contention that histone acetylation and chromatin activation are related events in rRNA gene transcription, whereas histone deacetylation represses transcription from certain promoters. The co-elution of the HAT with the Pol I holoenzyme is also consistent with the rDNA activation related to the nucleolar dominance in closely related species.

THE GROWTH SUPPRESSOR P53

p53, the product of another growth suppressor gene, can also inhibit rDNA transcription in vitro [Budde and Grummt, 1998]. In this study, pre-rRNA synthesis was measured in cell lines that express wild type or mutant p53. Consistent with the growth-suppressor activity, rRNA synthesis in the wild type cells was markedly inhibited as compared to that in the p53 mutant cells [Budde and Grummt, 1998]. The pre-rRNA levels were elevated in cells that express mutant p53 and in fibroblasts from p53 knockout mice. Minimal rDNA promoter was sufficient for the p53-mediated suppression of Pol I transcription. Recombinant p53 failed to repress Pol I transcription in vitro, which suggests lack of direct interaction of p53 with the basal Pol I transcriptional machinery. In this context, the molecular mechanism of action of p53 differs from that of Rb, and the p53-mediated repression of Pol I transcription is probably the result, rather than the cause, of growth arrest by the tumor/growth suppressor [Budde and Grummt, 1998]. Our study (A. Ghosh and S. Jacob, unpublished data) has confirmed the effect of wild type p53 on rRNA synthesis in vivo. Surprisingly, recombinant p53 can inhibit Pol III transcription in vitro [Chesnokov et al., 1996; Cairns and White, 1998] despite the very similar basal mechanisms of transcriptional regulation of class I and class III genes. Further study is required to elucidate completely the mechanism of Pol I transcription by p53, which should reveal the basic differences between the p53-mediated suppression of transcription by two classes of RNA polymerase. Based on the studies involving p53 and Rb, it is tempting to speculate that all tumor suppressors could inhibit rDNA transcription either by distinct interaction with the basal Pol I transcription machinery or by an indirect mechanism.

SV40 LARGE T ANTIGEN

In contrast with the inhibition of rRNA gene transcription by some viruses (e.g., adenovirus 2, poxvirus, and poliovirus), infection of cells with SV40 or polyomavirus, resulted in marked stimulation of Pol I transcription. Earlier studies suggested the probable role of SV40 large T antigen in the activation of rRNA gene transcription after SV40 virus infection [Learned et al., 1983; Soprano et al., 1981].

The ability of large T antigen to inactivate the tumor suppressors p53 and pRb suggested the potential role of T antigen in the control of cell growth [Nevins, 1994]. This observation explains the stimulation of rRNA synthesis in vivo and Pol I transcription in vitro by this protein. Recently, Comai and colleagues [Zhai et al., 1997] have demonstrated the T antigen-mediated rRNA promoter activation in vivo and Pol I transcription in vitro using a reconstituted system. Further, these investigators provided evidence for the interaction of T antigen with TBP as well as the Pol I specific TBP-associated factors $TAF_1$48 and $TAF_1$110. The segment of T antigen that binds to these proteins is also essential for the augmentation of Pol I transcription. More recent study [Zhai and Comai, 1999] from the same investigators has shown that (1) expression of large T antigen results in an increase in UBF phosphorylation; (2) this UBF modification in the C-terminal activation domain is mediated by the T antigen-associated protein kinase, and (3) UBF phosphorylation promotes the production of a stable UBF-SL1 complex. This study has established an important link among UBF phosphorylation by a defined protein kinase, formation of stable initiation complex and SV40-mediated stimulation of rRNA synthesis. It is logical to assume that the upregulation of Pol I transcription after infection with polyomavirus is also achieved by a similar mechanism.

CONCLUDING REMARKS

During the past few years, enormous progress has been made in the characterization of pro-

tein factors that are involved in the basal and regulated expression of ribosomal RNA gene. Recently, there has been considerable progress in the identification and characterization of a few repressors of rDNA transcription. The challenge in the coming years is to explore further the mechanisms of action of these key factors that participate in the downregulation of this transcriptional process. In this context, the discovery that the growth suppressors Rb and p53 inhibit rDNA transcription is significant. It should, however, be emphasized that Rb inhibits only the UBF-dependent transcription, and not the UBF-independent basal level of transcription. Further, p53 does not appear to interact directly with the basal transcriptional machinery. It would be of considerable interest to identify the repressors that block the basal level of Pol I transcription. Although two laboratories have shown that DNA-PK inhibits Pol I transcription in vitro, the physiological relevance of this observation has not been established.

