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Dynamic Organization of Pre-mRNA Splicing Factors

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Abstract Studies from several laboratories during the past few years have increased our understanding towards the dynamic organization of pre-mRNA splicing factors in the mammalian cell nucleus. Many well characterized splicing factors have been localized in a speckled pattern in the cell nucleus. Upon the activation of RNA polymerase II transcription, splicing factors are recruited to the sites of transcription from sites of reassembly and/or storage. Nascent intron-containing RNA transcripts are spliced at the sites of transcription. The speckled distribution of splicing factors in the nucleus is altered when either transcription or pre-mRNA splicing activities are interrupted suggesting that the organization of the splicing machinery in the interphase nucleus is a direct reflection of the transcriptional activity of the cell. © 1996 Wiley-Liss, Inc.

Key words: splicing factors, speckled distribution, interchromatin granule clusters, perichromatin fibrils, nuclear structure

The Localization of Splicing Factors in the Cell Nucleus

Pre-mRNA splicing, involving the excision of non-coding intervening sequences and the ligation of exons, is an essential modification for the majority of RNA transcripts transcribed by RNA polymerase II. The basic steps and many of the factors involved in this process have been extensively characterized both in vivo and in vitro. Among the well characterized splicing factors are the major small nuclear ribonucleoprotein particles (snRNP) such as U1, U2, U4, U5, and U6 and other non-snRNP proteins such as members of SR protein family (for reviews see Green, [1991]; Moore et al., [1993]). In spite of an elaborate understanding of the biochemical aspects of pre-mRNA splicing, much less is known regarding the spatial and temporal organization of this process in the mammalian cell nucleus. The localization of the major snRNP particles and the non-snRNP splicing factors such as SC35, SF2/ASF have been described to be a nuclear speckled pattern which consists of an average of 20–50 intensely stained speckles and less intensely stained areas occasionally interconnecting the larger speckles (for a review see Spector et al., [1993]). In addition to the speckled pattern, diffuse staining was also observed throughout the nucleoplasm. In situ hybridization studies using oligonucleotide probes complementary to the major snRNAs demonstrated a similar localization suggesting that the speckled pattern represented fully assembled snRNP particles [Carmo-Fonseca, et al., 1992; Huang and Spector, 1992; Matera and Ward, 1993; Visa et al., 1993]. These findings suggest that there is a well defined three-dimensional organization of the splicing machinery in the mammalian cell nucleus.

When cells enter mitosis, the speckled distribution of splicing factors dissociates concomitant with the condensation of chromatin and the subsequent break down of the nuclear envelope [Spector and Smith, 1986; Leser, et al., 1989; Spector, et al., 1991; Ferreira, et al., 1994]. It is possible that the dissociation of the speckled pattern during mitosis is partially regulated by phosphorylation. A serine kinase which phosphorylates SR proteins has recently been cloned and characterized [Gui et al., 1994; Gui et al., 1994]. The addition of SR protein kinase 1 (SRPK1) to permeabilized cells induces the break down of the speckled distribution of splicing factors [Gui et al., 1994]. At the end of mitosis, the speckled organization of splicing factors reforms in the daughter cell nuclei [Leser et al., 1989; Spector et al., 1991]. The reformation of

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nuclear speckles late in telophase seems to correlate closely with the onset of transcription in the newly formed daughter cell nuclei [Ferreira et al., 1994].

