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Nuclear Speckles

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Nuclear speckles, also known as interchromatin granule clusters, are nuclear domains enriched in pre-mRNA splicing factors, located in the interchromatin regions of the nucleoplasm of mammalian cells. When observed by immunofluorescence microscopy, they usually appear as 20–50 irregularly shaped structures that vary in size. Speckles are dynamic structures, and their constituents can exchange continuously with the nucleoplasm and other nuclear locations, including active transcription sites. Studies on the composition, structure, and dynamics of speckles have provided an important paradigm for understanding the functional organization of the nucleus and the dynamics of the gene expression machinery.

The mammalian cell nucleus is a highly compartmentalized yet extremely dynamic organelle (reviewed in Misteli 2001a; Spector 2006; Zhao et al. 2009). Many nuclear factors are localized in distinct structures, such as speckles, paraspeckles, nucleoli, Cajal bodies, polycomb bodies, and promyelocytic leukemia bodies and show punctate staining patterns when analyzed by indirect immunofluorescence microscopy (reviewed in Lamond et al. 1998; Spector 2001; Spector 2006).

In mammalian cells the pre-mRNA splicing machinery, including small nuclear ribonucleoprotein particles (snRNPs), spliceosome subunits, and other non-snRNP protein splicing factors, shows a punctate nuclear localization pattern that is usually termed "a speckled pattern" but has also been referred to as "SC35 domains (Wansink et al. 1993)" or "splicing factor compartments (Phair et al. 2000)" (Figs. 1 and 2). The first detailed description of the nuclear domains that we presently refer to as nuclear speckles was reported by Santiago Ramón y Cajal in 1910 (Ramón y Cajal 1910; reviewed in Lafarga et al. 2009). Ramón y Cajal used acid aniline stains to identify structures he referred to as "grumos hialinas" (literally "translucent clumps"). The term "speckles" was first put forth in 1961 by J. Swanson Beck (Beck 1961) upon examination of rat liver sections immunolabeled with the serum of individuals with autoimmune disorders. Although the connection was not made at the time, these speckles had been identified two years earlier by Hewson Swift (Swift 1959) at the electron microscopic level and called interchromatin particles. Swift observed that these particles were not randomly distributed but that they occurred in localized

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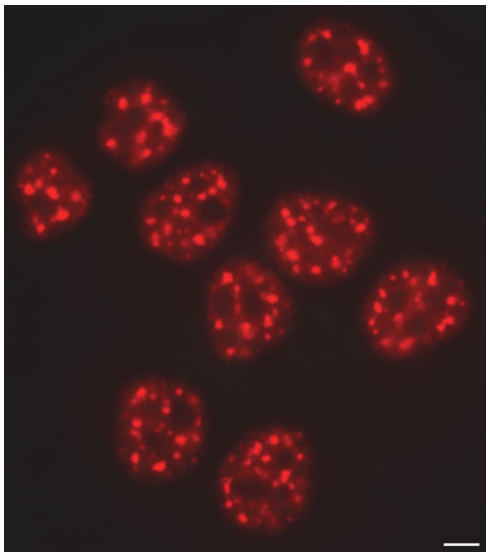

Figure 1. Speckles form in the interchromatin space. HeLa cells showing splicing factors localized in a speckled pattern as well as being diffusely distributed throughout the nucleoplasm. Bar = 5 μm.

"clouds," and cytochemical analysis indicated that they contained RNA (Swift 1959). However, the first link between pre-mRNA splicing and nuclear speckles or interchromatin granule clusters came from an examination of the distribution of snRNPs using anti-splicing factor-specific antibodies, demonstrating a speckled distribution pattern of snRNPs in cell nuclei (Lerner et al. 1981; Perraud et al. 1979; Spector et al. 1983).

It is now clear that much of the punctate localization of splicing factors observed by immunofluorescence microscopy corresponds to the presence of these factors in nuclear speckles of variable size and irregular shape that are revealed by electron microscopy as interchromatin granule clusters (IGCs) (Fig. 3). IGCs range in size from one to several micrometers in diameter and are composed of 20–25 nm granules that are connected in places by a thin fibril resulting in a beaded chain appearance (Thiry 1995b). These structures can be observed by electron microscopy without antibody labeling (Thiry 1995b). We will define "speckles" here specifically as the IGC component of the splicing factor labeling pattern, and distinguish this from other nuclear structures, including perichromatin fibrils and Cajal bodies, which also contain splicing factors (reviewed in Fakan 1994; Spector 1993).

