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Transcriptional regulation of human small nuclear RNA genes

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Abstract

The products of human snRNA genes have been frequently described as performing housekeeping functions and their synthesis refractory to regulation. However, recent studies have emphasized that snRNA and other related non-coding RNA molecules control multiple facets of the central dogma, and their regulated expression is critical to cellular homeostasis during normal growth and in response to stress. Human snRNA genes contain compact and yet powerful promoters that are recognized by increasingly well-characterized transcription factors, thus providing a premier model system to study gene regulation. This review summarizes many recent advances deciphering the mechanism by which the transcription of human snRNA and related genes are regulated.

Keywords

Human snRNA genes; transcription; Retinoblastoma protein; p53 tumor suppressor; RNA polymerase II; RNA polymerase III

1. Introduction

An extensive component of transcriptional output in eukaryotic organisms occurs from genes encoding non-coding (nc) RNA [1], and whose preponderance in the genome is only recently becoming appreciated [2]. The products of ncRNA genes can be divided into multiple functional categories. One category contains regulatory RNAs including small interfering and micro RNA that function in post-transcriptional regulation of gene expression. While not exhaustive, the other category encodes structural molecules, including ribosomal, transfer, small nucleolar, small cytoplasmic (sc), and small nuclear (sn) RNA that function in the processing, translation, and degradation of other RNA molecules (Table 1). However, recent roles for scRNA and snRNA molecules in chromosomal DNA replication [3], messenger RNA transcription [4-6], and global RNA polymerase III transcription [7,8] suggest that members of the structural RNA class can also be utilized for regulatory roles. The line between regulatory and structural classes of RNA has become further blurred by the discovery of RNA polymerase III-transcribed microRNA clusters [9] and of snRNA-like transcription units that encode anti-sense transcripts capable of post-transcriptional gene regulation [10]. The general preponderance of ncRNA gene products and of snRNA genes in particular, to the total cellular

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transcriptional profile, and their widespread function in every aspect of the central dogma indicates that their controlled expression is critically important to cellular homeostasis.

2. Human snRNA gene promoter structure and transcriptional machinery

Members of the snRNA and some related scRNA gene families are characterized by a diagnostic arrangement of promoter elements, minimally including a distal sequence element (DSE) that serves as an enhancer of transcription, and a proximal sequence element (PSE) that is located in the core promoter region upstream from the start site of transcription. Some genes additionally contain a companion TATA box located adjacently to the PSE (see Figure 1). In humans, the combination of the extragenic PSE and TATA elements directs recruitment of the RNA polymerase III-specific transcriptional machinery whereas the absence of a TATA box specifies recruitment of the RNA polymerase II-specific transcription apparatus. Indeed, mutation of the TATA box induces RNA polymerase II transcription from the U6 promoter normally receptive to RNA polymerase III, while insertion of a TATA box conversely causes RNA polymerase III transcription from the U2 promoter normally transcribed by RNA polymerase II [11,12]. Thus, relatively minor alterations in the core promoters radically change both the mechanism for transcription initiation and potential regulation of these genes. Other categories of RNA polymerase III-transcribed genes, such as vault RNA, contain a combination of extragenic PSE-like and TATA promoter elements along with canonical intragenic elements typically utilized for RNA polymerase III transcription of transfer RNA genes [13]. The combinatorial usage of promoter elements provides distinct regulatory options for different sets of snRNA genes.

The PSE sequence common to all human snRNA genes, irrespective of polymerase specificity, is recognized by the snRNA activating protein complex (SNAP_C) [14], also known as the PSE transcription factor (PTF) [15,16] or PSE binding protein (PBP) [17,18]. SNAP_C functions for both RNA polymerase II and III transcription of all snRNA genes thus far tested experimentally, consistent with the commonality of the PSE among human snRNA genes. SNAP_C is composed of at least five proteins SNAP190, SNAP50, SNAP45, SNAP43, and SNAP19 [19-25] that tend to bind to similar regions of the genome, as determined by chromatin immunoprecipitation and microarray analyses [26], suggesting the dominant role for each factor is through SNAP_C. This multi-protein complex plays a centrally important function in snRNA transcription through multiple roles in pre-initiation complex assembly, including direct promoter recognition, while serving as a target for numerous activators and repressors of transcription. For example, SNAP190 can interact with the Oct-1 activator [27] and p53 tumor suppressor [28]. SNAP43 can interact with both the Retinoblastoma (RB) and p53 tumor suppressors [28,29], whereas SNAP50 can interact with RB [29]. Thus, the various SNAP_C subunits have evolved specialized roles for communication with distinct sets of regulatory proteins likely to regulate snRNA transcription under differing growth conditions.

