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Review

Nuclear speckles: a fundamental layer of gene regulation

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Within the cell nucleus, non-DNA structures called nuclear bodies interact with chromatin to regulate gene expression and organize our genetic material. Among nuclear bodies, nuclear speckles are prominent. They interact with broad genomic regions, serve as major gene-activating structures, and are implicated in viral infection, cancer, neurodegeneration, stress response, and development. Advances that integrate genomics with imaging are leading to a deeper understanding of how nuclear speckles fit into our current knowledge of chromatin biology, genome organization, and the central dogma of biology. Collectively, these recent studies emphasize nuclear speckles as key gene regulatory structures within the cell nucleus, offering new perspectives on how gene expression dysregulation is linked to disease.

Highlights

Nuclear speckles are implicated across diverse physiological and disease conditions.

Nuclear speckle evolution is linked to genome organization in vertebrates.

Integration of genomics and imaging places nuclear speckles into the context of chromatin biology.

Functional studies establish speckle roles across RNA biogenesis.

Nuclear speckles vary across contexts, yet the full outcomes of their variation remain unresolved.

Nuclear speckles: a fundamental layer of gene regulation, critical for health and disease

During the genomics revolution in the 2000s–2020s, the gene regulation and **chromatin** (see Glossary) fields gained the ability to assay the entire genome, leading to an increased representation of the human genome in two dimensions. New technologies combine insights from genomics and imaging to place the genome in its 3D context [1–3]. This enhanced ability to connect genomic sequences with entities in space has now led to an explosion of research in the area of condensates (reviewed in [4]) and nuclear bodies.

The term 'condensate' refers to membraneless, nonstoichiometric cellular assemblies that concentrate certain biomolecules while excluding others [5]. Nuclear bodies are microscopically defined membraneless structures in the cell nucleus, characterized by specific protein and RNA compositions [6]. Nuclear bodies can exhibit properties of condensates; however, not all nuclear condensates are stable or large enough to meet the structural definition attributed to nuclear bodies.

The two most voluminous nuclear bodies are nucleoli, surrounded by repressive **heterochromatin**, and nuclear speckles, surrounded by active **euchromatin** (Figure 1). Nuclear speckles were first observed by Ramón y Cajal in 1910 as translucent clumps, or hyaline grumes, as seen through light microscopy (reviewed in [7,8]). These structures were later captured at the ultrastructural level and termed 'interchromatin granule structures', as observed via electron microscopy [9]. Speckles have been extensively referenced in the autoimmune literature to describe antinuclear autoimmune antibodies that display a 'speckled', rather than 'diffuse', nuclear staining pattern, leading to the term 'speckles' to describe these nuclear structures [10,11]. In the 1980s, specific proteins that label nuclear speckles were identified, enabling deeper investigations into nuclear speckle biology [12,13].

Structural integrity of nuclear speckles depends on the SON and SRRM2 speckle-resident proteins, which are considered the speckle organizational scaffolds and are widely used nuclear speckle-marking proteins [14,15]. In humans, heterozygous loss of function of either SON or

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SRRM2 leads to severe multisystem developmental disorders, called Zhu-Tokita-Takenouchi-Kim (**ZTTK**) and **SRRM2-related syndrome**, respectively [16–21]. Meanwhile, other speckle-resident proteins are compromised in a series of neurodevelopmental disorders, collectively termed **speckleopathies** [22]. In mice, **homozygous** deletion of *Son* is embryonic lethal, while *Son* **haploinsufficiency** results in organ development and hematopoiesis defects that resemble clinical features of **ZTTK syndrome** [23]. Beyond development, speckles are co-opted by viruses to assist viral replication, and speckle-resident proteins are often misregulated or mutated in cancer (reviewed in [24]). These data from genetics, virology, and cancer collectively point to pivotal roles of nuclear speckles for vertebrate biology. Based on technological advances in imaging and genomics, the molecular functions of nuclear speckles and how they drive physiological or pathogenic processes are becoming clearer.

A hallmark of nuclear speckles is the presence of high levels of proteins involved in RNA biogenesis. These proteins are organized into distinct subcompartments within speckles (Box 1 and Figure 1). Around half of speckle-resident proteins are pre-mRNA splicing factors [25]. Hence, a large focus of speckle research has been aimed at linking speckles to splicing. The remaining half of speckle-resident proteins are involved in other aspects of gene expression: transcription, polyadenylation, RNA nuclear export, and RNA covalent modifications. Thus, speckles have also been viewed as structures that streamline each step of the central dogma of biology, from transcription to translation [74,75]. While this is a compelling model, the field previously lacked functional evidence supporting speckles' role(s) in gene expression. Recent studies are helping bridge this knowledge gap, supporting a paradigm for nuclear speckles as a fundamental and distinct layer of gene regulation with implications across human health and disease. In this review article, we discuss our current knowledge of the principles of speckle-based gene regulation, emphasizing how they operate as an impactful mode of gene regulation.

Organization of chromatin at speckles

It has been recognized for decades that some, but not all, active chromatin resides adjacent to nuclear speckles [74] and that specific genes reposition to speckle-proximal regions upon cellular stress or differentiation [76–78]. When genomic methods to map chromatin–speckle association became available [79–81], a clearer picture emerged of which parts of the genome are positioned at speckles and how this varies between conditions. From a broad chromosome-wide view (~10–100 Mb), locations of speckle-associated genomic regions are remarkably consistent across cell lines [52,79,81–85], constituting 'global' speckle association. At smaller scales (~10–1000 kb), regulated speckle association does occur, and this 'context-dependent' speckle association correlates with boosted RNA expression [52,83,85–87] (Figure 1).

