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Genome Organization around Nuclear Speckles

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Abstract

Higher eukaryotic cell nuclei are highly compartmentalized into bodies and structural assemblies of specialized funcions. Nuclear speckles/IGCs are one of the most prominent nuclear bodies, yet their functional significance remains largely unknown. Recent advances in sequence-based mapping of nuclear genome organization now provide genome-wide analysis of chromosome organization relative to nuclear speckles. Here we review older microscopy-based studies on a small number of genes with the new genomic mapping data suggesting a significant fraction of the genome is near deterministically positioned near nuclear speckles. Both microscopy and genomic based approaches support the concept of the nuclear speckle periphery as a major active chromosomal compartment which may play an important role in fine-tuning gene regulation.

Discovery and rediscoveries of nuclear speckles

The “nuclear speckle” name is now reserved for one particular nuclear body, ranging in size from ~0.3 −3 um and in number from several to tens per nucleus (Fig. 1), as reviewed previously [1,2]. After nucleoli, these nuclear speckles represent the next largest nuclear body compartment in typical mammalian nuclei and are found over a wide range of metazoan species from plants to animals. Cajal first discovered nuclear speckles, which he named “hyaline grumes”, using a modified silver-staining protocol [3] (Fig. 1a). Nuclear speckles were rediscovered by electron microscopy (EM) as “Interchromatin Granule Clusters” (IGCs)- clusters of ~20–25 nm RNP granules lying between regions of chromatin [4] (Fig. 1b). Nuclear speckles were rediscovered yet again through the localization of various snRNP components, splicing factors (i.e. “SC35 domains”), and poly(A)$^+$ RNAs (“poly(A)$^+$ islands”) [5–7] (Fig. 2a).

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Nuclear speckles remain an operational definition dependent on the experimental detection method. While EM uniquely identifies IGCs, they likely miss a surrounding peripheral zone which together with the IGC define the actual nuclear speckle functional unit. EM of detergent extracted nuclei shows an amorphous zone of variable width, stained by heavy metals but distinctive from the surrounding chromatin (Fig. 1c, arrows), surrounding the IGC [8] (Fig. 1c, arrowheads). The long non-coding RNA (lncRNA) Malat-1 is highly enriched in nuclear speckles, with its distribution extending slightly outside the core of clustered granules into this peripheral zone with which active genes and their nascent transcripts may largely interact [9] (Fig. 1d). Many spliceosome and splicing factors are also enriched within and surrounding the IGC but are also found in a reticular pattern spreading throughout the nucleus and in Cajal bodies and other small foci [6] (Fig. 1d&e). Even potential scaffolding proteins (e.g. SON [10], Fig. 1e) and other splicing factor markers (pSC35, Figs. 1d,2a), which may be enriched 8–10-fold in the IGC core of the nuclear speckle, are present elsewhere in the nucleus and in a given cell type and species may not uniquely mark nuclear speckles. For instance, in mouse 2-cell embryos, immuno-EM showed localization of SC35 in IGCs but also in more numerous smaller foci [11]. Nearly all known nuclear speckle protein components are factors involved in transcription, post-transcriptional RNA processing, or nuclear RNA export and are therefore found to varying degrees as a diffusible nucleoplasmic pool and bound to active gene loci, nascent transcripts, pre-mRNAs, mRNAs, and/or other focal nuclear accumulations.

This has led to the common misconception that nuclear speckles form wherever there is strong transcription. In fact, only a subset of highly transcriptionally active endogenous genes or transgenes show a specific association with nuclear speckles [2,12,13].

Proposed nuclear speckle functions

To date, two main classes of models have been proposed for the possible physiological functions of nuclear speckles [1,2].

Model 1 suggests nuclear speckles are storage sites for RNA processing factors [1]. Proteomics of RNP granules purified from IGCs revealed hundreds of proteins involved in various stages of RNA processing and nuclear export [14], while EM shows no DNA or active transcription inside speckles. Additionally, after transcriptional or splicing inhibition, splicing factors accumulate within larger and rounder nuclear speckles. A newer and more nuanced variation of this model would be that nuclear speckles actively buffer nucleoplasmic levels of factors regulating aspects of transcription and RNA processing for homeostasis control (Yaron Shav-Tal, personal communication).