As promoters that contain SNAP_C binding sites are typically powerful, restricting inappropriate DNA binding by SNAP_C may be important to prevent high-level non-specific transcription. Correspondingly, promoter recognition by SNAP_C is influenced both by the intrinsic properties of the complex itself and by regulatory proteins. DNA binding by SNAP_C requires both SNAP190 and SNAP50, which directly bind to DNA via their Myb and zinc-finger DNA binding domains, respectively [23,30]. Neither factor is capable of DNA binding alone, but instead requires SNAP43 to facilitate the assembly of SNAP190 and SNAP50 into a DNA binding competent complex [31]. SNAP43 may make additional contacts with DNA as a member of the complex, as was experimentally observed for the orthologous SNAP43 in *Drosophila* [32,33]. Even after assembly into a functional complex, human SNAP_C does not bind well to many native PSE sequences in vitro (RWH, unpublished), but does bind synergistically with upstream activators, such as Oct-1 [15,34], and additional components of

the general machinery, notably TBP [35]. Interestingly, partial complexes containing SNAP43, SNAP50, and the N-terminal region of SNAP190 harboring the Myb DNA binding domain are fully functional for PSE-binding, TBP recruitment, and snRNA gene transcription by both RNA polymerases II and III [36]. These same subunits are also widely conserved throughout evolution, with homologues in vertebrate species, *Drosophila*, Trypanosomes, and plants [32,37-39]; correspondingly, these species maintain PSE-related promoter elements that are critical for snRNA gene transcription ([30] and references therein). In contrast, the human SNAP19 and SNAP45 subunits are dispensable for transcription in vitro [36], and are not as widely conserved, suggesting that these vertebrate-specific SNAP_C subunits may have adapted specialized regulatory roles for snRNA gene transcription. As SNAP45 can modulate PSE-binding by SNAP_C [36] and interact with TBP [21,24,31], this factor may contribute promoter recognition function for snRNA genes with differing PSE promoter sequences (see Figure 1 and ref. [40] for additional sequence comparisons). Alternatively, additional contributions to stabilized promoter binding may be important for putative snRNA genes containing non-canonical spacing arrangements of the PSE and TATA elements, as observed in some genes encoding antisense regulatory RNAs [10].

Beyond the initial promoter recognition events, subsequent steps in the pathways for pre-initiation complex assembly at the TATA-less and TATA-containing snRNA genes have diverged significantly (Figure 2). For TATA-less snRNA genes, SNAP_C binding is a prelude to the recruitment of traditional components of the general transcription machinery, including TBP, TFIIA, TFIIB, TFIIE, and TFIIIF [41], which are also used for mRNA transcription by RNA polymerase II. A role for human TFIIH is also suggested from in vitro transcription studies, but has not been conclusively established in humans [41]. This possibility seems likely given that TFIIH plays an indispensable role for transcription of the snRNA-like spliced leader genes in trypanosomes [42]. In principle, the shared usage of numerous general transcription factors for mRNA and snRNA gene transcription provides a common focal point for global regulation of RNA polymerase II transcription. Unexpectedly, U1 snRNA associates with TFIIH to stimulate RNA polymerase II transcription of certain mRNA genes [6], suggesting that U1 snRNA may contribute to RNA polymerase II regulation. An intriguing possibility is that different TFIIH complexes differing by the presence or absence of U1 snRNA participate in active transcription of mRNA and snRNA genes, respectively, providing the potential to differentially regulate these categories of RNA polymerase II transcribed genes at the level of preinitiation complex assembly. Interestingly, SNAP_C was found associated with sub-stoichiometric amounts of TBP in extracts from HeLa cells [19,24], and this complex, but not the TBP-TAF complex TFIID, reconstituted in vitro U1 snRNA transcription in extracts depleted of endogenous TBP [14], indicating that a complex of SNAP_C and TBP was the primary TBP-TAF complex functioning for transcription of these genes. In trypanosomes, SNAP_C tightly associates with both TBP and TFIIA [37,39], consistent with this idea. It is thus interesting that the TAF5 component of TFIID was detected at human U2 snRNA genes by chromatin immunoprecipitation [43], raising the possibility that SNAP_C may also function along with a subset of the canonical RNA polymerase II-specific TAFs. Although no homologues for SNAP_C have been described in yeast, it nonetheless appears that a role for SNAP_C in coordinating TBP activity at snRNA genes is evolutionarily conserved.

