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Review

RNA polymerase III repression by the retinoblastoma tumor suppressor protein ☆

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ABSTRACT

The retinoblastoma (RB) tumor suppressor protein regulates multiple pathways that influence cell growth, and as a key regulatory node, its function is inactivated in most cancer cells. In addition to its canonical roles in cell cycle control, RB functions as a global repressor of RNA polymerase (Pol) III transcription. Indeed, Pol III transcripts accumulate in cancer cells and their heightened levels are implicated in accelerated growth associated with RB dysfunction. Herein we review the mechanisms of RB repression for the different types of Pol III genes. For type 1 and type 2 genes, RB represses transcription through direct contacts with the core transcription machinery, notably Brf1–TFIIIB, and inhibits preinitiation complex formation and Pol III recruitment. A contrasting model for type 3 gene repression indicates that RB regulation involves stable and simultaneous promoter association by RB, the general transcription machinery including SNAPc, and Pol III, suggesting that RB may impede Pol III promoter escape or elongation. Interestingly, analysis of published genomic association data for RB and Pol III revealed added regulatory complexity for Pol III genes both during active growth and during arrested growth associated with quiescence and senescence. This article is part of a Special Issue entitled: Transcription by Odd Pols.

1. Introduction — the retinoblastoma tumor suppressor protein

Mutations in the RB1 gene, encoding the retinoblastoma (RB) tumor suppressor protein were originally identified as culpable in a rare childhood eye tumor known as retinoblastoma [1]. In early studies of retinoblastoma cell lines, tumor-associated mutations were correlated with either undetectable RB1 mRNA or mRNA of a decreased molecular size [2,3], consistent with the loss of RB expression in these pediatric tumors. Subsequently, RB1 mutations were observed in other classes of cancers, especially small-cell lung carcinomas [4], wherein genetic lesions at the RB1 locus are as penetrant as in retinoblastoma (reviewed in [5]). These early studies suggested an important role for RB in the prevention of adult-onset malignancies, and indeed, dysregulation of the RB pathway via direct mutation of RB or through indirect modulation of other regulatory components is so frequently observed as to be recognized as a hallmark of cancer [6,7].

The function of RB in tumor suppression has been an actively pursued topic with early studies linking RB function to cell cycle control. RB is a 110 kDa protein which becomes phosphorylated when cells are rapidly cycling [8–11] and dephosphorylated in growth arrested cells [12]. Microinjection of recombinant RB into nocodazole-synchronized cells early in the G1 phase resulted in a pronounced G1 cell cycle block that was not observed when RB was injected in asynchronously growing cells [13]. These studies linked the changes in RB phosphorylation status to cell cycle control, a process centrally important to regulated proliferation. RB post-translational modification is facilitated by the cyclin dependent kinase (cdk) 4/cyclin D and cdk2/cyclin E complexes, whose activities are important for G1 progression [14–16]. RB was found to associate with the E2F family of transcription factors and impede their function in the RNA polymerase (Pol) II activation of cell cycle target genes [17–20]. A prevalent model emerged that RB prevents the transcription of key genes required for DNA synthesis in early G1 and cyclin/cdk phosphorylation relieves RB repression (Fig. 1). In addition to cell cycle control, RB is dephosphorylated as cells differentiate, implicating RB function in developmental processes [9]. RB also participates in the regulation of apoptotic responses [21–23], and it is clear that RB governs multiple aspects of cell fate choices that are deregulated in tumorigenesis.

2. RNA polymerase III activity in cancer — the biosynthetic capacity hypothesis

The prevalent model for RB function in attenuating cancer progression attributes RB mutation with the loss of E2F repression and cell cycle control. However, this cell cycle-centric model does not account for a simple requirement that accelerated proliferation by cancer cells must also involve increased synthesis of cellular components to match their increased rate of division. In part, the requisite increased biosynthetic capacity for rapid proliferation is achieved by increased output by Pol III, which is responsible for the production of the 5S ribosomal (r) RNAs, all transfer (t) RNAs, and a multitude of other non-protein coding RNAs [24], many of which are involved in protein output

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(reviewed in [25,26]). Many of these well established genes and some newly identified loci have been recently characterized as bona fide Pol III targets in mammalian cells in genomic ChIP-seq experiments [27–29] that further revealed a surprising complexity to Pol III regulation and function.