The major snRNPs and SR proteins have been found to be associated with the residual insoluble fraction of the nucleus or nuclear matrix [Fu and Maniatis, 1990; Spector et al., 1983]. Nuclear matrix preparations involve the extraction of cells with detergent, salt and DNase I (for a review see Nickerson and Penman, [1992]). In such preparations, the population of splicing factors that is normally diffusely distributed throughout the nucleus is no longer detected. These observations demonstrate that splicing factors are distributed in the nucleus in at least two populations, a more soluble pool and an insoluble fraction, the nuclear speckles, which display a strong association with the nuclear matrix. However, the snRNPs and SR proteins appear to be associated with nuclear components through different molecular interactions. The speckled distribution of snRNP particles is sensitive to RNase A treatment, whereas the localization of SR proteins is not altered when cells are treated with RNase A [Spector et al., 1991]. The sensitivity of the speckled distribution of snRNPs to RNase A suggests that such localization is dependent upon RNA-protein or RNA-RNA interactions. In contrast, the localization of SR proteins is independent of interactions with RNA [Spector et al., 1991]. It has also been found that newly synthesized RNAs including pre- and mRNA are associated with the nuclear matrix [Mariman et al., 1982; Schroder et al., 1987a,b]. The localization of nascent RNA transcripts, using in situ hybridization, has also been shown to be tightly associated with the nuclear matrix [Xing and Lawrence, 1991]. Furthermore, studies by Zeitlin et al. [1987; 1989] have shown that the pre-mRNA associated with the nuclear matrix can be spliced in the presence of soluble nuclear extracts. Taken together, the close association of splicing factors and nascent RNA transcripts with the nuclear matrix as well as the reconstituted splicing activity of nuclear matrix preparations suggest that pre-mRNA splicing occurs in association with this nuclear structure. It is also possible that splicing factors are part of the structural constituents of the nuclear matrix.

In addition to being localized in a speckled pattern, snRNPs, but not SR proteins, are also found to be localized to nuclear bodies called coiled bodies (for reviews see Brasch and Ochs, [1992]; Lamond and Carmo-Fonseca, [1993]). Coiled bodies were first described by Ramon y Cajal [1903] as nucleolar accessory bodies. These generally round structures consist of coiled fibrillar strands measuring 0.5–1.0 μm in diameter [Monneron and Bernhard, 1969]. In addition to snRNPs, several nucleolar components and a coiled body specific protein, coilin, have been found in coiled bodies (for a review see Lamond and Carmo-Fonseca, [1993]). However, ^{3}H-uridine incorporation studies showed little to no labeling of these bodies after a short pulse [Moreno Diaz de la Espina et al., 1980] suggesting that coiled bodies are unlikely the sites of active RNA synthesis. In addition, the lack of essential splicing factors such as SC35 and SF2/ASF [Carmo-Fonseca et al., 1992; Huang and Spector, 1992; Raska et al., 1991; A. Krainer and D. Spector, unpublished] in these structures suggests that they are probably not the sites of active splicing. Coiled bodies are not present in all cell types. The number of coiled bodies per nucleus and the percentage of cells that contain coiled bodies increases dramatically in immortal cells or cancer cells as compared to primary cells [Spector et al., 1992]. In addition, coiled bodies have been found to be within the nucleoli in some cell lines derived from breast cancer tissues [Ochs et al., 1994]. The function of coiled bodies remains elusive.

The Ultrastructural Analysis of the Localization of Splicing Factors in the Cell Nucleus

When the localization of splicing factors was examined at the electron microscopic level, the speckled distribution pattern observed at the light microscopic level was found to correspond to two distinct nuclear structures, interchromatin granule clusters and perichromatin fibrils (Fig. 1), both of which have been previously defined according to their morphological characteristics (for a review see Fakan and Puvion, [1980]; Spector, [1993]). Interchromatin granule clusters are composed of granules measuring 20–25 nm in diameter which are linked by thin fibrils. The internal regions of the granule clusters do not incorporate ^{3}H-uridine after short pulses of labeling (for a review see Fakan and Puvion, [1980]), and do not immunolabel with anti-DNA antibodies [Turner and Franchi, 1987]. Based on these studies, it is unlikely that