As with TATA-less RNA polymerase II-transcribed snRNA genes, SNAP_C recruits TBP to RNA polymerase III-transcribed genes, in these cases to the TATA box, but whether this promoter recognition by these factors occurs serially, or that SNAP_C and TBP form a complex to concomitantly recognize the adjacent PSE and TATA promoter elements remains uncertain. Nonetheless, subsequent events in preinitiation complex assembly do not follow the TFIIB/TFIIA pathway. Instead, the SNAP_C/TBP juxtaposition results in the recruitment of a TFIIB-related factor called Brf2 [44-47]. Brf2 differs substantially from Brf1 used for transcription of other RNA polymerase III-transcribed genes in humans, and from the Brf used for U6

snRNA gene transcription in yeast [48], suggesting that this factor may have diverged extensively to permit additional regulatory flexibility. Indeed, additional alternatively spliced forms of Brf1 may contribute to snRNA gene transcription by RNA polymerase III [49], but the context for usage of these variants is not well understood. At least for Brf1 and Brf2, their distinct C-terminal extensions, which are not found within TFIIB, provide positive elements that select for RNA polymerase III usage [50]. These findings indicate that the promoter recruitment of TFIIB for RNA polymerase II transcription or of the TFIIB-related Brf2 factor for RNA polymerase III transcription, as dictated by the absence or presence of the TATA box, serves as a critical determinant for utilization of different RNA polymerases in humans. The assembly of SNAP_C, TBP, and Brf2 facilitates recruitment of the SANT-domain containing protein Bdp1 [30], a factor globally utilized for all RNA polymerase III transcription ([44, 51], reviewed in [52]).

3. Activation of snRNA gene transcription

The continued high-level expression of snRNA in cells depends upon the DSE [53,54], which is typically located approximately 200 bp upstream of the transcription start site. The DSE is typically compound in nature [55], containing an octamer motif recognized by the POU-domain activator protein Oct-1 [56,57] and an Staf-responsive element recognized by the snRNA transcriptional activating factor (Staf) [58-60], also known as SPH Binding Factor (SBF) [61-63]. Some snRNA genes also harbor Sp1 binding sites adjacent to the DSE (see also [40] for review). In addition, the proto-oncoprotein N-Myc globally activates RNA polymerase III transcription [64], and may regulate the U6 snRNA gene transcription via the E-box sequence located adjacently to the DSE of some U6 genes. Some evidence suggests that Oct-1 and Sp1 can bind the DSE cooperatively and act synergistically to stimulate U2 transcription [65] whereas Oct-1 and Staf are required for maximal U6 expression [62,66]. The combination of N-Myc, Oct-1, Sp1, and Staf may contribute to robust snRNA gene transcription under favorable growth conditions with an estimated initiation event occurring every 2-4 seconds at some snRNA genes [67], but whether all these factors can act synergistically has not been determined. Nonetheless, transcriptional activation by these regulatory proteins likely involves specialized interactions with the general transcription machinery as prescribed by the core promoters of the snRNA genes [68], with a likely possibility being that these activator proteins synergize to recruit SNAP_C. In this regard, the mechanism is best understood for Oct-1, wherein direct contacts between the Oct-1 POU domain and the SNAP190 subunit of SNAP_C contributes to increased SNAP_C recruitment to the PSE concomitant with stimulated snRNA gene transcription [27,34]. As SNAP_C has a relatively low affinity for most PSE sequences but a slow off-rate from DNA once bound [34], the activator-directed recruitment of SNAP_C represents a potential rate-limiting step for robust snRNA gene activation. Additional factors including HMGB1 [69], YY1 [70], and β -actin [71] may also contribute structural roles to improved preinitiation complex assembly during snRNA gene activation.

The intimate relationship between the DSE and PSE, and their respective binding factors further suggests that the coordinated assembly of a well-structured preinitiation complex is critical for full expression. Indeed, SNAP190 enhances DNA binding by Oct-1 activator and makes additional contacts with the phosphate backbone within the DSE [72], even though SNAP190 binds DNA specifically through the PSE. The strict spacing between the DSE and PSE is conserved in most, but not all, snRNA genes, and at least for U6 and 7SK snRNA gene expression, a nucleosome positioned between these promoter elements contributes to activated transcription [73-75]. A nucleosome at this location may spatially juxtapose the DSE and PSE to facilitate direct interactions between Oct-1 and SNAP190. The importance of the positioned nucleosome during activated snRNA gene transcription also suggests that factors modulating chromatin structure are important for regulated transcription. For example, the p300 and CBP histone acetyltransferases associate with snRNA gene promoters in vivo. Consistently, a higher

proportion of histone H3 at U6 promoters is acetylated in cells that maintain higher levels of RNA polymerase III transcription (GWJ, unpublished observations). As described in more detail below, the opposing activity of histone deacetylase (HDAC) factors is also important for transcriptional repression by the RB and p53 tumor suppressor proteins.