Considerable progress has been made in the elucidation of the mechanisms by which the termination factor (TTF-I/Reb-1p) mediates the termination of rRNA chain. An interesting aspect of these studies is the discovery that TTF-1 plays a key role in the chromatin reorganization around the rRNA gene promoter. It is important to pursue this lead and explore its potential role in the structural organization of rDNA at different levels and its other probable novel functions.

A major advance in recent years has been the complete characterization of the RNA polymerase I subunits and their genes in yeast. No doubt this has been a phenomenal undertaking that has paved way to explore the regulation of the synthesis of these subunits under different physiological conditions, their assembly mechanisms and their role in defining nucleolar structure. It is critical to study the probable post-translational modifications of some of these subunits and their effects on rDNA transcription. In this context, the close association of casein kinase/nuclear protein kinase II with RNA polymerase I is of utmost significance.

A question that one should pose in the light of recent development is the potential role of nucleolar structure itself in the processes leading to ribosome production. In addition to rDNA transcription and rRNA processing, the nucleolus participates in the formation of ribosomal subunits by association with the ribosomal proteins produced in the cytoplasm and transported to the nucleolus along with other factor(s) that facilitate this process. Clearly, additional biochemical and genetic studies are required to understand the relationship between the nucleolar structure and the myriad biochemical processes in the ribosome production, and consequently protein synthesis. The powerful genetic analysis that can be provided by the yeast system would be an invaluable tool to achieve this goal. Finally, the role of chromatin in rDNA transcription remains to be analyzed in detail. It is important to investigate how the different factors involved in the initiation and termination participate in rDNA transcription in the context of chromatin. This is the only way one can fully appreciate the physiological roles of the factors that have been well characterized biochemically.

ACKNOWLEDGMENTS

We regret being unable to cite all the relevant references due to constraint in the allotted space. Further, we have focused on a few areas that have received considerable attention in recent years. Because some of these topics have aroused some degree of controversy, the authors have tried to provide their own critical analysis of the published reports. Work done in the authors' laboratory was supported by grants from the National Institutes of Health (S.T.J.). We thank Cheryl Pulsinelli for assistance in the preparation of this article.

REFERENCES

Albert AC, Denton M, Kermekchiev M, Pikaard C. 1999. Histone acetyltransferase and protein kinase activities copurify with a putative *Xenopus* RNA polymerase I holoenzyme self-sufficient for promoter-dependent transcription. Mol Cell Biol 19:796–806.

Baylin SB. 1997. Tying it all together: epigenetics, genetics, cell cycle, and cancer. Science 277:1948–1949.

Brehm A, Miska EA, McCance DJ, Reid JL, Bannister AJ, Kouzarides T. 1998. Retinoblastoma protein recruits histone deacetylase to repress transcription. Nature 391: 597–601.

Bryk M, Banerjee M, Murphy M, Knudsen KE, Garfinkel DJ, Curcio MJ. 1997. Transcriptional silencing of Ty1 elements in the RDN1 locus of yeast. Genes Dev 11:255–269.

Budde A, Grummt I. 1998. p53 represses ribosomal gene transcription. Oncogene 19:1119–1124.

Cairns CA, White RJ. 1998. p53 is a general repressor of RNA polymerase III transcription. EMBO J 17:3112–3123.

Carles C, Riva M. 1998. Yeast RNA polymerase I subunits and genes. In: Paule MR, editor. Transcription of ribosomal RNA genes by eukaryotic RNA polymerase I. New York: Springer-Verlag/RG Landes. p 9–38.

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

Chen ZJ, Pikaard CS. 1997. Transcriptional analysis of nucleolar dominance in polyploid plants: biased expression/silencing of progenitor rRNA genes is developmentally regulated in *Brassica*. Proc Natl Acad Sci USA 94:3442–3447.

Chesnokov I, Chu W-M, Botchar MR, Schmid CW. 1996. p53 inhibits RNA polymerase III-directed transcription in a promoter-dependent manner. Mol Cell Biol 16:7084–7088.

Coté J, Quinn J, Workman JL, Peterson CL. 1994. Stimulation of GAL_4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. Science 265:53–60.

Datta PK, Ghosh AK, Jacob ST. 1995. The RNA polymerase I promoter-activating factor CPBF is functionally and immunologically related to the basic helix-loop-helix-zipper DNA-binding protein USF. J Biol Chem 270:8637–8641.

Dvir A, Stein LY, Calore BL, Dynan WS. 1993. Purification and characterization of a template-associated protein kinase that phosphorylates RNA polymerase II. J Biol Chem 268:10440–10447.

Fritze CE, Verschueren K, Strich R, Easton Esposito R. 1997. Direct evidence for SIR2 modulation of chromatin structure in yeast rDNA. EMBO J 16:6495–6509.