While much of our knowledge on chromatin–speckle contacts is based on studies in cell lines, studies in the mouse brain and during preimplantation development have been pivotal for expanding this knowledge to physiological contexts [81,85,88]. Similar to cell lines, speckle-associated regions are highly consistent between cell types in the mouse brain, and the cell-type-specific differences that occur correlate with differential gene expression [81,85]. In contrast, chromatin–speckle association is dynamic between the one-cell and **blastocyst stages** of mouse embryogenesis. In a recent study, speckle association in precleavage embryos was minimally detected at **pronuclear (PN) stage** 3 (PN3) and emerged at PN5 [88]. Importantly, PN5 occurs prior to **zygotic gene activation**, meaning that chromatin–speckle association is established in the absence of active transcription.

After zygotic gene activation, additional speckle-associated regions arise [88]. Between PN5 and the blastocyst stage, the genome coverage of chromatin–speckle association, as quantified from SON Cut&Tag-defined speckle-associated domains, increases from ~15 to ~30%. These large-

Glossary

Acidic activation domain (AAD): a protein domain found within transcription factors, characterized by an enrichment of acidic amino acids and the ability to activate transcription.
Blastocyst stage: a stage of mammalian development just prior to implantation, characterized by the presence of the trophectoderm, which will give rise to extraembryonic tissues, and the inner cell mass, which will give rise to the embryo.
Chromatin: the combination of DNA with its bound histones and proteins as it exists within a cell nucleus.
Cleavage and polyadenylation: RNA processing steps that help terminate transcription. Enzymatic cleavage of mRNAs occurs at a specific site at the 3′ end of a newly transcribed transcript. Then, a series of adenines are added to polyadenylate the mRNA.
Cornelia de Lange syndrome (CdLS): a neurodevelopmental disorder caused by mutations in the cohesin pathway.
CTCF/cohesin: a protein complex that facilitates chromatin looping.
Cytogenetic bands: parts of condensed chromosomes identifiable by microscopy after staining.
Euchromatin: chromatin characterized by active transcription, which is loosely packed and electron-poor when viewed under transmission electron microscopy.
Haploinsufficiency: a term in genetics referring to the loss of function of one of the two alleles.
Heterochromatin: chromatin characterized by inactive transcription, which is tightly packed and electron-dense when viewed under transmission electron microscopy.
Homozygous: a term in genetics referring to events that occur on both alleles.
Intrinsically disordered region (IDR): a protein region that lacks a stable structural conformation.
Lamin-associated domain: regions of the genome that are constitutively positioned at the lamin at the nuclear periphery.
Morula stage: the preimplantation stage of mammalian embryonic development, comprising approximately 16–32 cells, that precedes the blastocyst stage.
Principal component analysis: a dimensionality reduction method that

scale chromatin–speckle changes during early embryogenesis thus represent an exception to the consistency of speckle association observed in human cell lines and the adult mouse brain.

Outside of early embryonic development, speckle components dissolve during mitosis in a manner that depends on the kinase activity of DYRK3, a kinase implicated in the dissolution of multiple nuclear and cytoplasmic membraneless structures [89]. In metaphase, proteins that reside in interphase speckles are largely diffuse in the mitotic cytoplasm. As mitosis progresses, the DYRK3 kinase is degraded [89], and a subset of speckle constituents accumulates in mitotic bodies, termed 'mitotic interchromatin granules' [90,91], which increase in size and number from anaphase to telophase [92]. Speckle components sequentially re-enter the nucleus following nuclear envelope formation [93]. During this process, certain speckle components are initially diffuse before localizing to speckles [92]. Others localize to nonspeckle nuclear structures, such as Cajal bodies and nucleolus organizer regions, prior to redistributing to speckles [40,92]. The reassembly of nuclear speckles during G1 occurs after the reinitiation of transcription [92], as well as after SRRM2 has re-entered the nucleus [94]. Despite the coincidental timing of speckle formation after the onset of transcription, speckle assembly does not require active transcription [92].

Like during embryogenesis, the establishment of chromatin–speckle association during G1 does not require active transcription, nor is transcription required for the maintenance of speckle contacts in dividing cells [95]. These data support the idea that mechanisms outside of transcription are largely responsible for setting up and maintaining speckle association.

Other than early embryogenesis, it is unclear whether other contexts involve large-scale changes to chromatin–speckle association. Even cell lines with distinct speckle and nuclear morphologies show highly consistent global speckle association [96]. This consistent organization at large scales, with regulation at smaller scales, illuminates unique possibilities for how gene regulation is orchestrated within a cell (Figure 1). Nucleus-wide changes to speckle gene-boosting functions via altered speckle content or dynamics have the potential to coordinate the expression of large groups of genes within global speckle-associated chromatin. Meanwhile, context-dependent changes in chromatin–speckle association enable fine-tuning of gene regulatory programs.

condenses many variables into fewer variables to capture data variance.

Pronuclear (PN) stage: the stage of a one-cell embryo after fertilization and prior to the fusion of the nuclei from the oocyte and sperm.

Speckleopathies: a series of neurodevelopmental syndromes that involve genetic mutations in the genes encoding speckle-resident proteins.

SRRM2-related syndrome: a neurodevelopmental disorder characterized by mutations in the SRRM2 speckle protein.

Transcription export complex (TREX): a protein complex that assists with multiple RNA processing steps and facilitates the export of mRNAs from the nucleus to the cytoplasm.

ZTTK syndrome: a neurodevelopmental disorder characterized by mutations in the SON speckle protein.

Zygotic gene activation: initiation of transcription in a developing embryo. Prior to zygotic gene activation, the developing embryo relies on maternally deposited transcripts.