For some of the speckle components a speckle targeting signal has been identified. The arginine/serine-rich domain (RS domain) of some SR pre-mRNA splicing factors has been shown to be necessary and sufficient for the targeting of these factors to nuclear speckles (Caceres et al. 1997; Hedley et al. 1995; Li et al. 1991). In addition, the threonine-proline repeats of SF3b1 (Eilbracht et al. 2001) and the forkhead-associated domain in NIPP1 (Jagiello et al. 2000) have also been implicated in speckle-targeting. Most recently, Salichs et al. (Salichs et al. 2009) performed a genome-wide analysis of homopolymeric histidine tracts and identified 86 human proteins that contain stretches of five or more histidines. Of the 22

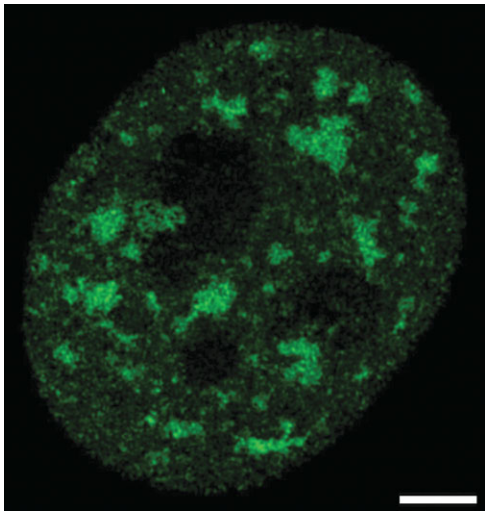

Figure 2. Structured illumination microscopy, using the OMX system (Applied Precision, Issaqua, Washington), of a HeLa cell expressing SC35-EYFP. At 100 nm resolution substructure can be observed within speckles. In addition, the diffuse population of SC35-EYFP is resolved as a granular distribution. Projection of twelve 0.125 μm optical sections through the center of a nucleus encompassing 1.5 μm. Image provided by Zsolt Lazar and R. Ileng Kumaran. Bar = 2 μm.

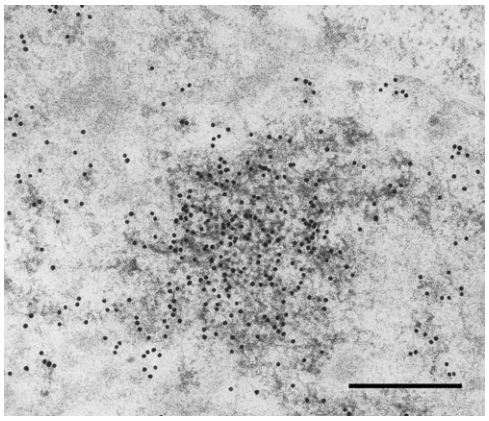

Figure 3. Nuclear speckles are equivalent to interchromatin granule clusters. Immunoelectron microscopy using a primary antibody against SC35 and a secondary antibody conjugated to 15 nm colloidal gold. IGCs are composed of a series of particles measuring 20–25 nm in diameter that are connected in places by a thin fibril resulting in a beaded chain appearance. Bar = 500 nm.

that were nuclear localized, 15 were shown to be present in nuclear speckles. Based on these data, the polyHis-repeats were proposed to act as a speckle-targeting signal that functions by acting as an interaction surface for resident nuclear speckle constituents. Interestingly, these targeting signals rely mostly on charge effects, being basic protein regions.

Interestingly, structures similar to nuclear speckles have been identified in the amphibian oocyte nucleus (Gall et al. 1999) and in *Drosophila melanogaster* embryos when transcription increases upon cellularization during cycle 14 (Segalat et al. 1992), but not in yeast (Potashkin et al. 1990). Importantly, not all nuclear proteins that show a speckle-like labeling pattern by immunofluorescence microscopy localize to IGCs. For example, the ER repeat protein YT521-B localizes in a speckled-like distribution that corresponds to YT bodies (Nayler et al. 2000), whereas PSPC1 localizes in approximately 5–20 punctate interchromatin structures termed "paraspeckles," which are also distinct from nuclear speckles (Fox et al. 2002; Fox and Lamond 2010). Therefore, it is essential to perform double-label immunofluorescence studies using an anti-splicing factor antibody to confirm the localization of any novel factors to nuclear speckles.