Georgel P, Demeler B, Terpening CM, Paule MR, Van Holde KE. 1993. Binding of the RNA polymerase I transcription complex to its promoter can modify positioning of downstream nucleosomes assembled in vitro. J Biol Chem 268:1947–1954.

Ghosh AK. 1997. Autoantigen Ku and its role in multiple cellular processes. Indian J Exp Biol 35:1261–1272.

Ghosh AK, Datta PK, Jacob ST. 1997. The dual role of helix-loop-helix-zipper protein USF in ribosomal RNA gene transcription in vivo. Oncogene 14:589–594.

Gottlieb TM, Jackson SP. 1993. The DNA-dependent protein kinase: requirement for DNA ends and association with KU antigen. Cell 72:131–142.

Grummt I. 1999. Regulation of mammalian ribosomal gene transcription by RNA polymerase I. Prog Nucleic Acids Res Mol Biol 62:109–154.

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

Hannan RD, Hempel WM, Cavanaugh A, Arino T, Dimitrov SI, Moss T, Rothblum L. 1998. Affinity purification of mammalian RNA polymerase I. J Biol Chem 273:1257–1267.

Hoff CM, Ghosh AK, Prabhakar BS, Jacob ST. 1994. Enhancer 1 binding factor, a Ku-related protein, is a positive regulator of RNA polymerase I transcription initiation. Proc Natl Acad Sci USA 91:762–766.

Hollingsworth RE Jr, Hensey CR, Lee W-H. 1993. Retinoblastoma protein and the cell cycle. Curr Opin Gen Dev 3:55–62.

Huang S, Lee W-H, Lee E Y-H-P. 1991. A cellular protein that competes with SV40 T antigen for binding to the retinoblastoma gene product. Nature 350:160–162.

Jacob ST. 1995. Regulation of ribosomal gene transcription. Biochem J 306:617–626.

Jones PL, Veenstra GJ, Wade PA, Vermaak D, Kass SU, Landsberger N, Strouboulis J, Wolffe AP. 1998. Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. Nature Genet 19:187–191.

Kass SU, Pruss D, Wolffe AP. 1997. How does DNA methylation repress transcription? Trends Genet 13:444–449.

Kuhn A, Stefanovsky V, Grummt I. 1993. The nucleolar transcription activator UBF relieves Ku antigen-mediated repression of the mouse ribosomal gene transcription. Nucleic Acids Res 21:2057–2063.

Kuhn A, Gottlieb TM, Jackson SP, Grummt I. 1995. DNA-dependent protein kinase: a potent inhibitor of transcription by RNA polymerase I. Genes Dev 9:193–203.

Kurl RN, Rothblum LI, Jacob ST. 1984. A purified fraction containing RNA polymerase I that can accurately transcribe rat ribosomal RNA gene. Proc Natl Acad Sci USA 81:6672–6675.

Labhart P. 1995. DNA-dependent protein kinase specifically represses promoter-directed transcription initiation by RNA polymerase I. Proc Natl Acad Sci USA 92:2934–2938.

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

Learned RM, Smale ST, Haltiner MM, Tjian R. 1983. Regulation of human ribosomal RNA transcription. Proc Natl Acad Sci USA 80:3558–3562.

Liu Z, Jacob ST. 1994. Characterization of a protein that interacts with the rat ribosomal gene promoter and modulates RNA polymerase I transcription. J Biol Chem 269: 16618–16626.

Lucchini R, Sogo JM. 1995. Replication of transcriptionally active chromatin. Nature 374:276–280.

Luo R, Postigo A, Dean D. 1998. Rb Interacts with histone deacetylase to repress transcription. 92:463–473.

Luo X, Sawadogo M. 1996. Antiproliferative properties of the USF family of helix-loop-helix transcription factors. Proc Natl Acad Sci USA 93:1308–1313.

Lusser A, Brosch G, Loidl A, Haas H, Loidl P. 1997. Identification of maize histone deacetylase HD2 as an acidic nucleolar phosphoprotein. Science 277:88–91.

Magnaghi-Jaulin L, Groisman R, Naguibneva I, Robin P, Lorain S, Le Villain JP, Troalen F, Trouche D, Harel-Bellan A. 1998. Retinoblastoma protein represses transcription by recruiting a histone deacetylase. Nature 391:601–604.

Moss T, Stefanovsky VY. 1995. Promotion and regulation of ribosomal transcription in eukaryotes by RNA polymerase I. In: Cohn WE, Moldave K, editors. Progress in nucleic acids and molecular biology. San Diego: Academic Press. p 25–66.

Nan X, Campoy FJ, Bird A. 1997. MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. Cell 88:471–481.