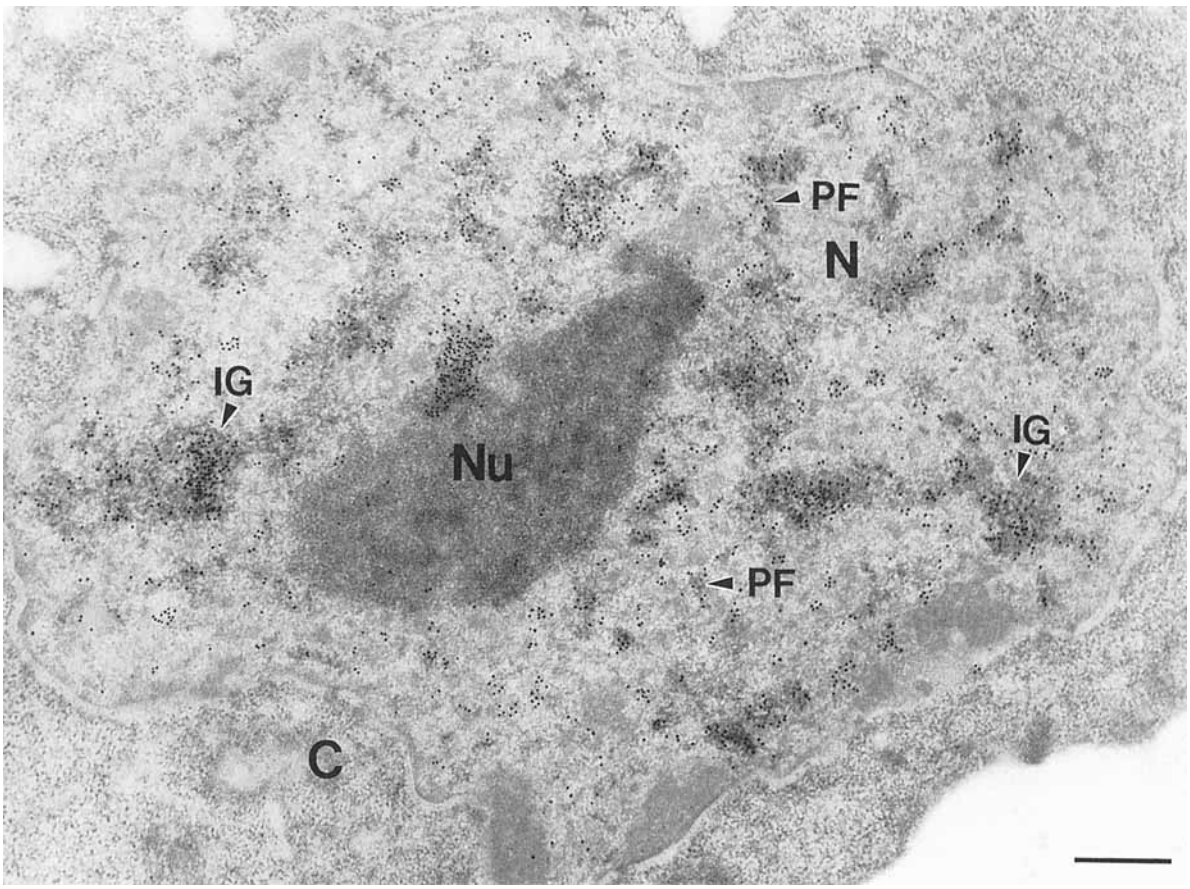

Fig. 1. The splicing factor SC35 is localized in a speckled nuclear pattern, which corresponds to interchromatin granule clusters and perichromatin fibrils, at the electron microscopic level. HeLa cells were immunogold labeled with monoclonal antibody specifically recognizing SC35 and post-stained with the EDTA regressive method [Bernhard, 1969] to reveal RNP enriched structures. IG = interchromatin granule clusters. PF = perichromatin fibrils. N = nucleus. Nu = nucleolus, C = cytoplasm. The scale bar represents 0.5 μm.

interchromatin granule clusters are sites of active transcription. Instead, they have been proposed to be sites of storage and/or reassembly of snRNPs and non-snRNP splicing factors [Jiménez-García and Spector, 1993; Spector et al., 1993]. More recently, the hyperphosphorylated form of RNA polymerase II, a form known to be involved in elongation during transcription, has been shown to be localized to the large speckles (mostly likely interchromatin granule clusters) [Bregman et al., 1995]. Since interchromatin granule clusters are not the sites of RNA synthesis, it is presently unclear if this population of RNA polymerase II is functionally active or if it is a storage form of the polymerase. In contrast to interchromatin granule clusters, perichromatin fibrils actively incorporate ^{3}H-uridine after short pulses (for a review see Fakan, [1994]) suggesting that these structures represent nascent transcripts, and therefore are the sites of active transcription.

