REVIEW

Like Attracts Like: Getting RNA Processing Together in the Nucleus

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Structures visible within the eukaryotic nucleus have fascinated generations of biologists. Recent data show that these structures form in response to gene expression and are highly dynamic in living cells. RNA processing and assembly require many factors but the nucleus apparently lacks any active transport system to deliver these to the RNAs. Instead, processing factors move by diffusion but are concentrated by transient association with functionally related components. At sites of high activity this gives rise to visible structures, with components in dynamic equilibrium with the surrounding nucleoplasm. Processing factors are recruited from this pool by cooperative binding to RNA substrates.

It has long been known that eukaryotic nuclei are not homogeneous but contain a variety of subnuclear structures often referred to as nuclear bodies. Most are involved in the synthesis, processing, and modification of RNA, and these will be discussed here. The cytoplasmic organelles, such as mitochondria, chloroplasts, and lysosomes, are largely discrete, membrane-bound structures. In contrast, the nuclear bodies, such as nucleoli, coiled (Cajal) bodies, and interchromatin granule clusters (IGCs or "speckles"), lack membrane boundaries. A long-standing, major question in the field has been whether these are relatively rigid structures that are required for RNA processing. Recent data indicate that these visible structures are highly dynamic and result from the way nuclear RNA processing is organized. No active transport system is known to be present in the nucleus, and current evidence indicates that both RNAs and RNA-processing factors move by diffusion through the interchromatin space (*1*–*4*). In the absence of active transport, the transient association of functionally related processing and transcription factors serves to increase local concentrations where they are needed, close to sites of high activity. In some conditions, this gives rise to structures visible by light microscopy. These are, however, the products of underlying RNA-processing activities, not a prerequisite for such activity. Similarly, large changes in the distribution of processing factors, seen for example on viral infection, are consequences of changes in transcription and processing, rather than the causes of such changes.

Coupling of transcription and pre-mRNA processing. The major steps in pre-mRNA processing—capping, splicing, 3′-end cleavage, and polyadenylation—are coupled to transcription (*5*, *6*). This is achieved by recruitment of the capping enzyme, splicing factors, and 3′-processing factors to the largest subunit of RNA polymerase II (pol II) forming a "transcriptosome" (*7*, *8*).

The unique COOH-terminal domain (CTD) of the large subunit of pol II has emerged as a key determinant in coupling transcription and RNA processing (*9*). The two major forms of the enzyme differ by phosphorylation of the CTD. Pol IIa has a low level of CTD phosphorylation and is associated with transcription initiation, whereas pol IIo has high CTD phosphorylation and is associated with transcription elongation. Truncation of the CTD in vivo causes a reduction in pre-mRNA capping (*10*, *11*) and impairs the efficiency of splicing and 3′-end formation (*12*). Similarly, overexpression of the CTD can inhibit pre-mRNA splicing (*13*). These data are discussed in more detail in note (*14*). Together they indicate that the pre-mRNA capping, splicing, and 3′-processing machinery are recruited to the CTD of pol II and then have a short "molecular hop" to the pre-mRNA at their sites of action. This represents an efficient system for delivering processing factors to the nascent pre-mRNA as and when they are needed, a rationale similar to that for the recruitment of factors to other nuclear structures (see below).

Localization of pre-mRNA processing. One of the most critical questions in interpreting the many reported analyses of RNA processing by microscopy is how to identify the active versus inactive populations of transcription and processing factors. A useful analogy is perhaps to consider the flux of bed linen (*15*). This is found in a functionally active form (in the bedroom), undergoes recycling (in the laundry), and is also found in a storage compartment (the closet). From an analysis of the steady-state distribution, it would be difficult to assess which is the functional pool. The greatest concentration is normally in the storage compartment, but increased substrate (arrival of guests) leads to rapid relocation to the active sites, whereas viral infection can lead to abnormal distribution (dirty sheets accumulate in the laundry). Similarly, the greatest concentrations of RNA-processing factors may, or may not, correspond to sites of function, dependent on metabolic activity.

