NUCLEAR SPECKLES: A MODEL FOR NUCLEAR ORGANELLES

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Speckles are subnuclear structures that are enriched in pre-messenger RNA splicing factors and are located in the interchromatin regions of the nucleoplasm of mammalian cells. At the fluorescence-microscope level they appear as irregular, punctate structures, which vary in size and shape, and when examined by electron microscopy they are seen as clusters of interchromatin granules. Speckles are dynamic structures, and both their protein and RNA–protein components can cycle continuously between speckles and other nuclear locations, including active transcription sites. Studies on the composition, structure and behaviour of speckles have provided a model for understanding the functional compartmentalization of the nucleus and the organization of the gene-expression machinery.

SPECKLE
An irregularly shaped nuclear domain that is visualized by immunofluorescence microscopy, typically by using anti-splicing-factor antibodies. Usually 25–50 speckles are observed per interphase mammalian nucleus.

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doi:10.1038/nrm1172

The nucleus was one of the first intracellular structures to be identified by microscopy, but its functional organization is still poorly understood. In recent years it has become apparent that the nucleus is highly compartmentalized but extremely dynamic (for reviews, see REFS 1,2). Many nuclear factors are localized in distinct structures, such as SPECKLES, PARASPECKLES, nucleoli, CAJAL BODIES, GEMS and PROMYELOCYTIC LEUKAEMIA BODIES, and show a punctate staining pattern when analysed by indirect immunofluorescence microscopy[2,3].

In mammalian cells, the pre-messenger RNA splicing machinery — including the small nuclear ribonucleoprotein particles (snRNPs), spliceosome subunits and other non-snRNP protein splicing factors — shows a dynamic, punctate nuclear-localization pattern that is usually termed a 'speckled pattern', but has also been referred to as 'SC35 domains'[4] or 'splicing-factor compartments'[5] (FIG. 1). The term 'speckles' was first introduced in 1961 by J. Swanson Beck[6], on the examination of rat-liver sections that had been immunolabelled 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 at the electron-microscope level and named interchromatin particles[7]. Interestingly, Swift observed that these particles were not randomly distributed, but that they occurred in localized 'clouds', and cytochemical analysis indicated that they contained RNA[7]. However, the first connection between pre-mRNA splicing and nuclear speckles came from the examination of the distribution of snRNPs using antibodies specific to these splicing factors[8–10].

It is now clear that much of the punctate localization of splicing factors that is observed by immunofluorescence microscopy corresponds to the presence of these factors in nuclear speckles of variable size and irregular shape, which are seen by electron microscopy as 'INTERCHROMATIN GRANULE CLUSTERS' (IGCs) (FIG. 2). IGCs range in size from one to several micrometres 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[11]. These structures can be observed by electron microscopy without antibody labelling[11]. Depending on the splicing factor examined, speckles show an average enrichment of 5–10-fold above their diffuse nucleoplasmic distribution[12] (J. Swedlow and D. L. S., unpublished observations). We define 'speckles' here as specifically the IGC component of the splicing-factor labelling pattern, and distinguish them from other nuclear structures, including PERICHROMATIN FIBRILS, Cajal bodies and INTERCHROMATIN-GRANULE-ASSOCIATED ZONES[13], which also contain splicing factors (for reviews, see REFS 14,15).

PARASPECKLE
A subnuclear structure that is distinct from speckles. Typically, 10–20 paraspeckles are present in the interchromatin nucleoplasmic space, and they are often located adjacent to speckles. So far, three proteins — paraspeckle proteins 1 and 2 and p54/nrb — have been localized to these nuclear domains.

CAJAL BODY
A nuclear structure that contains newly assembled small nuclear ribonucleoprotein particles that are involved in pre-messenger RNA splicing, and small nucleolar ribonucleoprotein particles that are involved in ribosomal RNA processing. Also contains Cajal-body-specific guide RNAs. Cajal bodies are usually identified as foci labelled with antibodies against the autoantigen p80 coilin.

GEMS
'Gemini of Cajal bodies' are nuclear structures that are usually localized either coincident with or adjacent to Cajal bodies, depending on the cell line examined. Gems are characterized by the presence of the 'survival of motor neurons' (SMN) protein.

PROMYELOCYTIC LEUKAEMIA (PML) BODY
A subnuclear structure that is also known as nuclear domain 10, promyelocytic leukaemia oncogenic domain or Kr body. These bodies are characterized by the presence of the promyelocytic leukaemia protein and there are typically 10–30 per nucleus.

