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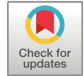
Oncogenic fusion proteins and their role in three-dimensional chromatin structure, phase separation, and cancer



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Three-dimensional (3D) chromatin structure plays a critical role in development, gene regulation, and cellular identity. Alterations to this structure can have profound effects on cellular phenotypes and have been associated with a variety of diseases including multiple types of cancer. One of several forces that help shape 3D chromatin structure is liquid–liquid phase separation, a form of self-association between biomolecules that can sequester regions of chromatin into subnuclear droplets or even membraneless organelles like nucleoli. This review focuses on a class of oncogenic fusion proteins that appear to exert their oncogenic function via phase-separation-driven alterations to 3D chromatin structure. Here, we review what is known about the mechanisms by which these oncogenic fusion proteins phase separate in the nucleus and their role in shaping the 3D chromatin structure. We discuss the potential for this phenomenon to be a more widespread mechanism of oncogenesis.

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Current Opinion in Genetics & Development 2022, **74**:101901

This review comes from a themed issue on **Cancer Genomics**

Edited by **Luciano di Croce** and **Jane Skok**

https://doi.org/10.1016/j.gde.2022.101901

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Introduction

Three-dimensional (3D) chromatin structure plays a critical role in gene regulation by connecting distant regulatory elements to gene promoters and modulating gene expression. Modifications to this structure have been associated with the development of human diseases including cancer. Several molecular forces have been identified for establishing and/or modulating the 3D chromatin structure, such as those directed by CCCTC-Binding Factor (CTCF) and multisubunit cohesin complexes, and liquid–liquid phase separation (LLPS), a form of self-association between biomolecules that can sequester regions of chromatin fibers into subnuclear droplets or even membraneless organelles like nucleoli. This review focuses on an emerging class of oncogenic fusion proteins that appear to exert their oncogenic function at least partly, via phase-separation-driven alterations to 3D chromatin structure. Despite the relatively small number of such fusions that have been characterized, this phenomenon is potentially a much more widespread mechanism of oncogenesis and further identification and characterization of such fusions could have important implications for diagnostic and therapeutic development.

Three-dimensional chromatin organization

3D chromatin structure plays a fundamental role in gene regulation and cellular identity by rewiring contacts between regulatory loci and gene promoters. Current theories suggest that this organization is driven largely by two coexisting, dynamic, yet sometimes opposing forces: compartmentalization and loop extrusion [1–6]. Although the exact mechanisms driving compartmentalization are still under investigation, this phenomenon is thought to be largely mediated by affinity interactions between genomic regions with similar epigenetic marks and levels of transcription. In contrast, loop extrusion is mediated by cohesin, a ring-like protein complex that extrudes DNA until it encounters CTCF proteins bound in a convergent orientation, at which point it stabilizes point-to-point interactions called chromatin loops. The combined forces of loop extrusion and compartmentalization produce contact domains, also known as 'topologically associated domains' (TADs), which are broad regions of self-interacting chromatin. The interplay of these forces in shaping 3D chromatin architecture is nicely discussed by Rowley and Corces [7].

Increasing evidence suggests that LLPS — long known for forming membraneless nuclear bodies such as nucleoli, nuclear speckles, and Cajal bodies — may play a broader role in 3D chromatin organization than previously suspected. Indeed, the association of chromatin-bound proteins in these nuclear bodies is thought to play a role in shaping 3D chromatin architecture [8]. LLPS is mostly driven by a collection of weak, multivalent interactions between proteins that contain intrinsically disordered regions (IDRs) [9,10]. Transcription factors (TF) and coregulators implicated in chromatin remodeling and gene regulation, such as Mediator and RNA polymerase II (RNA Pol II), are enriched with IDRs and are thought to exercise their function in part via phase separation [11–16]. Additionally, although not the focus of this review, RNA has been proposed to have a prominent role in chromatin organization in a way that is compatible with the concept of LLPS [17–20].

Despite mounting evidence supporting the role of LLPS in 3D chromatin organization, it remains unclear exactly what types of chromatin structures LLPS can generate and in which biological contexts. One hypothesis that has gained support in recent years proposes LLPS as one of the driving forces for chromatin compartmentalization [19,21–25]. Moreover, while DNA loops are thought to be formed largely via the Adenosine triphosphate (ATP)-dependent process of extrusion [3,26,27], recent evidence suggests that cohesin itself may undergo LLPS [28]. Finally, a model proposing phase separation as a general mechanism driving super-enhancer-mediated gene regulation has also gained extensive support [29,30], extending the potential role of phase separation to all levels of chromatin organization.