In the interphase nucleus, individual chromosomes occupy discrete compartments, termed chromosome territories (*16*). Actively transcribed genes are generally localized on the surface of these chromosome territories, forming "perichromatin fibers" (*17*). Transcription by pol II in mammalian cells has been localized to 2000 to 3000 sites (*18*, *19*), substantially fewer than the estimated 70,000 to 100,000 nascent transcripts. Similar observations have been made for RNA pol III (*20*), leading to the proposal that RNA polymerases associate into transient complexes or "transcription factories" (*21*). This would act to increase the local concentration of transcription factors and RNA polymerase at sites of active transcription.

Consistent with the coupling of pre-mRNA processing to transcription, spliceosomal snRNAs (small nuclear RNAs) and other general splicing factors show dispersed nucleoplasmic staining that matches the distribution of nascent transcripts (*16*, *22*). Splicing also involves a large family of related "SR proteins," and different pre-mRNAs bind specific subsets of SR proteins (*23*). SRp20 (*24*) and other SR proteins [(*15*) and Fig. 1] are concentrated at a subset of sites of active transcription that presumably correspond to the nascent pre-mRNAs that bind these proteins.

Most, but not all, pre-mRNA–processing factors are also enriched in 20 to 50 "speckles" (*22*, *25*, *26*) that correspond to the IGCs as visualized by electron microscopy (*27*). Inhibition of either splicing or transcription causes the speckles to coalesce into 5 to 10 larger speckles (*18*, *28*). The use of photobleaching techniques in living cells shows that green fluorescent protein (GFP)–tagged splicing factors move rapidly in and out of speckles (*4*). These data suggest that the speckles (IGCs) represent sites where free snRNPs (the complexes between spliceosomal snRNAs and associated proteins) transiently assemble before recruitment by the CTD of RNA pol II and transfer to the nascent transcripts (*29*, *30*). Speckles are frequently localized close to highly transcribed genes, suggesting the stockpiling of processing factors ready

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for use. A class of long-lived polyadenylated RNA is associated with IGCs (*31*) and may act to nucleate this assembly. This would resemble the proposed nucleation of pre-nucleolar bodies by persistent pre–ribosomal RNAs (pre-rRNAs) (see below).

The nucleolus. The problem of delivering components where and when they are needed is particularly acute during ribosome synthesis. A growing HeLa cell synthesizes around 7500 ribosomal subunits per minute, using up some 300,000 ribosomal proteins, and each pre-rRNA must associate with ~150 different species of small nucleolar RNA (snoRNA). How is this vast array of components to be marshaled by diffusion? The answer appears to be self-assembly. The nucleolus self-assembles in association with the ribosomal DNA (rDNA) in response to transcription of the pre-rRNA, gathering together, and thereby concentrating, all of the components needed for ribosome synthesis.

Within the nucleolus the 18*S*, 5.8*S* and 25/28*S* rRNAs are cotranscribed as a large pre-rRNA and processed to the mature rRNAs by a complex series of endonuclease cleavages and exonuclease digestion (*32*). In addition, the rRNAs assemble with the ~80 ribosomal proteins and undergo extensive covalent nucleotide modification, sites of which are selected by guide snoRNAs (Fig. 2).

Formation of the normal nucleolus requires ongoing transcription of the rDNA. Inhibition of pre-rRNA transcription, in yeast or human cells, leads to dissociation of nucleolar components (*33*, *34*). Conversely, the assembly of the nucleolus after mitosis in vertebrates, or in developing *Xenopus* embryos, coincides with resumption of pre-RNA synthesis (*35*). Ectopic expression of an rDNA unit in *Drosophila* (*36*), or pre-rRNA transcription from a pol II promoter on a replicating plasmid in yeast (*37*), results in formation of nucleoplasmic mininucleoli. In each case functional ribosomes are produced in the absence of morphologically normal nucleoli (*36*, *38*).