INTERCHROMATIN GRANULE CLUSTER
(IGC). A structure seen by electron microscopy that is equivalent to the speckles that are seen by fluorescence microscopy. Each IGC is composed of a series of particles, 20–25 nm in diameter, that seem to be connected in places by a thin fibril.

PERICHROMATIN FIBRILS
Fibrils observed by the electron microscope that are detected at transcription sites and shown to coincide with the incorporation of tritiated-uridine or 5-bromouridine 5′-trisphosphate, indicating that they are nascent transcripts.

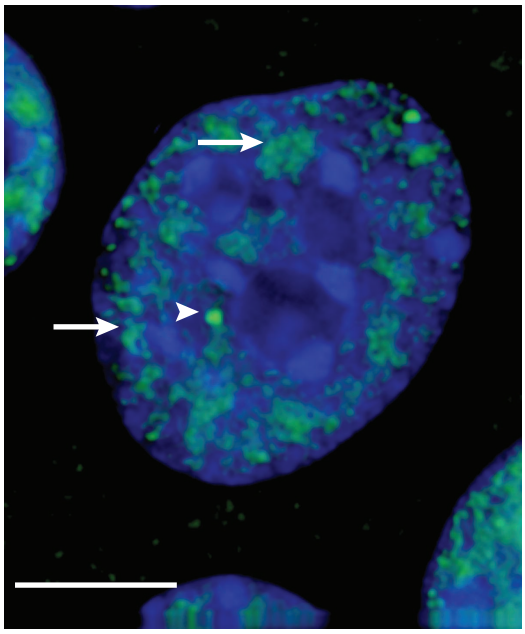

Figure 1 | **Speckles form in the interchromatin space.** Splicing factors (green labelling) using the Y12 antibody[9], which recognizes small nuclear ribonucleoprotein particles, localize specifically in the nucleus in a speckled pattern (arrows), in Cajal bodies (arrowhead) and diffusely in the nucleoplasm. The speckles occur in nuclear regions containing little or no DNA, as judged by 4,6-diamidino-2-phenylindol (DAPI) staining (blue labelling), and are excluded from nucleoli. Scale bar, 10 μm.

A speckle-targeting signal has been identified for some of the speckle components. The arginine/serine-rich domain (RS domain) of certain SR PROTEIN pre-mRNA splicing factors has been shown to be necessary and sufficient for targeting these factors to nuclear speckles[16–18]. Interestingly, structures similar to nuclear speckles have been identified in the amphibian oocyte nucleus[19] ('B SNURPOSOMES') and in *Drosophila melanogaster* embryos, when transcription increases on CELLULARIZATION during cycle 14 (REF. 20), but not in yeast[21]. Significantly, not all nuclear proteins that show a speckle-like labelling pattern by immunofluorescence microscopy localize to IGCs that contain splicing factors. For example, paraspeckle protein 1 (PSP1) localizes to paraspeckles, which, although they look similar to speckles, are distinct structures[22]. It is therefore essential to carry out double-label immunofluorescence studies using an antibody against splicing factors to confirm that any new factors localize to nuclear speckles.

In this review, we discuss the structure, composition and function of nuclear speckles, and describe how they provide an important model for understanding the organization and dynamics of nuclear organelles.

Structure and location of speckles

As judged by both light and electron microscopy, the IGCs that constitute speckles form throughout the nucleoplasm in regions that contain little or no DNA[11]. Unlike nucleoli, speckles do not seem to assemble around specific chromatin loci, and *in situ* hybridization studies have localized active genes predominantly at the periphery of, rather than within, speckles. However, although they apparently contain few, if any, genes, speckles are often observed close to highly active transcription sites. This indicates that they might have a functional relationship with gene expression, and some genes have been reported to localize preferentially close to speckles[23–27], although this does not seem to be obligatory for transcription/pre-mRNA splicing.

Several lines of evidence point to speckles functioning as storage/assembly/modification compartments that can supply splicing factors to active transcription sites. For example, live-cell studies show that splicing factors are recruited from speckles to sites of transcription[28]; conversely, splicing factors accumulate in enlarged, rounded speckles when transcription[10] or pre-mRNA splicing[29] are inhibited. Significant results were obtained from high-resolution pulse-labelling experiments analysed at the electron-microscopic level, studying the incorporation of either tritiated uridine or 5-bromouridine 5′-trisphosphate after short pulses. It was shown that nascent pre-mRNA is predominantly localized outside nuclear speckles (IGCs) in fibrillar structures, 3–5 nm in diameter, which are known as perichromatin fibrils[30–33]. It is likely that most co-transcriptional splicing is associated with these perichromatin fibrils, rather than within IGCs. Perichromatin fibrils can occur both on the periphery of IGCs and in nucleoplasmic regions away from IGCs[15].

