The RNA Polymerase I Transcription Machinery: An Emerging Target for the Treatment of Cancer

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rRNA genes, transcription, nucleolus, signaling, drugs, apoptosis

Abstract

The RNA polymerase I (Pol I) transcription machinery in the nucleolus is the key convergence point that collects and integrates a vast array of information from cellular signaling cascades to regulate ribosome production that in turn guides cell growth and proliferation. Cancer cells commonly harbor mutations that inactivate tumor suppressors, hyperactivate oncogenes, and upregulate protein kinases, all of which promote Pol I transcription and drive cell proliferation. The intimate balance between Pol I transcription and growth-factor signaling is perturbed in cancer cells, indicating that upregulation of rRNA synthesis is mandatory for all tumors. Though the emerging picture of transcriptional regulation reveals an unexpected level of complexity, we are beginning to understand the multiple links between rRNA biogenesis and cancer. In this review, we discuss experimental data and potential strategies to downregulate rRNA synthesis and induce an antiproliferative response in cancer cells.

INTRODUCTION

Nucleolus: a nuclear organelle composed of protein and nucleic acids where rRNA genes are located and transcribed

Ribosomal RNA (rRNA): rRNA the central component of the ribosome, is required for decoding mRNA into amino acids and for interaction with transfer RNAs (tRNAs) during translation by providing peptidyl transferase activity

RNA polymerase I (Pol I): one of three classes of eukaryotic nuclear DNA-dependent RNA polymerases that is located in the nucleolus and is responsible for the synthesis of pre-rRNA

Transcription factor: a protein that binds to specific DNA sequences and controls gene expression by promoting or blocking the recruitment of RNA polymerases to regulatory gene sequences

The biogenesis of ribosomes is a highly coordinated multistep process that largely takes place in the nucleolus, where ribosomal RNA (rRNA) is synthesized, processed, modified, and assembled into ribosomal subunits. Because ribosome biogenesis consumes a tremendous amount of cellular energy, the process is tightly linked to cell growth and proliferation. The rate of protein synthesis strongly correlates with the cellular content of rRNA and transfer RNA (tRNA), and therefore cells need to sustain high rates of RNA Pol I and Pol III transcription to produce 1–2 million ribosomes per cell generation. The synthesis of rRNA, the first event in ribosome synthesis, is efficiently regulated, responding to a variety of external signals such as nutrient availability, growth factors, and cellular stress. Indeed, transcription of rRNA genes and maturation of rRNA play a central role in the complex network that controls cell growth and proliferation. The current model for transcriptional regulation of ribosomal DNA (rDNA), which is composed of hundreds of copies of rRNA genes, proposes two overlapping mechanisms. For short-term regulation, the transcription rate at euchromatic active rDNA is altered by reversible modification of Pol I transcription factors that affect the efficiency of transcription initiation and/or the rate of transcription from active rRNA genes, whereas long-term regulation during development and differentiation is mediated by epigenetic mechanisms. In the latter case, specific chromatin modifications alter the ratio of active to silent copies of rRNA genes, thereby adjusting the number of genes that are involved in transcription. Both types of regulation have been reviewed in the past, and readers are referred to recent reviews for further information (1–4).

Recent evidence suggests that quantitative and qualitative changes in rRNA synthesis may be among the most important molecular alterations in cancer cells (5). These findings open a new, and largely unexplored, field of research aimed at clarifying the relationship between rDNA transcription and cell proliferation (reviewed in 6–9). Understanding this intimate link is required for the development of new strategies for the molecular characterization and subsequent therapy of neoplastic diseases. Moreover, comprehensive analysis of the connection between Pol I transcription, cell proliferation, and growth-factor signaling pathways will not only expand our understanding of the molecular mechanisms that control rRNA synthesis, but will also drive the design and development of novel drugs to combat cancer through targeted downregulation of Pol I transcription.

Basal Components of the RNA Pol I Transcription Machinery

Mammalian cells contain several hundred copies of tandemly repeated rRNA genes per haploid genome, and these genes are transcribed in the nucleolus with high efficiency to meet the cell's metabolic activity and demand for ribosomes. Mammalian rDNA clusters are characterized by multiple alternating modules of a long intergenic spacer of approximately 30 kb and a precursor rRNA (pre-rRNA) coding region of approximately 14 kb. Each active rRNA gene is transcribed by a specialized nucleolar DNA-dependent RNA polymerase, Pol I, to generate a 47S pre-rRNA. After synthesis, this primary transcript is subsequently processed and modified to generate one molecule each of mature 18S, 5.8S, and 28S rRNA, which together with 5S rRNA (which is transcribed by Pol III) forms the RNA backbone of the ribosome.

Initiation of rDNA transcription requires assembly of a specific multiprotein complex at the rDNA promoter containing Pol I and a surprising number of auxiliary proteins (**Figure 1**). In mammals, the synergistic action of two Pol I–specific factors that bind to the rDNA promoter, the upstream binding factor (UBF) (10), and the promoter selectivity factor, termed SL1 in humans and TIF-IB in mice (1, 11–15), leads to the assembly of the preinitiation complex. UBF belongs to

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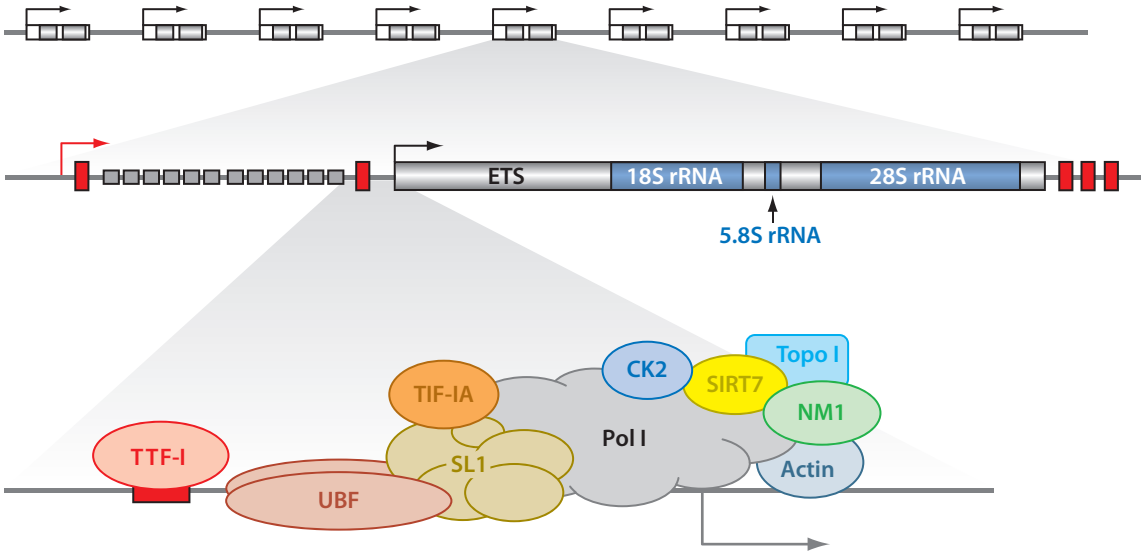

Figure 1

The structural organization of mammalian rDNA repeats and the basal factors required for transcription initiation. The diagram shows the arrangement of tandemly repeated rRNA genes. The site of transcription initiation of 47S pre-rRNA (*black arrow*) and intergenic transcripts from the spacer promoter (*red arrow*) are indicated. Terminator elements are located downstream of the transcription unit (T_{1-10}), downstream of the spacer promoter (T_{SP}), and upstream of the gene promoter (T_o) (*red boxes*). Repetitive enhancer elements (*gray boxes*) are located between the spacer promoter and major gene promoter. The ellipsoids show the factors that are associated with the rDNA promoter and Pol I, respectively. TTF-I is associated with the upstream terminator T_o. Synergistic binding of UBF and TIF-IB/SL1 to the rDNA promoter is required for the recruitment of RNA polymerase I (Pol I)—together with multiple Pol I-associated factors—to the transcription start site to initiate pre-rRNA synthesis.

the class of sequence-nonspecific high mobility group proteins that contain high mobility group (HMG) boxes, a protein motif known to bend DNA. The HMG boxes enable UBF to loop approximately 140 base pairs (bp) of DNA into a single turn, thereby inducing a nucleosome-like structure, called enhancosome (16). UBF activates rRNA gene transcription by several means, for example, by recruiting Pol I to the rDNA promoter, by stabilizing binding of TIF-IB/SL1, and by displacing nonspecific DNA binding proteins such as histone H1 (17, 18). More recently, additional roles have been ascribed to UBF, including regulation of Pol I promoter escape (19) and transcription elongation (20). Importantly, the association of UBF with rDNA is not restricted to the promoter but extends across the entire transcribed region, suggesting that UBF binding may contribute to the active chromatin state of rDNA. Indeed, UBF levels decrease during differentiation, and depletion of UBF leads to reversible methylation-independent compaction of chromatin and reduction in the number of actively transcribed rDNA repeats (21). These results suggest that modulation of UBF levels may be an important determinant of the relative proportion of active and silent rRNA genes during growth and differentiation.