Three-dimensional chromatin structure, phase separation, and cancer

Alterations in 3D chromatin structure via a variety of different mechanisms has been linked to the development of various cancers. Mutations in cohesin, which are among the most common mutations found in cancer, have been shown to result in misregulation of intrachromosomal DNA looping, affecting genome organization and gene expression (recently reviewed by Waldman [31]). Point mutations and somatic structural variations found in multiple cancer types have been shown to disrupt TADs and alter transcription levels of the surrounding genes [32]. One example of this phenomenon is the Isocitrate dehydrogenase (IDH) gain-of-function mutation in gliomas, which alters TAD boundaries, resulting in the induced expression of the Platelet Derived Growth Factor Receptor Alpha (PDGFRA) oncogene [33].

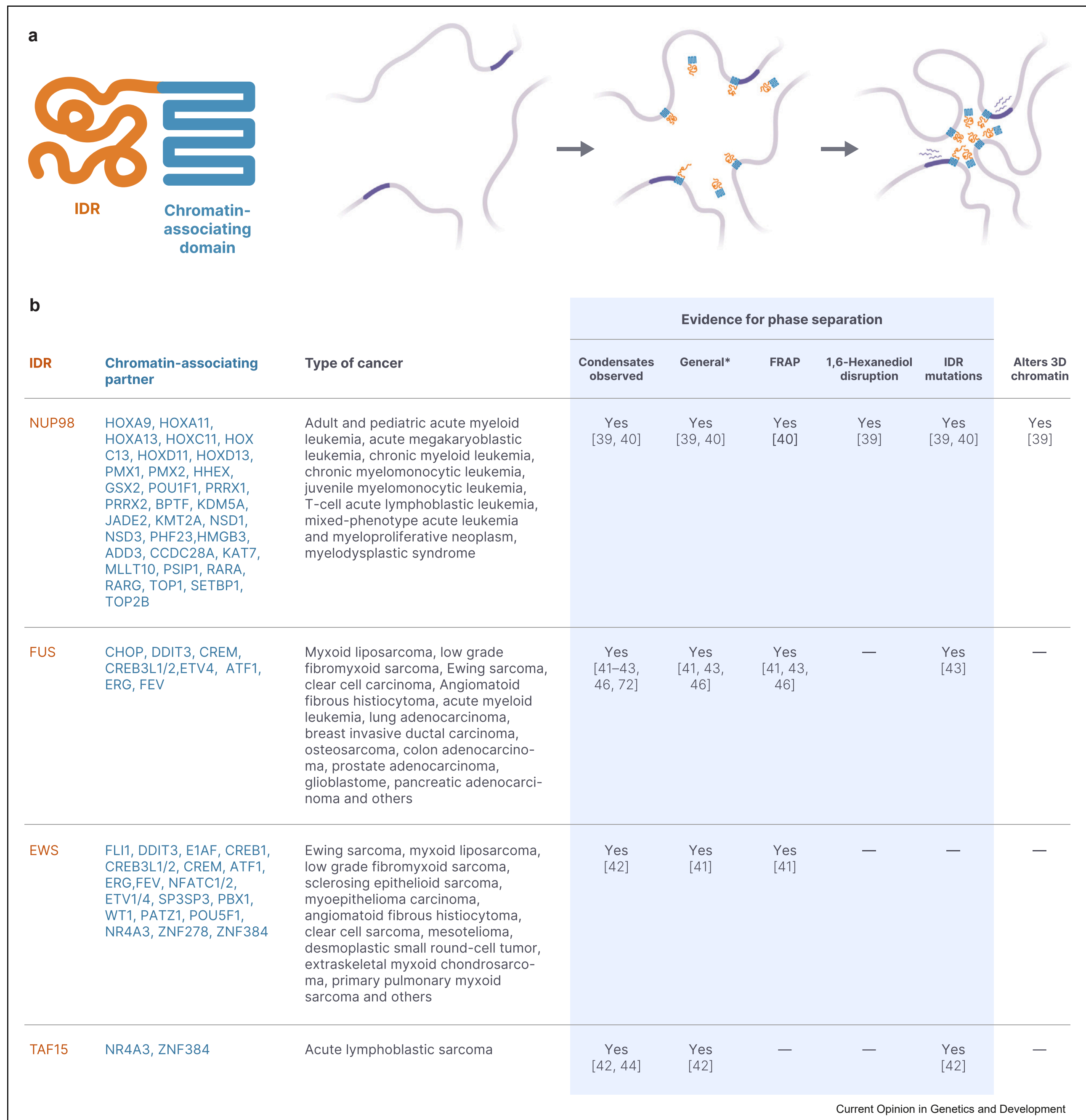
LLPS has also been associated with cancer, independently from 3D chromatin conformation. For example, several oncogenes and tumor suppressor genes exert their normal activity via LLPS which when disrupted — via alterations of either gene expression or LLPS capability — can promote cancer development. For example, p53 binding protein 1 (53BP1) uses LLPS to recruit DNA repair complexes to sites of DNA damage. Under expression of 53BP1 in various contexts promotes DNA instability and the development of cancer [34]. In another example, mutations disrupting the phase separation capacity of the IDR in the histone H3K27 demethylase UTX (also known as KDM6A) abolish its tumor suppression capabilities [35]. In contrast, a mutation in the histone acetylation reader ENL, commonly found in pediatric kidney cancer, confers novel phase separation capacity and has been linked to alterations in transcription and oncogenesis [36].