Transcription and Pre-mRNA Splicing Are Temporally and Spatially Linked

More recently, several studies have demonstrated that the elaborate organization of splicing factors in a speckled pattern is functionally related to the transcriptional and splicing activities of the cell [Jiménez-García and Spector, 1993; O'Keefe et al., 1994]. When the transcription of RNA polymerase II is inhibited by α-amanitin, the distribution of splicing factors including snRNPs and non-snRNP splicing factors are reorganized into larger and fewer round clusters. These clusters represent fused interchromatin granule clusters when examined using electron microscopy [Spector et al., 1993]. During the inhibition of RNA polymerase II

activity, few perichromatin fibrils, which represent the newly synthesized RNA, are observed. The inhibition of transcription significantly reduces the number of transcripts, and consequently, splicing factors accumulate in the fused interchromatin granule clusters further supporting the idea that these structures are storage and/or reassembly sites for splicing factors. Furthermore, inhibition of pre-mRNA splicing by microinjecting into cells antisense oligonucleotides complementary to snRNAs results in a similar reorganization of splicing factors [O'Keefe et al., 1994]. In this case, cellular transcription was also inhibited. The lack of either pre-mRNA splicing substrate or functional splicing components (snRNPs) resulted in splicing factors accumulating in the fused interchromatin granule clusters implicating that the localization of splicing factors at perichromatin fibrils is a reflection of the need for splicing factors at the sites of transcription.

The suggestion that transcription and pre-mRNA splicing are closely linked has been supported by several earlier studies. An examination of nascent RNA transcripts of early *Drosophila* embryo genes [Beyer and Osheim, 1988] has shown that pre-mRNA splicing occurred co-transcriptionally. Other studies of genes in mouse and *Drosophila* somatic cells [Fakan et al., 1986], puffs and Balbiani rings in *Chironomus* polytene chromosomes [Kiseleva et al., 1994; Sass and Pederson, 1984], as well as the loops of lampbrush chromosomes in amphibian germinol vesicles (Wu et al., 1991) have shown that splicing factors were localized to the active transcription sites. Several nascent cellular RNAs in mammalian cells such as c-fos, fibronectin, and neurotensin have been localized with splicing factors at their sites of transcription [Huang and Spector, 1991; Xing et al., 1993]. Additional work by Zhang et al. [1994] demonstrated that both actin pre-mRNA and mRNA were localized to the actin gene loci suggesting that this RNA was spliced at the sites of its transcription. Furthermore, our recent studies [Huang and Spector, in press] using a transient transfection system have shown that the spatial association between splicing factors and nascent RNA is intron-dependent. Intron-containing RNAs are associated with splicing factors and are spliced at the sites of transcription. Together, these findings clearly demonstrate that transcription and splicing are closely associated both temporally and spatially.

Splicing Factors Are Recruited to the Sites of Intron-Containing RNA Transcription

An interesting question is whether splicing factors are recruited to the sites of transcription or the active genes and/or transcripts move to pre-existing regions in the nucleus that are enriched in splicing factors. This question has been addressed using two different systems. One system uses cells that have been infected with adenovirus [Jiménez-García and Spector, Pombo et al., 1994; 1993] and another system examines transiently transfected cells [Huang and Spector, in press]. When HeLa cells are infected with adenovirus, the newly established viral replication and transcription centers form ring-shaped structures which have been morphologically characterized at both the light and electron microscopic level [Martinez-Palomo et al., 1967; Pombo et al., 1994; Puvion-Dutilleul and Puvion, 1990; Reich et al., 1983; Sugawara et al., 1977; Voelkerding and Klessig, 1986]. When the localization of splicing factors such as snRNPs were examined in the infected cells, the distribution of these factors was found to be colocalized with the adenoviral RNA in the ring structures [Jiménez-García and Spector, 1993; Pombo et al., 1994]. Along with increasing numbers of ring structures of the viral replication and transcription centers, the speckled organization of splicing factors was reduced. In addition to splicing factors, RNA polymerase II, Br-UTP incorporation, and hnRNP C protein have also been shown to localize to these structures with the viral RNA confirming that the ring structures represent the sites of viral RNA transcription [Jiménez-García and Spector, 1993; Pombo et al., 1994]. These findings suggest that splicing factors are recruited to the sites of new RNA synthesis in the infected cells.