Pol I promoter specificity is conferred by TIF-IB/SL1, a protein complex containing the TATA binding protein and five TATA binding protein–associated factors, including $TAF_I110/95$, TAF_I68, TAF_I48, TAF_I35, and TAF_I12 (22–25). TAF_I proteins perform important tasks in transcription complex assembly, mediating specific interactions between the rDNA promoter and Pol I, thereby recruiting Pol I—together with a collection of Pol I–associated factors—to rDNA. Recruitment of Pol I to the pre-initiation complex requires the interaction of UBF with SL1/TIF-IB and with PAF53, a 53-kDa protein that is associated with Pol I (26). In mammals, Pol I exists in

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two distinct forms, Pol Iα and Pol Iβ. Both forms are catalytically active, but only Pol Iβ can assemble into productive transcription initiation complexes (27). Numerous proteins, including the growth-dependent transcription initiation factor TIF-IA, PAF53, protein kinase CK2, the chromatin modifiers PCAF and G9a, nuclear actin, and myosin (NM1), as well as proteins involved in DNA repair and replication, such as topoisomerases I and IIα, Ku70/80, PCNA, TFIIH, and CSB, were shown to be associated with Pol Iβ. These findings are compatible with a mechanism by which Pol I is recruited to the rDNA promoter as a massive multiprotein complex that acts as a scaffold to coordinate rRNA synthesis and maturation as well as chromatin modification and DNA repair.

The Activity of Basal Pol I Factors Is Regulated by Posttranslational Modifications

Transcription of rRNA genes is efficiently regulated to be responsive to both general metabolism and specific environmental challenges. Signaling pathways that affect cell growth and proliferation regulate rRNA synthesis by modulating individual steps of the transcription cycle. Conditions that impair cellular metabolism, such as nutrient starvation, oxidative stress, inhibition of protein synthesis, or cell confluence, downregulate rDNA transcription, whereas growth factors and agents that stimulate growth and proliferation upregulate Pol I transcription (**Figure 2**). Short-term regulation occurs at the level of transcription initiation, elongation, and RNA processing, and many of the proteins participating in these processes can serve as the convergence point for regulatory pathways. In many cases, the proliferation rate of cancer cells is proportional to the level of UBF (28, 29). The analysis of 17 hepatocellular carcinoma samples demonstrated that UBF was overexpressed in 12 of them when compared with matched healthy liver tissue from the same patients. Additionally, overexpression of UBF was sufficient to activate rRNA synthesis and accelerate growth of human cells, whereas antisense-mediated knockdown of UBF suppressed cell proliferation and induced nonapoptotic death of hepatoma cells (30).

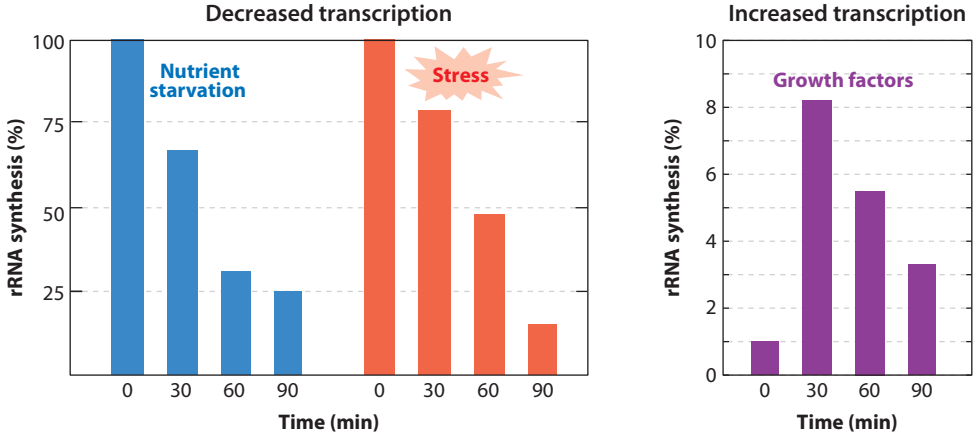

Figure 2

Regulation of Pol I transcription in response to external signals. The bar diagrams show the relative levels of pre-rRNA upon exposure of cells to amino acid starvation (*left*), exposure to oxidative stress (*middle*), and growth factor stimulation (*right*).

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In addition, the activity of UBF is intricately controlled by phosphorylation, such that transcriptional silencing in quiescent cells correlates with hypophosphorylation of UBF (31, 32). Conversely, activation of Pol I transcription upon serum stimulation involves phosphorylation of UBF at a specific serine residue (Ser484) by the G1-specific kinase complexes cdk4/cyclin D1 and cdk2/cyclin E. During S-phase, an increase in UBF activity is achieved through cdk2/cyclin E– and cdk2/cyclin A–dependent phosphorylation at another serine residue, Ser388, and this phosphorylation increases the interaction of UBF and Pol I (33, 34). Upon mitogenic stimulation, extracellular signal-regulated kinase (ERK) phosphorylates UBF at two threonine residues (Thr117 and Thr201), and these phosphorylations increase the interaction of UBF with rDNA and facilitate Pol I transcription through chromatin (20, 35, 36). Given that ERK is often hyperactivated in cancers, upregulation of UBF activity augments ribosome and protein synthesis, an important step in tumorigenesis.

Acetylation is another posttranslational modification that regulates the activity of basal Pol I transcription factors, including UBF and SL1/TIF-IB. For example, acetylation of UBF by the CREB-binding protein (CBP) precludes inhibition of UBF activity by the tumor suppressor pRb (37, 38). Another histone acetyltransferase, PCAF, targets TAF_I68, a subunit of the promoter selectivity factor SL1/TIF-IB. PCAF-dependent acetylation of TAF_I68 stimulates transcription complex formation and transcription initiation (39). Interestingly, PCAF-dependent acetylation of TAF_I68 is counteracted by SIRT1 (mSir2), the founding member of a family of highly conserved NAD^+-dependent histone deacetylases that links the cellular energy status with metabolic activity. SIRT1-dependent deacetylation of TAF_I68 leads to transcriptional repression, underscoring the functional relevance of reversible acetylation in regulating Pol I transcription. These results suggest that at least part of the growth-inhibiting and anticancer action of resveratrol, a drug that activates SIRT1, may be due to repression of aberrant Pol I transcription in cancer cells.

Aside from growth-dependent regulation, Pol I transcription also oscillates during cell cycle progression (**Figure 3**). Transcription is maximal during S- and G2-phase, subsides during mitosis, and then slowly recovers during G1-phase (40). Mitotic silencing of Pol I transcription is caused by Cdk1/cyclin B-dependent phosphorylation of a single threonine residue (Thr852) at TAF_I110 that impairs the interaction of SL1/TIF-IB with UBF (41, 42). At the end of mitosis, Cdc14B, a phosphatase that is sequestered in an inactive state in the nucleolus during interphase and is released from rDNA during mitosis, dephosphorylates Thr852, thereby activating SL1 and relieving mitotic repression of Pol I transcription (I. Tumurbaatar and R. Voit, personal communication). Thus, deregulation of Pol I transcription may have severe consequences on cell cycle progression, providing a molecular explanation for the uncontrolled growth of cancer cells.

Most importantly, fluctuations in rRNA synthesis in response to external signals correlate with the activity of the basal transcription initiation factor TIF-IA (43–46). TIF-IA interacts with RPA43, a unique subunit of Pol I, and with two Pol I–specific TAF_Is, thereby serving as a bridge between Pol I and the pre-initiation complex at the rDNA promoter (27, 47, 48). TIF-IA is phosphorylated at multiple sites, and signals that affect cell proliferation and metabolism alter the phosphorylation pattern of TIF-IA. In growth-arrested cells TIF-IA is hypophosphorylated and incapable of binding to Pol I, indicating that signaling cascades directly target TIF-IA. Mitogenic stimulation causes a transient tenfold increase in pre-rRNA synthesis that correlates with phosphorylation of TIF-IA at two specific serine residues by ERK and RSK kinases (49). Mutations of these phosphorylation sites abolish TIF-IA activity, impair Pol I transcription, and retard cell growth, demonstrating that ERK- and RSK-mediated phosphorylation of TIF-IA regulates rRNA synthesis and nucleolar activity. A dominant-negative mutant of TIF-IA suppresses cell cycle progression, presumably by restricting ribosome production and thereby halting growth. An even more striking result was obtained upon expression of an activated version of TIF-IA