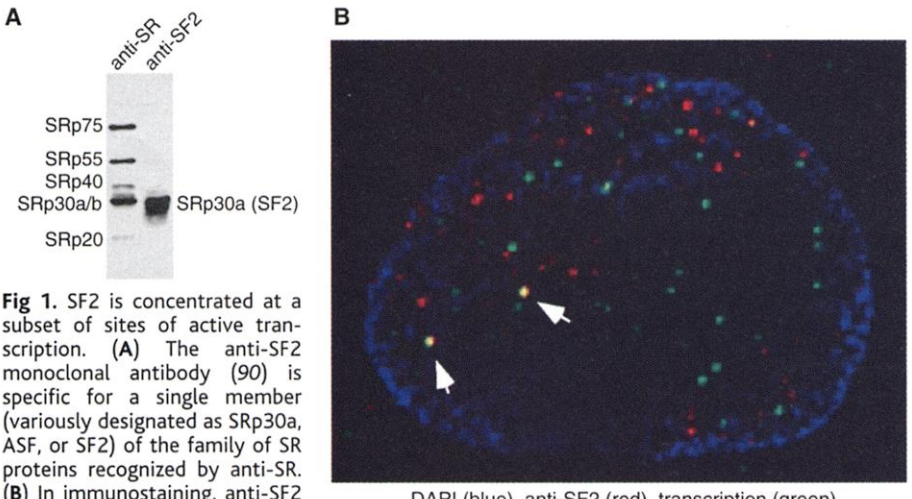

Fig. 1. SF2 is concentrated at a subset of sites of active transcription. (**A**) The anti-SF2 monoclonal antibody (*90*) is specific for a single member (variously designated as SRp30a, ASF, or SF2) of the family of SR proteins recognized by anti-SR. (**B**) In immunostaining, anti-SF2 decorates many particles distributed throughout the nucleoplasm (red channel). The green channel shows sites of bromouridine 5′-triphosphate (BrUTP) incorporation. Arrowheads indicate prominent points of red and green coincidence, indicating that SF2 is concentrated at these sites of active transcription. A single optical section of a HeLa cell nucleus is shown, following deconvolution. [Figure generously provided by K. Neugebauer]