IDR–DNA binding fusion proteins in cancer

In addition to the observations mentioned above that independently link phase separation and 3D chromatin structure to cancer, several recent studies describe a novel paradigm in which phase separation of cancer-related fusion proteins promotes oncogenesis by directly inducing changes to 3D chromatin structure. Chromosomal translocations, a common feature of cancers, can produce chimeric genes that encode fusion proteins, which have been shown to influence diverse functions including cellular cycle, cell shape, cell mobility, and RNA metabolism, among others [37]. Intriguingly, IDRs are enriched in cancer-associated fusion proteins (43.3% versus 20.7% in all human proteins) [38] and a subset of these fuse the IDR of one protein to a chromatin-associated domain of another (Figure 1a). This unique combination of domains provides, in theory, the potential to alter 3D chromatin structure by pulling a set of bound regions into condensates formed via IDR LLPS, and as a result of this process, alter the expression of surrounding genes (Figure 1a).

We recently showed that a cancer-associated fusion between an IDR and a DNA binding domain could induce oncogenic transcription programs at least partly via *de novo* loop formation [39••]. This study focused on the NUP98–HOXA9, a classic example for a set of TF fusion oncoproteins found recurrently in human hematological malignancies such as acute myeloid leukemia. The two components of this chimera are: (1) NUP98, a constitutively expressed nucleoporin protein that contains a phenylalanine-glycine (FG)-repeat-rich IDR, and (2) HOXA9, a TF that contains a DNA binding homeodomain regulating gene expression essential for proper cell proliferation and differentiation during embryogenesis. Expression of this fusion in primary hematopoietic stem/progenitor cells (HSPCs) is sufficient to transform cells and activate leukemic gene-expression profiles. In contrast, a phase-separation-incompetent mutant of