STRUCTURE AND LOCATION OF SPECKLES

As determined by both light and electron microscopy, the clusters of interchromatin granules that constitute speckles form throughout the nucleoplasm in regions containing little or no DNA (Thiry 1995b). Although they apparently contain few, if any, genes speckles are often observed close to highly active transcription sites. This suggests that they likely have a functional relationship with gene expression, and some specific genes have been reported to preferentially localize near speckles (Brown et al. 2008; Huang et al. 1991; Johnson et al. 2000; Moen et al. 2004; Smith et al. 1999; Xing et al. 1993; Xing et al. 1995), although this does not appear to be obligatory for transcription/pre-mRNA splicing. Interestingly, Shopland et al. (Shopland et al. 2003) found that gene-rich chromosomal regions (R-bands) are more frequently found along the edge of nuclear speckles than gene-poor regions (G-bands). In addition, coordinately expressed active genes can be found in association with the same nuclear speckle. Based on these findings, Shopland et al. (2003) suggested that nuclear speckles act as functional centers that organize active genes on their periphery to form euchromatic neighborhoods.

Several lines of evidence point to speckles acting as storage/assembly/modification compartments that can supply splicing factors to active transcription sites (reviewed in Lamond and Spector 2003). For example, a series of high resolution pulse-labeling experiments analyzed at the electron microscopic level, studying the incorporation of either tritiated uridine or Br-UTP after short pulses, have shown that nascent pre-mRNA is predominantly localized outside of nuclear speckles (IGCs) in fibrillar structures, 3–5 nm in diameter, which are termed perichromatin fibrils (PFs) (Cmarko et al. 1999; Fakan et al. 1971; Fakan et al. 1978; Monneron et al. 1969). It is likely that most of the cotranscriptional splicing

is associated with these PFs, rather than within IGCs. PFs can occur both on the periphery of IGCs and in nucleoplasmic regions away from IGCs (Fakan 1994).

Some apparent discrepancies in the literature concerning the possible direct role of speckles as splicing sites may have arisen because the PFs can show a close topological relationship with the periphery of IGCs. Using the fluorescence microscope it is difficult to distinguish these PFs from the IGCs. In addition, as highly expressed genes will recruit a significant amount of pre-mRNA splicing factors (Huang et al. 1996), these regions of highly active transcription will be indistinguishable from IGCs at the fluorescence microscopy level. Although many in the field do not view speckles as direct transcription/pre-mRNA splicing centers, others suggest that they may have a more direct role relating to the splicing and transport of pre-mRNA (reviewed in Hall et al. 2006; Melcak et al. 2000; Shopland et al. 2002; Wei et al. 1999).

COMPOSITION OF SPECKLES

Many pre-mRNA splicing factors, including snRNPs and SR proteins (Fu 1995), have been localized to nuclear speckles by either immunofluorescence, fluorescent protein-tagging, and/or immunoelectron microscopy. In fact, this speckled localization pattern is highly diagnostic for proteins involved in pre-mRNA splicing. In addition, several kinases (Clk/STY, hPRP4, and PSKHI) (Brede et al. 2002; Colwill et al. 1996; Ko et al. 2001; Kojima et al. 2001; Sacco-Bubulya et al. 2002) and phosphatases (PP1) (Trinkle-Mulcahy et al. 1999; Trinkle-Mulcahy et al. 2001) that phosphorylate/dephosphorylate components of the splicing machinery have also been localized to nuclear speckles. This supports the idea that speckles may be involved in regulating the pool of factors that are accessible to the transcription/pre-mRNA processing machinery (reviewed in Misteli et al. 1997b).