Model 2 instead suggests nuclear speckles are “gene expression hubs”, enhancing transcription, post-transcriptional splicing, and/or other RNA processing activities for a subset of genes through rapid recycling of components between speckles and genes [2]. Immuno-FISH revealed ~half of 20–25 active genes localize near the nuclear speckle periphery [2], while certain co-expressed genes co- localized at the nuclear speckle periphery with frequencies higher than expected by chance [15–17]. Entire gene-rich chromosome bands localize near nuclear speckles at increased frequency [18]. Moreover, the

pre-mRNA and/or mRNAs of a few of these active genes (COLA1, MyHC) overlap with and enter within nuclear speckles, while poly(A)+ RNA accumulates inside and at the periphery of IGCs [2,19,20] (Fig. 2a). Local decondensation of chromatin near nuclear speckles has been visualized by EM (Fig. 2b), and a concentration of active transcription surrounding speckles has been visualized by pulse-labeling and RNA pol2 staining [21–24](Fig. 2c–e). Splicing defects and inhibition of either splicing or nuclear export lead to accumulation of RNAs inside speckles [25–28], while microinjected RNAs with functional splice sites and/or splicing enhancers localize and, after export inhibition, accumulate inside nuclear speckles [27,29–31].

Despite healthy debate for over 20 years, little progress has been made in distinguishing between the above two models. Two main questions need to be answered to judge the significance of the data on which Model 2 is based. How prevalent are genes that position at high frequency near nuclear speckles? Given the higher density of both nuclear speckles and active transcription in the nuclear interior, what is the actual mean speckle distance that should be considered unusually “close” to a nuclear speckle?

A subset of the active genome organizes around speckles

Missing until recently have been genome-wide methods capable of mapping chromosome regions relative to nuclear speckles. Several methods measuring molecular proximity have failed to identify speckle-associated chromosome regions. The high compaction of chromatin observed near nuclear speckles (Fig. 1b, c, Fig. 2b) predicts that speckle-associated chromosome regions should be large domains, 100s of Kbp to Mbp in size. However, ChIP against speckle-enriched factors (SRSF2/SC35, SON) shows localized peaks over promoters and gene bodies of active genes [32,33]. Similarly, CHART (capture hybridization analysis of RNA targets) and RAP (RNA anti-sense purification) show MALAT1 focally enriched over transcriptionally active and spliced gene bodies, possibly via interaction with pre-mRNAs since the association is transcription-elongation dependent [34,35]. Molecular proximity methods are likely detecting the small fraction of marker proteins/RNAs interacting at nascent transcripts and active genes, independent of speckle localization but within cross-linking distance of the DNA. Conversely, they fail to detect DNA within microscopic but not molecular proximity of these same marker proteins/RNAs concentrated within speckles.

Recently, three orthogonal methods have been applied to identify nuclear speckle associated chromosome regions genome-wide [24,36,37] (Fig. 3). MARGI (Mapping of RNAGenome Interactions) is a ligation-based method that reads the sequences of RNA-DNA molecules linked by a bridging adaptor oligonucleotide and exploits the known concentration of particular RNAs (snRNAs, 7S RNA, MALAT1) within nuclear speckles and their periphery [37] (Fig. 3a). SPRITE (Split-Pool Recognition of Interactions by Tag Extension) identifies DNA and potentially RNA molecules found in the same molecular complexes isolated after sonication of nuclei [36] (Fig. 3b). The molecular interaction distances producing a MARGI signal are unknown. For SPRITE, complexes containing increasing numbers of fragments should probe longer interaction distances, but these actual microscopic distances are unknown. TSA (Tyramide Signal Amplification)-Seq instead uses a cytological staining

method producing a diffusible signal to label chromosome regions proportionally to their distance, up to ~ 1 um, from nuclear speckles [24]. TSA-Seq maps distances over cytological size scales and a physical model derived from the diffusion equation accurately converts sequence read count enrichment into an actual physical distance (Fig. 3c).