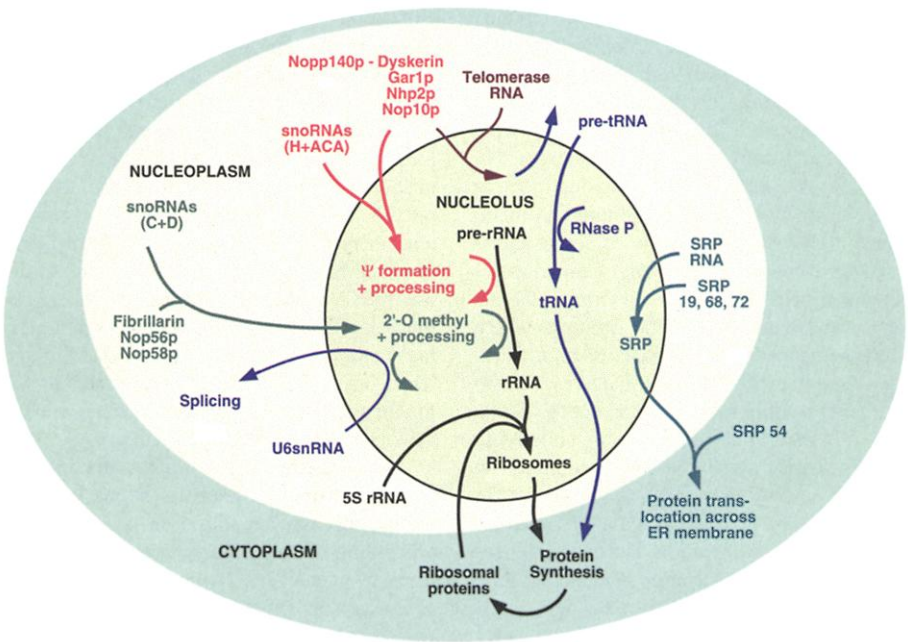

Fig. 2. RNA processing in the nucleolus. Within the nucleolus the pre-rRNAs are processed to the mature rRNAs by endonuclease cleavage and exonuclease digestion; see (*32*). During this processing, the rRNAs assemble with the approximately 80 ribosomal proteins and undergo extensive covalent nucleotide modification. The box C + D class of snoRNAs select sites of 2′-*O*-methylation (*91*, *92*) and associate with three common proteins including Nop1p/fibrillarin, the putative rRNA 2′-*O*-methylase (*93*). The box H + ACA class of snoRNAs select sites of pseudouridine (Ψ) formation (*94*, *95*) and associate with four common proteins including Cbf5p/dyskerin/NAP57 the probable Ψ-synthase (*96*–*98*). The snoRNAs not only carry the modifying enzymes to the pre-rRNA but, by specific base-pairing, create the enzyme recognition site. A small number of snoRNAs of each class, including the U3 snoRNA, are required for processing of the pre-rRNA, probably functioning in the structural reorganization of the pre-rRNA. Ψ formation and 2′-*O*-methylation of the U6 snRNA is also nucleolar, and methylation is directed by box C + D snoRNAs (*99*, *100*). Moreover, there are "orphan" snoRNAs that are predicted to select sites of RNA modification but for which no known target exits (*101*), suggesting that other RNA species, possibly including mRNAs, are modified in the nucleolus and/or CBs (see Fig. 3). The RNA component of human telomerase is targeted to the nucleolus by a 3′ domain that closely resembles the box H + ACA class of snoRNAs (*102*, *103*). Like other H + ACA snoRNAs, human telomerase RNA is associated with dyskerin, mutations in which are associated with the hereditary disease dyskeratosis congenita and with reduced telomerase activity (*102*, *104*). In contrast, the yeast telomerase RNA associates with the Sm-proteins characteristic of the spliceosomal snRNAs (*105*). Initial assembly of another RNA-protein complex, signal recognition particle (SRP) may also occur in the nucleolus. The SRP RNA and three of the six SRP proteins, SRP19, SRP68, and SRP72, are detected in the nucleolus, but not the later assembling SRP54 protein (*106*, *107*). Another major class of RNA, pre-tRNAs, are localized to the yeast nucleolus, together with the pre-tRNA–processing enzyme RNase P (*108*). Human RNase P was similarly found to be localized in the nucleolus and also in coiled bodies (*109*), indicating that pre-tRNA processing is a conserved nucleolar function.

In the absence of pre-rRNA synthesis, components of the ribosome synthesis machinery associate with each other, but not with the rDNA or RNA polymerase I, forming prenucleolar bodies. This association is probably nucleated by long-lived, unprocessed pre-rRNAs that persist throughout mitosis (*39*, *40*) and during *Xenopus* development (*41*). A class of nucleolar proteins (exemplified by human nucleolin and yeast Nsr1p) can bind to nuclear localization signals, and may act to recruit ribosomal proteins to the nucleolus (*42*). Together, these data indicate that nucleolar components have a propensity to associate with each other even in the absence of pre-rRNA transcription and are recruited by association with the nascent pre-rRNAs, regardless of where these are transcribed. In images of interphase cells, the nucleolus appears stable, but it is actually a surprisingly dynamic structure. Photobleaching experiments show that pre-rRNA–processing factors very rapidly exchange with the surrounding nucleoplasm (*4*); the maximum mean residence time of fibrillarin (and presumably the box C + D class of snoRNAs with which it is associated) is less than 40 s. This indicates that these associate transiently, probably by multiple weak interactions, making them available for transfer to the nascent transcripts.

The observed, compact nucleolar structure probably also involves packaging of the rDNA. A partially condensed chromatin state is established by Sir2p, a nicotinamide adenine dinucleotide (NAD)–dependent histone deacetylase (*43*); this also results in suppression of recombination in the rDNA and transcriptional silencing of pol II genes in the rDNA repeats (*44*).