The protein composition of nuclear speckles has been assessed by proteomic analysis of an enriched IGC fraction purified from mouse liver nuclei. This approach identified 146 known proteins, as well as numerous uncharacterized proteins (Mintz et al. 1999; Saitoh et al. 2004). The proteomic information, together with additional localization studies, has revealed that speckles contain many other proteins apart from pre-mRNA splicing factors. Of particular interest is the localization of transcription factors (Larsson et al. 1995; Mortillaro et al. 1996; Zeng et al. 1997), 3′-end RNA processing factors (Krause et al. 1994; Schul et al. 1998), eukaryotic translation initiation factor eIF4E (Dostie et al. 2000), eif4AIII, a protein involved in translation inhibition (Li et al. 1999), and structural proteins (Jagatheesan et al. 1999; Nakayasu et al. 1984; Sharma et al. 2010). Consistent with these findings, proteomic analyses of in vitro assembled spliceosomes indicate that they may also contain transcription and 3′-end RNA processing factors, together with splicing factors, in a higher order complex (Rappsilber et al. 2002; Zhou et al. 2002). However, they do not contain factors primarily involved in ribosome subunit biogenesis or tRNA production, and the protein composition of speckles underlines their close relationship with mechanisms of gene expression by RNA Pol II.

Although transcription does not take place within the majority of nuclear speckles (Cmarko et al. 1999) and DNA is not localized to these nuclear regions (reviewed in Thiry 1995b), a population of the serine-2-phosphorylated form of the RNA polymerase II (RNAPII) large subunit (LS) that is involved in elongation has been localized to these regions by immunofluorescence microscopy (Bregman et al. 1995; Mortillaro et al. 1996). In addition, biochemical characterization of the IGC proteome has identified several subunits of RNAPII (Mintz et al. 1999; Saitoh et al. 2004), supporting the presence of a pool of RNAPII in speckles. However, other studies have not observed an enrichment of RNAPII in speckles (Grande et al. 1997; Kimura et al. 2002; Zeng et al. 1997) and it is not present in B snurposomes (Doyle et al. 2002).

The Cdk9-cyclin T1 complex, also known as TAK/P-TEFb, is thought to be involved in transcriptional elongation via phosphorylation of the RNAPII LS (reviewed in Price 2000). This complex was found diffusely distributed throughout the nucleoplasm, but not in nucleoli

(Herrmann et al. 2001). In addition, a significant overlap between cyclin T1 and nuclear speckles was observed. However, although Cdk9 was present in the vicinity of nuclear speckles, the degree of overlap was limited (Herrmann et al. 2001; Matera et al. 1993).

Further evidence for a link between transcription and speckles comes from observations on FBI-1, a cellular POZ-domain-containing protein that binds to the HIV-1 long terminal repeat and associates with the HIV-1 transactivator protein Tat (Pessler et al. 1997). FBI-1 has been found to partially colocalize with Tat and its cellular cofactor, P-TEFb at nuclear speckles (Pendergrast et al. 2002). In addition, the nucleosome binding protein HMG-17, which can alter the structure of chromatin and enhance transcription, has been localized in a similar pattern to FBI-1 (Hock et al. 1998). Therefore, although little or no transcription takes place in nuclear speckles, a subset of proteins involved in this process are associated with these nuclear regions in addition to being present at transcription sites. Although it is currently unclear what determines the subset of transcription factors that are localized to nuclear speckles, their presence may relate to the assembly of higher-order complexes and/or to regulatory steps affecting either the modification state or accessibility of specific transcription factors.

In addition to transcription factors, a population of poly(A)$^+$ RNA has been localized to nuclear speckles (Carter et al. 1991; Huang et al. 1994; Visa et al. 1993). This population of poly(A)$^+$ RNA does not chase to the cytoplasm when transcription is blocked with α-amanitin, as would be expected if these species represented nascent mRNA (Huang et al. 1994). Interestingly, Hutchinson et al. (Hutchinson et al. 2007) identified MALAT1 (metastasis-associated lung adenocarcinoma transcript 1), a long nuclear retained noncoding RNA to be enriched in nuclear speckles (reviewed in Wilusz et al. 2009). Recent studies have implicated MALAT1 in the recruitment of SR splicing factors from nuclear speckles to sites of transcription (Bernard et al. 2010) and in the regulation of alternative splicing by modulating SF2/ASF phosphorylation (Tripathi et al. 2010).