MARGI detects peaks of 100kb-13 Mbp width essentially over all active chromosomal regions (the A Hi-C compartment) as enriched in speckle-associated RNAs. Resolution currently is limited by the relatively low read count of DNA fragments linked to nuclear speckle associated RNAs. It remains unclear whether MARGI, like RAP and CHART, is simply detecting the focal enrichment of Malat1 and snRNAs near active gene bodies or whether it is actually measuring those active genes near nuclear speckles. SPRITE complexes containing large numbers of DNA fragments show increased numbers of inter-chromosomal contacts. Two distinct subsets, or “hubs”, of chromosome regions with unusually high numbers of such inter-chromosomal contacts emerge from analysis of these large clusters: transcriptionally inactive regions and gene-rich, transcriptionally active regions. Regions within each hub contact each other at high frequency, but show low contacts with the opposite hub. Contact frequencies of 1 Mbp-size chromosome regions with this active hub correlated with Malat1 lncRNA localization measured by RAP-RNA-Seq [35], and showed a linear correlation with the fraction of alleles (20–80%) mapping within 0.5 um from a nuclear speckle by FISH [35]. TSA-Seq currently maps speckle proximity at high resolution, estimating mean distance to nuclear speckles with an accuracy of ~50 nm. Most active chromosomal regions localize closer to speckles than the median distance across the genome.

Strikingly, in human K562 cells TSA-Seq reveals Speckle-Associated Domains (SPADs) corresponding to ~5% of the genome showing deterministic positioning within 500 nm of nuclear speckles with near 100% frequencies (estimated mean distance < 0.32 um to speckle periphery). More generally, the ~20% of the genome mapping closest to nuclear speckles correlates closely to the A1 HI-C subcompartment, with the A2 Hi-C subcompartment mapping instead at intermediate mean distances to speckles. This 20% of the genome closest to speckles shows high gene density and contains ~70% of super-enhancers and the top 5% expressed genes. TSA-Seq suggests that as an ensemble-average, interphase chromosomes traverse from nuclear periphery to interior and back to periphery, with gene expression “hot zones” located at the apex of these chromosome trajectories into the nuclear interior. These gene expression hot zones are either near deterministically positioned close to nuclear speckles (Type I) or located at intermediate distances (Type II). Type I gene expression hot zones contain the most highly expressed genes and have the higher gene density, the greater fraction of housekeeping genes, and the greater enrichment of genes with low RNA polymerase pausing, which may be linked to the increased concentration of factors involved in transcriptional pause release and elongation in nuclear speckles [38,39].

How MARGI, SPRITE and TSA-Seq predictions compare awaits data generation from the identical cell lines. Whereas MARGI and SPRITE, through cross-linking to speckle-enriched RNAs (MARGI) or active chromosomal hubs (SPRITE), are conceptualized as measuring the fraction of alleles within close distance to speckles, TSA-Seq instead measures mean speckle distances. Both A1 and A2 Hi-C subcompartments are identified as

speckle-interacting by MARGI and SPRITE, but only A1 is identified as close to speckles by TSA-Seq. An important question is whether chromosome regions showing a low contact frequency by SPRITE and an intermediate distance to speckles by TSA-Seq are positioned persistently away from speckles (Fig. 4a), or might show frequent speckle associations over a physiologically meaningful time frame (either through gene movements to speckles or speckle protrusions and/or movements to genes (Fig. 4b)), or are positioned near smaller speckles giving rise to a proportionally lower TSA-Seq signal (Fig. 4c).

Beyond correlation- is there a functional significance to gene association with speckles?

These new genomic methods demonstrate that the nuclear speckle colocalization observed years ago for ~50% of ~25 protein-coding genes now extends to a comparable fraction of ALL expressed protein-coding genes. This high prevalence of nuclear speckle association across the genome greatly revives interest in the possible functional significance of speckle association, though it still remains only a correlation that does not distinguish cause and effect. However, what is also new has been the demonstration of the existence of an active mechanism for moving genes towards nuclear speckles. Using the Hsp70 HSPA1A gene as a model system, live cell microscopy revealed an actin-dependent, directional movement of plasmid HSPA1A transgene arrays, over distances of up to 4–5 um towards nuclear speckles with an average velocity of ~ 1 um/min after heat-shock induction [40]. The existence of an active mechanism for moving these Hsp70 transgene arrays to nuclear speckles strongly suggests the existence of active mechanism for positioning endogenous chromosomal regions near nuclear speckles.