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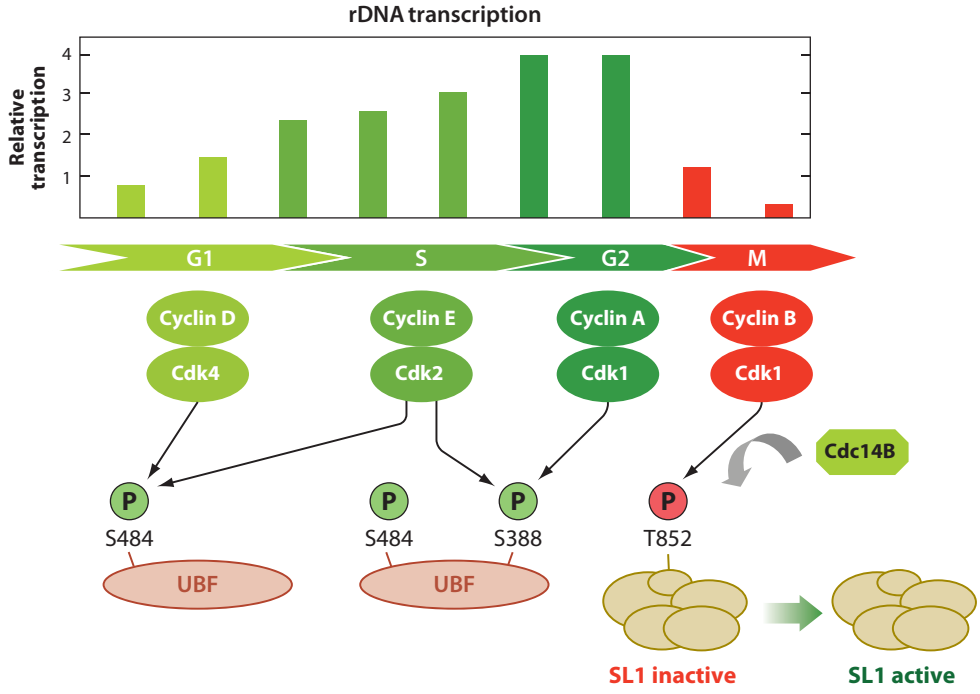

Figure 3

Regulation of Pol I transcription during cell cycle progression. UBF is activated during interphase by phosphorylation of serine 484 (S484) by Cdk4/cyclin D and phosphorylation of serine 388 (S388) by Cdk2/cyclin E and A. At the entry into mitosis, phosphorylation of TAF_I110 at threonine 852 (T852) by Cdk1/cyclin B inactivates TIF-IB/SL1. At the exit from mitosis, Cdc14B dephosphorylates T852, leading to recovery of TIF-IB/SL1 activity. Activating phosphorylations are marked in green, inhibiting ones in red.

carrying a single serine-aspartate substitution within the ERK phosphorylation site that mimics constitutive phosphorylation. Despite the high rate at which HEK293T cells proliferate, increased proliferation and profound effects on cell behavior were observed as a result, indicating that rRNA synthesis is limiting for proliferation of these transformed cells.

In addition to ERK signaling, TIF-IA activity is regulated by mTOR, the mammalian target of rapamycin (50, 51). mTOR is a sensor for nutrient availability and controls diverse readouts, all of which are related to cell growth. Inhibition of mTOR signaling inactivates TIF-IA by decreasing phosphorylation at Ser44 and by enhancing phosphorylation at Ser199, and these changes in TIF-IA phosphorylation impair transcription complex formation. Thus, reversible modification of basal components of the Pol I transcription machinery is an effective way to rapidly and efficiently regulate rDNA transcription in response to growth factor and nutrient availability. Given that TIF-IA is targeted by several kinase cascades and integrates multiple signaling pathways to regulate rDNA transcription either globally or in a cell-type specific fashion, it represents a promising molecular target for drugs designed to block cell proliferation.

Perturbation of Nucleolar Structure and Function Triggers Apoptosis

Cancer-associated morphological changes of the number and size of nucleoli were recognized long ago as a reliable marker of the proliferative state of cancer cells (52). The increase of nucleolar size

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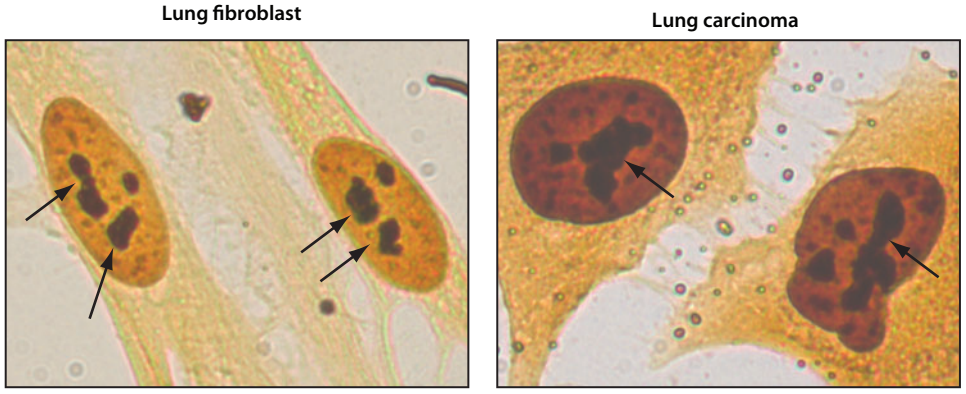

Figure 4

Cancer cells have enlarged nucleoli. The figure shows silver-stained normal human fibroblasts (MRC-5) and human lung carcinoma (A549). Nucleoli are marked by arrows.

correlates with elevated levels of rRNA and protein synthesis and with uncontrolled growth of cancer cells (28). Indeed, a specific staining technique, called AgNOR (**Figure 4**), that can identify hypertrophic nucleolar structures is used by pathologists for cancer diagnostics (53). The nucleolus, long regarded as a mere factory for ribosome assembly, has recently attracted much attention because it actively contributes to the control of cellular survival and proliferation. Disruption of one or more of the steps that control ribosome biosynthesis has been associated with alterations of cell growth. Several studies have correlated deregulation of ribosome biosynthesis with cancer and other diseases, suggesting that overexpression of rRNA could be an initiating step in tumorigenesis (reviewed in 6–9, 54). For example, the gene encoding ribosomal protein S19 is mutated in Diamond-Blackfan anemia (55), and many ribosomal protein genes act as haploinsufficient tumor suppressors in zebra fish (56). A dominant-negative allele of Bob1, a nucleolar protein essential for pre-rRNA processing, causes nucleolar stress and leads to cell cycle arrest in a p53-dependent manner (57). The importance of ribosome synthesis in cell cycle control and proliferation has been emphasized by the finding that mouse liver cells in which ribosome synthesis was impaired by conditional knockout of the ribosomal protein S6 were able to grow but failed to proliferate. Moreover, overexpression of Brat, a repressor of rRNA synthesis in *Drosophila*, was shown to drastically decrease rRNA synthesis and cell division without affecting cell size (58). Thus, inhibition of rRNA synthesis, and hence ribosome biogenesis, leads to uncoupling of cell growth and cell proliferation. However, despite the increasing appreciation of the role of the nucleolus in the regulation of cell growth and apoptosis, the molecular mechanisms that link ribosome biosynthesis and nucleolar structure to cell survival are still poorly understood.

There is increasing evidence that degradation of p53 by the ubiquitin pathway is intimately linked with nucleolar structure and function, which firmly places the nucleolar transcription machinery at the center of control pathways that are influenced by the abundance of p53. Microinjection of antibodies to UBF led to shut-down of pre-rRNA transcription, nucleolar disruption, p53 stabilization, and apoptosis (59). Likewise, disruption of the *TIF-IA* gene by Cre-dependent homologous recombination not only abrogated Pol I transcription, but also led to disintegration of the nucleolus, cell cycle arrest, and p53-dependent apoptosis. Mice that are homozygous for the mutant *TIF-IA* allele die before or at embryonic day 9.5 (60). *TIF-IA*$^{-/-}$ embryos are extremely small, with retarded, discordant development and an increased rate of apoptosis. Significantly, in TIF-IA–deficient cells the MDM2-p53 feedback pathway that controls p53 stability is severely

AgNOR proteins: silver-stainable nucleolar proteins whose amount is directly related to nucleolar activity and cell proliferation

Apoptosis: process of programmed cell death in multicellular organisms, involving nuclear fragmentation and chromosomal DNA fragmentation, leading to cell death