Some apparent discrepancies in the literature, concerning the possible direct role of speckles as splicing sites, might have arisen because in cultured mammalian cells the perichromatin fibrils can show a close topological relationship with the periphery of IGCs. Using the fluorescence microscope, it is difficult to distinguish these perichromatin fibrils from the IGCs. In addition, as highly expressed genes recruit a significant amount of pre-mRNA splicing factors[34], these regions of highly active transcription are indistinguishable from IGCs at the fluorescence-microscope level. Nonetheless, although the majority view in the field at present is that speckles are not direct transcription/pre-mRNA splicing centres, other workers argue that they might still have an important function that relates to the splicing and transport of pre-mRNA[12,35,36]. A key point that remains to be established clearly is whether speckles, defined as IGCs, contain any nascent mRNA in transit to the cytoplasm (see below).

Composition of speckles

Many pre-mRNA splicing factors — including snRNPs and SR proteins[37] — have been localized to nuclear speckles by immunofluorescence, fluorescent protein tagging and/or immuno-electron microscopy (for a review, see REF. 38). In fact, this speckled localization pattern is highly diagnostic for proteins that are involved in pre-mRNA splicing. In addition, several kinases (such as CLK/STY, PRP4 and PSKH1)[39–43] and phosphatases (protein phosphatase 1; PP1)[44,45] that can phosphorylate/dephosphorylate components of the splicing machinery have

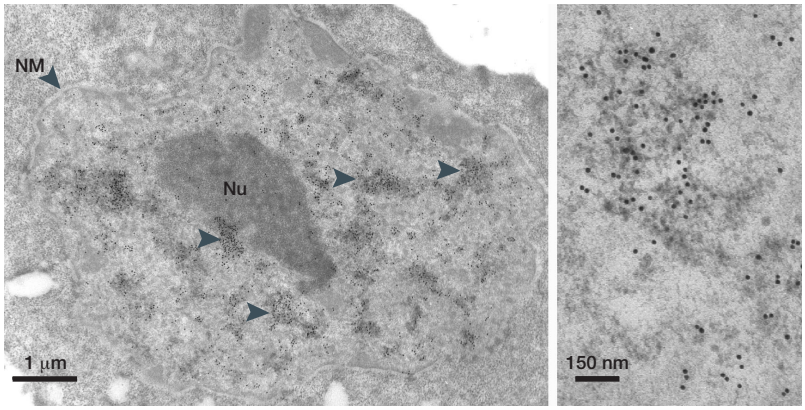

Figure 2 | **Immuno-electron microscopic localization of pre-mRNA splicing factors.** Splicing factors localize to interchromatin granule clusters (IGCs/speckles; left panel, arrowheads) and perichromatin fibrils (transcription sites). IGCs are composed of a series of particles measuring 20–25 nm in diameter that are connected in places by a thin fibril, resulting in a beaded chain appearance (right panel). Sections are immunolabelled with 3C5 antibody[96], which recognizes the SR family of pre-messenger RNA splicing factors, and 15-nm colloidal-gold-conjugated secondary antibody. NM, nuclear membrane; Nu, nucleolus.

also been localized to nuclear speckles. This supports the idea that speckles might be involved in regulating the pool of factors that are accessible to the transcription/pre-mRNA processing machinery[46].

In an attempt to characterize in detail the protein composition of speckles, proteomic analysis of an enriched IGC fraction purified from mouse liver nuclei has been carried out — 136 known proteins, as well as numerous uncharacterized proteins have been identified[47] (N. Saitoh, P. Sacco-Bubulya and D. L. S., unpublished observations). The proteomic information, together with further localization studies, has revealed that speckles contain other proteins apart from pre-mRNA splicing factors. Of particular interest is the localization of transcription factors[48–50], 3′-end RNA-processing factors[51,52], the eukaryotic translation-initiation factor eIF4E[53], a protein involved in translation inhibition (eIF4Aiii)[54], and structural proteins[55,56]. Consistent with these findings, the most recent proteomic analyses of *in-vitro*-assembled spliceosomes indicate that they might also contain transcription and 3′-end RNA-processing factors, together with splicing factors, in a higher-order complex[57,58].

