Organization of RNA polymerase II transcription and pre-mRNA splicing within the mammalian cell nucleus

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Introduction

Splicing is an essential step in the processing of intron-containing pre-mRNAs before their transport out of the nucleus (for a review see [1]). During the last few years, a significant number of studies have addressed the relationship of pre-mRNA splicing to nuclear structure (for a review see [2]). Small nuclear ribonucleoprotein particles (snRNPs) as well as several non-snRNP splicing factors have been shown by immunofluorescence microscopy to be localized in a speckled pattern that is distributed in various regions throughout the nucleoplasm. At the electron microscopic level, the speckled pattern corresponds to interchromatin granule clusters and perichromatin fibrils [3–6]. After pulse-labelling with $^{3}H$uridine, incorporation is observed preferentially by electron microscopy at perichromatin fibrils, with little to no labelling of internal regions of interchromatin granule clusters (for a review see [7]). Based on these autoradiography and immunolocalization studies, it has been suggested that perichromatin fibrils may represent the sites of active transcription and interchromatin granule clusters, sites of splicing factor storage and/or assembly. We have examined the relationship of the organization of pre-mRNA splicing factors to the transcriptional and splicing activities of the cell. We have found that splicing factors shuttle between storage and/or assembly sites (interchromatin granule clusters) and sites of active transcription (perichromatin fibrils). Our data suggest a nuclear recruiting mechanism that regulates the distribution of splicing factors.

Splicing factors and RNA polymerase II in transcriptionally active cells

We have examined the effects of changes in transcriptional activity on the nuclear distribution of splicing factors, to test whether splicing factor-enriched nuclear regions are static structures or are responsive to changes in transcriptional activity. Transcriptionally active cells exhibit a characteristic, speckled nuclear immunostaining pattern when cells are immunolabelled with anti-SC-35 [8] monoclonal antibodies or antibodies to several other splicing factors (for a review see [2]). The 20–50 irregularly shaped speckles are present at various places throughout the nucleoplasm and connections can be observed between some of the speckles. However, no immunostaining is found within the nucleoli. At the electron microscopic level the speckled pattern corresponds to interchromatin granule clusters and perichromatin fibrils [6]. To identify the nuclear regions that are active or will be potentially active in RNA polymerase II transcription, at the time of fixation, we localized RNA polymerase II at the electron microscopic level using an antibody that reacts with the heptapeptide repeat of the largest subunit [9]. We found RNA polymerase II to be enriched at perichromatin fibrils and we found little or no labelling of interchromatin granule clusters [10]. The localization of RNA polymerase II and sites of incorporation of $^{3}H$uridine (for a review see [7]) are distributed throughout the nucleoplasm and are not restricted to the nuclear periphery. Since the sublocalization of RNA polymerase II at perichromatin fibrils coincides with previous studies that showed $^{3}H$uridine incorporation to occur at these sites [11], it is likely that these fibrils represent nascent transcripts. Since there is little or no labelling of RNA polymerase II, $^{3}H$uridine incorporation [12, 13] or single- and double-stranded DNA [14, 15] in the interchromatin granule clusters, it is unlikely that these clusters represent sites of high transcriptional activity as was suggested by Carter et al. [16, 17] based on poly(A)$^+$ RNA localization. Perhaps the poly(A)$^+$ RNA localized to the interchromatin granule clusters represents stable RNA species, while the poly(A)$^+$ RNA localized to perichromatin fibrils represents nascent transcripts.

Inhibition of RNA polymerase II transcription

Since SC-35 functions in the processing of pre-mRNA molecules, which are synthesized by RNA polymerase II, we were interested in examining the effect of inhibiting RNA polymerase II on the distribution pattern of this splicing factor. Cells were incubated with α-amanitin at a concentration of 5
μg/ml, which specifically inhibits RNA polymerase II [18]. Cells incubated with the drug for 5 h show an alteration in the shape of the speckles [10, 19]. The speckles appear round, extremely bright in fluorescence intensity and connections between speckles are not observed, compared with control cells. The large round clusters of splicing factors correspond to interchromatin granule clusters. At this length of drug incubation there is a significant decrease in the level of RNA polymerase II transcription, as seen by a dramatic decrease in the number of nucleoplasmic autoradiographic grains.

To be sure that the effects we observed with α-amanitin treatment were specifically due to the inhibition of RNA polymerase II and not to a secondary effect of drug treatment, we performed similar experiments in a Chinese-hamster-ovary cell line, Ama1, which is resistant to α-amanitin [20]. The enzyme from mutant cells has a decreased affinity for binding α-amanitin. Ama1 cells were incubated in 5 μg/ml of α-amanitin for as long as 18–24 h, and the distribution of snRNPs was examined using anti-SC-35 monoclonal antibodies. When compared with control cells that had not been drug-treated, α-amanitin-treated Ama1 cells showed no alteration in the distribution of SC-35 nor in the level of transcription [10]. This finding supports the idea that the drug effect observed in non-resistant Chinese-hamster-ovary cells is due to the inhibition of RNA polymerase II and the subsequent decrease in pre-mRNA transcripts directly. These experiments demonstrated that, on inhibition of RNA polymerase II, interchromatin granule clusters increase in size and become uniform in shape, supporting their role as storage and/or assembly sites (interchromatin granule clusters) where they may be disassembled, recycled and/or await the initiation of new pre-mRNA synthesis. These data suggest that pre-mRNA splicing occurs at the site of transcription (perichromatin fibrils) and that factors shuttle between storage and/or assembly sites (interchromatin granule clusters) and sites of active transcription.