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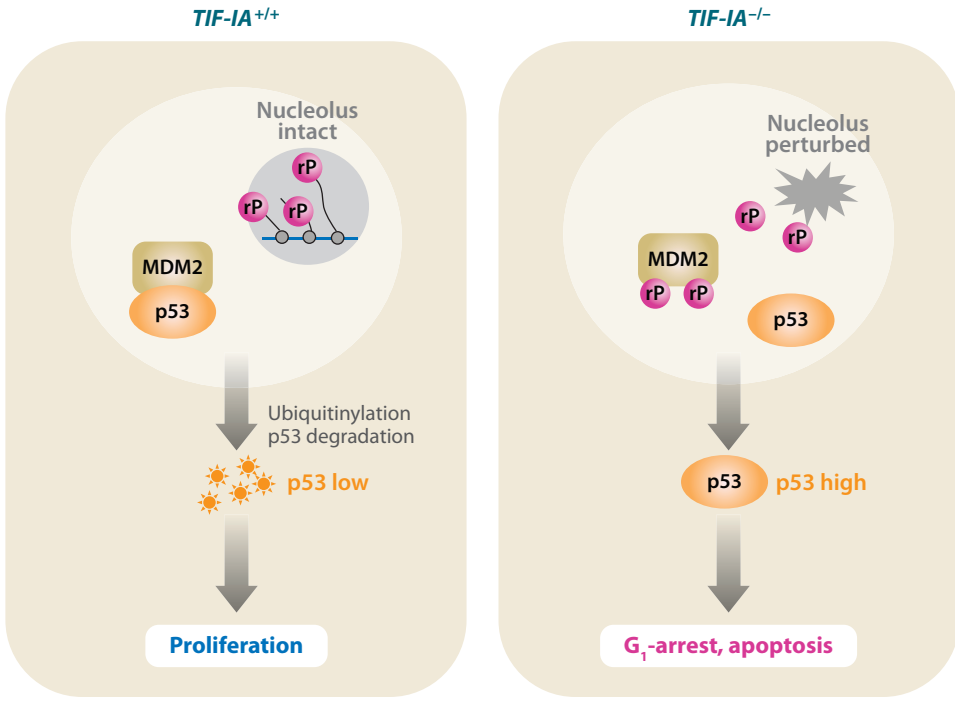

Figure 5

Ablation of TIF-IA leads to cell cycle arrest and apoptosis. In TIF-IA–containing cells, the nucleolus is transcriptionally active, and p53 is maintained at low levels through ubiquitination by MDM2 and degradation by proteasomes. In TIF-IA–deficient cells, the nucleolar structure is perturbed and ribosomal proteins (rP) are released into the nucleoplasm, where they associate with MDM2 to inhibit its activity. As a consequence, the amount and activity of p53 is enhanced, leading to cell cycle arrest and apoptosis.

impaired. Stabilization of p53 in response to TIF-IA deficiency is most likely due to inhibition of MDM2/HDM2. In unstressed cells, MDM2 interacts with p53 and controls p53 abundance by ubiquitination that marks p53 for nuclear export, proteolysis by the proteasome, or both. MDM2 activity is suppressed by interaction with nucleolar proteins such as p14ARF in humans (p19ARF in mice) or ribosomal proteins (reviewed in 61).

Under conditions of nucleolar stress, for example, after inhibition of Pol I transcription by actinomycin D (62) or genetic inactivation of TIF-IA, the p53-MDM2 complex is disrupted and p53-dependent pathways are activated. One possible mechanism for the regulation of p53 in response to nucleolar stress is that perturbations of the nucleolus release proteins that have been shown to interact with MDM2, including p14ARF or ribosomal proteins such as L5, L11, or L23, and thus stabilize p53 by inhibiting the E3 ligase activity of MDM2 (62–66) (**Figure 5**). Thus, drugs that target the Pol I transcription machinery should be powerful tools not only to selectively inhibit growth and proliferation, but also to trigger apoptosis in cancer cells.

The finding that inhibition of Pol I transcription induces the apoptotic program raises the exciting possibility that cell-specific inactivation of TIF-IA in proliferating cells may be a powerful approach to trigger cell- or tissue-specific cell suicide. Indeed, targeted disruption of the TIF-IA gene in the developing nervous system has been shown to lead to chronic neurodegeneration in mice. In this study, mice carrying the floxed allele of the TIF-IA gene were crossed with *NestinCre* transgenic mice that express the Cre recombinase under the control of the *Nestin*

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promoter, a gene that is specifically expressed in neural and glial progenitor cells. Interestingly, these *TIF-IA*^{NesCre} mutants are born alive but die shortly after birth, lacking the entire brain because of selective activation of the apoptotic machinery in neural and glial progenitors (67) (**Figure 6**). Moreover, preliminary studies indicate that Cre-loxP–mediated excision of the TIF-IA gene in dopaminergic neurons leads to mice displaying a remarkable spectrum of Parkinson's disease with relentless chronic progression, neurodegeneration correlating with increased levels of p53, and apoptosis in dopaminergic neurons (C. Rieker et al., unpublished results). Thus, targeted inactivation of TIF-IA may represent a novel and successful strategy not only to establish animal models

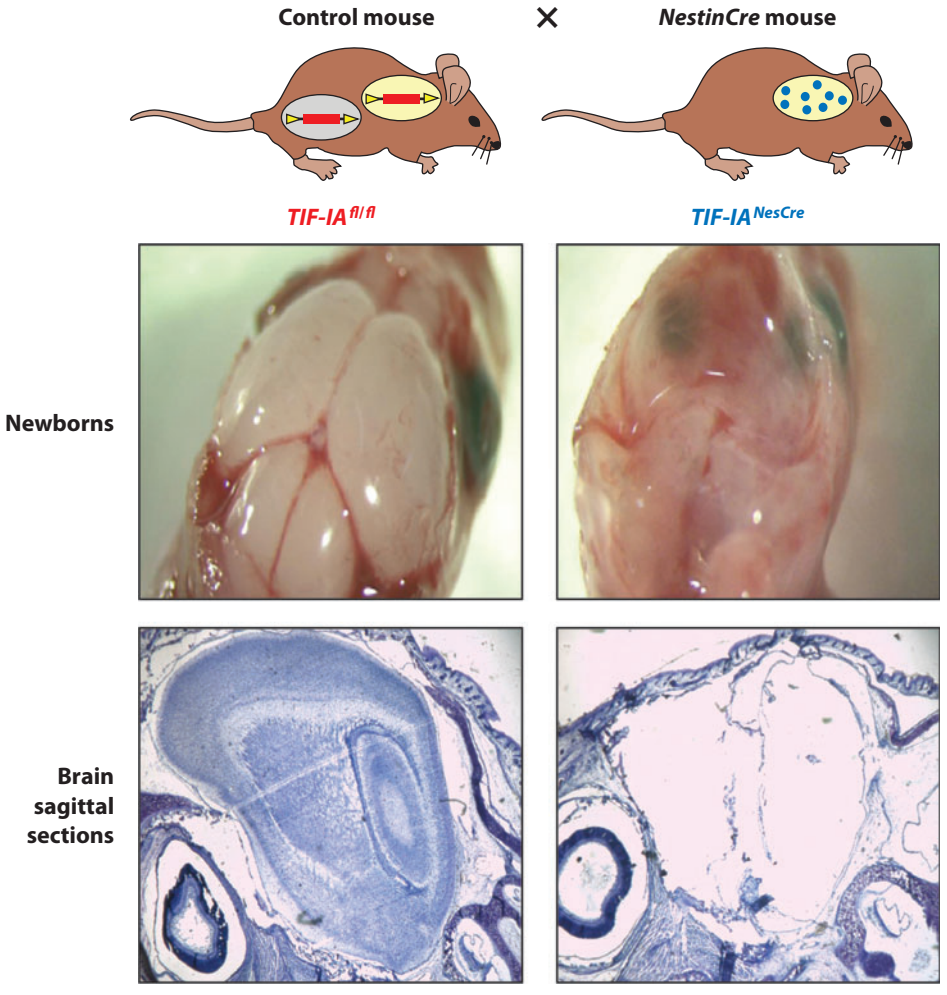

Figure 6

Neural progenitor cell–specific knockout of *TIF-IA* leads to mice without a brain. Mice carrying the floxed allele of the *TIF-IA* gene were crossed with *NestinCre* transgenic mice that express the Cre recombinase under the control of the *Nestin* promoter, a gene that is specifically expressed in neural and glial progenitor cells. *TIF-IA*^{NesCre} mutants are born alive but die shortly after birth. The upper two panels show that newborn *TIF-IA*^{NesCre} mice lack the entire brain because of activation of the apoptotic machinery in neural and glial progenitors. The lower panels show stained sagittal paraffin sections of the brains of control and *TIF-IA*^{NesCre} mice.

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for specific diseases, but also to specifically perturb nucleolar function and to induce apoptosis in defined cells and tissues.

Protein Kinases Regulate rDNA Transcription

Cancer cells use many different mechanisms to increase rRNA synthesis and ribosome biogenesis. Whereas in normal cells the rate of rRNA synthesis is tightly linked to nutritional availability, tumor cells acquire self-sufficiency resulting from activation of downstream mediator kinases that is independent of extracellular signaling events. Several protein kinases, including CK2, ERK, and mTOR, have been shown to be hyperactivated during carcinogenesis and to upregulate Pol I transcription. In this section, we discuss the contribution of protein kinases to rDNA transcription and proliferation of cancer cells.