Moreover, live-cell imaging of Hsp70 transgenes demonstrates a strong likelihood of functional significance of speckle association by showing that nascent transcripts of these transgenes become visible only after, and not before, first contact of the transgene with a nuclear speckle. Using a plasmid transgene HSPA1A array expressing MS2-tagged HSPA1A transcripts, visible accumulations of nascent transcripts after heat shock were observed only 0 to several minutes following first contact with nuclear speckles [40]. Like most multi-copy plasmid transgene arrays, this HSPA1A transgene array is heterochromatic and shows greatly delayed and asynchronous heat-shock induced expression, raising questions of physiological significance. However, more recent work using a BAC Hsp70 transgene system that more closely recapitulates normal Hsp70 gene expression points to a model in which amplification of gene expression is linked to nuclear speckle association (IMN Kim et al, **bioRxiv 604298; doi:** https://doi.org/10.1101/604298). Several fold higher levels of nascent transcripts are seen after heat shock using smRNA FISH for speckle-associated versus non-associated endogenous Hsp70 alleles, while live-cell imaging of Hsp70 BAC transgenes shows association of the transgene with nuclear speckles precedes the appearance of a MS2-nascent HSPA1B transcript signal by up to several minutes, while separation from nuclear speckles precedes by several minutes downregulation and disappearance of these MS2 signals.

This transcriptional amplification phenomenon appears to require actual physical contact, if only transient, with the nuclear speckle. Nuclear speckles behave as liquid phase-separated bodies, with measured viscosities comparable to other liquid-phase separated RNP bodies [41]. Biophysical analysis suggests liquid condensates may greatly enhance rates of certain reactions by concentrating factors that favor the reaction [42]. Moreover, interactions among components of nuclear speckles may lead to a multi-layer organization concentrating certain components, including MALAT1 and snRNAs, to the speckle periphery in position to interact with active chromosomal regions [9].

Nuclear speckles have also been suggested as playing a distinct role in post-transcriptional splicing and nuclear export of RNAs and changes in expression of a many nuclear speckle proteins are linked to human disease states, particularly cancer, and altered alternative splicing of particular mRNAs [43,44]. Both unspliced RNAs and spliced RNAs without Exon Junction Complex (EJC) proteins accumulate within nuclear speckles, suggesting that speckles may act as a quality control for mRNAs prior to export [25–31,45]. The liquid phase condensation behavior of nuclear speckles could enhance the functions of speckles to regulate RNA processing activities both by accelerating reactions within speckles and by buffering concentrations of speckle components in the nucleoplasm.

Conclusions and perspectives

Gene expression in the nucleus is regulated at many levels including transcription, splicing, mRNA modification and export. Two of these at least- transcription and splicing-already are known to be tightly coupled [46]. Components related to all of these levels of gene expression are concentrated in nuclear speckles, including for example transcription (p300/CBP, pTEF-beta), splicing (snRNPs, SR proteins), and RNA capping, poly-adenylation, and export factors. The recent genome-wide demonstration of the prominent localization of a significant fraction of active gene loci near nuclear speckles revives old models of nuclear speckles and their periphery acting as a central hub to coordinate multiple steps of gene expression. Genomic analysis also reveals a linear gradient of gene expression as a function of mean speckle distance [24]. Years ago, models were presented by which speckle protrusions or a transport network might link the contents of nuclear speckles with sites of active transcription and RNA processing [47,48]. More recently, live-cell imaging has revealed directional movements of transgenes towards nuclear speckles and dynamic changes in distances of transgenes from speckles due to the mobility of both. It is fascinating to speculate that the dynamics of gene loci, speckles, and interchromatin granules might lead to a merging of both the older “storage site” and “transcriptional hub” models of nuclear speckle function. Live-cell imaging technologies should allow future exploration of how nuclear speckle and gene dynamics may contribute to the fine-tuning of gene expression.