Additional support for the recruitment of splicing factors to sites of active transcription comes from recent studies using transiently transfected cells [Huang and Spector, in press]. When cells express transiently transfected templates which encode intron-containing RNA at high levels, splicing factors are localized to the sites of RNA transcription and RNAs are spliced at these loci. The localization of the splicing factors appears to be in areas that are similar in size and shape as the localization of the RNA when examined by optical sectioning using confocal laser scanning microscopy. When the RNAs are expressed at very high levels, the nuclear re-

gions occupied by both splicing factors and RNA are much larger than a regular speckle observed in non-transfected cells. Such unusually large clusters of splicing factors at the sites of RNA transcription suggest that splicing factors are recruited from elsewhere in the nucleus to the active sites of new RNA synthesis. Splicing factors do not colocalize with sites of transcription of intron-less RNAs.

There are at least two different mechanisms by which splicing factors can be recruited to the transcription sites of intron-containing RNA. First, the transcription of intron-containing RNA may trigger a signal transduction system which initiates the movement of splicing factors to the sites of transcription. Such a mechanism could be an elaborate process. The triggering signal could be the newly synthesized intron-containing RNA, or the transcription apparatus itself, such as the binding of transcription factors and/or polymerases to the promoter sequences. Splicing factors which are normally in excess at their sites of storage and/or reassembly would respond to the activation of transcription and move to those sites. One possible way of regulating the localization of splicing components could be via phosphorylation. Recent studies have shown that splicing factors undergo phosphorylations and dephosphorylations at different stages of the pre-mRNA splicing process [Mermoud et al., 1992; Mermoud et al., 1994]. In addition, a serine kinase, SRPK1, has been characterized to be involved in the phosphorylation of the SR protein family [Gui et al., 1994; Gui et al., 1994]. Secondly, it is also possible that there is a soluble pool of splicing components which are ready to function in the nucleoplasm, the continuously generated intron-containing RNAs would bind to those factors and deplete the soluble pool. The excess splicing factors at the sites of storage and/or reassembly would then be released to supplement the soluble pool so that a certain concentration of the soluble factors in the nucleoplasm can be maintained. However, continuous and rapid transcription could eventually exhaust the soluble and the insoluble storage form of splicing factors in the nucleus. Both of these models could explain the recruitment of pre-mRNA splicing factors to sites of active transcription. Future experiments are needed to distinguish between these two or other possibilities.

We hypothesize that the amount of splicing factors being recruited to the sites of transcription is dependent upon the kinetic equilibrium of at least three parameters, the level of transcription, the efficiency of RNA splicing and the dissociation of RNA from the splicing complex. When the level of transcription is low and the splicing efficiency is relatively high, the amount of splicing factors recruited to or localized at the transcription sites could be very small and they may not be detectable. Such was probably the case with the localization of actin RNA where the splicing factor SC35 was not detected at the sites of transcription in over 50 percent of the cases although the RNA was shown to be spliced at those loci [Zhang et al., 1994]. However, when the level of transcription is very high, a large number of splicing factors are recruited to fulfill the splicing task. Such is the case when one examines the sites of transcription of transiently transfected templates or highly expressed endogenous RNAs such as collagen subunits in young fibroblasts [Xing et al., 1995; Huang and Spector, unpublished]. Careful examination using electron microscopy has shown that the large accumulation of splicing factors at the transcription sites of highly expressed templates gives a granular appearance mixed with swirling fibrils which closely resembles the interchromatin granule clusters [Huang and Spector, in press]. Our interpretation is that the granular appearance in interchromatin granule clusters may be due to the presence of the high concentration of splicing factors. The recruitment of splicing factors to a highly active transcription site gives rise to a granular appearance probably due to the high concentration of splicing factors. However, this cluster differs from a typical interchromatin granule cluster in that the splicing factors at the sites of transcription are most likely actively engaged in splicing whereas the factors in the typical interchromatin granule clusters are likely to be stored and/or reassembled at those sites.