Although transcription does not take place within most nuclear speckles[33], and DNA is not localized to these nuclear regions[11], a population of the serine-2-phosphorylated form of the RNA polymerase II (Pol II) large subunit, which is involved in elongation, has been localized to these regions by immunofluorescence microscopy[49,59]. In addition, biochemical characterization of the IGC proteome has identified several subunits of Pol II[47] (N. Saitoh, P. Sacco-Bubulya and D. L. S., unpublished observations), supporting the presence of a pool of Pol II in speckles. However, some studies have not observed an enrichment of Pol II in speckles[50,60,61], and it is not present in B snurposomes[62].

The cell division protein kinase 9 (Cdk9)–cyclin T1 complex (which is also known as TAK/P-TEFb) is thought to be involved in transcriptional elongation through the phosphorylation of the Pol II large subunit[63]. This complex was found to be distributed diffusely throughout the nucleoplasm, but not in nucleoli[64]. In addition, a considerable overlap between cyclin T1 and nuclear speckles was observed. However, although Cdk9 was present in the vicinity of nuclear speckles, the degree of direct overlap was limited[64,65].

FBI-1 is a cellular POZ-domain-containing protein that binds to the HIV-1 long-terminal repeat and associates with the HIV-1 transactivator protein Tat[66]. FBI-1 has been found to partially colocalize with Tat and its cellular cofactor, P-TEFb, at nuclear speckles[67]. 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 (REF 68). Therefore, although little or no transcription takes place in nuclear speckles, a subset of proteins that are involved in this process are associated with speckles, in addition to being present at transcription sites. Although, at present, it is unclear what determines the subset of transcription factors that are localized to nuclear speckles, their presence might relate to the assembly of higher-order complexes and/or to regulatory steps affecting either the modification state or the accessibility of specific transcription factors.

In addition to transcription factors, a population of poly(A)$^+$ RNA has been localized to nuclear speckles[69–71]. It is not known if these RNAs encode proteins or if they represent non-coding RNAs[72]. Interestingly — as shown by a pulse–chase experiment — this population of poly(A)$^+$RNA is not transported to the cytoplasm when transcription is blocked with α-amanitin, as would be expected if these species represented nascent mRNAs[71]. In this regard, an earlier study found that a considerable fraction of nuclear poly(A)$^+$ RNA consisted of sequences that were not detected in the cytoplasm[73]. Therefore, unless mRNA export is inhibited when transcription is inhibited, as was recently proposed[36], either these speckle-associated RNAs might be defective transcripts destined never to leave the nucleus, or, more likely, they represent species with a specific nuclear function. Studies are underway to identify and characterize this class of nuclear RNA (K. V. Prasanth and D. L. S., unpublished observations). Of particular interest is whether any of these RNAs represent a structural RNA that is involved in the organization of speckles.

Although an underlying scaffold that would function as a platform on which to organize IGCs has not been identified[42], several proteins with possible structural roles in the nucleus — such as a population of lamin A[56] and snRNP-associated actin[55] — have been detected in nuclear speckles. Furthermore, a lipid that regulates actin-binding proteins[74], phosphatidylinositol-4,5-bisphosphate, and several phosphatidylinositol phosphate kinase (PIPK) isoforms have also been localized to nuclear speckles[75]. Interestingly, the addition of a dominant-negative, amino-terminal lamin mutant, either to baby hamster kidney cells, or to nuclei from *Xenopus laevis*, results in an inhibition of Pol II, but not Pol I and Pol III, transcription[76]. Future studies are

INTERCHROMATIN-GRANULE-ASSOCIATED ZONE
A region that is adjacent to interchromatin granule clusters, which contains U1, but not U2, small nuclear RNAs.

SR PROTEINS
A family of pre-messenger RNA splicing factors that are characterized by repeats of arginine–serine dipeptides at their carboxyl termini.

SNURPOSOME
A nuclear structure, identified in amphibian oocytes, that contains splicing small nuclear ribonucleoprotein particles. Three classes — known as A, B and C snurposomes — have been defined, and they differ in their composition. B snurposomes are most closely related to speckles in their composition, and could represent oocyte forms of the speckles that are found in somatic-cell nuclei.

CELLULARIZATION
The transition from a syncytium to distinct cells which occurs at the fourteenth round of cell division in the *Drosophila melanogaster* embryo.

CLK/STY
A kinase family, the members of which are characterized by having the serine residues in the arginine–serine domain of SR proteins as their primary substrates.