Recent data have shown that many other RNAs also undergo processing and assembly in the nucleolus (see Fig. 2). Moreover, in yeast, both exit from mitosis and the checkpoint for recombination and synapsis at pachytene during meiosis requires the regulated formation and dissociation of nucleolar-associated protein complexes involving Sir2p (*45*–*48*). In mammalian cells, the activity of the tumor suppressor protein p53 can similarly be controlled by the regulated binding and release of the regulatory factor Mdm2 from the nucleolar ARF protein (*49*, *50*). There is little evidence that the nucleolus functions as a corral, that is, a physical barrier that prevents contact with nucleoplasmic components. Rather, nucleolar components may provide a framework for the assembly and organization of other protein complexes and RNA-processing activities.

Coiled (Cajal) bodies. The coiled body (CB) was first identified as the nucleolar accessory body by Ramón y Cajal (*51*, *52*), and the name "Cajal body" has recently been advocated for this structure (*7*).

The protein p80 coilin is commonly used as a marker for coiled bodies; although in most cell types, much of the coilin is dispersed in the nucleoplasm and is slightly enriched in regions associated with IGCs (*53*). Despite their highly structured appearance in electron microscopy, CBs are dynamic, active structures when visualized in living cells (*54*). Cells entering interphase have small or no CBs, which appear and coalesce with time (*54*, *55*) and are particularly prominent in neurons, which have high metabolic activity but do not divide (*51*, *52*).

The synthesis of small nucleolar RNPs (snoRNPs) and snRNPs, and probably the recycling of snRNPs following splicing, may take place in association with complexes involving p80 coilin and the SMN complex (Fig. 3). However, these activities may not require morphological structures visible as CBs. Microinjection of anti-coilin antibodies leads to loss of visible coiled bodies, but the cells lacking CBs are viable and competent for mRNA splicing (*56*). CBs frequently localize adjacent to nucleoli (*51*, *52*), and CB components can be driven to nucleolar association by treatment with a protein phosphatase inhibitor (*57*; *58*). However, we do not think that the CBs represent a system for the physical delivery of snoRNPs or snRNPs to their sites of action. Photobleaching shows that CB components exchange very rapidly with the surrounding nucleoplasm (*59*, *60*), making the CBs ill-suited to act as a transport vehicle. The large number of different RNA species seen in association with CBs has led to the suggestion that they may function in sorting different classes of RNPs (*59*).