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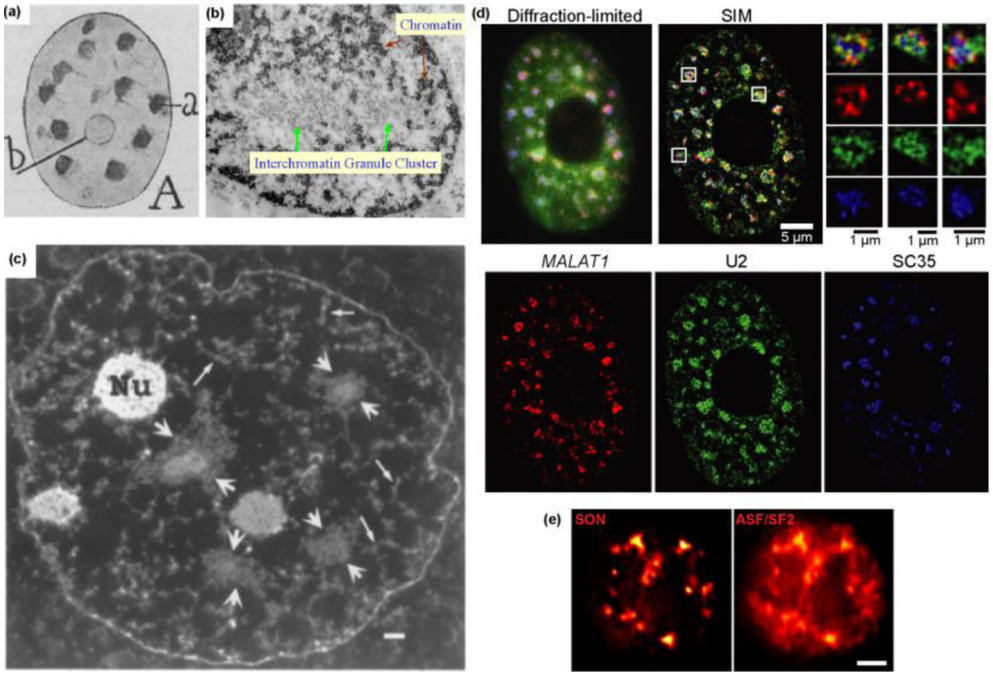
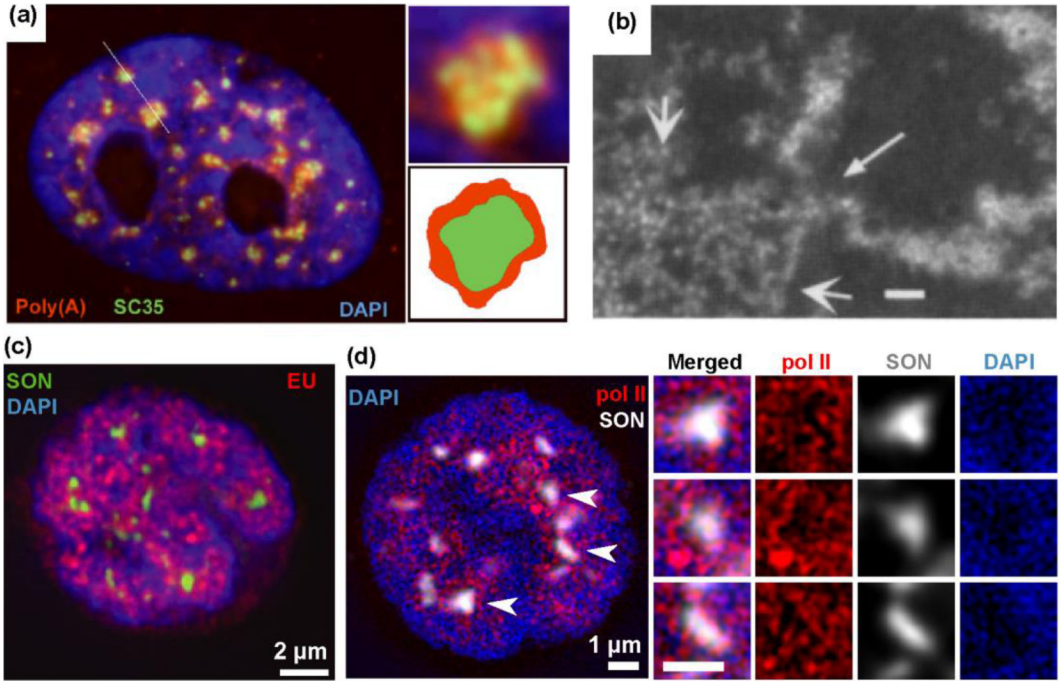