In addition to stimulated preinitiation complex assembly, transcription of RNA polymerase II-transcribed snRNA genes may also be activated by the linkage of transcription with downstream RNA processing events. Indeed, appropriate snRNA termination depends upon the 3' box (see Figure 2), which is recognized by RNA polymerase II when recruited to PSE-containing genes [76-78], and suggests that an intimate relationship exists between factor recruitment by SNAP_C bound to the PSE and downstream RNA termination and processing events dictated by the 3' box. One candidate target in this process is the Integrator complex, which is involved in coupling snRNA transcription to subsequent 3' end processing events of U1 and U2 snRNA [79], and may function in additional steps during snRNA transcription analogous to services provided by the Mediator complex during mRNA gene transcription. The Integrator complex is composed of at least twelve polypeptides and associates with the carboxy terminal domain (CTD) of the RNA polymerase II largest subunit. Efficient 3' end processing depends upon RNA polymerase II CTD phosphorylation [80,81]. Furthermore, mutation of serine 5 or serine 2 within the context of the CTD results in diminished snRNA gene transcription, as was similarly observed for RNA polymerase II transcription of a mRNA transcript, and of 3' end formation [82], suggesting that CTD kinases, including TFIIF and P-TEFb, could contribute to efficient snRNA gene expression. Intriguingly, serine 7 of the CTD is also specifically required for both snRNA gene transcription and downstream 3' end formation with no effects on mRNA transcription and is also critical for recruitment of the Integrator complex to U1 and U2 snRNA genes [82].

Similarly, activated transcription of RNA polymerase III-transcribed human snRNA genes may also involve promoter association by La [83], a factor initially characterized as a human autoantigen, and subsequently shown to play a role in RNA polymerase III termination, possibly also acting as a chaperone for RNA polymerase III transcripts during snRNP biogenesis [84]. However, in vitro RNA polymerase III transcription assays indicate that La is not essential for transcription [85], although any function in transcription may only be revealed in a cellular context. In addition, the protein kinase CK2 can phosphorylate RNA polymerase III to stimulate transcription [86,87], perhaps in an analogous fashion to the role performed by the elongation factor P-TEFb, which phosphorylates RNA polymerase II. Although the largest subunits of RNA polymerase II and III are very similar, RNA polymerase III does not contain a CTD [88], and whether the role of RNA polymerase III phosphorylation is analogous to that for RNA polymerase II phosphorylation for recruitment of elongation and processing factors is unknown.

4. Cell cycle regulation of human snRNA gene transcription

Human snRNA gene transcription was thought to be independent of cell cycle constraints [89], in part due to their high rates of transcription and stable structures leading to a highly abundant presence of these RNAs during all stages of the cell cycle. However, the demand for snRNA and other non-coding RNAs fluctuates depending on the metabolic state of the cell, and therefore their transcription is regulated during changes in cell growth and cell cycle progression [90]. Recent evidence suggests that transcription of snRNA genes is dynamic, as described below, with expression restricted at various points during the cell cycle by the protein kinase CK2 and the RB tumor suppressor protein. It is also apparent that independently of cell cycle constraints, human U6 transcription can be repressed in response to cell stress and nutrient levels by MAF1, a downstream effector of the mTOR signaling pathway, through a direct association between MAF1 and RNA polymerase III [91]. The association between MAF1 and

RNA polymerase III in *Saccharomyces cerevisiae* is inhibited by the protein kinase PKA and stimulated by the protein phosphatase PP2A [92,93], and presumably this relationship is also maintained in human snRNA gene regulation providing a link to cAMP-mediated signaling pathways.

Currently, the cell cycle regulation of RNA polymerase III transcription is better understood and is more thoroughly described herein (Figure 3). RNA polymerase III transcription is most active during the late G1, S and G2 phases of the cell cycle and is repressed during the M and G0/early G1 phases [94-97]. The protein kinase CK2 is suggested to play a major role in M-phase repression of snRNA transcription, even though CK2 stimulates snRNA transcription by RNA polymerase III during S phase [87,98]. CK2 associates with multiple components of the RNA polymerase III transcriptional apparatus, including the polymerase itself [86], and SNAP_C [99], and phosphorylates both the Bdp1 component of the Brf2-TFIIB complex [86] and the SNAP190 component of SNAP_C [99]. Consistent with its role in cell cycle regulated expression, Bdp1 is phosphorylated predominately during M-phase, which also correlates with its reduced promoter association at this stage of the cell cycle [87,94]. The loss of promoter association by Bdp1 is sufficient to explain M-phase repression of snRNA expression. Although less pronounced, SNAP_C promoter association is also diminished during M-phase [87], and may be attributable to CK2 phosphorylation of SNAP190 at a serine-rich region located adjacently to its Myb-DNA binding domain, which inhibits SNAP_C recognition of the PSE [100].