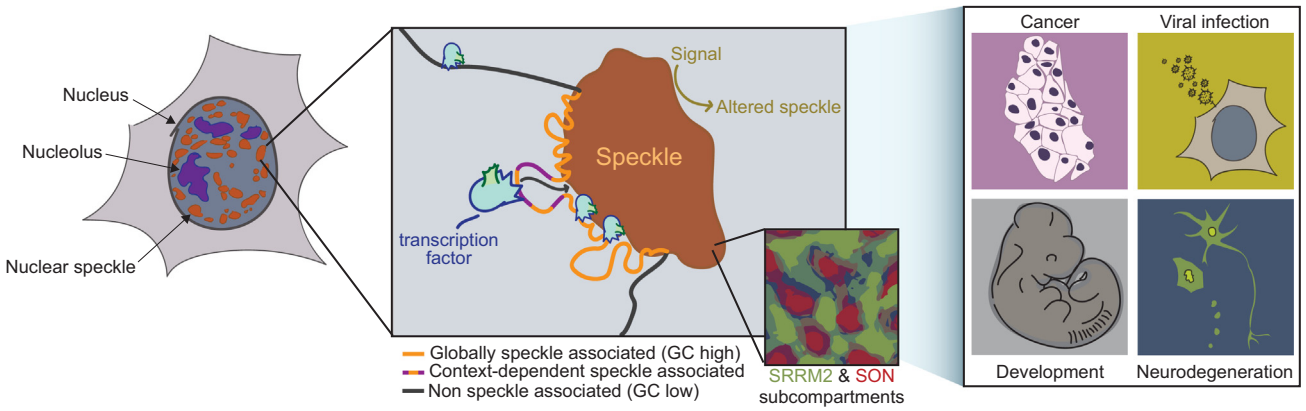

Figure 1. Regulation at nuclear speckles. Regulation at nuclear speckles can occur through altered chromatin–speckle association or altered speckles, both of which respond to a wide variety of stimuli. Across cell lines and conditions, the pattern of broad chromosomal regions that are most speckle-associated is largely invariable, constituting global speckle association, which is GC-rich. At smaller scales, cell type- and condition-specific differences occur, constituting context-dependent speckle association. Both global and context-dependent speckle associations are controlled by transcription factors. Alterations of speckles, which are composed of intermingling substructures of the SON (red) and SRRM2 (green) speckle scaffolds, can occur in a variety of ways to impact speckle characteristics. A list of known conditions that alter speckles is provided in Table 1 in the main text.

Box 1. Compartmentalization within nuclear speckles

Proteomic and imaging-based studies show that nuclear speckles contain a myriad of factors involved in transcription, splicing, mRNA capping, polyadenylation, and mRNA nuclear export [25–29]. Additionally, speckles host noncoding RNAs such as *MALAT1*, a long noncoding RNA that recruits certain proteins to speckles [30] but is not essential for speckle structure [31], and small nuclear RNAs such as *U1* and *U2* that facilitate mRNA splicing. These constituents are not homogeneously distributed within speckles but rather form distinct substructures [32]. At the speckle core are SON and SRRM2 [33], which are essential for the speckle occupancy of many speckle components [14]. SON and SRRM2 form distinct substructures from one another within speckles, each with its distinct molecular clients [15]. While SON and SRRM2 reside at the speckle core, *MALAT1*, U1, U2, and U2B show enrichment at the speckle periphery, suggesting a distinct molecular environment at the edges versus the center of nuclear speckles [33]. Supporting this, members of the exon junction complex, a critical complex for posttranscriptional RNA control, are also localized at the edges rather than the core of nuclear speckles [34].

The mechanisms of speckle molecular organization are an area of active research. Of particular interest is what facilitates cohesion within and between speckle substructures. Speckle substructures are punctate in form, with distinct structures intermingling with one another within a speckle [15,33]. Manipulation of speckle constituents can lead to demixing, where the components of speckles become spatially segregated. For example, SRSF1 knockdown results in a distinct *MALAT1* and U1 small nuclear ribonucleoprotein particle (snRNP) ring around the SRRM2 component of speckles [33]. However, the intermingling of SON and SRRM2 speckle substructures within speckles is resilient to a wide variety of stressors and consistent across cell lines [15], arguing for a degree of robustness in the molecular interactions between speckle substructures.

SON and SRRM2 have distinct biophysical properties, with dynamic protein exchange for SRRM2, but not SON [15]. The SRRM2 component of speckles requires the SRRM2 arginine/serine (RS) domains, RS1 and RS2, which can be replaced by other oligomerizable modules to rescue nuclear speckle assembly [15]. RS1 and RS2 flank the SRRM2 IDR. Hence, SRRM2 follows the 'stickers-and-spacers' framework described for biomolecular condensates [35], where the RS domains are the 'stickers' with oligomerization functions, and the IDR is the spacer. Phosphorylation of the SRRM2 IDR modulates the material properties of cellular SRRM2 condensates without impacting the condensation threshold [36], adding to the growing evidence that speckle properties are regulated by post-translational modifications (see also Table 1 in the main text).

Global speckle-associated regions cover large, but specific, genomic regions

Global speckle-associated regions encompass large stretches of the genome, including entire **cytogenetic bands** and chromosome arms. For example, in chromatin–speckle mapping experiments conducted thus far, human chromosome 19 is highly speckle associated, while chromosome 18 has very little speckle association [52,79,81–85]. These speckle association patterns are remarkably correlated with GC content [79,97]. Large regions of the human genome are either GC-rich or GC-poor and are subject to distinct splice-site recognition strategies [98–100]. While mammalian, bird, reptile, and amphibian genomes show this heterogeneity in GC content across genomic segments, this feature is not present in invertebrates or fishes, which have more uniformly low GC content across their genomes [101]. Interestingly, speckles appear to have evolved with vertebrate territorialization—they are evident in snakes, turtles, birds, and mammals but absent from eels, zebrafish, and *Drosophila* [97,102,103]. The coevolution of speckles and GC content, together with the organization of high-GC genomic regions next to speckles, supports the leading theory that speckles may have evolved to accommodate high-GC-content gene architecture (also see *RNA processing*, below) [97].

Speckle-associated, high-GC-content genomic regions are gene-dense and enriched for shorter genes. In contrast, longer genes tend to be less speckle-associated [97]. This organization suggests that speckles influence large but selective gene sets. Top among genes enriched within speckle-associated chromatin are those involved in protein translation, whereas active genes that do not associate with speckles are enriched for other functional categories [82,85]. The compartmentalization of genes into speckle and nonspeckle nuclear locations may therefore be a mechanism to coordinate broad regulatory programs via changes to speckles.