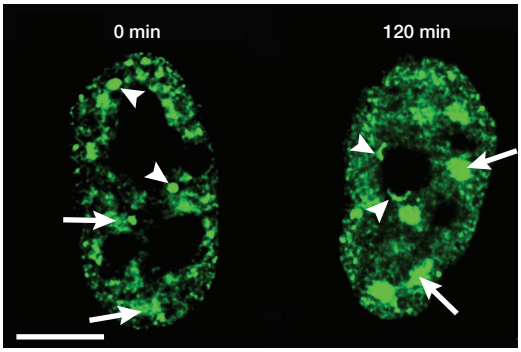

Figure 3 | **Modulation of transcription affects speckle organization.** In transcriptionally active cells, pre-messenger RNA splicing factors localize in a speckled distribution pattern (left panel, arrows), as well as being diffusely distributed throughout the nucleoplasm. In certain cell types, these factors are also present in Cajal bodies (left panel, arrowheads). On transcriptional inhibition (actinomycin D 0.5 μg per ml, 120 min), speckles increase in size and 'round up' (right panel, arrows). In addition, some factors form a 'cap' on the surface of the nucleolus (right panel, arrowheads). Immunolabelling was carried out using Y12 antibody[9], which recognizes small nuclear ribonucleoprotein particles. Scale bar, 8 μm.

required to address these intriguing observations and the role of these proteins in the organization of IGCs or individual interchromatin granules, and their relationship to transcription/pre-mRNA processing.

Dynamic behaviour of speckles

Speckles are dynamic structures and 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 metabolic and environmental signals that influence the available pools of active splicing and transcription factors. 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[10,35,77] (FIG. 3). That nuclear speckles become round and increase in size on transcriptional inhibition supports the view that speckles might function in the storage/assembly/modification of splicing factors, and that they are not direct sites of splicing. Furthermore, when the expression of intron-containing genes increases[28,34], or when transcription levels are high during viral infection[78,79], the accumulation of splicing factors in speckles is reduced and they redistribute instead to nucleoplasmic transcription sites. Individual speckle components can therefore shuttle continually between speckles and active gene loci. As discussed below, speckles are also regulated during the cell-division cycle.

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 that express splicing factors fused with green fluorescent protein (GFP)[28]. Speckles in such cells show transcription-dependent peripheral movements, although individual speckles remain in their neighbourhoods. Photobleaching techniques have also been used to measure the flux of some speckle components, and have shown that their exchange rate is very rapid[5,80]. 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 ~30 s, with half recovery in ~3–5 s. The movement rates for splicing factors were measured to be slow (~1%) compared with free GFP, and this reduction in movement was proposed to result from numerous transient interactions of splicing factors with nuclear binding sites, both within and outside speckles. Kinetic modelling indicated that the maximal mean residence time for GFP–SF2/ASF in speckles was less than 50 s (REF. 5). It is a remarkable feature of nuclear organization that the overall structure of speckles persists, despite the large flux of components

The speckle cell cycle

On entry into mitosis, and after breakdown of the nuclear envelope/lamina, proteins that are associated with nuclear speckles become diffusely distributed throughout the cytoplasm[81–84] (FIG. 4). During metaphase, these proteins continue to localize in a diffuse cytoplasmic pattern and also localize within one to three small structures that are known as MITOTIC INTERCHROMATIN GRANULES (MIGs)[83,85–87]. MIGs seem to be structurally analogous to IGCs[84,85,88]. As mitosis progresses from anaphase to early telophase, the MIGs increase in number and size. During mid–late telophase, and after deposition of the nuclear envelope/lamina, pre-mRNA splicing factors enter daughter nuclei and, concomitantly, their localization in MIGs decreases, showing that these factors are recycled from the cytoplasm (MIGs) into daughter nuclei[87]. Live-cell studies have indicated that most of these factors enter daughter nuclei within 10 min[87].

Although MIGs have been proposed to be the mitotic equivalent of nuclear speckles[11,83–85], their function in mitotic cells is unclear. In telophase cells, some MIGs have been found in close proximity to the newly formed nuclear envelope[84,87]. The 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, indicate that the MIGs might be directly transported into the nuclei[84,85]. However, colocalization of SF2/ASF and a hyperphosphorylated form of Pol II (H5) in the MIGs of late telophase cells has indicated that this might not be the case. For example, SF2/ASF and other pre-mRNA processing factors were shown to enter daughter nuclei, whereas a subpopulation of SC35 and Pol II (H5) remained in MIGs until G1 phase, showing that various components of MIGs are differentially released for their subsequent entry into daughter nuclei[87].