Upon exit from M phase, U6 snRNA gene expression remains low, in part due to the action of the RB tumor suppressor protein, which silences RNA polymerase III transcription at the G0/early G1 stage of the cell cycle [97,101]. Although tenuous, the link between RB and RNA polymerase III expression was suggested because RB activity during the cell cycle is inversely correlated with that observed for RNA polymerase III transcription. Hypo-phosphorylated RB is active in the early G1 phase, and is switched off by increased phosphorylation as cells progress through middle and late G1 into the S phase concomitant with increased RNA polymerase III transcription. The related RB family members, p107 and p130, also repress U6 transcription [102], but it is not clear whether these different factors provide redundant cell cycle regulation of RNA polymerase III transcription, or regulate snRNA transcription at different points during the cell cycle or in distinct cellular contexts. Interestingly, a role for RB family members for the regulation of snRNA gene transcription by RNA polymerase II has not been observed [103], suggesting that the distinct usage of SNAP_C and Brf2-TFIIB specifies RB targeting of RNA polymerase III-transcribed snRNA genes.

As the products of these non-coding RNA genes contribute substantially to the biosynthetic capacity of the cell, the repression of snRNA gene transcription and other RNA polymerase III transcripts by RB likely plays an important role in growth control limiting tumor progression [104-107]. Indeed, cancer cells that lack RB function also exhibit elevated levels of RNA polymerase III transcripts [108]. Structure-function studies of RB for snRNA repression support a role for RNA polymerase III regulation as an integral component of tumor suppression. Growth suppression by RB is dependent on the pocket region, which extends from amino acids 379-792 and contains the A and B domains. The A/B pocket domain is required but not sufficient for tumor suppression, which additionally requires the C-terminal region. This extended pocket domain of RB rescues the lethal phenotype during embryogenesis associated with RB loss, allowing RB^{-/-} mice to develop normally [109,110]. The A/B pocket plus the C-terminal region of RB is required for interactions with many components of the RNA polymerase III general transcription machinery, including the SNAP43 and SNAP50 subunits of SNAP_C and the TBP and Brf1 subunits of TFIIB complexes, and is necessary for repression of both U6 snRNA and tRNA-like gene transcription [103,108].

Although RB represses global RNA polymerase III transcription, the extended family of cognate genes transcribed by this polymerase contain diverse promoter elements and utilize distinct general transcription factors [52]. Consequently, the mechanism of RB repression of snRNA gene transcription has necessarily diverged from that for other classes of RNA polymerase III transcribed genes (Figure 4). The 5S rRNA and tRNA genes transcribed by RNA polymerase III contain intragenic promoter elements that are recognized by TFIIB and TFIIC (5S rRNA) or by TFIIC alone (tRNA). For both classes of genes, TFIIC recruits the Brf1-TFIIB complex, which contains TBP, Brf1, and Bdp1, and functions as an initiation factor for RNA polymerase III recruitment [44]. RB interacts with the Brf1 component of TFIIB complex, disrupting the TFIIB-TFIIC interaction to prevent preinitiation complex assembly and subsequent recruitment of RNA polymerase III [111,112], although this aspect of the model has not been thoroughly examined in living cells. Nonetheless, the disruption of Brf1-TFIIB function indicates that the proposed mechanism for 5S rRNA and tRNA repression by both the p53 (see next section) and RB tumor suppressor proteins is similar.

In contrast, RB utilizes a different mechanism to regulate U6 snRNA gene expression. During U6 repression, RB stably associates with the promoter, both in vitro and in vivo as measured by cross-linking immunoprecipitation assays, via protein-protein interactions with components of SNAP_C and Brf2-TFIIB [103]. This aspect of the RB/SNAP_C pathway involving stable promoter association is similar to the classical RB-E2F mechanism wherein RB interacts with E2F complexes for promoter association [113]. However, unlike both the RB/E2F and RB/Brf1-TFIIB repression pathways, wherein RB disrupts pre-initiation complex assembly and RNA polymerase recruitment [114,115], RB maintains its association with SNAP_C, TBP, and RNA polymerase III at the promoter [103]. Thus, during snRNA gene repression, RB likely inhibits steps subsequent to RNA polymerase III recruitment, potentially including promoter escape, open complex formation, first dinucleotide bond formation, elongation, and termination.