There is a growing list of conditions that influence speckle form and content (Table 1). However, the relationship between speckle phenotype and the expression of genes within the large gene

Table 1. Conditions that alter nuclear speckles[1]

Condition	Speckle phenotype	Details	Refs
Hypoxia	Decreased speckle cohesion	Increased SR protein phosphorylation, leading to decreased mRNA retention in speckles	[37]
Hypoxia	Nuclear speckle dispersal	Poison exon cassette induced downregulation of SRSF6, leading to global exon skipping	[38]
Hypoxia	Enrichment of SRSF1 near speckles	Recruitment of SRSF1 by MALAT1 speckle-resident long non-coding RNA, leading to alternative splicing	[30]
Oxidative stress (sodium arsenite)	Increased speckle cohesion and smaller speckle size	Decreased SR protein phosphorylation, leading to mRNA retention in speckles	[36,37]
Oxidative stress (H_2O_2)	Compromised nuclear speckle coarsening	Disrupted interaction between CK2 kinase and SRRM2, leading to reduced SRRM2 phosphorylation and altered splicing efficiency	[36]
Heat shock	Increased speckle cohesion and smaller speckle size	Decreased SR protein phosphorylation, leading to mRNA retention in speckles	[36,37]
DNA damage (UV)	Smaller speckle size	N/D	[36]
Proteosome inhibition (MG132)	Smaller speckle size	N/D	[36]
Transcription inhibition (JQ1-VHL)	Enlarged nuclear speckles	The TREX mRNA export complex facilitates nascent transcript retention in speckles	[39]
Transcription inhibition (alpha amanitin and/or DRB)	Rounded nuclear speckles with altered RNA and protein content	Speckles fuse via long-range directional movements	[13,31,40–42]
Splicing inhibition	Enlarged, rounded speckles with altered speckle content	mRNA retention in speckles	[43–45]
Neuronal stimulation (seizure induction in rats)	Altered speckle morphology, formation of compact domains within speckles, altered speckle protein composition	Implicates speckles in learning and memory	[46]
Neuronal stimulation (long-term potentiation induction in rats)	Increased nuclear speckle number and surface area; increased number of small and large speckles with reduced number of medium-sized speckles	The ARC protein increases nuclear levels during long-term potentiation. ARC regulates both basal and activity dependent formation of nuclear speckles	[47]
Tau aggregates	Altered speckle content	SRRM2 and other speckle constituents are displaced from nuclear speckles to cytoplasmic Tau aggregates, leading to mRNA splicing and processing defects	[48]
Tau aggregates in the context of HSV-1 infection	Nuclear speckle decondensation	Implicates speckles in neurodegenerative disease	[49]
(GGGGCC)n repeat expansion in C9ORF72	Altered speckle phase separation properties and granule dynamics with (GGGGCC)n RNA expression	(GGGGCC)n repeat RNA binds nuclear speckle proteins, leading to mRNA splicing defects. Implicates speckles in frontotemporal dementia and amyotrophic lateral sclerosis	[50]
(CAG)n repeat RNA	Accumulation of (CAG)n repeat RNA in nuclear speckles	Implicates speckles in Huntington's disease	[51]
Variation in human cancer	Centralized versus dispersed SON-labelled nuclear speckles	Altered RNA expression of genes within speckle-associated chromatin. Implicates speckles in clear cell renal cell carcinoma patient survival and response to therapy	[52]
Tubercidin	Enlarged and reduced number of nuclear speckles	Altered expression of apoptotic genes. Implicates speckles in cardiotoxicity	[53]
Ribotoxic stress	P38 MAPK-dependent relocalization of TIAR to nuclear speckles	Higher splicing efficiency of immediate early genes. Implicates speckles in rapid stress response	[54]
Ultradian rhythm	12 h fluctuations in SON protein levels and nuclear speckle dynamics	Amplified gene expression when SON is high and speckles are fluid. Implicates speckles in proteostasis	[55]

(continued on next page)

Table 1. (continued)

Condition	Speckle phenotype	Details	Refs
Pyrvinium pamoate (FDA-approved compound)	Increased nuclear speckle sphericity, perimeter, and altered biophysical properties	Suggests a potential speckle therapeutic for proteostasis disorders	[56]
HIV infection	HIV induces CPSF6 puncta fusion with nuclear speckles. HIV replication complexes accumulate within nuclear speckles and integrate into speckle-associated DNA	Fusion between CPSF6 puncta and nuclear speckles depends on intrinsically disordered domain of SRRM2	[57–60]
Influenza A infection or gene products	Influenza M1 and M2 mRNAs accumulate in nuclear speckles	The influenza M1 mRNA is targeted to nuclear speckles by the NS1 protein to enable splicing into M2. This process depends on the host speckle-resident kinase, TAO2. Speckle resident NS1 causes dysregulated host transcriptional termination	[61–65]
HSV-1 infection	Larger speckle size, reduced speckle number, redistribution of speckle and paraspeckle components	HSV replication compartments coalesce at and reorganize nuclear speckles, where HSV-1 immediate-early viral transcripts accumulate. Speckle component SRSF2 colocalizes with paraspeckle components, such as NEAT1 and PSPC1. Speckles lose *MALAT1* and increase dynamics of SRRM2	[66–68]
Kaposi's sarcoma-associated herpesvirus (KSHV)	Enlarged and fewer nuclear speckles located at the nucleus periphery and with altered protein and RNA content.	KSHV viral RNA *Kaposin* is necessary and sufficient for speckle remodeling, leading to speckle-like structures at the site of viral RNA production	[69,70]
Epstein Barr virus (EBV)	EBV causes nuclear speckle dispersal and reorganization	Viral and cellular RNA splicing and export factors form new nodular structures, which is accompanied by blocked export of host mRNA and selective processing and export of viral mRNA	[71]
Human cytomegalovirus (HCMV)	HCMV viral transcripts populate nuclear speckles	The HCMV protein, IE86, connects HCMV immediate early transcription sites to nuclear speckles	[72]
Adenovirus	Enlarged nuclear speckles	Spliced viral late RNAs accumulate in nuclear speckles prior to nuclear export	[73]

[1]JQ1-VHL - JQ1 BRD4 bromodomain inhibitor fused to VHL; DRB - 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole; MAPK - Mitogen-Activated Protein Kinase; TIAR - TIA-1 Related Protein; HSV - Herpes Simplex Virus

neighborhoods that surround them remains poorly understood. In our recent study, we identified two major speckle states in cancer and found that they are associated with gene expression differences across entire cytogenetic bands [52]. These shifts closely matched the degree to which those regions contact speckles, suggesting that speckle states influence expression at the scale of large genomic neighborhoods. While these are exciting correlational observations, the causal relationship between speckle phenotypes and RNA expression has yet to be fully resolved and requires extensive functional study.