The Dynamic Nature of Pre-mRNA Splicing: The Speckle Concept

In summary, we present a unifying model attempting to explain the functional organization of pre-mRNA splicing in the cell nucleus (Fig. 2). We propose that the speckled distribution of splicing factors is a dynamic reflection of the transcriptional status of a typical interphase nucleus. The splicing factors concentrated in the majority of the interchromatin granule clusters are likely to be stored or reassembled at these sites. In contrast, the factors at the peri-

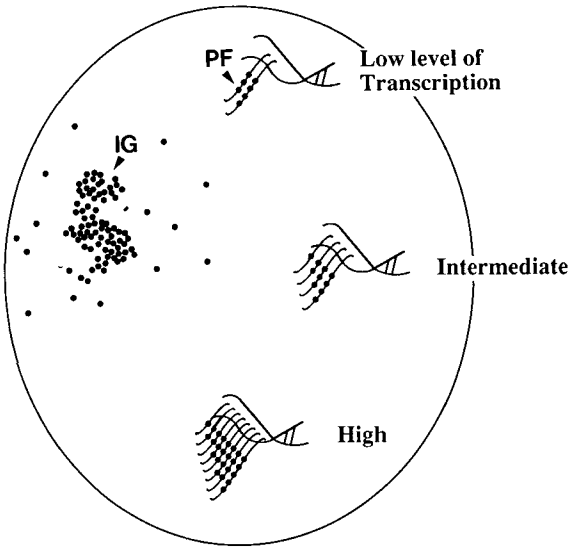

Fig. 2. A model for the functional organization of pre-mRNA splicing in the cell nucleus. IG = interchromatin granule clusters. PF = perichromatin fibrils. When the transcription level is low or intermediate, a small amount of splicing factors are localized at the sites of transcription and these sites are observed as perichromatin fibrils. When the transcription level is very high, a substantial amount of splicing factors are present at the sites of transcription resulting in a granular appearance which closely resembles, but is functionally different from, interchromatin granule clusters.

chromatin fibrils are likely to be actively engaged in splicing as these structures represent the sites of RNA synthesis. Splicing factors are constantly shuttling between the sites of active splicing and the sites of storage and/or reassembly in response to transcriptional activity. When the level of transcription is low or moderate, the splicing factors and nascent RNAs are probably visualized as perichromatin fibrils throughout the nucleoplasm and on the surface of some of the interchromatin granule clusters (Fig. 2). When the level of transcription is very high, the large amount of splicing factors together with nascent RNAs at the transcription site probably results in a morphological structure which closely resembles an interchromatin granule cluster (Fig. 2). Inhibition of either transcription or splicing abolishes the need of the shuttling of splicing factors which results in an accumulation of these factors in the interchromatin granule clusters which become larger and more uniform in shape. We conclude that the distribution of splicing factors and the organization of interchromatin granule clusters is highly regulated and dynamic in response to the transcriptional activity of the cell.

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REFERENCES

Bernhard W (1969): A new staining procedure for electron microscopical cytology. J Ultrastruc Res 27:250–265.

Beyer AL, Osheim YN (1988): Splice site selection, rate of splicing, and alternative splicing on nascent transcripts. Genes Dev 2:754–765.

Brasch K, Ochs RL (1992): Nuclear bodies (NBs): A newly "rediscovered" organelle. Exp Cell Res 202:211–223.

Bregman DB, Du L, van der Zee S, Warren SL (1995): Transcription-dependent redistribution of the large subunit of RNA polymerase II to discrete nuclear domains. J Cell Biol 129:287–298.

Fakan S (1994): Perichromatin fibrils are in situ forms of nascent transcripts. Trends Cell Biol 4:86–90.

Fakan S, Puvion E (1980): The ultrastructural visualization of nucleolar and extranucleolar RNA synthesis and distribution. Int Rev Cytol 65:255–299.

Fakan S, Leser G, Martin TE (1986): Immunoelectron microscope visualization of nuclear ribonucleoprotein antigens within spread transcription complexes. J Cell Biol 103: 1153–1157.

Ferreira JA, Carmo-Fonseca M, Lamond AI (1994): Differential interaction of splicing snRNPs with coiled bodies and interchromatin granules during mitosis and assembly of daughter cell nuclei. J Cell Biol 126:11–23.

Fu X-D, Maniatis T (1990): Factor required for mammalian spliceosome assembly is localized to discrete regions in the nucleus. Nature 343:437–441.

Green MR (1991): Biochemical mechanisms of constitutive and regulated pre-mRNA splicing. Annu Rev Cell Biol 7:559–599.

Gui JF, Lane WS, Fu X-D (1994): A serine kinase regulates intracellular localization of splicing factors in the cell cycle. Nature 369:678–682.