RB has been demonstrated to utilize a wide variety of co-repressor proteins to enact repression for RNA polymerase II transcription. While not much is known about the RB co-repressor proteins for regulating RNA polymerase III transcription, several protein partners are associated with RB mediated repression of the E2F-stimulated RNA polymerase II target genes [116], and these have served as candidates for co-repressors of human snRNA gene transcription. In particular, RB associates with the ATP dependent chromatin remodeling factor SWI/SNF, a complex that repositions nucleosomes during transcriptional regulation possibly to occlude factor access to promoter elements or to prevent DNA unwinding required for transcription factor binding [117,118]. Interestingly, the Brg1 subunit of SWI/SNF associates with human U6 snRNA gene promoters in cells that maintain functional RB. During repression of in vitro U6 transcription, RB stimulates promoter recruitment of Brg1, but not the related BRM protein, suggesting that Brg1-specific SWI/SNF complexes are utilized during RNA polymerase III regulation (GWJ, unpublished). As in vivo mapping studies have demonstrated the presence of a positioned nucleosome between the DSE and PSE of human U6 and 7SK snRNA genes [73,75], the altered positioning of this nucleosome may interfere with the cooperative binding of Oct1 and SNAP_C. Alternatively, RB could remodel nucleosomes near the start site of transcription or within the gene itself. However, RNA polymerase III can transcribe through a nucleosome [119], and hence a positioned nucleosome in the U6 promoter region that disrupts factor binding is more likely to be effective as target for repression.

RB also associates with chromatin modifying proteins, including the class I HDACs consisting of HDAC1, HDAC2, and HDAC3 [120-122] that remove acetyl groups from lysine residues in histone tails resulting in condensation of chromatin. Interestingly, RB can associate with both HDAC and SWI/SNF activities as part of the same complex during repression [117], and indeed both HDAC1 and HDAC2 can associate with snRNA gene promoters in vivo, although

autologous RB expression in RB deficient cells preferentially results in HDAC2, and less obviously, HDAC1 promoter recruitment (GWJ, unpublished). RB repression of U6 transcription from chromatin templates is sensitive to HDAC inhibitors, whereas repression of naked DNA templates is refractory (GWJ, unpublished), suggesting that histones are the primary target for HDAC function during RB repression. RB repression of other RNA polymerase III-transcribed genes is not dependent upon HDAC activity [115], consistent with the differing mechanisms for repression of RNA polymerase III. These observations also indicate that RB repression of snRNA gene transcription involves multiple steps with transcriptional repression functionally separable from events governing chromatin structure, although the precise orders of events during RB repression remain unclear. Nonetheless, the human snRNA gene transcription has become a premier model system to investigate the multi-step mechanism for RB repression.

5. Human snRNA gene transcription in response to genomic stress

Although human snRNA genes are highly transcribed, it has become clear that transcriptional repression is critical for the regulated expression of these genes in response to a variety of growth conditions, both during the cell cycle, as described previously, and in response to DNA damage. One initial hint of such an activity arose from studies demonstrating that the transcription of numerous snRNA genes by both RNA polymerase II and III is sharply down regulated in response to UV light treatment for an extended period beyond the time required for repair of lesions that might impair transcription [123-128]. This initial finding suggested that a factor activated in response to DNA damage was capable of restricting snRNA gene transcription or alternatively that an essential factor was lost after DNA damage.

Additional studies of adenovirus strain 12 infected cells provided some clues to the identity of potential regulatory factors capable of carrying out this role. Infection of cells by this virus induces four chromosomal fragile sites [129], which are observed as regions of incompletely condensed chromatin during metaphase. The three most prominent fragile sites map to the U1 snRNA, U2 snRNA, and 5S rRNA loci [130]. The fourth site maps to the U1 pseudogene loci. Except for the U1 pseudogene locus, the other loci each contain multiple tandem repeats of potent transcription units with the U1 and U2 genes transcribed by RNA polymerase II and the 5S rRNA genes transcribed by RNA polymerase III. Chromosomal fragile site formation at sites of U1 and U2 genes requires the adenovirus E1B protein [131], as well as the cellular tumor suppressor protein p53 [132,133]. Interestingly, these sites also become constitutively fragile in cells that lack expression of the Cockayne syndrome group B protein (CSB) [134], and as CSB and p53 can physically interact [135], these cellular factors could function together at snRNA gene loci.

Additional studies indicated that a functional U2 promoter was required for the metaphase fragility [136-138], together suggesting that CSB and the p53 tumor suppressor might regulate snRNA genes, both during adenovirus infection and potentially in response to UV light, as described above. A role for CSB is plausible given that CSB plays a positive role in rRNA gene transcription by RNA polymerase I [139]. Additional experimental evidence also indicates that p53 does repress U1 transcription by RNA polymerase II and that endogenous p53 association with the U1 and U2 snRNA loci is stimulated by UV light treatment of cells [28]. A model for p53 repression of U1 transcription is shown in Figure 5. Whether p53 represses snRNA gene transcription in response to other types of DNA damage has not been determined. However, it seems likely that p53 regulation would be invoked during viral challenge, such as by certain strains of adenovirus, as well as by cytoside arabinoside and actinomycin D, both of which activate the p53 response pathway and induce chromosomal fragile sites at the U1 and U2 loci [140].