Context-dependent speckle association involves moderately sized gene neighborhoods

Context-dependent speckle association often occurs within global speckle-associated regions—that is, quantitative differences in speckle contacts of a portion of the genome that already has some degree of speckle association. During p53 activation, about one-third of p53 target genes are repositioned to speckles, and speckle association at baseline is highly predictive of which p53 target genes experience regulated speckle association [86]. Similarly, heat shock-regulated genes that increase speckle association also generally show speckle pre-positioning [83], and the *β-globin* gene is modestly speckle-associated prior to its enhanced speckle association during erythropoiesis [104]. Moreover, tethering of speckle-targeting transcription factors to a TetO-tagged locus led to stronger repositioning to nuclear speckles when the TetO repeat array was integrated between two

lamin-associated domains, which have modest speckle contact, as compared to a lamin-associated domain, which has minimal speckle contact [95]. These observations suggest that not all genes have equal opportunity to become positioned at speckles, with larger distances to speckles at steady state being a potential barrier to speckle utilization by genes.

To date, the observed context-dependent speckle-associated regions have been smaller in scale than global speckle-associated regions. Nonetheless, they encompass multiple genes. Regions of up to 1.5 Mb have speckle association regulated by Hyoxia-Inducible Factor 2α (HIF-2α), including a region with one HIF-2α binding site and nine HIF-2α-responsive genes [52]. At the *HSPA1A* genomic locus, speckle positioning is accompanied by an expression boost of *HSPA1A* and *HSPA1B* flanking genes, *VARS*, *LSM2*, *C6ORF48*, and *MSH5* [87]. These data support the idea that speckle association boosts expression of moderately sized gene neighborhoods. However, additional data are needed to definitively demonstrate speckle control over gene neighborhoods. Still, these findings highlight context-dependent speckle association as a potential mechanism to regulate groups of flanking genes.

Transcription factors mediate chromatin–speckle association

It has recently been uncovered that transcription factors mediate both global and context-dependent chromatin–speckle association (Figure 1). This is a fundamental discovery because, through structure–function studies, it establishes speckle targeting as a distinct molecular function of transcription factors. Initially, three speckle-targeting transcription factors/complexes were identified: the **CTCF/cohesin** complex [82], p53 [86], and HIF-2α [52]. The involvement of CTCF/cohesin was suggested by correlational data that found strong enrichment of CTCF and cohesin protein RAD21 binding sites within speckle-associated chromatin [79,82]. This concept was further supported in a study that investigated the consequences of DNA methyltransferase inhibitors: inhibition of DNA methylation enabled new CTCF chromatin binding and enhanced chromatin–speckle association at the newly CTCF-bound sites in a CTCF-dependent manner [105]. Another study utilized inducible degradation of CTCF or RAD21 to demonstrate the requirement of CTCF and RAD21 for global chromatin–speckle association [82]. In a third study, the cohesin-loading protein NIPBL has also been implicated [88]. Knockdown of *Nipbl* in mouse embryos led to reduced chromatin–speckle association of the speckle-associated regions that were established after zygotic gene activation. *Nipbl* knockdown had minimal effect on speckle-associated regions established before zygotic gene activation, suggesting potential involvement of other factors. Both *Rad21* and *Nipbl* are frequently mutated in a neurodevelopmental disorder called **Cornelia de Lange syndrome (CdLS)** [106]. Interestingly, speckleopathies and CdLS share symptoms such as developmental delay, cognitive impairment, and craniofacial anomalies. Chromatin–speckle mapping experiments in CdLS patient-derived cells show reduced global speckle association [82], suggesting that CdLS and speckleopathies could have shared defects in speckle-based gene regulation.

In contrast to CTCF/cohesin, which are expressed at high levels across cell types, the p53 and HIF-2α speckle-targeting factors are stress-responsive, with their protein levels controlled by environmental and cellular cues. p53 and HIF-2α drive context-dependent speckle association of a subset of their target genes, leading to boosted target gene expression [52,86]. For both p53 and HIF-2α, speckle-associating target genes are enriched in distinct functional categories compared with nonspeckle-associating targets. Many transcription factors, including p53 and HIF-2α, elicit vastly different cellular responses depending on context. Thus, the organization of functional pathways into speckle-associating versus nonspeckle-associating chromosomal regions could provide a mechanism to skew transcription factor-driven functional outcomes. In support of this, altered HIF-2α-mediated DNA–speckle association or altered speckle states

shift which HIF-2α target genes are preferentially expressed [52]. This suggests that speckle-based gene regulation could skew outcomes of transcription factor activity through altered speckles or altered ability of transcription factors to drive chromatin–speckle association (Figure 1).