CK2. The protein kinase CK2 is a constitutively active serine threonine kinase that is overexpressed in many cancers, including leukemias and solid tumors (reviewed in 68). Elevated CK2 levels have been linked to malignant transformation and aggressive tumor behavior. CK2 plays an important role in different steps of tumorigenesis, upregulating oncogenes, downregulating tumor suppressors, promoting cell cycle progression, and inhibiting apoptosis. Consistent with a direct role in Pol I transcription, CK2 is associated with Pol Iβ, the initiation-competent subclass of Pol I (**Figure 7**). CK2 phosphorylates a number of proteins involved in Pol I transcription and pre-rRNA processing, including UBF, TIF-IA, SL1/TIF-IB, topoisomerase IIa, nucleolin, and nucleophosmin (69–71). Phosphorylation by CK2 affects the nucleolar localization or the interaction of these proteins with the Pol I transcription apparatus (72). UBF is phosphorylated by CK2 at serine residues within the C-terminal acidic domain, which contributes to, but is not sufficient for, transcriptional activation. CK2-dependent phosphorylation stabilizes both binding of UBF to rDNA and the interaction of UBF with SL1/TIF-IB. Moreover, a recent study has demonstrated that phosphorylation of TIF-IA by CK2 and dephosphorylation by FCP1 at two serine residues (Ser 170/172) is necessary for sustaining multiple rounds of Pol I transcription. Phosphorylation by CK2 triggers the release of TIF-IA after transcription initiation, a process that is a prerequisite for transcription elongation (71). Thus, CK2 regulates Pol I transcription at multiple levels, affecting the assembly of the transcription initiation complex, transcription initiation, promoter escape, transcription elongation, and reinitiation of Pol I transcription. Consistently, inhibition of CK2 represses rDNA transcription, leading to perturbation of nucleolar structure, cell cycle arrest, and apoptosis.

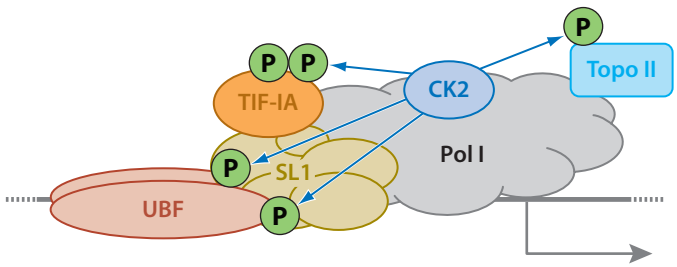

Figure 7

Regulation of Pol I transcription by CK2. Pol I–associated CK2 phosphorylates several components of the Pol I transcription machinery, including TIF-IA, UBF, SL1/TIF-IB, and topoisomerase IIα.

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ERK. Extracellular signal-regulated kinase (ERK) and its downstream effector kinase ribosomal S6 kinase (RSK) are members of a group of serine/threonine protein kinases termed mitogen-activated protein kinases (MAPKs). ERK is a downstream component of an evolutionarily conserved signaling pathway that transduces extracellular signals into the cell nucleus to modulate transcription. ERK responds to mitogenic signals that activate receptors at the cell surface. Upon mitogen stimulation, the epidermal growth factor receptor (EGFR) triggers a signaling cascade involving the GTPase Ras, the kinases Raf, MEK, ERK, and RSK (reviewed in 73). This signaling pathway is frequently hyperactivated in cancer (reviewed in 74). Consistent with its positive effect on cell growth and proliferation, MAPKs were found to activate rRNA synthesis by targeting the transcription factors TIF-IA and UBF (**Figure 8**). Transcription activation upon mitogenic stimulation correlates with ERK-dependent phosphorylation of UBF at two threonine residues (Thr117 and Thr201), both being essential for Pol I transcription elongation (35). Phosphorylation by ERK influences the interaction of UBF with DNA, suggesting that dynamic phosphorylation

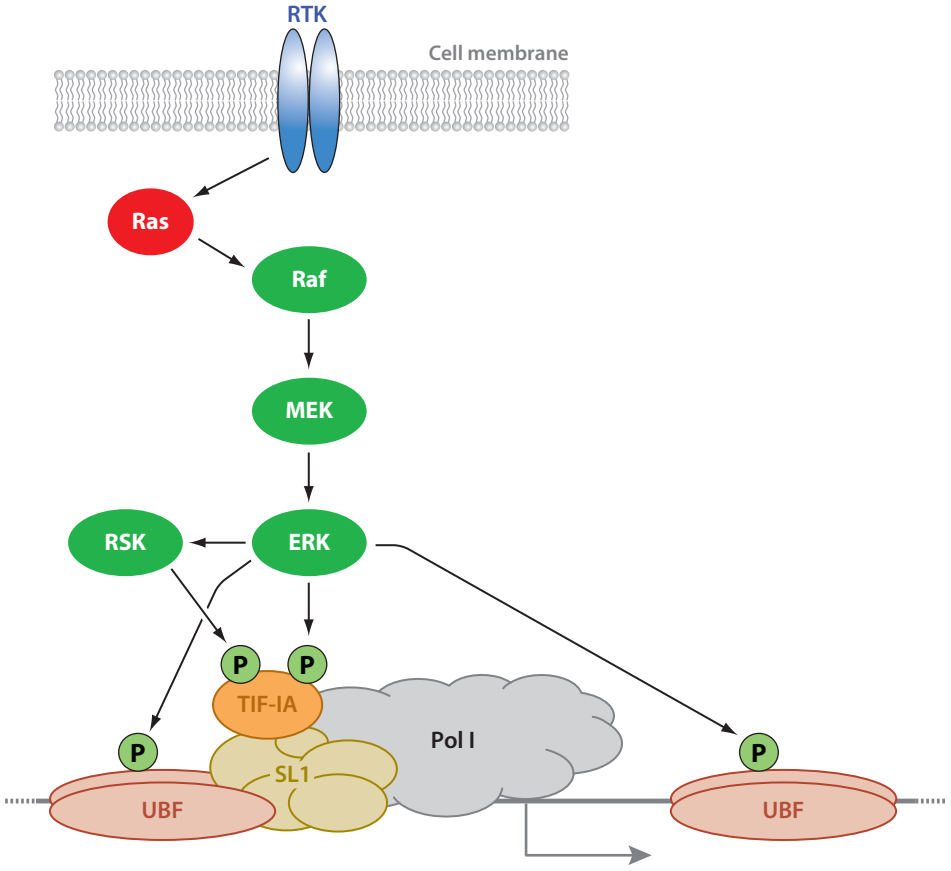

Figure 8

Regulation of Pol I transcription by MAPK signaling. The diagram shows the chain of events by which growth factors activate ERK kinase via the receptor tyrosine kinase (RTK), Ras, MEK cascade. ERK and RSK phosphorylate TIF-IA at two serine residues, and these phosphorylations are required for TIF-IA activity. ERK also phosphorylates UBF, thereby activating Pol I transcription. This signaling pathway is frequently hyperactivated in cancer cells, allowing maximal Pol I transcription in the absence of growth factors.

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and dephosphorylation events promote the passage of Pol I through an altered UBF-DNA complex, presumably immediately downstream of the transcription start site. In addition, ERK and RSK phosphorylate TIF-IA at two serine residues (Ser633 and Ser649). Replacement of Ser649 by aspartic acid activates TIF-IA and accelerates cell proliferation, whereas the respective alanine mutation suppresses cell cycle progression in proliferating tumor cells, presumably by restricting ribosome production and restraining cell growth (49). Thus, the MAPK signaling cascade targets two basal Pol I transcription factors, TIF-IA and UBF, leading to upregulation of rDNA transcription, which in turn is necessary for the proliferative advantage of cancer cells. Several drugs that target this pathway are currently being investigated in clinical trials (reviewed in 75, 76).

mTOR. The mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase that regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis, and transcription (77). Although discovered almost two decades ago in genetic screens, the precise function of the TOR proteins (called TOR in yeast and mTOR in mammalian cells) remains to be fully defined. Dysregulation of the mTOR pathway is implicated as a contributing factor to various human diseases, especially various types of cancer (reviewed in 78). The mTOR pathway is involved in the cellular response to nutrients as well as mitogenic growth factors, regulating ribosome biogenesis in response to nutrients, especially amino acid availability. It affects the synthesis of ribosomal components, including transcription and processing of pre-rRNA, expression of ribosomal proteins, and the synthesis of 5S rRNA. mTOR controls Pol I transcription mainly via the transcription factor TIF-IA (50, 51). Inhibition of mTOR signaling by rapamycin inactivates TIF-IA by decreasing phosphorylation at serine 44 (Ser44) and enhancing phosphorylation at serine 199 (Ser199). Phosphorylation of Ser44 and Ser199 affects TIF-IA activity in opposite ways. Whereas Ser44 phosphorylation is required for TIF-IA activity, phosphorylation at Ser199 inactivates TIF-IA. This indicates that mTOR-responsive kinases and phosphatases modulate the activity of TIF-IA in different ways, implying that antagonizing phosphorylations may play a key role in mTOR-dependent regulation of Pol I transcription. Importantly, rapamycin treatment abolishes the association of TIF-IA with both Pol I and TIF-IB/SL1, thereby abrogating transcription initiation complex formation. In addition, mTOR stimulates phosphorylation of UBF in its C-terminal domain, thereby promoting its interaction with SL1/TIF-IB (79). Given the potential of rapamycin to impair pre-rRNA synthesis, it is not surprising that mTOR inhibitors act as powerful tumor-suppressive drugs (80).