Further support for the differential release of factors from MIGs comes from an earlier study[83], which reported the nuclear import of snRNPs, whereas cytoplasmic MIGs were still labelled with anti-SR protein and anti-SC35 antibodies. On the basis of

PRP4
A kinase that localizes to nuclear speckles and interacts with CLK/STY, as well as several proteins that are involved in pre-mRNA splicing (SF2/ASF, U5 snRNP) and chromatin remodelling (BRG1, N-CoR deacetylase complexes).

PSKH1
A human kinase that is localized to nuclear speckles but that does not directly interact with SR proteins.

MITOTIC INTERCHROMATIN GRANULES
(MIGS). The speckles (interchromatin granule clusters) that form in the cytoplasm of cells undergoing mitosis, and that increase in number from metaphase to telophase.

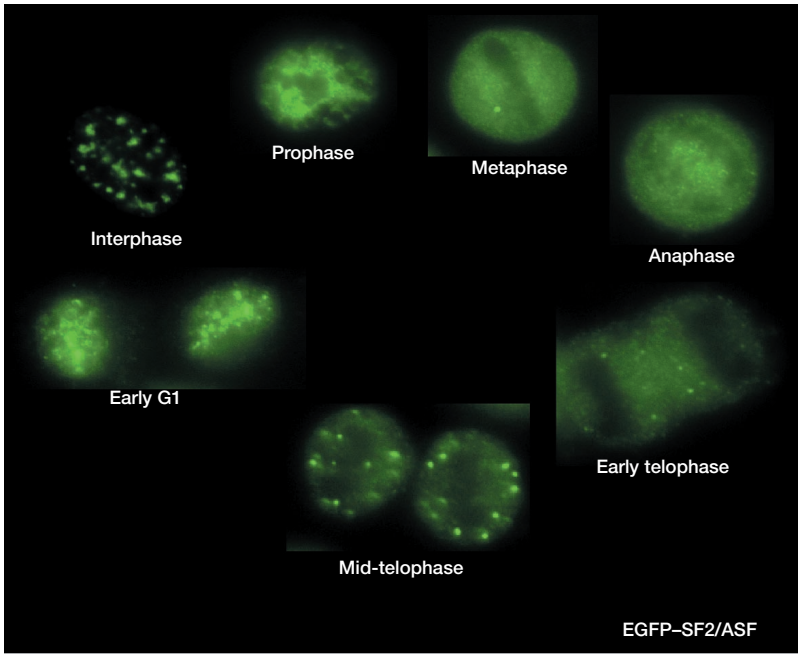

Figure 4 | **The speckle cell cycle.** The interphase speckled pattern disperses as cells enter prophase of mitosis. From metaphase to mid-telophase, mitotic interchromatin granules (MIGs) appear in the cytoplasm and increase in number and size. During mid–late telophase, factors leave MIGs and enter the daughter nuclei, and speckles form during early G1 phase. HeLa cells that are expressing enhanced green fluorescent protein (EGFP)–pre-messenger RNA splicing factor/alternative splicing factor (SF2/ASF) are shown. This image was kindly provided by P. Sacco-Bubulya, Cold Spring Harbor Laboratory, USA.

these findings, it was suggested that MIGs might have a role in the modification of the components of the splicing machinery before their nuclear entry; or that alternatively they might function as enriched populations of these factors, allowing for protein–protein interactions between subsets of proteins before their nuclear entry[87].

Interestingly, splicing factors are competent for pre-mRNA splicing immediately after their entry into daughter nuclei[87], supporting the possibility that MIGs might be responsible for splicing-factor modification, allowing for the immediate targeting of modified (phosphorylated) pre-mRNA-processing complexes to transcription sites in telophase nuclei. As daughter nuclei that are in late telophase have not yet assembled nuclear speckles, cytoplasmic MIGs probably function as their counterparts to provide competent pre-mRNA splicing factors to the initial sites of transcription in newly formed nuclei[87]. Perhaps splicing factors are released from MIGs through hyperphosphorylation, as has been shown for their release from nuclear speckles in interphase nuclei.

Perspective

We propose below a 'REGULATED-EXCHANGE' MODEL, on the basis of the known dynamic properties of speckles, to account for the basic principles of speckle formation and organization. The key features of this model are based on the following main points. First, we believe that most evidence points to the fact that speckles form through a process of self-assembly, and might not depend on an underlying scaffold structure. Therefore, transient macromolecular interactions are likely to form the basis of speckle morphogenesis. Second, under steady-state conditions, the respective rates of association and disassociation of individual speckle components define their exchange rates and the sizes of their bound and soluble pools in the nucleus. Third, 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.