Although an underlying scaffold that would serve as a platform on which to organize IGCs has thus far not been identified (Sacco-Bubulya et al. 2002), several proteins with possible structural roles in the nucleus, such as a population of lamin A (Jagatheesan et al. 1999) and snRNP-associated actin (Nakayasu et al. 1984), have been detected in nuclear speckles. However, another study failed to detect an alteration in nuclear speckles in LMNA-/- cells (Vecerova et al. 2004). In addition to actin, phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2], a lipid that regulates actin-binding proteins (Zhao et al. 1998), as well as multiple phosphatidylinositol phosphate kinase (PIPK) isoforms, have also been localized to nuclear speckles (Boronenkov et al. 1998). Recently, Sharma et al. (Sharma et al. 2010) have implicated the 2530-amino acid Son protein in the organization of nuclear speckles. Son contains a concentrated region of multiple tandem repeat sequences including multiple serine-rich repeats and an RS domain. Based on RNAi depletion experiments, Son was proposed to act as a scaffolding protein for RNA processing factors in nuclear speckles (Sharma et al. 2010).

DYNAMICS OF NUCLEAR SPECKLES

Speckles are dynamic structures; their size, shape, and number can vary, both between different cell types and within a cell type, according to the levels of gene expression and in response to signals that influence the pools of active splicing and transcription factors available. When transcription is halted, either by the use of inhibitors, or as a result of heat shock, splicing factors accumulate predominantly in enlarged, rounded speckles (Melcak et al. 2000; Spector et al. 1991; Spector et al. 1983). The fact that nuclear speckles become round and increase in size upon transcriptional inhibition supports the view that speckles may function in the storage/assembly/modification of splicing factors, and that they are not direct sites of splicing. Furthermore, when expression of intron-containing genes increases (Huang et al. 1996; Misteli et al. 1997a), or during viral infection when transcription levels are high (Bridge et al.

1995; Jiménez-García et al. 1993), the accumulation of splicing factors in speckles is reduced, and they redistribute to nucleoplasmic transcription sites. Individual speckle components can therefore shuttle continually between speckles and active gene loci. Speckles are also dynamically regulated during mitosis.

The movement of factors into and out of speckles can be directly visualized by fluorescence microscopy as fluctuations in the shape and intensity of speckles in live cells expressing splicing factor/fluorescent protein fusions (Misteli et al. 1997a). Speckles in such cells show transcription-dependent peripheral movements, although individual speckles remain in their neighborhoods. Photobleaching techniques have also been used to measure the flux of some speckle components and have shown that their exchange rate is very rapid (Kruhlak et al. 2000; Phair et al. 2000). Complete recovery for GFP-SF2/ASF (a member of the SR-family of pre-mRNA splicing factors), after photobleaching of the fluorescence signal in speckles, was apparent in approximately 30 seconds with half recovery in approximately 3–5 seconds. The movement rates for splicing factors through the nucleoplasm were measured to be slow compared with free GFP; this reduction in movement was proposed to result from multiple transient interactions of splicing factors with nuclear binding sites, both within and outside of speckles. Kinetic modeling indicated that the maximal mean residence time for GFP-SF2/ASF in speckles was less than 50 seconds (Phair et al. 2000). It is a remarkable feature of nuclear organization that at steady-state the overall structure of speckles, as well as other nuclear domains, persists despite the large flux of their components.

THE SPECKLE CELL CYCLE

Upon entry into mitosis and following breakdown of the nuclear envelope/lamina, proteins associated with nuclear speckles become diffusely distributed throughout the cytoplasm (Ferreira et al. 1994; Reuter et al. 1985; Spector et al. 1986; Thiry 1995a). During metaphase, these proteins continue to localize in a diffuse cytoplasmic pattern and also accumulate within one to three small structures called mitotic interchromatin granules (MIGs) (Ferreira et al. 1994; Leser et al. 1989; Prasanth et al. 2003; Verheijen et al. 1986). MIGs appear to be structurally analogous to IGCs (Leser et al. 1989; Thiry 1993; Thiry 1995a). As mitosis progresses from anaphase to early telophase, the MIGs increase in number and size. During mid- to late-telophase and after re-formation of the nuclear envelope/lamina, pre-mRNA splicing factors enter daughter nuclei and, concomitantly, their localization in MIGs decreases, demonstrating that these factors are recycled from the cytoplasmic MIGs into daughter nuclei (Prasanth et al. 2003). Live-cell studies have indicated that the majority of these factors enter daughter nuclei within 10 minutes (Prasanth et al. 2003).