CDKs. The family of cyclin-dependent kinases (CDKs) plays a central role in cell cycle regulation and rDNA transcription. Fluctuations in rRNA synthetic activity during cell cycle progression are brought about by CDK-dependent phosphorylation of components of the Pol I transcription machinery (**Figure 3**). As described above, SL1/TIF-IB is inactivated during mitosis by Cdk1/cyclin B–dependent phosphorylation of TAF_I110, which inhibits the interaction with UBF and prevents transcription complex formation (41, 42). After mitosis, rRNA synthesis is reactivated in G1-phase through the actions of cdk4/cyclin D1 and cdk2/cyclin E that phosphorylate Ser484 of UBF (33). Later, in S-phase, Pol I transcription is enhanced via phosphorylation of Ser388 on UBF by cdk2/cyclin E and cdk2/cyclin A (34). CDK activities are frequently upregulated in cancer, typically through overexpression of their cyclin partners that control the activity and specificity of CDKs, or through inactivation of CDK inhibitors. In several types of cancer, overexpression of CDKs was also documented (81). The redundancy and dispensability of most members of the CDK family, as well as their cyclin partners, for normal cell proliferation make CDKs very promising targets for downregulation of cell proliferation. Not surprisingly, several inhibitors of members of the CDK family are currently being pursued in clinical trials (reviewed in 82).

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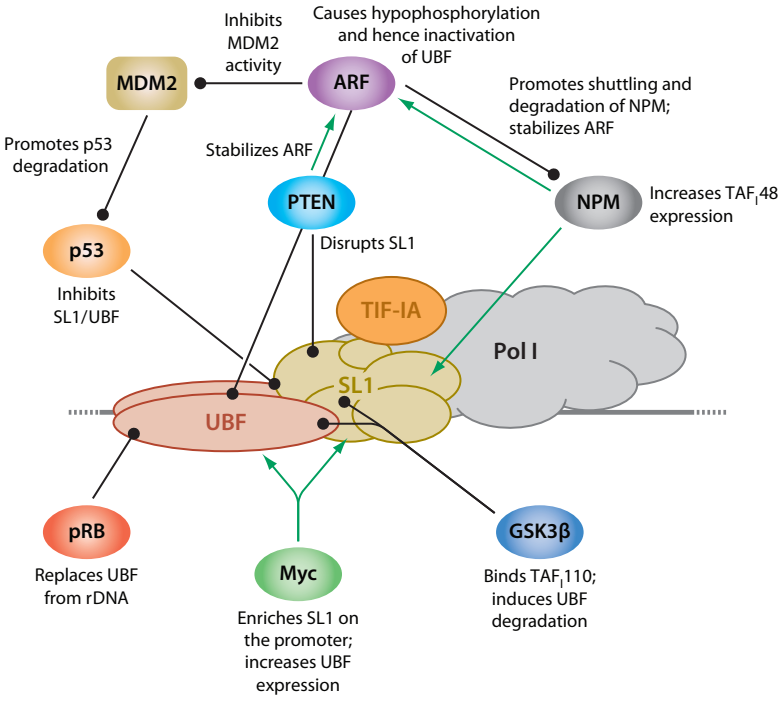

Figure 9

Oncogenes and tumor suppressors control Pol I transcription. Oncogenes activate rRNA synthesis by upregulating the level of transcription factors and/or stabilizing protein-protein or protein-DNA interactions (*green arrows*), whereas tumor suppressors inhibit rRNA synthesis by interfering with macromolecular interactions required for transcription initiation complex assembly (*black lollipops*).

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ONCOGENES AND TUMOR-SUPPRESSORS CONTROL rRNA SYNTHESIS

In addition to kinase signaling, Pol I transcription is regulated by an interplay between oncogenes and tumor suppressors. With more than 100 oncogenes and 30 tumor suppressors identified to date, several of the most prominent members of both families were found to regulate rRNA biogenesis, thereby cementing the link between the hyperactivation of Pol I transcription and malignant transformation. In this section we discuss several of the best-studied examples (**Figure 9**).

Oncogenes Upregulate rRNA Synthesis

Oncogenes were initially identified as genes carried by viruses that can cause transformation of their target cells. It was later demonstrated that the majority of viral oncogenes have cellular counterparts that, if activated through mutation or aberrant signaling, can lead to tumorigenesis. Several oncogenes have been demonstrated to directly regulate rRNA biogenesis, whereas others affect signaling pathways that control Pol I transcription. Thus, it is plausible to propose that a developing cancer might achieve a proliferative advantage by elevating the level of specific oncogenes to raise the production of rRNA.

c-Myc. Proteins of the *myc* proto-oncogene family (c-*myc*, N-*myc*, and L-*myc*) are often upregulated in a wide range of cancers (reviewed in 83). In normal cells, Myc proteins appear to integrate environmental signals to modulate a diverse, and sometimes opposing, group of cellular processes, including proliferation, growth, apoptosis, energy metabolism, and differentiation. Binding of c-Myc to numerous genes recruits multiple coactivator or corepressor complexes that affect chromatin structure and transcriptional activity. Myc proteins activate genes implicated in cell growth, ribosome biogenesis, protein synthesis, and metabolism (reviewed in 84). c-Myc was also shown to localize in nucleoli at sites of rRNA synthesis, to interact with specific consensus elements at rRNA genes, and to associate with SL1/TIF-IB. Activation of c-Myc in fibroblasts or human B-cells led to enrichment of SL1/TIF-IB at the rDNA promoter and increased pre-rRNA synthesis (85, 86). In addition, c-Myc appears to enhance ribosome synthesis indirectly by upregulating expression of UBF, several ribosomal proteins, and proteins involved in rRNA maturation (87–90). Thus, by facilitating recruitment of the Pol I machinery to rDNA, c-Myc appears to promote cell growth at least in part through production of components required for ribosome biogenesis.

Nucleophosmin (B23). Nucleophosmin (also termed NPM or B23) is an abundant nucleolar phosphoprotein that has both oncogenic and tumor suppressor activities (reviewed in 91, 92). The *nucleophosmin* gene (*NPM*) is frequently overexpressed in tumors, and chromosomal translocations of *NPM* have been implicated in the development of hematological malignancies. More than 30% of patients with acute myeloid leukemia have mutations in nucleophosmin that cause aberrant cytoplasmic localization of the protein. Nucleophosmin possesses intrinsic endoribonuclease and histone chaperone activity and affects multiple cellular processes, including pre-rRNA processing, response to genotoxic stress, control of cellular ploidy, DNA repair, maintenance of chromatin structure, and regulation of the activity and/or stability of the tumor suppressors p53 and p14ARF. Overexpression of nucleophosmin was shown to upregulate TAF_I48 levels and to stimulate proliferation of transformed cells (93). On the other hand, an expression of nucleophosmin mutant lacking histone chaperone activity decreased rRNA synthesis (94).

Tumor Suppressors Downregulate rRNA Synthesis

In contrast to oncogenes, loss or mutation of tumor suppressor genes leads to tumorigenesis. Tumor suppressor proteins are common targets for genetic alteration in human cancers and have been implicated as key mediators for suppression of cell transformation (reviewed in 95). Although definitive evidence is still lacking, it appears that tumor suppressors can use repression of Pol I to achieve growth restraint. Tumor suppressor genes fall into two groups: the caretakers, which include repair genes that protect the integrity of the genome, and the gatekeepers, which induce apoptosis and senescence. Because of their pleiotropic roles in the control of cell division, genome stability, or programmed cell death, tumor suppressors have a dampening or repressive effect on cell cycle progression or promote apoptosis, and sometimes do both.

pRb and p130. The retinoblastoma susceptibility (*Rb*) gene was the first tumor suppressor to be identified in a human cancer. pRb and the related pocket proteins p107 and p130 restrict cell growth and proliferation. The *Rb* gene is often mutated in tumors, and the function of pRb is compromised in almost all types of cancer. Originally, it was thought that pRb performs its antiproliferative function solely through interaction with the E2F family of transcription factors, thereby overriding progression through the G1 restriction point (96). This simplified model was challenged by the discovery that UBF is the target for pRb-induced repression of Pol I