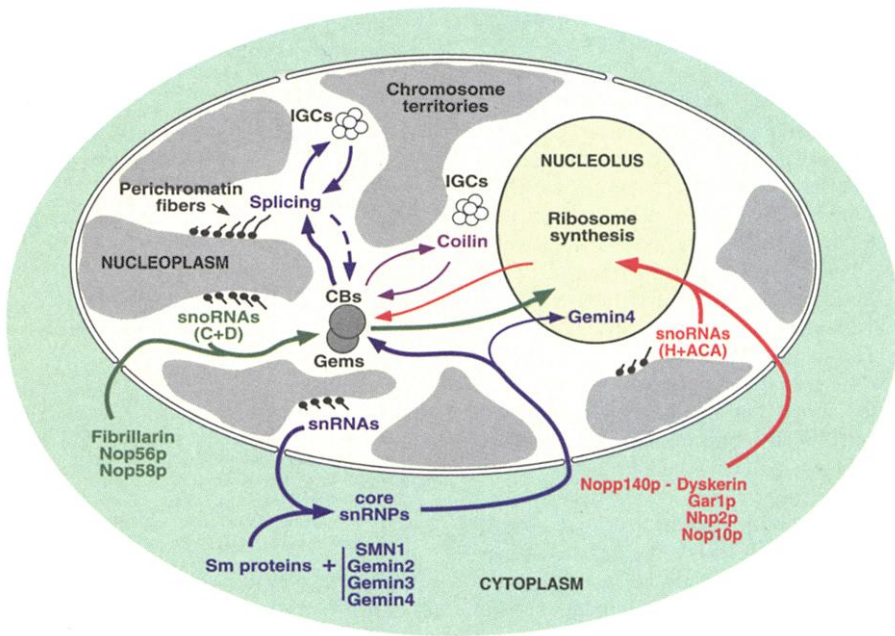

Fig. 3. Coiled (Cajal) bodies and their Gems. In vertebrates, the newly synthesized snRNAs are exported to the cytoplasm where they assemble with the seven common Sm proteins and undergo 3′ processing and cap-trimethylation to form core snRNPs that are reimported into the nucleus. Microinjected snRNAs (*110*, *111*) and transiently expressed Sm proteins (*59*) are observed in CBs before their appearance in other nucleoplasmic regions. In contrast, mature snRNPs do not initially localize to CBs on nuclear reentry after mitosis, suggesting that late snRNP maturation and assembly steps take place in the CBs; for example, covalent modification of the snRNAs by Ψ formation and 2′-*O*-methylation and association with the large numbers of species-specific snRNP proteins. In the cytoplasm, the Sm proteins are associated with the SMN complex, which includes oligomers of the SMN protein (survival of motor neurons), together with Gemin2, Gemin4, and a putative DEAD-box RNA helicase, Gemin3 (*112*–*116*). Each of these proteins also concentrates in nuclear structures called Gems (Gemini of coiled bodies) (*117*). In many cell types, Gems show partial or complete coincidence with CBs (*118*), and their functional distinction is unclear. This suggests that newly synthesized snRNPs are escorted to the nucleus by the SMN complex. This complex may also function in recycling snRNPs following splicing (*119*). Yeast lacks both morphological CBs and an obvious coilin homolog, but does have a Gemin2 homolog, Brr1p. Like Gemin2, Brr1p interacts with Sm proteins and *brr1* mutations inhibit snRNA 3′ processing, suggesting that this pathway is conserved (*113*, *120*). CBs and SMN are also implicated in snoRNP synthesis. Microinjected box C + D snoRNAs localize transiently to CBs before appearing in the nucleoli (*103*, *111*), and mutant snoRNAs that lack an intact box C + D region (the likely protein-binding sites) are retained in the CBs (*111*). In contrast, the H + ACA snoRNAs, and the associated Nopp140p protein, accumulate first in nucleoli and then in CBs (*121*). Multiple interactions between snoRNA-associated proteins, coilin, and SMN have been observed (*117*, *121*, *122*), and Gemin4, at least, is also associated with nucleoli (*116*).

Although this is an attractive idea, the rapid exchange of CB components also makes it hard to see how this would function. Instead, we envisage that the processing, assembly, and probably the modification of many different species of snRNA and snoRNA take place in association with a set of common components including the SMN complex and coilin. This may serve to bring together the RNA and protein components of the particles and the numerous processing and assembly factors.

In *Xenopus* oocytes at least, CBs are also implicated in 3′-end formation of the cell cycle–regulated histone mRNAs, a process involving the U7 snRNA and a stem-loop binding protein. Both of these are present in CBs (*61*, *62*), and coilin binds directly to the U7 snRNP (*63*). The oocyte histone gene cluster is often associated with CBs, and overexpression of U7 snRNA induced the appearance of additional CBs. This suggests that the local concentration of U7 engaged in histone 3′-end formation was able to induce the formation of visible CBs (*64*). In addition to the nucleoli and histone cluster, CBs have a statistically significant tendency to localize near genes encoding snRNAs and snoRNAs in somatic cells (*65*–*68*). Sites of high concentration of snRNAs or snoRNAs may favor association of p80 coilin and the SMN complex into supramolecular structures, visible as coiled bodies.

This model for CB function clearly resembles the model for nucleolar assembly presented above, but also has similarities with current models for nuclear-cytoplasmic transport. This too, is envisaged to involve multiple weak interactions, in this case between transport receptors and nuclear pore components, which concentrate the transport receptor–cargo complexes in the vicinity of the pore and facilitate diffusion through the pore structure.