In addition to RNA polymerase II-transcribed snRNA genes, p53 represses U6 snRNA gene transcription by RNA polymerase III, and was proposed to act as a gene-specific repressor of transcription by this polymerase [141]. Subsequent experimentation demonstrated that p53 acts as global repressor of RNA polymerase III transcription, including that of the 5S rRNA gene locus [142]. Elevated levels of RNA polymerase III transcripts were observed in primary fibroblasts from Li-Fraumeni patients, whose predisposition to cancer is ascribed to inherited mutations in p53, many of which disable DNA binding by p53 [143]. However, both U1 and U6 transcription was repressed by p53 comprised solely of the C-terminal domain from amino acids 301-393, lacking the central DNA binding domain [28]. Although surprising, this result is at least consistent with the ability of DNA-binding defective p53 to initiate fragile site formation at the U1 locus [134]. Human U1 genes harbor a high affinity p53 element located adjacently to the PSE [28], and it remains a distinct possibility that p53 directly recognizes this site during repression in the natural context. Nonetheless, for many human snRNA genes, promoter targeting by p53 depends substantially upon protein-protein contacts, such as those observed between p53 and SNAP_C [28], as well as with TBP.

A reliance on protein-protein contacts for targeted gene regulation is also observed for other classes of RNA polymerase III-transcribed genes, such as tRNA and 5S rRNA, which lack identifiable p53 binding elements, even though, paradoxically, the p53 DNA binding domain is required for their repression [144]. This observation also hints that p53 employs different mechanisms to repress the various categories of RNA polymerase III-transcribed genes. For most RNA polymerase III-transcribed genes that contain intragenic promoter elements, p53 interacts with Brf1-TFIIB to impede recognition of promoter bound TFIIC [145], preventing subsequent RNA polymerase recruitment. This mechanism of repression explains the apparent lack of p53 association at tRNA and 5S rRNA genes (A. Gridasova, unpublished), in contrast with observed robust p53 association along with SNAP_C at U1, U2, and U6 snRNA genes [28]. The snRNA promoter association by p53 also correlates with increased HDAC recruitment as similarly observed for RB repression of U6 transcription. However, p53 repression invokes a more obvious recruitment of both HDAC1 and HDAC2 as compared to the preferential HDAC2 recruitment shown by RB at U6 promoters (A. Gridasova, unpublished), suggesting that p53 may use differing co-repressor complexes to repress snRNA gene transcription as compared to that used by RB. It is thus interesting that p53 appears to disrupt U6 promoter association by RNA polymerase III, in contrast with RB that apparently co-occupies the U6 promoter along with RNA polymerase III during repression [103]. Despite these mechanistic differences, the functional convergence of both the p53 and RB tumor suppressors for snRNA gene repression highlights the importance of this gene family to cancer progression, and these genes may represent novel targets for therapeutic intervention. Furthermore, while much of the initial research has focused on U1 transcription by RNA polymerase II and U6 transcription by RNA polymerase III, the vast array of cellular functions influenced by other members of this gene family portends the full scope of regulatory control remaining to be discovered.

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Figure 1. Sequence comparison of human snRNA gene core promoters

Human snRNA gene core promoters contain a proximal sequence element (blue) located approximately 42-44 bp upstream from the transcriptional start site (green). Differences at conserved positions within the PSE are indicated. The U12 promoter contains a degenerate PSE (dashed underline) located adjacently to the start site. Those genes transcribed by RNA polymerase III contain an additional TATA box (red) located at variable positions from the 3' border of the PSE. Except for U1, U2, U6, and 7SK, the transcriptional start sites are predicted but have not been experimentally confirmed. The location of the experimentally determined p53-binding site within the U1 promoter is indicated (pink) with the brackets representing the region protected during Dnase I footprinting [28] and the p53 element quarter sites indicated by arrows. The database accession numbers for the indicated sequences are U1 (J00318), U2 (U57614), U3 (X14945), U4B (M15956), U5C (NT 010194), U11 (X58716), U12 (NT 035014), Y5 (NR 001571), U6 (M14486), 7SK (X05490), MRP (X51867), Y1 (NT 007914), Y4 (L32608), U6atac (NT 035014), Y3 (NT 007914), and H1 (X16612).