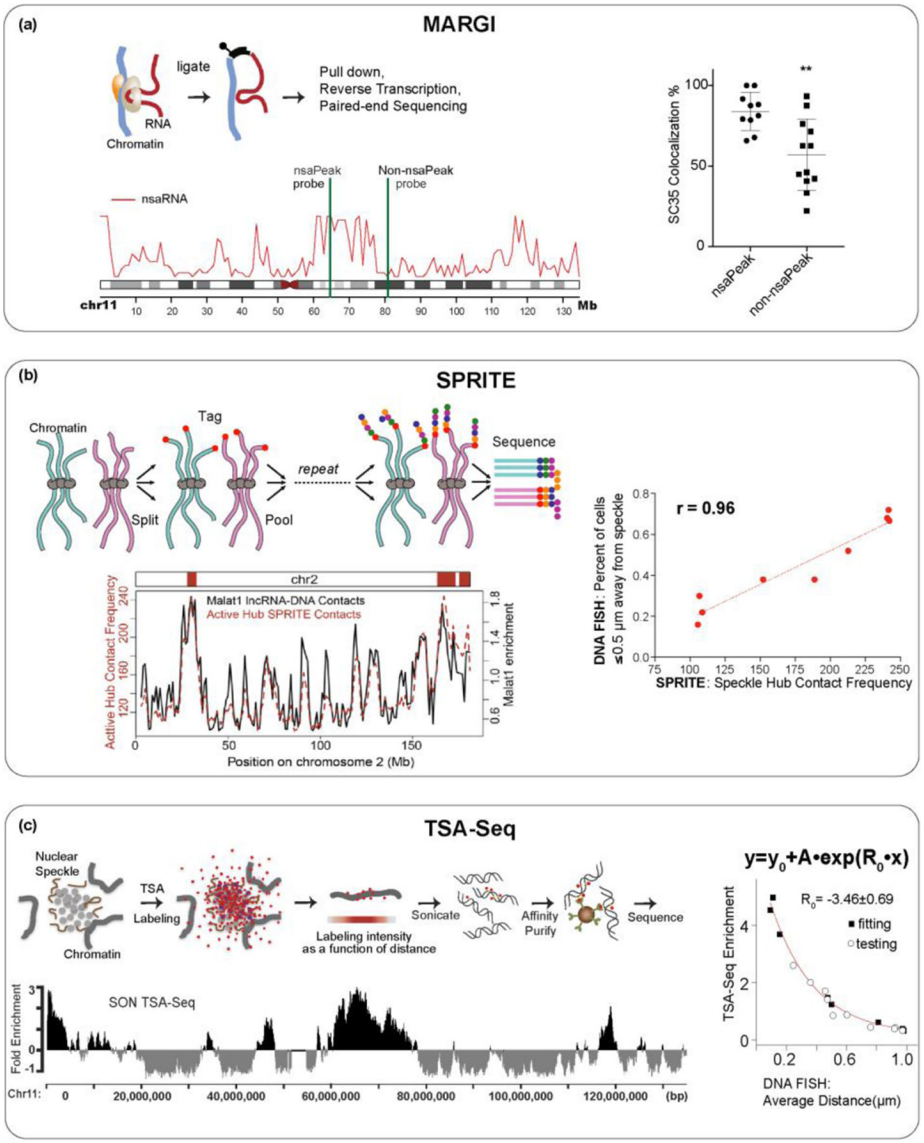
1. Variable Identification/Visualization of Nuclear Speckles.