Mechanistically, gene–speckle targeting by p53 does not require p53 transactivation functions but does require DNA binding and the p53 proline-rich region [86]. This separation of transactivation and speckle-targeting functions of p53 demonstrates that regulation of chromatin–speckle association is a distinct transcription factor molecular function. Subsequent studies of RAD21 and HIF-2α revealed homologous proline-rich regions, which were essential for chromatin–speckle targeting in both cases [52,82]. These protein regions have thus been coined 'speckle targeting motifs' (STMs). Over 500 gene regulators contain putative STMs [52], indicating that speckle targeting could be a widespread mechanism of gene regulation. In preimplantation mouse embryos, STM-containing transcription factors are expressed in a stage-specific manner that corresponds to the establishment of stage-specific speckle-associated regions [88]. For example, STM-containing transcription factors encoded by maternally deposited transcripts have enriched DNA-binding motifs within speckle-associated regions that arise before zygotic gene activation. Meanwhile, STM-containing transcription factors expressed after zygotic gene activation have enriched DNA-binding motifs within later-established speckle-associated domains. GATA6 is one such transcription factor that is expressed at the **morula stage** and shows strong enrichment of binding sites within morula-established speckle-associated regions. Knockdown of *Gata6* compromises speckle association, supporting that GATA6 is yet another transcription factor that drives chromatin–speckle association.

STMs represent one starting point for the *de novo* discovery of novel speckle-targeting proteins. However, they have been defined based on just a few transcription factors, and the exact amino acid requirements within STMs have not been determined. Moreover, proline-rich STMs do not represent the sole mechanism for speckle targeting. In a recent study, transcription factor **acidic activation domains (AADs)** were shown to be sufficient to direct chromatin–speckle association [95]. This study identified a group of AAD-containing transcription factors that facilitate chromatin–speckle targeting and a second group of transcription factors without AADs that did not direct chromatin–speckle association. Here, global inhibition of transcription compromised speckle targeting by transcription factor AADs. Global transcription inhibition also disrupts speckle content and dynamics (Table 1). Thus, the observed disruption of AAD-mediated speckle association could be an indirect effect of transcription inhibition. Nonetheless, the authors demonstrate that global speckle association is largely insensitive to transcription inhibition. Moreover, the authors show that domains outside of AADs within two transcription factors, ZNF384 and ZNF460, accomplish speckle targeting even with global transcription inhibition. Additionally, the *HSPA1* locus repositions to speckles under heat shock, which also involves speckle alterations that mimic global transcription inhibition [41], indicating that chromatin–speckle association can be resilient to major speckle changes. These are foundational discoveries because they demonstrate that chromatin–speckle targeting by transcription factors occurs through multiple mechanisms. Additionally, the study illustrates that not all transcription factors direct chromatin association with nuclear speckles. These findings emphasize that speckle targeting is a specialized, yet widespread, transcription factor mechanism.

Speckles impact multiple steps of RNA production

Given the intricate organization of chromatin around nuclear speckles, it is essential to understand how speckles fit into the central dogma of biology. The process of producing translation-ready RNA bridges chromatin mechanisms, transcription, co-transcriptional RNA processing,

RNA stabilization, and nuclear export. Nuclear speckles contain factors involved in multiple steps of this process [25–27] (Box 1). Hence, the chromatin surrounding speckles may be subject to a potent gene-activating environment that enhances the entire process of RNA production, making it more efficient. Several publications provide important insights that illustrate multifaceted roles for speckles in boosting gene expression.

Chromatin mechanisms

It is critical to first place speckles in the context of the other studied chromatin mechanisms of gene regulation. Covalent modifications of histones and histone variants impact chromatin accessibility, transcription factor binding, and the recruitment of co-activators or co-repressors. Certain regions of chromatin also participate in phase-separated transcriptional condensates. Nuclear speckles are also condensates with phase-separation properties (Box 1) but are distinct entities from transcriptional condensates [15,94,107]. There are clear correlations between histone properties, transcriptional condensates, and nuclear speckles; however, the causal direction of these relationships is not yet resolved (Box 2). The field is also in the process of untangling how speckles relate to chromatin architecture, particularly regarding chromatin architectural insights that have emerged from chromatin conformation capture methodologies (Box 3). While much of the interaction between speckles and histone modifications, transcriptional condensates, and chromatin conformation is still under investigation, the data available indicate that these are distinct layers of gene regulation that work together to accomplish the gene regulatory landscape within a cell (see Boxes 2 and 3 for more details).

Transcription

While speckle-associated chromatin is enriched for highly active genes, sequencing and imaging-based chromatin–speckle mapping studies demonstrate that speckle association of genes is not essential for transcription—many active genes are not positioned at speckles [79–81,113]. Indeed, a recent imaging-based study shows prominent active RNA Pol II foci away from speckles at active neuronal genes, supporting speckle–distal gene activation [85]. Reciprocally, gene positioning at speckles is not sufficient to instigate transcription. Not all genes positioned at speckles are transcriptionally active. Moreover, when p53's transactivation domains were

Box 2. The activating environment of speckle-associated chromatin

Enhancers, super-enhancers, and activating histone modifications are highly enriched within speckle-associated chromatin [79]. However, the causal relationships between speckles and histone modifications are not yet clear. Several key histone-modifying enzymes, histone-binding proteins, and co-activator complexes contain putative speckle-targeting motifs (STMs), including histone acetyltransferases (EP300, EP400, CREBBP, KAT6A, KAT6B, KAT4, and KAT8), histone 3 lysine 4 methyltransferases (KMT2A, KMT2B, KMT2C, and KMT2D), bromodomain-containing proteins (BRD2, BRD3, and BRD4), and mediator complex members (MED1, MED12, MED13, MED13L, MED14, MED15, and MED26) [52]. While the speckle-targeting functions of these putative STMs have yet to be demonstrated, their presence within these factors hints at a mechanism beyond direct DNA-binding transcription factors that could link active chromatin to nuclear speckles. Alternatively, histones or histone modifications could directly interface with nuclear speckles. Biochemical studies show that nuclear speckles are tightly associated with chromatin [108]. In round spermatids, a spermatogenesis stage prior to transcriptional shutdown, histone variant H2A.B.3 interacts with several speckle proteins and resides within speckles [109]. H2A.B.3 is a specialized histone variant with high tissue specificity, presenting an opportunity to impact speckle-mediated gene expression in a tissue-specific manner. Outside of H2A.B.3 during spermatogenesis, the extent to which specific histone variants or histone covalent modifications interact with speckles and whether these interactions are direct or mediated by protein linkers are major open questions.