Gui JF, Tronchere H, Chandler SD, Fu X-D (1994): Purification and characterization of a kinase specific for the serine- and arginine-rich pre-mRNA splicing factors. Proc Natl Acad Sci USA 91:10824–10828.

Huang S, Spector DL (1991): Nascent pre-mRNA transcripts are associated with nuclear regions enriched in splicing factors. Genes Dev 5:2288–2302.

Huang S, Spector DL (1992): U1 and U2 small nuclear RNAs are present in nuclear speckles. Proc Natl Acad Sci USA 89:305–308.

Jiménez-García LF, Spector DL (1993): In vivo evidence that transcription and splicing are coordinated by a recruiting mechanism. Cell 73:47–59.

Kiseleva E, Wurtz T, Visa N, Daneholt B (1994): Assembly and disassembly of spliceosomes along a specific premessenger RNP fiber. EMBO J 13:6052–6061.

Lamond AI, Carmo-Fonseca M (1993): The coiled body. Trends Cell Biol 3:198–204.

Leser GP, Fakan S, Martin TE (1989): Ultrastructural distribution of ribonucleoprotein complexes during mitosis. snRNP antigens are contained in mitotic granule clusters. Eur J Cell Biol 50:376–389.

Mariman EC, van Eekelen CAG, Reinders RJ, Berns AJM, van Venrooij WJ (1982): Adenoviral heterogeneous nuclear RNA is associated with the host nuclear matrix during splicing. J Mol Biol 154:103–119.

Martinez-Palomo A, LeBuis J, Bernhard W (1967): Electron microscopy of adenovirus 12 replication. 1. Fine structural changes in the nucleus of infected KB cells. J Virol 1:817–827.

Matera AG, Ward DC (1993): Nucleoplasmic organization of small nuclear ribonucleoproteins in cultured human cells. J Cell Biol 121:715–727.

Mermoud JE, Cohen P, Lamond AI (1992): Ser/Thr-specific protein phosphatases are required for both catalytic steps of pre-mRNA splicing. Nucleic Acids Res 20:5263–5269.

Mermoud JE, Cohen PTW, Lamond AI (1994): Regulation of mammalian spliceosome assembly by a protein phosphorylation mechanism. EMBO J 13:5679–5688.

Monneron A, Bernhard W (1969): Fine structural organization of the interphase nucleus in some mammalian cells. J Ultrastruc Res 27:266–288.

Moore JM, Query CC, Sharp PA (1993): Splicing of precursors to mRNA by the spliceosome. In Gesteland RF, Atkins JF (eds): "The RNA World." Cold Spring Harbor, NY: Cold Spring Harbor Press, pp 303–358.

Moreno Diaz de la Espina S, Sanchez Pina A, Risueno MC, Medina FJ, Fernandez-Gomez, ME (1980): The role of plant coiled bodies in the nuclear RNA metabolism. Electron Micros 2:240–241.

Nickerson JA, Penman S (1992): The nuclear matrix: Structure and involvement in gene expression. In Stein G, Lian J (eds): "Molecular and Cellular Approaches to the Control of Proliferation and Differentiation." New York: Academic Press, pp 343–380.

O'Keefe RT, Mayeda A, Sadowski CL, Krainer AR, Spector DL (1994): Disruption of pre-mRNA splicing in vivo results in reorganization of splicing factors. J Cell Biol 124:249–260.

Ochs RL, Stein TWJ, Tan EM (1994): Coiled bodies in the nucleolus of breast cancer cells. J Cell Sci 107:385–399.

Pombo A, Ferreira J, Bridge E, Carmo-Fonseca M (1994): Adenovirus replication and transcription sites are spatially separated in the nucleus of infected cells. EMBO J 13:5075–5085.

Puvion-Dutilleul F, Puvion E (1990): Replicating single-stranded adenovirus type 5 DNA molecules accumulate within well-delimited intranuclear areas of lytically infected HeLa cells. Eur J Cell Biol 52:379–388.

Ramon y Cajal S (1903): Un sencillo metodo de coloracion selectiva del reticulo protoplasmico y sus efectos en los diversos organos nerviosos. Trab Lab Invest Biol 2:129–221.