Figure 2. Factors involved in human snRNA gene transcription

The transcription of human snRNA genes by RNA polymerases II (A) and III (B) involves a combination of shared factors, including the Staf and Oct-1 activators and the general transcription factors SNAP_C and TBP along with additional factors specialized for transcription by only one polymerase. These polymerase-specific factors include TFIIA, TFIIB, TFIIE, TFIIF, and TFIIH for RNA polymerase II transcription, and the Brf2 and Bdp1 components of the Brf2-TFIIB complex for RNA polymerase III transcription. For genes harboring snRNA core promoter elements, RNA polymerase II and III termination is directed by the 3'box or by the TTTT terminator, respectively. The numbers within SNAP_C represent the apparent molecular weights of each subunit as measured by SDS-PAGE, and relative positioning within the complex and towards DNA is suggested from in vitro protein-protein interaction

studies [31,178]. Studies performed with *Drosophila* SNAP_C suggest that SNAP50 makes DNA contacts towards the 3' end of the PSE [32,33].

Figure 3. Model for cell cycle regulation of snRNA gene transcription by RNA polymerase III

RNA polymerase III transcription is elevated during late G1, S, and G2 phases and becomes repressed during M and early G1 phases. A role for the protein kinase CK2 in M phase repression and S phase activation is indicated whereas RB is proposed to act predominately during early G1 phase. Other RB family members, including p107 and p130, may influence snRNA gene transcription during the cell cycle whereas MAF1, a downstream effector of the mTOR pathway, can also repress snRNA gene transcription in response to cell stress and changes in nutrient levels (not shown).

A. RB/SNAPc Model **B. RB/TFIIIB Model** **C. RB/E2F Model** **Figure 4. Models for gene regulation by RB**

During RNA polymerase III repression, RB can inhibit transcription through stable association with the general transcriptional machinery and RNA polymerase at snRNA promoters (A. RB/SNAP_C model) or through disrupted preinitiation complex formation by blocking communication between TFIIC and Brf1-TFIIIB (B. RB/TFIIIB model) disabling polymerase recruitment. Stable promoter association by RB at E2F target genes is also suggested to occur during RNA polymerase II repression (C. RB/E2F model). In this model, the preinitiation complex is disrupted either by RB interference with E2F signaling or through co-repressor effects on chromatin structure, such as enacted by HDAC and/or SWI/SNF containing complexes. A role for co-repressors that alter the snRNA gene chromatin environment is also

suggested, although repression can occur independently of histone deacetylase activity on naked U6 DNA templates, suggesting that the repression of polymerase activity occurs downstream from chromatin modification events.

Figure 5. Model for U1 snRNA gene regulation by p53

In response to DNA damage, such as caused by UV light, p53 is activated and can associate with the U1 promoter, possibly through direct interactions with the SNAP190 and SNAP43 subunits of SNAP_C or through association with a high affinity p53 response element located adjacently to the start site of transcription for this gene. Promoter association by SNAP_C is not apparently affected either by DNA damage or by p53 binding in vitro, although downstream events in the preinitiation complex assembly are likely affected by p53 promoter association. RNA polymerase II is also polyubiquitinated in response to UV light contributing to decreased U1 expression. The promoter association of p53 also directs additional cofactor recruitment,

such as HDAC1 and HDAC2, which may interfere with chromatin structure and activator signaling.

Table 1
Characteristics and function of selected snRNA and related scRNA family members

RNA	Species	Polymerase		Function(s)
		specificity	Size (nt)	
7SK snRNA	Human	III	330-332	inhibition of P-TEFb and RNAPII elongation [4, 5]
H1 RNA	Human	III	341-344	tRNA maturation [159-161], RNAP III transcription [7,8]
U1 snRNA	Human	II	164	mRNA splicing [146-148], stimulation of TFIIH and RNAPII transcription [6]
U2 snRNA	Human	II	186	mRNA splicing [149,150], stimulation of RNAPII elongation [151]
U4 snRNA	Human	II	141-145	mRNA splicing [152]
U5 snRNA	Human	II	116	mRNA splicing [153,154]
U6 snRNA	Human	II	106-107	mRNA splicing [155,156]
U3 snRNA	Human	II	217-241	rRNA processing [157,158]
MRP RNA	Human	III	267	rRNA processing [166], mitochondrial DNA replication [162-165]
MRP RNA	Yeast	III	340	cell cycle progression [167,168]
Y1 scRNA	Human	III	~112	
Y3 scRNA	Human	III	~101	
Y4 scRNA	Human	III	~94	non-coding RNA degradation [169-173], chromosomal DNA replication [3]
Y5 scRNA	Human	III	~84	
Vault RNA	Human	III	86-142	nucleocytoplasmic trafficking, multidrug resistance [174-177]