While speckle-associated regions generally have low levels of repressive histone modifications, such as histone 3 lysine 9 trimethylation (H3K9me3), H3K9me3 does occur within certain speckle-associated chromatin during mouse embryogenesis [88]. Thus, repressive chromatin is not mutually exclusive with speckle association. As embryogenesis progresses, speckle-associated chromatin progressively decreases levels of repressive histone modifications and increases activating histone modifications [88], supporting the idea that speckles could poise genes for future activation. In total, these studies suggest that histone modifications combine with speckle association to orchestrate gene regulatory programs.

Box 3. Relationship between nuclear speckles and chromatin conformation studies

With the advent and widespread adoption of chromatin conformation capture [110], the chromatin architecture field has built a conceptual framework based on the frequency with which chromatin is crosslinked with other chromatin. This framework spans scales of chromatin organization. At the larger scales are compartments and subcompartments defined by splitting the genome into two or more groups based on **principal component analysis** of crosslinking frequencies. At smaller scales are topologically associated domains (TADs), defined by higher contact frequencies within a stretch along the linear genome, and 'loops', defined by high contact frequencies between two genomic points. Compartments, TADs, and loops are useful definitions for describing and quantifying chromatin conformation capture data. However, they should be interpreted in the context of the cell nucleus, where nuclear bodies occupy substantial real estate. It has been suggested that discoveries from chromatin–chromatin contact mapping, in part, reflect how chromatin is organized at non-DNA structures such as nuclear speckles [111], with recent studies providing new clarification. The A1 subcompartment defined by Hi-C data is highly speckle associated and interactions within the A1 subcompartment depend on SRRM2 [79,80,112], indicating that Hi-C-defined subcompartments reflect chromatin interactions with speckles. At the level of TADs, speckle-associated regions are established prior to TAD detection during mouse embryogenesis [88], supporting the idea that speckle association on its own does not result in TAD formation. However, in favor of speckles informing the establishment of TADs, the boundaries of early speckle-associated regions significantly overlap with TAD boundaries once they are established. At the level of chromatin loops, degradation of SON and SRRM2 disrupts speckles without impacting CTCF loop strength or genomic binding [105], indicating that CTCF loops and speckle association are distinct. Additionally, deletion of the RAD21 STM compromised speckle association without impacting the distance between two adjacent TADs [82], showing that TAD–TAD interactions can also be independent of speckle positioning. Thus, nuclear speckles are responsible for larger-scale subcompartment organization observed in Hi-C data, may be implicated in helping establish TADs during early development, and are independent of certain smaller-scale chromatin architectural features such as CTCF looping.

While these studies help reconcile findings from chromatin–chromatin mapping studies with the existence of nuclear bodies, how chromatin architectural features interface with nuclear speckles to impact gene expression remains a key open question. For example, changes to chromatin loops or TADs could have different functional consequences if they occur at versus away from speckles and therefore pose an important consideration for the interpretation of past and future investigations into the role(s) of chromatin architecture.

mutated, the p53 target gene, *p21*, was positioned at speckles but transcriptionally silent [86], demonstrating that gene positioning at speckles is insufficient to activate transcription.

Speckle positioning of the *HSPA1A* genomic locus correlates with a higher RNA amount within active transcription sites compared with non-speckle-associated *HSPA1A* alleles, without influencing the fraction of active sites within the cell population [87]. This finding indicates that speckles boost, rather than initiate, gene expression. Further supporting the role of speckles as gene expression boosters, disruption of p53-driven speckle targeting of *p21* compromised the amount of RNA within active transcription sites without impacting the proportion of cells with active transcription [86]. Experiments with HIF-2α similarly show that disrupting HIF-2α-driven speckle association diminishes the amount of RNA within active transcription sites [82]. Together, these data indicate that speckle proximity amplifies RNA production. For overall gene regulation, this is a critical distinction because it means that speckles function on top of the existing transcriptional landscape of a cell.

The mechanisms by which speckles amplify RNA expression, and whether this involves amplification of transcription, have yet to be fully resolved. Arguing against major roles of speckles in transcriptional amplification, induced degradation of SON and SRRM2 has a mild impact on pulse-labeled nascent RNA levels, with more extreme effects on polyadenylated mRNA [97]. While this finding indicates a stronger role of speckles in RNA stability, discussed in more detail in RNA processing, a role for speckles in boosting transcription should not be discounted. Degradation of SON and SRRM2 has the drawback of releasing other speckle components from speckle structures, which could confound gene expression measurements [97,114]. Speckle degradation experiments to date have also been performed in a couple of cancer cell lines [97,105,114]. Thus, the impact of speckle loss on transcription under different cellular contexts remains to be determined.

Arguing for potential roles for speckles in transcriptional amplification, members of the Positive Transcription Elongation Factor b (P-TEFb) complex—CDK9, Cyclin T1, and HEXIM1—are localized within speckles [115]. P-TEFb has been implicated in transcriptional amplification by facilitating the release of paused RNA Pol II to enable productive transcriptional elongation [116,117]. Since the active phosphorylated form of CDK9 is enriched within speckles [118], the release of active CDK9 from speckles is a hypothetical mechanism by which speckles could amplify transcription. Additionally, because heat shock, p53, and HIF-2α experiments directly visualize sites of transcription inside the nucleus, the speckle-mediated expression boost likely occurs during or shortly after transcription [52,86,87]. RNA fluorescence in situ hybridization studies of HIF-2α-mediated speckle association show that speckles boost both mature and immature (unspliced) RNA levels within transcription sites [52], indicating that speckles enhance RNA production before splicing has occurred. Because splicing generally occurs co-transcriptionally, this finding suggests a potential role for transcription in the speckle-mediated boost of RNA expression.