Raska I, Andrade LEC, Ochs RL, Chan EKL, Chang CM (1991): Immunological and ultrastructural studies of the nuclear coiled body with autoimmune antibodies. Exp Cell Res 195:27–37.

Reich NC, Sarnow P, Duprey E, Levine AJ (1983): Monoclonal antibodies which recognize native and denatured forms of the adenovirus DNA-binding protein. Virology 128:480–484.

Sass H, Pederson T (1984): Transcription-dependent localization of U1 and U2 small nuclear ribonucleoproteins at major sites of gene activity in polytene chromosomes. J Mol Biol 180:911–926.

Schroder HC, Trolltsch D, Friese U, Bachmann M, Muller WE (1987a): Mature MRNA is selectively released from the nuclear matrix by an ATP/dATP-dependent mechanism sensitive to topoisomerase inhibitors. J Biol Chem 262:8917–8925.

Schroder HC, Trolltsch D, Wenger R, Bachmann M, Diehl-Seifert B, Muller WE (1987b): Cytochalasin B selectively releases ovalbumin mRNA precursors but not the mature ovalbumin mRNA from hen oviduct nuclear matrix. Eur J Biochem 167:239–245.

Spector DL (1993): Macromolecular domains within the cell nucleus. Annu Rev Cell Biol 9:265–315.

Spector DL, Schrier WH, Busch H (1983): Immunoelectron microscopic localization of snRNPs. Biol Cell 49:1–10.

Spector DL, Fu X-D, Maniatis T (1991): Associations between distinct pre-mRNA splicing components and the cell nucleus. EMBO J 10:3467–3481.

Spector DL, Lark G, Huang S (1992): Differences in snRNP localization between transformed and nontransformed cells. Mol Biol Cell 3:555–569.

Spector DL, O'Keefe RT, Jiménez-García LF (1993): Dynamics of transcription and pre-mRNA splicing within the mammalian cell nucleus. Cold Spring Harb Symp Quant Biol 58:799–805.

Sugawara K, Gilead Z, Wold M, Green M (1977): Immunofluorescence study of the adenovirus type 2 single-stranded DNA-binding protein in infected and transformed cells. J Virol 22:527–539.

Turner BM, Franchi L (1987): Identification of protein antigens associated with the nuclear matrix and with clusters of interchromatin granules in both interphase and mitotic cells. J Cell Sci 87:269–282.

Visa N, Puvion-Dutilleul F, Bachellerie J-P, Puvion E (1993): Intranuclear distribution of U1 and U2 snRNA visualized by high resolution in situ hybridization: Revelation of a novel compartment containing U1 but not U2 snRNA in HeLa cells. Eur J Cell Biol 60:308–321.

Voelkerding K, Klessig DF (1986): Identification of two nuclear subclasses of the adenovirus type 5-encoded DNA-binding protein. J Virol 60:353–362.

Wu Z, Murphy C, Callan HG, Gall JG (1991): Small nuclear ribonucleoproteins and heterogeneous nuclear ribonucleoproteins in the amphibian germinal vesicle: Loops, spheres and snurposomes. J Cell Biol 113:465–483.

Xing Y, Lawrence JB (1991): Preservation of specific RNA distribution within the chromatin-depleted nuclear substructure demonstrated by in situ hybridization coupled with biochemical fractionation. J Cell Biol 112:1055–1063.

Xing Y, Johnson CV, Dobner PR, Lawrence JB (1993): Higher level organization of individual gene transcription and RNA splicing. Science 259:1326–1330.

Xing Y, Johnson CV, Moen PT Jr., McNeil JA, Lawrence JB (1995): Nonrandom gene organization: structural arrangements of specific pre-mRNA transcription and splicing with SC-35 domains. J Cell Biol 131:1635–1647.

Zeitlin S, Parent A, Silverstein S, Efstratiadis A (1987): Pre-mRNA splicing and the nuclear matrix. Mol Cell Biol 7:111–120.

Zeitlin S, Wilson RC, Efstratiadis A (1989): Autonomous splicing and complementation of in vivo–assembled spliceosomes. J Cell Biol 108:765–777.

Zhang G, Taneja KL, Singer RH, Green MR (1994): Localization of pre-mRNA splicing in mammalian nuclei. Nature 372:809–812.