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transcription. pRb accumulates in the nucleoli of differentiated or cell cycle–arrested cells (97) and causes downregulation of rRNA synthesis in confluent cells (98). pRb represses Pol I transcription by interacting with UBF (99, 100). Binding of pRb to UBF does not affect the interaction of UBF with SL1/TF-IB or with Pol I, but leads to dissociation of UBF from rDNA, therefore impairing transcription complex formation (99). Thus, inactivation of UBF appears to be an effective way for pRb to shut down rRNA synthesis and inhibit cell growth. Analysis of other members of the pocket protein family revealed that p130, but not p107, can bind to UBF and repress Pol I transcription, indicating that pRb and p130 functionally overlap in regulation of Pol I transcription (99–101). Consistent with their ability to repress rRNA synthesis, both pRb and p130 are frequently inactivated in cancer cells, whereas p107 is not affected. This finding raises the possibility that pRb and p130 exert their tumor-suppressor activity—at least partially—through the interaction with UBF rather than with E2F transcription factors.

p53. Similar to pRb and p130, the tumor suppressor p53 has been shown to repress Pol I transcription. p53, widely dubbed as the guardian of the genome, is a transcription factor that serves as a genotoxic stress sensor and triggers growth arrest, apoptosis, and cell senescence in response to DNA damage (102). Mutations of *p53* and loss of its function are probably the most common alterations in human cancer (103). Neoplastic cells lacking functional p53 may pass through the restriction point regardless of the metabolic state. Cancers with deleted *Rb* and mutated *p53* exhibit much higher rates of rRNA synthesis and are generally more aggressive than those with intact *Rb* and *p53*, thus underscoring their regulatory function in rRNA synthesis (104, 105). Under normal conditions, p53 is a short-lived protein present in cells at a very low level. On exposure to various forms of exogenous stress, such as DNA damage and heat shock, p53 becomes stabilized and gives rise to a cascade of events resulting in either cell cycle arrest or apoptosis. Functional characterization of p53 revealed a variety of biochemical activities, including its function as a sequence-specific transcription factor that triggers activation of genes that suppress cell proliferation.

In accord with its role in restricting cell proliferation, p53 inhibits ribosome biogenesis by repressing Pol I and Pol III transcription (106–108). Mutations in p53 that affect its growth-suppressing activity also abolish its ability to inhibit rRNA synthesis. p53 interacts with TATA binding protein (TBP) and TAF_I110, and therefore p53 interferes with the formation of preinitiation complexes consisting of SL1/TIF-IB and UBF. These findings reveal a novel mechanism by which the tumor suppressors pRb and p53 inhibit cell proliferation, namely by direct inhibition of cellular rRNA synthesis. Given that many tumor cells harbor mutations that affect both pRb and p53, the combined effect of both mutations has an added impact on Pol I activity and tumor progression. Conversely, transient activation of p53 upon DNA damage or early stages of tumor development could exert a beneficial effect in preventing tumorigenesis.

PTEN. The tumor suppressor PTEN (phosphatase and tensin homolog deleted on chromosome ten), one of the most frequently deleted or mutated genes in human cancer, has been shown to regulate cell growth by its ability to regulate Pol I transcription as well (109). PTEN is a phosphatase that downregulates phosphatidylinositol 3-kinase (PI3K), the enzyme that is essential for antiapoptotic, protumorigenic AKT activation. PTEN acts upstream of mTOR, triggering the activation of various downstream signaling events that inhibit cell proliferation. Inactivation of PTEN results in constitutive activation of the PI3K/AKT pathway and tumorigenesis. Expression of PTEN in PTEN-deficient cells represses Pol I transcription, whereas decreased PTEN levels correlate with enhanced rRNA synthetic activity (110). Inhibition of rRNA synthesis requires the lipid phosphatase activity of PTEN and is independent of the cellular p53-status. Significantly, PTEN triggers disruption of the TATA binding protein–containing promoter selectivity

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factor SL1/TIF-IB, thereby preventing the assembly of transcription initiation complexes. In Ras-transformed cells, PTEN was found at the rDNA promoter in a complex with another potential tumor-suppressor, glycogen synthase kinase (GSK)3β. Inhibition of GSK3β upregulates rRNA synthesis, whereas a constitutively active GSK3β mutant inhibits rDNA transcription by interaction with SL1/TIF-IB (111). Thus, the interplay between PTEN and GSK3β represents a powerful mechanism the cell uses to ensure that ribosome biogenesis is coupled to growth control.

Nucleolus organizer region (NOR): clusters of genes, arrayed head-to-tail, that encode the precursor for rRNAs (pre-rRNA)

p14ARF. The tumor suppressor p14ARF is a small basic protein that is produced from the same gene that encodes another tumor suppressor, $p16^{INK4A}$. Consistent with its growth-restricting function, p14ARF downregulates rDNA transcription by interfering with UBF phosphorylation, which in turn impairs pre-initiation complex formation (112). Most importantly, p14ARF is a potent cell cycle inhibitor that regulates the abundance of p53 by binding to and inhibiting MDM2. MDM2 (HDM2 in humans) is an E3 ligase that ubiquitinates p53 to mark it for nuclear export and proteolysis. In normal conditions, nucleolar sequestration of p14ARF allows MDM2 to continuously promote p53 degradation. However, upon exposure to stress or oncogenic pathways, for example, any treatment that affects nucleolar activity, the nucleolar structure is perturbed and nucleolar components, among them p14ARF, are released into the nucleoplasm, where it binds to MDM2 to inhibit its activity. As a consequence, p53 levels are elevated and genes triggering apoptosis are activated. p14ARF interacts with nucleophosmin, and binding to nucleophosmin can trap p14ARF in the nucleolus and suppress its function. Overexpression of p14ARF inhibits processing of pre-rRNA, possibly by lowering the endonuclease activity of nucleophosmin (113). In support of this, rDNA transcription and pre-rRNA processing are increased in p19ARF-deficient murine fibroblasts (114).

EPIGENETIC CONTROL OF rDNA TRANSCRIPTION

Given the repetitive nature of rRNA genes, two strategies for regulating rRNA synthesis are conceivable. Pol I transcription can be controlled either by changing the rate of transcription from each active gene or by adjusting the number of genes that are involved in transcription. Although there is evidence for both options, the majority of short-term regulation affects the rDNA transcription cycle, for example, pre-initiation complex assembly, initiation, promoter escape, and transcription elongation or termination (for review, see 4, 15). However, even in metabolically active cells, only a subset of rDNA copies is transcribed, and therefore rDNA silencing must occur in a cell- and/or tissue-specific manner. The number of active rRNA genes varies between different cell types, indicating that the fraction of active gene copies changes during development and differentiation (115). There are two classes of nucleolus organizer regions (NORs): active and inactive. Several epigenetic characteristics distinguish potentially active from inactive mammalian rRNA genes. Generally, an open chromatin structure that is characterized by DNA hypomethylation, acetylation of histone H4, and dimethylation of histone H3 at lysine 4 (H3K4me2) correlates with transcriptional activity, whereas CpG hypermethylation, histone H4 hypoacetylation, and methylation of H3K9 and H4K20 correlate with transcriptional silencing (116). This posttranslational marking system, known as the histone code, represents a fundamental regulatory mechanism that has an impact on most, if not all, chromatin-mediated processes.

Epigenetic gene regulation plays an essential role in many aspects of tumor biology, including cell growth, differentiation, cell cycle control, DNA repair, and recombination. Epigenetic mechanisms involving DNA methylation and histone modifications cause the heritable silencing of genes without changing their coding sequence. Disruption of the balance of epigenetic networks has been implicated in many human diseases, including imprinting disorders, mental retardation,

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chromosomal instabilities, aging, and uncontrolled cell growth. All human cancers have epigenetic abnormalities, and changes in DNA methylation and aberrant histone-modification profiles can be used for detection and prognosis as well as therapeutic targets. Analysis of the methylation profile of human hepatocellular carcinomas showed significant hypomethylation of the rDNA promoter in tumors compared with matched normal tissues, consistent with the elevated rRNA synthetic activity of rapidly proliferating cells (117). Additionally, low-level rDNA methylation was associated with poor prognosis for patients with endometrial carcinoma and ovarian cancer (118, 119). Importantly, hypomethylation of rRNA genes correlates with decreased genomic stability, suggesting that silencing entails the assembly of a generally repressive chromatin domain that is less accessible to the cellular recombination machinery. Apparently, silencing individual rDNA transcription units by specific methylation contributes to the stable propagation of a subset of genes during cell proliferation and differentiation. Therefore, maintaining a fraction of rRNA genes in a stable, heterochromatic state that represses both transposition and recombination is clearly a challenge and a necessity. Deciphering the mechanisms that establish and maintain heterochromatin and rDNA silencing will reveal whether epigenetic defects cause human diseases and senescence. This is of practical relevance because inhibitors of DNA methyltransferases and histone deacetylases are increasingly used clinically.