According to this view, the entry of splicing factors into late-telophase nuclei results in the association of a subset of these factors with initial transcription/pre-mRNA-processing sites[87]. As the population of factors increases, there is an increased probability of protein–protein interactions among those factors that are not engaged in transcription/pre-mRNA processing, resulting in the formation of nuclear speckles. These initial speckles seem to form predominantly in nucleoplasmic regions that are devoid of chromosome territories and/or other nuclear organelles. They could initiate either at random locations, or in the vicinity of genes that are transcribed at high levels during the telophase/G1 phase transition.

The size and shape of interphase speckles is a reflection of the steady-state dynamics of the protein constituents that are both arriving at and leaving from these structures[5,80]. Although photobleaching analyses have indicated rapid recovery kinetics of splicing factors in speckles, consistent with a diffusion-based process[5,80], the relative size of speckles remains constant throughout interphase. This indicates that there might be a sensing mechanism that maintains these domains. In support of this possibility, 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[89], nor does simple treatment of unfixed cells with detergent[90]. Therefore, turnover by simple diffusion cannot account for the presence of nuclear speckles.

Our regulated-exchange model (FIG. 5) proposes that the cell-type-specific basal exchange rate of factors in speckles is directly responsible for maintaining a particular steady-state level of factors that are associated with these structures. As the on/off rate of these factors is similar — although not identical — in the presence or absence of transcription[5], the observed basal dynamics might 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 might result from a non-uniform release and/or delivery of factors, which is related to the location of active genes in their vicinity[28]. Consistent with this possibility, speckles tend to round up, when Pol II transcription is inhibited by α-amanitin[91] or when pre-mRNA splicing is inhibited using an antisense approach[29], indicating that there is a uniform exchange rate of factors in all directions.

REGULATED-EXCHANGE MODEL
A model proposed in this review to account for the basic principles of speckle formation and their dynamic properties.