We have previously used adenovirus 2 as a model system to study the organization of splicing factors in the cell nucleus upon the introduction of new DNA templates [21]. The localization of adenoviral RNA sequences was examined throughout the infection process by fluorescence in situ hybridization, and compared with the distribution of several host cell factors that are involved in transcription, pre-mRNA splicing and in packaging RNA transcripts. We have found that, on introduction of new DNA templates into the nucleus, transcription factors, splicing factors and RNA packaging proteins are recruited to these new sites of active viral transcription. Furthermore, when Cos-1 cells were transiently transfected with a plasmid containing a portion of the rat β-tropomyosin gene directed by the simian virus 40 promoter [22] a similar recruitment of splicing factors to new sites of RNA synthesis was observed [21]. Therefore, the recruitment of factors to sites of active transcription seems to be a general occurrence [21, 23–25].

Inhibition of pre-mRNA splicing in vivo
To assess the effect of the inhibition of pre-mRNA splicing on the organization of splicing factors, antisense DNA probes that would specifically interfere with pre-mRNA splicing were microinjected into living cells. An oligonucleotide probe of 20 bases (5'-CTCCCTGCCCAGTAGTAT-3'), complementary to the 5' end of U1 snRNA that base-pairs with the 5' splice site of pre-mRNA and that encompasses sequences that inhibit pre-mRNA splicing in vitro, was microinjected into the cytoplasm of HeLa cells. The oligonucleotide entered the nucleus and within 1 h changes were observed in the organization of the speckled pattern. Similar to the effects observed using drugs that inhibit RNA polymerase II, antisense oligonucleotides targeted to U1 snRNA caused the interchromatin granule clusters to round up and increase in size, and the connections between clusters were no longer visible [10]. However, when a control oligonucleotide (5'-TCCGTTACCAAGCG-3') that has no complementarity to splice sites or regions of interaction between snRNAs was microinjected into cells, no change in the organization of splicing factors was observed [10]. Therefore, interfering with the interaction of U1 snRNP with the 5' splice site of pre-mRNAs resulted in a specific reorganization of splicing factors. Splicing factors return to storage and/or assembly sites (interchromatin granule clusters) where they may be disassembled, recycled and/or await the initiation of new pre-mRNA synthesis. These data suggest that pre-mRNA splicing occurs at the site of transcription (perichromatin fibrils) and that factors shuttle between storage and/or assembly sites (interchromatin granule clusters) and sites of active transcription.

Conclusions
In summary, we have examined the effect of the inhibition of RNA polymerase II on the organization of splicing factors within the interphase nucleus. We have found that in actively transcribing cells splicing factors are localized at active sites of transcription (perichromatin fibrils) and at storage and/or assembly sites (interchromatin granule clusters). On inhibition of RNA polymerase II tran-
scription or pre-mRNA splicing, splicing factors are concentrated at interchromatin granule clusters, which become larger and uniform in shape. When new DNA templates whose pre-mRNA must be spliced are introduced into the cell nucleus, cellular splicing factors are recruited to these new sites of transcription. Our studies have shown a direct correlation between the transcription of RNA polymerase II and the nuclear organization of splicing factors. We propose a recruiting model to account for the dynamic localization of splicing factors. In this model, factors are associated with specific storage and/or assembly sites in the nucleus. Before transcription or at the initiation of transcription, these factors are recruited to the active sites of transcription by another factor or by a chaperone molecule. Evidence to support this model comes from studies that showed that a subpopulation of splicing factors are localized to interchromatin granule clusters in mammalian cell nuclei (for a review see [2]). However, these clusters contain little labelled RNA after short pulses with [3H]uridine (for a review see [7]) and little or no RNA polymerase II, single-stranded or double-stranded DNA [14, 15], suggesting that they probably do not represent active sites of transcription. Therefore, in cell nuclei splicing factors are localized both to sites of active transcription (perichromatin fibrils) and to storage and/or assembly sites (interchromatin granule clusters). Furthermore, upon the introduction of new transcription sites into the cell nucleus, a concomitant decrease in the signal intensity of splicing factors at host cell speckles is observed, with an increase at new active sites of viral transcription, while the overall level of snRNP proteins remains constant [21]. These findings suggest strongly that there are signals generated in the nucleus that regulate the compartmentalization of factors to the nuclear regions where they will be functioning. Identification of these signalling mechanisms will be critical to understanding the integration of a variety of functional events that occur within the boundaries of the nuclear envelope.

The work presented at this meeting was supported by grants from the National Institute of Health (GM42694, SP30CA45508-06) to D.L.S.


Received 26 July 1993