RNA processing

Intricately coordinated with transcription are the co- and post-transcriptional processes of mRNA capping, splicing, cleavage, and polyadenylation, all of which ensure mRNAs are stabilized and prepared for translation [119]. A recent study made use of transgenes integrated within speckle-associated or nonspeckle-associated chromatin to examine the role of gene–speckle association in splicing efficiency [84]. The authors show more efficient splicing of genes transcribed at speckles, particularly for a high-GC transgene. This provides an important line of evidence demonstrating a role for chromatin–speckle association in a specific step of RNA processing. The more dramatic boost in splicing efficiency for a high-GC-content transgene compared to one with low-GC content suggests that speckles may be particularly suited to boost splicing of high-GC-content genes. In support of this idea, another study found that high-GC-content transcripts are more stably enriched in speckles as compared to lower-GC transcripts [100]. These high-GC transcripts have weak splicing features, experience less efficient splicing compared with the lower-GC transcripts, and depend on nuclear speckles for intron excision. Similarly, speckle disruption by inducible SON and SRRM2 degradation leads to chaotic splicing of the speckle-associating GC-rich transcripts, with subsequent defects in RNA nuclear export and increased mRNA degradation [97]. As major effects on nascent transcription were not evident in this study, the observed speckle-dependent changes in polyadenylated mRNA levels can be largely attributed to increased degradation of mis-spliced GC-rich transcripts [97]. Together, these data indicate that speckles boost splicing efficiency and ensure splicing fidelity, particularly for the types of genes that tend to reside within speckle-associated chromatin.

The speckle-resident long noncoding RNA, *MALAT1*, while not required for speckle integrity [31], also plays key roles in splicing fidelity and transcription [120,121]. A recent study found that *MALAT1* regulates alternative splicing during hypoxia, which occurs at genes that are positioned at speckles [122]. In the context of influenza infection, trafficking of an influenza mRNA to speckles enables its splicing to generate an alternative protein needed for the viral replication cycle [61–63]. These data support the idea that speckles are utilized in different contexts to facilitate gene regulation through splicing.

Beyond splicing, nuclear speckles have also been implicated in **cleavage and polyadenylation** and nuclear export of mRNAs [123,124]. A critical cleavage and polyadenylation protein, RBBP6, localizes to nuclear speckles via its **intrinsically disordered region (IDR)** [123]. Disruption of the RBBP6 IDR compromises RBBP6 speckle localization and leads to less efficient cleavage and polyadenylation in cells, despite the mutant version being fully competent in an *in vitro* cleavage assay. Disruption of nuclear speckles also leads to defects in 3′ mRNA processing,

underscoring a role for speckles in cleavage and polyadenylation. In further support of splicing-independent speckle functions, intronless RNAs transit through nuclear speckles, where they bind the **transcription export complex (TREX)** for TREX-dependent nuclear export [124]. Because these studies focus on RNA–speckle interactions, the extent to which chromatin–speckle positioning impacts the efficiency of cleavage and polyadenylation and nuclear export of RNAs expressed from speckle-associated chromatin remains unclear. Nonetheless, these studies support the idea that speckles streamline multiple steps of RNA processing.

Collectively, efficient RNA processing at nuclear speckles boosts gene expression by assisting the process of making RNAs competent for translation. Transcription and RNA processing are tightly coupled processes [125,126]. Hence, efficient RNA processing at speckles may feed back to boost transcription. Moreover, efficient RNA processing and packaging of RNAs by RNA-binding proteins, which predominate in speckles, could stabilize RNAs produced at speckles to further boost RNA amounts. Experiments that deplete the RNA-degrading exosome complex member RPP40 increase RNA amounts from nonspeckle-associated transcription sites without impacting speckle-associated sites [87]. This finding supports a role for nuclear speckles in protecting RNAs from degradation. However, the mechanisms by which nuclear speckles stabilize RNAs as they are produced and the degree to which this involves speckle-resident RNA-binding proteins are still unclear.

Concluding remarks and future perspectives

In total, growing evidence indicates that speckles boost the efficiency of multiple stages of RNA production. The use of well-controlled functional experiments has carried this field beyond correlational data to demonstrate tangible gene-expression-boosting functions of nuclear speckles. With these new insights, major open questions remain (see Outstanding questions). The mechanisms underlying speckle gene regulatory functions and how this relates to the compartmental structure of speckles (Box 1) remain unclear. As multiphase, multicomponent assemblies, loss-of-function studies on one or two components disrupt the nuclear distribution and/or biophysical properties of other speckle components, making it challenging to discern the molecular events that occur at individual speckle substructures [14,15,97]. Single-molecule microscopy studies that evaluate RNA biogenesis together with speckle substructures may advance understanding of the roles of the SON and SRRM2 speckle substructures. Additionally, a more detailed understanding of the molecular interactions within and between speckle structures will advance the design of structure–function studies to pointedly query the role of specific interactors.

Critically, it is imperative to consider that speckles can vary. Numerous studies indicate that speckle variation is, in and of itself, a major mode of gene regulation that is co-opted by viruses and cancer, utilized as cellular defense mechanisms against stress, and harnessed as a part of physiological cell signaling responses (Table 1). These studies suggest that speckles can take on distinct molecular functions depending on context. As the field continues to uncover the molecular functions of nuclear speckles, it is essential to keep the potential context-dependent molecular functions in mind. Functional experiments to date have been performed on a limited number of cell lines. Without ways to classify cell lines by speckle function, the field risks arriving at opposing conclusions about what speckles do, depending on which lines are selected for study. Thus, understanding how speckles vary and the consequences of such variation is paramount for continued progress and is a priority for future research. Excitingly, speckle variation presents a potentially distinct mechanism for regulating gene expression programs within a cell. As the field continues to explore how speckles vary and impact gene expression, this will open new avenues for understanding physiological and pathogenic processes.

Outstanding questions

Do nuclear speckles coordinate the expression of gene neighborhoods?

Which transcription factors and coactivators control chromatin positioning at nuclear speckles, and what molecular mechanisms accomplish this?

How do nuclear speckles orchestrate multiple steps of gene expression?

How do nuclear speckles vary across normal and pathogenic processes?

To what extent are gene-boosting molecular functions of speckles context dependent?

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Declaration of interests

The authors have no conflicts of interest to declare.

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