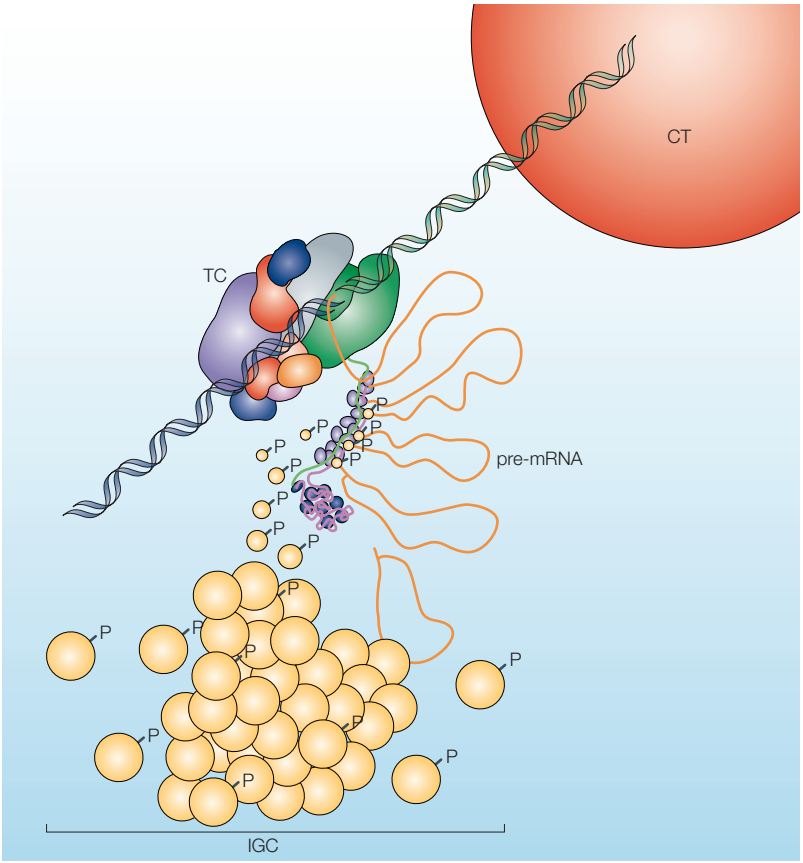

Figure 5 | **A 'regulated-exchange' model accounts for the dynamics of nuclear speckles.** Nuclear speckles (interchromatin granule clusters; IGCs) form as the result of protein–protein interactions among pre-messenger RNA splicing factors and other constituents at the telophase/G1-phase transition. A basal level of factor exchange occurs between the speckles and the nucleoplasmic pool that is regulated by phosphorylation/dephosphorylation in a cell-type-specific manner. Modulation of the phosphorylation level of speckle proteins results in an increased release and recruitment to transcription sites. The model is not drawn to scale, and is modified with permission from REF. 97 © Saunders (2002). CT, chromosome territory; IGC, interchromatin granule cluster; TC, transcription complex; pre-mRNA, pre-messenger RNA.

How might this exchange rate of speckle factors be regulated? One possibility is through phosphorylation/dephosphorylation. The concept that speckle dynamics might be regulated by phosphorylation/dephosphorylation resulted from an unbiased search for an enzymatic activity that could release splicing factors from nuclear speckles[92]. This search resulted in the identification, purification and cloning of the kinase SRPK1 (REFS 92,93). Subsequently, phosphorylation of the RS domain of SR splicing factors has been shown to be necessary for the recruitment of SR proteins from nuclear speckles to sites of transcription/pre-mRNA processing[94], and for their association with the forming spliceosome[95]. Several kinases that are involved in this phosphorylation (such as CLK/STY[39,42] and PRP4[41]), as well as a kinase that is proposed to be involved in phosphorylation of the carboxy-terminal domain of Pol II *in vitro*[40], have been localized to nuclear speckles. This leaves open the possibility that phosphorylation/dephosphorylation has a role in determining the basal rate of factor exchange.

As well as the basal activities, a further level of control can be exerted by modulating phosphorylation events. For example, the rapid induction either of a gene[34] or group of genes (viral infection)[78,79] can result in an increased outward flow of factors from speckles. Depending on the location of the gene or genes, such an increased flow can, in some cases, seem to be directional[28], resulting in depletion of the pool of factors that are present in speckles. An extreme example of this can be observed on overexpression of CLK/STY kinase or addition of SRPK1 kinase to permeabilized cells[92,93], which results in the complete redistribution of splicing factors from speckles to the diffuse nuclear pool[39,42]. Significantly, this redistribution did not reveal any underlying scaffold[42], supporting the idea that macromolecular interactions (protein–protein and protein–RNA) are mainly responsible for the formation and maintenance of nuclear speckles.

Interestingly, the expression of a mutant form of CLK/STY that lacks 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[42]. Consistent with this observation, the addition of kinase inhibitors to cells resulted in an inhibition of the dynamic movements on the periphery of speckles[28]. Also, the use of PP1 inhibitors resulted in enlarged, irregularly shaped speckles with less well-defined edges, probably resulting from the inability of factors to be released from perichromatin fibrils on the periphery of IGCs, which is also consistent with a modulating effect on the exchange rate[89].

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 required factors, in the correct phosphorylation state, are available to pre-mRNA transcripts at the sites of transcription. In addition, this mechanism ensures that a considerable population of factors, which are not functionally needed, are sequestered out of the soluble nuclear pool, representing a basic mechanism for the organization of non-membrane-bound nuclear organelles.

Although much progress has been made with regard to the role of nuclear speckles in gene expression, several important questions remain. Does signalling from the gene to the speckle directly modulate factor release, or is such release indirectly controlled by a simple decrease or increase in the ratio of factors (soluble pool/speckle), owing to changes in the level of transcription/pre-mRNA processing? What is the detailed mechanism of speckle formation? Do speckles initiate randomly in daughter nuclei, or is their position established together with particular chromosome territories, gene loci or other structures? How is the number and size of speckles determined? What is the role of the poly(A)$^+$ RNA in speckles? We look forward to insights into these areas over the next few years as we continue to unravel the inner workings of the nucleus and the interplay between structure and function.

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Acknowledgements

We thank members of the Lamond group, and K.V. Prasanth and P. Sacco-Bubulya in the Spector group for their helpful comments. We also appreciate the comments of S. Fakan, J. Gall, G. Matera and J. Swedlow. We thank A. Fox and Y. Wah Lam (University of Dundee, UK) for their help in preparing figures 1 and 3, and P. Sacco-Bubulya (Cold Spring Harbor Laboratory) for providing figure 4. A. I. L. is a Wellcome Trust Principal Research Fellow. D. L. S. is funded by the National Institute of General Medical Sciences/ National Institutes of Health.

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