Consistent with their essential role in active growth, Pol III transcripts are frequently elevated in tumor cells [30–33], and many of these same cells exhibit defects in the RB pathway. Indeed, the RB tumor suppressor was shown to repress global Pol III transcription both in vitro and in vivo [34]. From these early studies, a biosynthetic capacity hypothesis was proposed that Pol III inhibition by tumor suppressors, including RB, limits cellular proliferation [34,35]. Supporting the concept that Pol III activity is limiting to robust cancer proliferation, the forced reduction of Pol III activity in mouse xenograft assays could inhibit tumor formation [36]. An integral connection between enhanced Pol III activity and cellular dysfunction in human cancer is also suggested from the observation that scleroderma patients who exhibit autoantibodies against Pol III also are more likely to be diagnosed with some forms of cancer than similar patients who don't express elevated Pol III antibodies [37]. These observations hint that Pol III levels or activity may be a prelude to a clinically evident disease in a subset of cancer patients. Pol III-associated structures and activity may also serve as useful biomarkers for cancer diagnosis and disease staging as some Pol III transcripts are localized to nuclear structures termed perinucleolar compartments (PNC) [38], and increased PNC prevalence has been positively correlated with tumor progression and poor prognosis for patient survival [39–43]. While the function of the PNC remains enigmatic, these observations provide support of the hypothesis that increased steady state levels of Pol III transcripts are an important contributing factor in cancer progression.

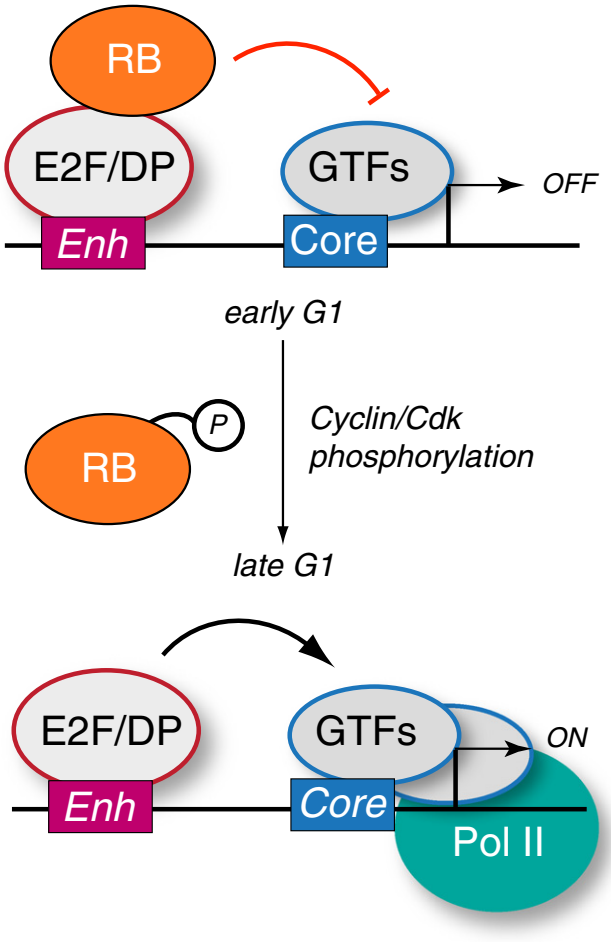

Fig. 1. RB repression of cell cycle target genes is relieved by cyclin/cdk phosphorylation. During early G1, RB can form stable complex at cell cycle target gene promoters through interactions with the E2F/DP transcriptional activator, interfering with transcriptional activation. Repression can be mediated via disruption of the general transcription factors (GTFs) or via recruitment of chromatin modifying factors. Later in G1, RB is phosphorylated by the cyclin D/cdk4 and cyclin E/cdk2 kinases to disrupt E2F interaction and relieve repression.