(a) Cajal identified nuclear speckles (handwritten “a”) using different histochemical stains, including silver staining; also shown are nucleoli (handwritten “b”). Original drawing at the Cajal Institute, CSIC, Madrid. Reprinted from Fig. 2a, Ref. [3]. (b) Energy loss EM visualization of nuclear speckles in Hela cells as Interchromatin Granule Clusters (green arrows) surrounded by chromatin (red arrows); energy loss signal (black) is proportional to presence of nucleic acid (phosphorus). Image courtesy of Michael Hendzel. (c) EM section showing nuclear speckles in detergent-extracted nucleus stained with heavy metals in CHO cells; a peripheral zone (large arrowheads), which appears distinct from the surrounding chromatin (narrow arrows), surrounds the granular core of the speckle. Negative image is shown, with heavy metal staining appearing white and low staining black. Scale bar= 0.5 μm. Reprinted from Fig. 5B, Ref. [8]. (d) lncRNA Malat1 (red) and U2 snRNA (green) concentrate at the speckle periphery surrounding SC35 (blue) speckle core as visualized by 3D SIM light microscopy; U2 snRNA also localizes to small foci outside of speckles. Diffraction-limited image (top left) is shown for comparison. Reprinted from Fig. 1A, Ref. [9]. (e) The SON speckle marker (left) shows a higher, more localized concentration over nuclear speckles than SR splicing factors such as ASF/SF2 (right) which stains a larger region surrounding speckles, regions connecting speckles, and other focal accumulations. Scale bar= 2 μm. Reprinted from Fig. S1A, Ref. [24].



2. Poly(A)+ RNA, Decondensed Chromatin, and Transcription Sites at the Nuclear Speckle Periphery.

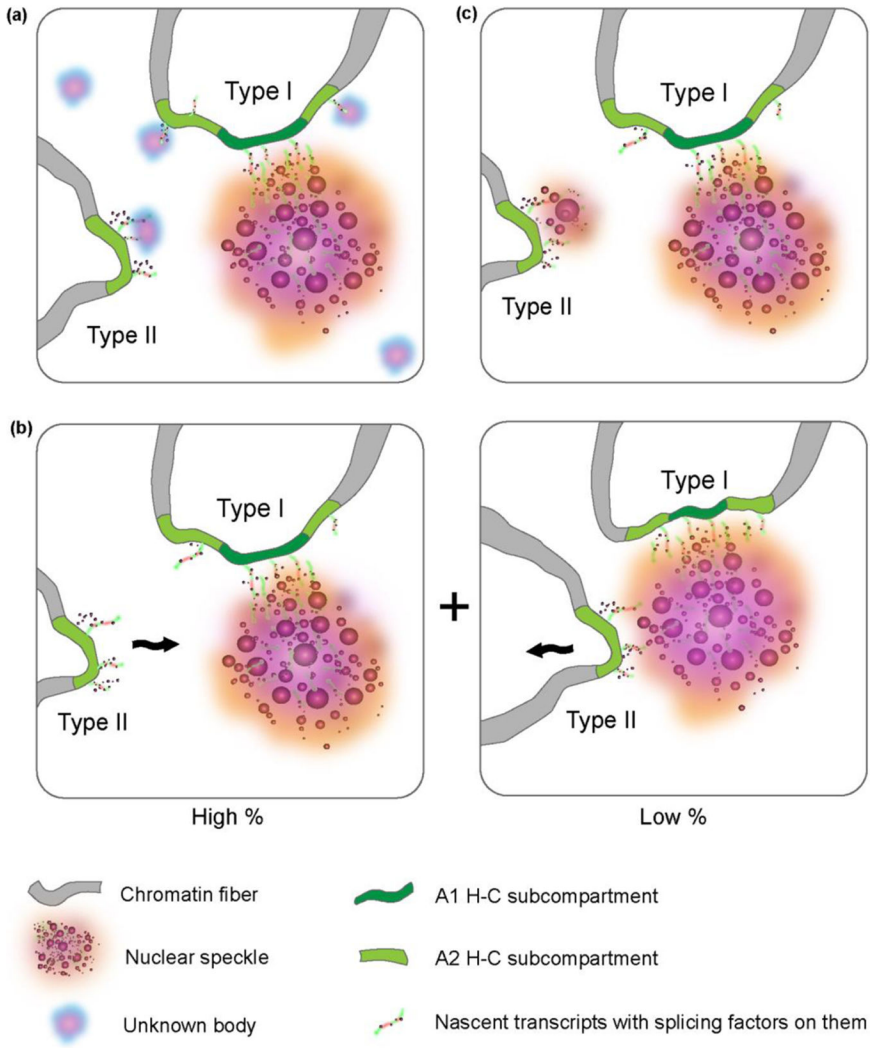
(a) Nuclear speckles were identified as “Poly(A)+ RNA islands”(red) and “SC35 domains” (green); the Poly(A)+ RNA signal overlaps but is also peripheral to the SC35 domain. Reprinted from Fig. 2A,E,F, Ref. [2]. (b) Serial-section EM reconstruction of detergent-extracted nucleus stained with heavy metals in CHO cells. Negative image is shown, with heavy metal staining appearing white and low staining black. Locally decondensed large-scale chromatin fibers (small arrowheads) frequently mapped to the Interchromatin Granule Cluster periphery (large arrowheads). Scale bar= 120 nm Reprinted from Fig. 8E, Ref. [8]. (c-e) Active sites of transcription and Ser5p- RNA pol2 staining foci surround nuclear speckle periphery. (d-e) 5 min EU pulse-labeling of transcription (d) or Ser5p-RNA pol 2 staining (e) (red) relative to nuclear speckle marker (SON) (green in (c) and white in (d)). 2x enlarged views corresponding to regions surrounding speckles (arrows). Reprinted and Modified from Fig. 5C&D, Ref. [24, with permission from Rockefeller University Press.