CURRENTLY APPROVED CANCER THERAPEUTICS THAT INHIBIT POL I TRANSCRIPTION

With rRNA biogenesis being regulated by multiple processes, several approved anticancer therapeutics are proposed to exert their activity at least in part through interference with rRNA synthesis (**Table 1**). One of the most intriguing examples is cisplatin, a platinum-based chemotherapy drug used to treat various types of cancer, including sarcomas, some carcinomas, lymphomas, and germ cell tumors. Platinum complexes bind and crosslink DNA, thereby interfering with cell division. For a long time it was believed that cisplatin works primarily through induction of DNA repair mechanisms, activating apoptosis when DNA repair proves impossible. However, further studies demonstrated that cisplatin is capable of selectively inhibiting Pol I transcription both in vitro and in vivo. In contrast, its clinically ineffective analog transplatin, although still being able to damage DNA, had no effect on rRNA synthesis (120, 121). Analysis of the molecular mechanism

Table 1 Approved anticancer drugs that may partially exhibit their therapeutic potential through inhibition of rRNA biogenesis

Drug	Target	Mode of action	Ref.
Actinomycin D	GC-rich duplex DNA	Intercalates in GC-rich regions of rDNA and inhibits at low concentrations elongation of Pol I transcription	(126)
Cisplatin	Adjacent guanosines in DNA	Forms crosslinks in DNA that possess high affinity for HMG-containing proteins. Hijacks UBF from its site of action, thus inhibiting Pol I transcription	(120)
Irinotican/Topotican	Topoisomerase I	Traps Topoisomerase I to rDNA leading to DNA strand breaks and inhibition of Pol I transcription	(127)
Mitomycin C	Guanosines in 5′-CpG-3′motifs	Inhibits Pol I transcription by alkylating guanosines and inducing interstrand crosslinks in rDNA	(128)
5-Fluorouracil	Thymidylate synthase	Incorporation of 5-FU in 47S pre-rRNA inhibits processing of pre-rRNA	(129)
Temsirolimus	mTORC1	Inhibits rRNA synthesis by interfering with mTORC1 activity	(130)

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underlying inhibition of Pol I transcription revealed that intrastrand cisplatin-DNA crosslink possesses an extraordinary affinity for UBF (K_d = 60 pM), and therefore can hijack UBF away from rDNA (122, 123). Moreover, the link between the UBF expression and sensitivity of cancer cells to cisplatin was identified, making it a possible marker for patient selection (30). Together, the tight link between the expression levels of UBF and enhanced rRNA synthetic activity of cancer cells, on the one hand, and the sensitivity of cancer cells to cisplatin, on the other hand, implies that UBF is a promising target for screening anticancer drugs and chemotherapeutic reagents.

DEVELOPMENT OF DRUGS THAT TARGET THE POL I TRANSCRIPTION MACHINERY

As with all anticancer therapies, the goal is to knock out a critical source of sustained support that is preferentially required by cancer cells. Given that aberrant Pol I transcription is a hallmark of cancer, targeting components of the Pol I machinery should be a promising approach to combat cancer. In support of this concept, a recent study has revealed that inhibition of rRNA synthesis by low doses of actinomycin D (ActD) leads to growth arrest of normal, that is, pRb/p53-containing cells, while triggering apoptosis in pRb/p53-deficient cells (124). In an attempt to specifically target the nucleolus and the Pol I transcription apparatus, several product candidates have been generated that are being used as antineoplastic agents based on their ability to selectively inhibit rRNA synthesis and kill cancer cells. The most advanced drug, CX-3543 (also known as quarfloxin), was designed to bind to quadruplex DNA, that is, higher-order structures that can form within G-rich regions of rDNA. CX-3543 inhibits elongation of Pol I transcription through disruption of nucleolin–G-quadruplex complexes on the nontemplate strand of transcribed rDNA, leading to translocation of nucleolin into the nucleoplasm and induction of apoptosis (124a). CX-3543 successfully completed two separate phase I clinical trials that utilized different dosing schedules in patients with solid tumors. During these trials, blood levels of quarfloxin were obtained that were within the range that exerted antiproliferative potency in vitro. CX-3543 was well tolerated and displayed clinical benefits in patients with carcinoid and neuroendocrine tumors (125). Based on these scientific and clinical findings, CX-3543 was advanced into phase II clinical development for the treatment of carcinoid and neuroendocrine tumors.

Another drug, the nucleolar targeting agent CX-5461, was designed as an oral inhibitor of Pol I transcription. CX-5461 was selected for its potency to inhibit Pol I transcription in cell-free systems (127a). CX-5461 was shown to impair binding of SL1/TIF-IB to the rDNA promoter, thereby preventing initiation of Pol I transcription and inhibiting cell proliferation. The antiproliferative potency of CX-5461 was demonstrated in preclinical trials. In support of selective targeting of the Pol I transcription machinery, CX-5461 inhibited rRNA synthesis in cancer cells with an IC_{50} of 50–100 nM, concentrations that are ~200-fold lower than those required to inhibit Pol II transcription. In addition, CX-5461 demonstrated antitumor activity in human cancer xenograft mouse models.

PERSPECTIVES

Although significant advances have been made toward understanding the mechanism and regulation of Pol I transcription, and the links between increased ribosome production and cancer have been explored, the question remains as to whether the deregulation of rRNA synthesis itself could trigger cell transformation or whether increased rRNA synthesis plays a secondary, but necessary, part in tumorigenesis. Certainly, deregulation of rRNA synthesis can have an enormous impact on the ability of cells to sustain life. Therefore, changes in pre-rRNA transcription and processing

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that accompany or precede malignant transformation are not only of great scientific interest, but offer unique possibilities to combat cancer by selectively targeting proteins that are involved in ribosome biogenesis. Potential targets for anticancer therapeutic strategy are protein kinases, such as ERK/RSK, mTOR, and CK2, which are often hyperactivated in cancer cells and are known to be required for rDNA transcription. Alternatively, the concept of targeting key components of the machineries that produce rRNA seems quite obvious. Indeed, several approved anticancer drugs have been shown to inhibit rRNA synthesis, albeit not necessarily with the required selectivity. In contrast, selective inhibition of Pol I transcription was demonstrated to trigger apoptosis or autophagy in cancer cells. As cells with compromised p53 and pRb functions are more sensitive to transient inhibition of rRNA synthesis, they show promise for a practical therapeutic window in the clinic and in defining strategies for patient selection. Discovery of specific and selective inhibitors of rRNA synthesis can be a daunting but rewarding task, and some progress has already been made in this direction. As certain drugs rely on the structure rather than on the primary sequence of DNA, specific binding of UBF to structured DNA opens the door for the design of therapeutics that specifically target rDNA.

Additionally, structure-based virtual ligand screening technology will facilitate the design of drugs that specifically inhibit components of the Pol I machinery, thus offering unexplored opportunities for therapeutic intervention. Recent advances in techniques to solve the crystal structure of large nucleoprotein complexes make the resolution of the crystal structure of human Pol I an achievable goal. Moreover, the involvement of numerous protein-protein and protein-DNA interactions makes the Pol I transcription apparatus a ripe field for the design and functional screening of small molecules that target protein-protein or transcription factor–DNA interactions. Thus, although the area of targeting anticancer drugs to the Pol I transcription machinery is still in its infancy, it promises to be a provocative and emerging field. Comprehensive future studies will provide a better understanding of the key events in Pol I transcription that drive the transformation process. This detailed knowledge will be required to develop diagnostic tools to characterize specific molecular lesions in individual neoplastic tissues. The challenge is now to develop new classes of improved targeted strategies to selectively inhibit Pol I transcription in rapidly proliferating cells and to eliminate cancer cells without harming healthy tissues or organs.

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SUMMARY POINTS

1. Hallmarks of cancer include excessive cellular proliferation, deregulated signaling, and abnormalities of the nucleolus.
2. The size of nucleoli correlates with rRNA synthetic activity, and nucleolar hypertrophy is a diagnostic marker for cancer.
3. Mutations in signaling pathways lead to excessive ribosome synthesis to support the excessive protein synthesis that is required for cell growth and proliferation of cancer cells.
4. Several classic cancer chemotherapies have been discovered to mechanistically act on or through the nucleolus and to selectively kill cancer cells.
5. Oncogenes and kinase signaling pathways that positively regulate Pol I transcription are often hyperactivated in cancer cells, whereas tumor suppressors exert an inhibitory effect on Pol I transcription.

6. Inhibition of Pol I transcription leads to disruption of nucleoli, cell cycle arrest, upregulation of p53, and induction of apoptosis.
7. rRNA synthesis and cell proliferation are enhanced in cells carrying mutations in tumor suppressor genes, and such cells are more sensitive to inhibition of rRNA synthesis.
8. Drugs that target the Pol I transcription machinery and selectively inhibit rRNA synthesis will enable the development of novel and promising therapeutic strategies to combat cancer.

DISCLOSURE STATEMENT

D.D. and W.G.R. are employees of Cylene Pharmaceuticals. Two of the experimental drugs (CX-3543, CX-5461) used as examples are the property of Cylene Pharmaceuticals.

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