3. RNA polymerase III transcription during cell cycle progression

The products of Pol III target genes play essential roles in cell growth, and consequently they are often maintained at very high levels during periods of active proliferation. Many of these RNAs exhibit long half lives, and the combination of high steady state levels and long half lives has often led to the assumption that these "housekeeping genes" are constitutively active. However, it has become clear that Pol III transcription is dynamically regulated both during development and throughout the cell cycle (Fig. 2). The highest levels of Pol III activity are typically observed in late G1, S, and G2 phases and lowest levels prevalent during early G1 and M phases [44–48], as determined using extracts prepared from staged cells. While global Pol III activity fluctuates during the cell cycle, Pol III target genes exhibit distinct factor requirements, and its activity at specific loci is governed by stage-specific and gene-specific mechanisms, dependent upon underlying promoter structures, as described in more detail below.

Pol III transcribed genes are grouped into three general types (Fig. 3). Both type 1 (5S rRNA) and type 2 (tRNA) Pol III responsive genes contain intragenic promoter elements that recruit TFIIIA plus TFIIIC, or just TFIIIC, respectively (reviewed in [25,26]). In both types, promoter recognition is followed by recruitment of the Brf1–TFIIIB complex, composed of the TATA box binding protein (TBP) [49–52], Brf1 [53–57], and Bdp1 [56,58–60]. In contrast, type 3 genes contain extragenic promoter elements, and promoter recognition is accomplished by a multiprotein complex called the small nuclear RNA activating protein complex (SNAPc) [61–66], also known as the PSE transcription factor (PTF) [67–70]. SNAPc cooperates for promoter recognition with a related but different TFIIIB complex that contains TBP and Bdp1, but Brf2 instead of Brf1 [56,57,71,72]. Pol III is directly recruited by TFIIIB complexes [50,73–75], and these complexes have emerged as the preeminent signaling nexus governing Pol III activity (Fig. 4).

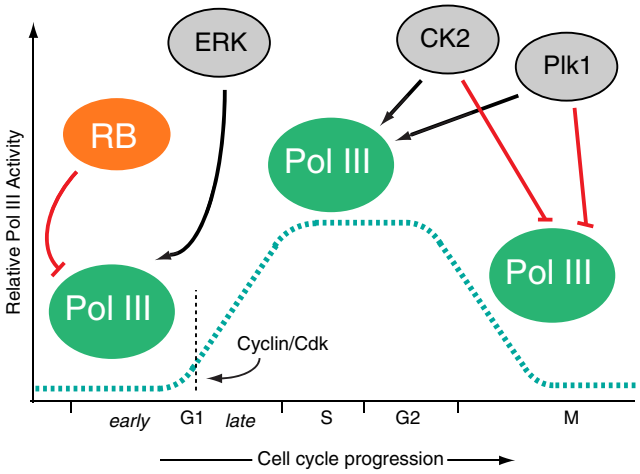

Fig. 2. Pol III activity is dynamically regulated during cell cycle progression. During G0 and early G1, Pol III transcription is repressed by RB and its related family members, p107 and p130. Upon RB phosphorylation by cyclin/cdk, Pol III activity increases until peak activity is achieved during late G1 and S phases. In some contexts, Pol III activity increases in early G1 after serum stimulation via Ras activation and ERK stimulation of Brf1–TFIIIB in a process that precedes cyclin/cdk activation. The protein kinase CK2 and Polo-like kinase both exhibit positive and negative effects on Pol III transcription, stimulating activity in the S phase but inhibiting activity during mitosis. The mechanism for Pol III regulation by each of these factors is different for the distinct types of Pol III genes, as dictated by underlying differences in promoter architecture and factors involved.