Nan X, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN, Bird A. 1998. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. Nature 393:386–389.

Nevins JR. 1994. Cell cycle targets of the DNA tumor viruses. Curr Opin Genet Dev 4:130–134.

Niu H, Jacob ST. 1994. Enhancer 1 binding factor (E1BF), a Ku-related protein, is a growth-regulated RNA polymerase I transcription factor: association of a repressor activity with purified E1BF from serum-deprived cells. Proc Natl Acad Sci USA 91:9101–9105.

Paule MR. 1998. Transcription of ribosomal RNA genes by eukaryotic RNA polymerase I. New York: Springer-Verlag/RG Landes.

Peterson CL, Dingwall A, Scott MP. 1994. Five SWI/SNF gene products are components of a large multisubunit complex required for transcriptional enhancement. Proc Natl Acad Sci USA 91:2905–2908.

Reeder RH. 1985. Mechanisms of nucleolar dominance in animals and plants. J Cell Biol 101:2013–2016.

Rogalsky VM, Todorov G, Moran D. 1993. Translocation of retinoblastoma protein associated with tumor growth inhibitors. Biochem Biophys Res Commun 192:1139–1146.

Rose KM, Stetler DA, Jacob ST. 1981. Protein kinase activity of RNA polymerase I purified from a rat hepatoma: probable function of M_r 42,000 and 24,600 polypeptides. Proc Natl Acad Sci USA 78:2833–2837.

Saez-Vasquez J, Pikaard CS. 1997. Extensive purification of a putative RNA polymerase I holoenzyme from plants that accurately initiates rRNA gene transcription in vitro. Proc Natl Acad Sci USA 94:11869–11874.

Seither P, Iben S, Grummt I. 1998. Mammalian RNA polymerase I exists as a holoenzyme with associated basal transcription factors. J Mol Biol 275:43–53.

Shan B, Zhu X, Chen PL, Durfee T, Yang Y, Sharp D, Lee WH. 1992. Molecular cloning of cellular genes encoding retinoblastoma-associated proteins: identification of a gene with properties of the transcription factor E2F. Mol Cell Biol 12:5620–5631.

Sharma K, Tollervey D. 1999. Base pairing between U3 small nucleolar RNA and the 5′ end of 18S rRNA is required for pre-rRNA processing. Mol Cell Biol 19:6012–6019.

Shou W, Seol JH, Shevchenko A, Baskerville C, Moazed D, Chen ZWS, Jang J,

Shevchenko A, Charbonneau H, Deshaies RJ. 1999. Exit from mitosis triggered by Tem1-dependent release of the protein phosphatase Cdc14 from nucleolar RENT complex. Cell 97:233–244.

Siegert JL, Robbins PD. 1999. Rb Inhibits the intrinsic kinase activity of TATA-binding protein-associated factor $TAF_{II}250$. Mol Cell Biol 19:846–854.

Song CZ, Hanada K, Yano K, Maeda Y, Yamamoto K, Muramatsu M. 1994. High conservation of subunit composition of RNA polymerase I(A) between yeast and mouse and the molecular cloning of mouse RNA polymerase I 40-kDa subunit RPA40. J Biol Chem 269:26976–26981.

Soprano K, Jonak G, Galanti N, Flores J, Baserga R. 1981. Identification of an SV40 DNA sequence related to the reactivation of silent rRNA genes in human greater than mouse hybrid cells. Virology 109:127–136.

Straight AF, Shou W, Dowd GJ, Turck CW, Deshaies RJ, Johnson AD, Moazed D. 1999. Net1, a Sir2-associated nucleolar protein required for rDNA Silencing and nucleolar integrity. Cell 97:245–256.

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

Weinberg RA. 1995. Purification and characterization of a novel factor which stimulates rat ribosomal gene transcription in vitro by interacting with enhancer and core promoter elements. Cell 81:323–330.

White RJ. 1997. Regulation of RNA polymerases I and III by the retinoblastoma protein: a mechanism for growth control? Trends Biochem Sci 22:77–80.

Yoshida M, Horinouchi S, Beppu T. 1995. Trichostatin A and trapoxin: novel chemical probes for the role of histone acetylation in chromatin structure and function. BioEssays 17:423–430.

Zhai W, Tuan J, Comai L. 1997. SV40 large T antigen binds to the TBP-TAF(I) complex SL1 and coactivates ribosomal RNA transcription. Genes Dev 11:1605–1617.

Zhai W, Comai L. 1999. A kinase activity associated with simian virus 40 T antigen phosphorylates upstream binding factor (UBF) and promotes formation of a stable initiation complex between UBF and SL1. Mol Cell Biol 19:2791–2802.