Although MIGs have been proposed to be the mitotic equivalent of nuclear speckles (Ferreira et al. 1994; Leser et al. 1989; Thiry 1995a; Thiry 1995b), their function in mitotic cells is unclear. In telophase cells, some MIGs were found to be in close proximity to the newly formed nuclear envelope (Prasanth et al. 2003; Thiry 1995a). This close proximity of MIGs to the nuclear periphery and the disappearance of MIGs in late telophase cells with the concomitant appearance of IGCs in daughter nuclei have suggested that the MIGs might be directly transported into the nuclei (Leser et al. 1989; Thiry 1995a). However, colocalization of SF2/ASF and a hyperphosphorylated form of RNAPII LS in MIGs of late telophase cells has suggested that this may not be the case. For example, SF2/ASF and other pre-mRNA processing factors were shown to enter daughter nuclei while a subpopulation of SC35 and RNAPII LS remained in MIGs until G1, demonstrating that various components of MIGs are differentially released for subsequent entry into daughter nuclei (Prasanth et al. 2003).

Further support for differential release of factors from MIGs comes from an earlier study that reported the nuclear import of snRNPs while cytoplasmic MIGs were still labeled with anti-SR protein and anti-SC35 antibodies (Ferreira et al. 1994). Based on these findings, it was suggested that MIGs may play important roles

either in the modification of the components of the splicing machinery before their nuclear entry, or as enriched populations of these factors, allowing for protein-protein interactions to occur between subsets of proteins before their nuclear entry (Prasanth et al. 2003).

Interestingly, splicing factors were shown to be competent for pre-mRNA splicing immediately upon entry into daughter nuclei (Prasanth et al. 2003), supporting the possibility that MIGs may be responsible for splicing factor modification, allowing for immediate targeting of modified (phosphorylated) pre-mRNA processing complexes to transcription sites in telophase nuclei. Because daughter nuclei late in telophase have not yet assembled nuclear speckles, cytoplasmic MIGs are likely to function as their counterparts to provide competent pre-mRNA splicing factors to the initial sites of transcription in newly formed nuclei (Prasanth et al. 2003). Perhaps splicing factors are released from MIGs via hyperphosphorylation, as has been shown for their release from nuclear speckles in interphase nuclei.

SPECKLE BIOGENESIS

Nuclear speckles are one of the most prominent nuclear compartments, and their study has served as a paradigm for understanding the biogenesis of nuclear bodies. Most evidence points to the fact that nuclear speckles form through a process of self-assembly (reviewed in Misteli 2001b) whereby transient macromolecular interactions form the basis of speckle morphogenesis. Under steady-state conditions, the respective rates of association and disassociation of individual speckle components will define their exchange rates and the sizes of their bound and soluble pools in the nucleus. Regulatory mechanisms can influence these association and/or disassociation rates, thereby changing the fraction of bound and soluble speckle components in response to specific cellular signals.

In this view, the entry of splicing factors into late-telophase nuclei results in an association of a subset of these factors with initial transcription/pre-mRNA processing sites (Prasanth et al. 2003). As the population of factors increases, there is an increased probability of protein-protein interactions among those factors not engaged in transcription/pre-mRNA processing, resulting in the formation of nuclear speckles. These initial speckles appear to form predominantly in nucleoplasmic regions that are devoid of chromosome territories and/or other nuclear organelles. They may initiate either at random locations, or in the vicinity of genes that are transcribed at high levels during the telophase/G1 transition. Interestingly, in this regard Brown et al. (Brown et al. 2008) examined the position of various erythroid genes in erythroblasts and found that the majority of associations between erythroid genes occurred at a nuclear speckle. Interestingly, the associations were predominantly observed in regard to active genes. Based on their findings, the authors proposed that active genes can be brought into close proximity by the nucleation of splicing factors into nuclear speckles.

The size and shape of interphase speckles is a reflection of the steady-state dynamics of protein constituents that are both arriving at and leaving from these structures (Kruhlak et al. 2000; Phair et al. 2000). Although photobleaching analyses have indicated rapid recovery kinetics of splicing factors in speckles, consistent with a diffusion-based process (Kruhlak et al. 2000; Phair et al. 2000), the relative size of speckles remains constant throughout interphase. In fact, the incubation of permeabilized cells with a nuclear extract containing an ATP-regenerating system maintains transcriptional activity and does not result in a loss of speckles (Misteli et al. 1996), nor does simple treatment of unfixed cells with detergent (Spector et al. 1992).