Conclusions. RNA-processing factors diffuse rapidly in the nucleoplasm but can be concentrated by relatively weak, transient association with the transcription machinery and functionally related components. This system provides increased local concentrations of components that must function together, allowing their efficient recruitment by the RNAs. At sites of high activity this can give rise to visible structures, the nuclear bodies, with components that exchange rapidly with pools in the surrounding nucleoplasm.

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14. The CTD of RNA polymerase II plays a key role in coupling pre-mRNA transcription and processing. The CTD consists of a basic heptad repeat (consensus YSPTSPS repeated 27 times in *S. cerevisiae* and 52 times in human) and is the major site of reversible phosphorylation. Transcription by pol II can be resolved into initiation and elongation phases. Pol IIa, with low CTD phosphorylation, is recruited to the preinitiation complex and generates short transcripts by nonprocessive transcription (*69*). Phosphorylation of the CTD generates pol IIo and is associated with capping of the transcript and transition to processive elongation (*6*). In the initiation complex, the polymerase is blocked from entering elongation mode by the concerted activity of two complexes, termed DSIF and NELF, that interact with pol IIa (*70*, *71*). Phosphorylation of the CTD by P-TEFb (a complex of CDK9 + cyclin T) (*72*) clears these inhibitory factors, as neither can bind pol IIo (*70*, *71*). The mammalian capping enzyme can interact directly with hSPT5 (a component of DSIF) (*73*) and with the phosphorylated CTD. Both interactions result in a severalfold stimulation of guanylyltransferase activity (*73*, *74*). Taken together, these data suggest that the capping enzyme is recruited to the initiation complex by an association with DSIF and then transfers to the phosphorylated CTD, ensuring efficient cotranscriptional capping of the pre-mRNA. The cap plays key roles in subsequent RNA metabolism, in splicing, nuclear export, stability, and translation, and this mechanism ensures that all pol II transcripts are capped before the polymerase enters the elongation phase. The CTD also functions in pre-mRNA splicing and 3′-end formation. Antibodies against the CTD inhibit in vitro splicing as do peptides containing several heptad repeats (*75*). Similarly, exogenously added pol IIa inhibits splicing, whereas pol IIo increases splicing efficiency (*76*). Spliceosomal snRNPs and a subset of the SR-protein splicing factors show preferential binding to the phosphorylated CTD (*77*–*80*), and a discrete CTD-binding domain is present in other splicing factors (*75*). These CTD-binding proteins may act as adapters, directly coupling splicing to transcription. The cap is bound by the nuclear cap-binding complex (CBC), which facilitates efficient splicing of the cap proximal intron (*81*). The reduction of pre-mRNA capping in CTD mutants probably contributes to reduced splicing of the cap proximal intron in vivo. Because the splicing efficiency of cap distal introns is also reduced by CTD truncation, the splicing defect may be directly attributed to the inability of mutant pol II to interact efficiently with these factors. In an in vitro mRNA 3′-processing system, the CTD of RNA pol II was required for efficient cleavage of the pre-mRNA but not for polyadenylation (*82*), and the phosphorylated CTD is approximately two times as active. The two major pre-mRNA cleavage factors, CPSF and CstF, can bind directly to the CTD, independently of its phosphorylation state (*72*). CPSF is recruited to the preinitiation complex via an interaction with the transcription factor TFIID and is subsequently transferred to the phosphorylated CTD (*83*). Both visualization of 3′-end processing in situ (*84*, *85*) and other analyses (*86*–*88*) show that 3′-end formation is also coupled to pre-mRNA splicing. The above data suggest that the CTD acts to bind and concentrate RNA-processing factors in the vicinity of the nascent transcript (*89*), thereby facilitating the efficient processing of the RNA.
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123. We would like to thank A. Bird, M. Carmo-Fonseca, I. Davis, A. I. Lamond, T. Misteli, K. Neugebauer, T. Pederson, and lab members for unpublished data and helpful comments. J.D.L. was supported by the Medical Research Council and D.T. by the Wellcome Trust.