3. Genomic assays for speckle localization and correlation with speckle localization measured by FISH.

(a) MARGI uses an oligonucleotide adaptor to ligate RNA and DNA fragments (top left). Counts of ligations with nuclear-speckle associated RNAs (saRNAs) show peaks and valleys across chromosomes (bottom left). FISH measurements show an increased level of SC35 colocalization with probes selected from peaks (nsaPeak) versus valleys (non-nsaPeak). Adapted under Creative Commons Attribution License (CC BY) from Graphical Summary, Fig. 4A,D, Ref. [37], (b) SPRITE measures colocalization of DNA and RNA fragments in isolated, chromatin complexes (top left). Large complexes with many DNA fragments show unusually high numbers of inter-chromosome interactions with a small number of active chromosomal regions, defining an “active chromosomal hub”. The frequency of chromosome interactions with this active hub (red, y-axis) correlate with Malat1 RAP-RNA-Seq (black, y-axis) (bottom left). FISH of different probes showed a linear correlation in percentage of alleles mapping <0.5 μm from a nuclear speckle (y-axis) with this active hub

contact frequency (x-axis) (right). Adapted from Graphical Summary, Figs. 5A, 6D, Ref. [36]. (c) TSA-Seq measures spreading of diffusible tyramide free-radical, generated by a peroxidase localized near nuclear speckles by SON immunostaining, producing biotin-DNA labeling proportional to speckle distance. DNA purification is followed by biotinylated-DNA pull-down and sequencing (top left). TSA-Seq score uses log2 ratio of observed versus average read count (y-axis, log2 ratio of fold-enrichment), with peaks predicting regions near speckles (bottom left). An exponential relationship was observed between probe TSA-Seq fold-enrichment and the average FISH-signal distance from a speckle (right). Adapted from Figs. 1A, 2A,B, 3A,B, Ref. [24] with permission from Rockefeller University Press.



4. Models for explaining variable speckle proximity/distance observed by genomic methods. Variable size peaks observed for SPRITE [36], TSA-Seq [24], and MARGI [37] have been interpreted differently. (a) Model 1: SON TSA-Seq identified two types of transcription hot-zones mapping near TSA-Seq local maxima: Type I (large peaks, correlated with A1 Hi-C subcompartment) positioned close, on average, to nuclear speckles and Type II peaks (smaller peaks, correlated with A2 Hi-C subcompartment), positioned at intermediate distances, on average, from speckles, and possibly interacting with an unknown nuclear body. (b) Model 2: Larger TSA-Seq (Type I), MARGI, and SPRITE peaks could correspond to a larger fraction of alleles localizing close to nuclear speckles, with smaller TSA-Seq (Type II), MARGI and SPRITE peaks showing a smaller fraction of alleles close to speckles. (c) Model 3: Larger (Type I) versus smaller (Type II) TSA-Seq peaks could correspond to association of chromosome region with larger versus smaller nuclear speckles, respectively. Further work is needed to determine which model best describes chromosome positioning relative to nuclear speckles.