The observed basal exchange rate may be directly related to the maintenance of speckles, rather than indirectly related to an involvement in transcriptional/pre-mRNA processing events. In addition, the irregular shape of individual nuclear speckles in interphase nuclei may result from a nonuniform release and/or delivery of factors, related to the location of active genes in their vicinity (Misteli et al. 1997a). Consistent with this possibility, either upon inhibition of RNAPII transcription by α-amanitin

(Spector et al. 1993), or inhibition of pre-mRNA splicing using an antisense approach (O'Keefe et al. 1994), speckles tend to round-up, suggesting a uniform exchange rate of factors in all directions.

This exchange rate of speckle factors may be regulated through phosphorylation/dephosphorylation events. For example, phosphorylation of the RS domain of SR splicing factors has been shown to be necessary for recruitment of SR proteins from nuclear speckles to sites of transcription/pre-mRNA processing (Misteli et al. 1998) and for their association with the forming spliceosome (Mermoud et al. 1994). Several kinases (i.e., Clk/STY [Colwill et al. 1996; Sacco-Bubulya et al. 2002] and hPRP4 [Kojima et al. 2001]) involved in this phosphorylation, as well as a kinase proposed to be involved in the phosphorylation of the carboxy-terminal domain of RNAPII LS in vitro (Ko et al. 2001), have been localized to nuclear speckles, leaving open the possibility that phosphorylation/dephosphorylation plays a role in determining the basal rate of factor exchange.

However, in addition to the basal activities, an additional level of control can be exerted by modulating phosphorylation events. For example, the rapid induction either of a gene (Huang et al. 1996), or group of genes such as during viral infection (Bridge et al. 1995; Jiménez-García et al. 1993), can result in an increased outward flow of factors from speckles. An extreme example of this can be observed upon overexpression of Clk/STY kinase, or addition of SRPK1 kinase to permeabilized cells (Gui et al. 1994a; Gui et al. 1994b), which results in the complete redistribution of splicing factors from speckles to the diffuse nuclear pool (Colwill et al. 1996; Sacco-Bubulya et al. 2002).

Interestingly, expression of a mutant form of Clk/STY that lacks its catalytic activity resulted in an increased accumulation of factors in highly concentrated foci on the periphery of speckles, possibly a reflection of their inability to be released (Sacco-Bubulya et al. 2002). Consistent with this observation, the addition of kinase inhibitors to cells resulted in an inhibition of the dynamic movements on the periphery of speckles (Misteli et al. 1997a). Also, protein phosphatase 1 (PP1) inhibitors resulted in enlarged irregularly shaped speckles with less well defined edges, probably resulting from the inability of factors to be released from PFs, on the periphery of IGCs, also consistent with a modulating effect on the exchange rate (Misteli et al. 1996).

In summary, a basal exchange rate of factors, coupled with a mechanism to modulate this rate (that is, providing a stimulus-induced burst), ensures that the needed factors, in the correct phosphorylation state, are available to pre-mRNA transcripts at the sites of transcription. In addition, such a mechanism ensures that a significant population of factors, which are not functionally needed, are sequestered out of the soluble nuclear pool, in this case in nuclear speckles, representing a basic mechanism for the organization of nonmembrane-bound nuclear organelles.

CONCLUSIONS

Nuclear speckles are organelles located in the interchromatin nuclear space and are among the most widely studied nuclear domains. They are best known for accumulating high local concentrations of snRNPs and other non-snRNP protein splicing factors. However, the presence in speckles of many other factors involved in mRNA production by RNA polymerase II further supports their intimate relationship with gene expression. Although most speckles apparently do not contain DNA in an analogous way to the rRNA gene repeats within nucleoli, nonetheless highly expressed genes can be found associated with speckles, consistent with an important role for speckles in coordinating the supply and/or recycling of pre-mRNA processing and transcription factors. The analysis of nuclear speckles has helped to establish some key paradigms and principles for the dynamic assembly of membrane-free organelles in the nucleus. We anticipate that future work will refine our understanding of speckle composition and of mechanisms involved in targeting proteins to speckles and regulating their formation. It is likely that building a detailed model of gene expression in vivo will require further

characterization of nuclear speckles to define how the complex events required for transcription and RNA processing are efficiently coordinated within the nucleus.

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