Figure 1



IDR	Chromatin-associating partner	Type of cancer	Evidence for phase separation					Alters 3D chromatin
			Condensates observed	General*	FRAP	1,6-Hexanediol disruption	IDR mutations	
NUP98	HOXA9, HOXA11, HOXA13, HOXC11, HOX C13, HOXD11, HOXD13, PMX1, PMX2, HHEX, GSX2, POU1F1, PRRX1, PRRX2, BPTF, KDM5A, JADE2, KMT2A, NSD1, NSD3, PHF23,HMGB3, ADD3, CCDC28A, KAT7, MLLT10, PSIP1, RARA, RARG, TOP1, SETBP1, TOP2B	Adult and pediatric acute myeloid leukemia, acute megakaryoblastic leukemia, chronic myeloid leukemia, chronic myelomonocytic leukemia, juvenile myelomonocytic leukemia, T-cell acute lymphoblastic leukemia, mixed-phenotype acute leukemia and myeloproliferative neoplasm, myelodysplastic syndrome	Yes [39, 40]	Yes [39, 40]	Yes [40]	Yes [39]	Yes [39, 40]	Yes [39]
FUS	CHOP, DDIT3, CREM, CREB3L1/2,ETV4, ATF1, ERG, FEV	Myxoid liposarcoma, low grade fibromyxoid sarcoma, Ewing sarcoma, clear cell carcinoma, Angiomatoid fibrous histiocytoma, acute myeloid leukemia, lung adenocarcinoma, breast invasive ductal carcinoma, osteosarcoma, colon adenocarcinoma, prostate adenocarcinoma, glioblastome, pancreatic adenocarcinoma and others	Yes [41–43, 46, 72]	Yes [41, 43, 46]	Yes [41, 43, 46]	—	Yes [43]	—
EWS	FLI1, DDIT3, E1AF, CREB1, CREB3L1/2, CREM, ATF1, ERG,FEV, NFATC1/2, ETV1/4, SP3SP3, PBX1, WT1, PATZ1, POU5F1, NR4A3, ZNF278, ZNF384	Ewing sarcoma, myxoid liposarcoma, low grade fibromyxoid sarcoma, sclerosing epithelioid sarcoma, myoepithelioma carcinoma, angiomatoid fibrous histiocytoma, clear cell sarcoma, mesotelioma, desmoplastic small round-cell tumor, extraskeletal myxoid chondrosarcoma, primary pulmonary myxoid sarcoma and others	Yes [42]	Yes [41]	Yes [41]	—	—	—
TAF15	NR4A3, ZNF384	Acute lymphoblastic sarcoma	Yes [42, 44]	Yes [42]	—	—	Yes [42]	—

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IDR/chromatin-associating fusion proteins in cancer. **(a)** The proposed mechanism of action of IDR/chromatin-associating fusion proteins in cancer. Fusion proteins bind chromatin at enhancers and gene promoters and recruit bound loci into phase separation-dependent condensates activating oncogenic transcriptional profiles. **(b)** A list of IDR/DNA-binding fusion proteins frequently found in cancer that have been described to phase separate in the nucleus. **In vitro/in vivo* assays for coalescence, fusion/fission, concentration dependency. FRAP: fluorescence recovery after photobleaching.

NUP98–HOXA9, or one harboring a shorter IDR incapable of inducing LLPS, were unable to transform HSPCs, highlighting a critical requirement of IDR-mediated phase separation for cancerous transformation. ChIP-seq and Hi-C analysis revealed that NUP98–HOXA9-expressing cells formed DNA loops whose anchors were bound by NUP98–HOXA9, but not by CTCF, supporting a model that proposes that phase separation but not loop extrusion is the driving force for the formation of *de novo* chromatin contacts in the

context of this fusion. Genes whose promoters overlap the anchors of the NUP98–HOXA9-bound loops were upregulated, including proto-oncogenes such as PBX3 and HOX cluster genes. These findings suggest phase separation as the driver of aberrant chromatin looping and the resulting changes in gene expression in leukemias harboring this fusion. Some of these results were confirmed by Chandra et al., who demonstrated the phase separation capabilities of NUP98-HOXA9 and other leukemia-related fusion oncoproteins [40]. It is important to mention that NUP98-HOXA9-induced alterations to 3D chromatin structure have only been investigated in HEK 293T cells and moving forward it will be important to confirm these changes in leukemia-relevant cell types.

Several other cancer-associated fusion proteins that have a similar composition — including an IDR and a chromatin interaction domain — are likely to impart oncogenic properties via similar alterations to 3D chromatin structure (Figure 1b). One group of likely candidates is the FUS/EWS/TAF15 (FET) fusion oncoproteins [44–47,72]. These FET oncogenic fusions form multiple large nuclear condensates in cells similar to the ones observed when the aforementioned oncogenic fusions are present. FET fusion proteins are essential oncogenic drivers in various cancers including myxoid liposarcoma, Ewing sarcoma, and adenocarcinomas. They were shown to undergo phase separation at target binding loci and form phase-separated transcriptional condensates recruiting RNA Pol II and other cofactors (e.g. BRD4), which results in the increase in gene expression [41*–43]. Chong et al. also identified IDR-dependent nuclear hubs of FET fusion oncoproteins and demonstrated their role in transcriptional regulation; however, they propose that such hubs are formed via a non-LLPS-driven mechanism [44]. Finally, several studies have reported that the Switch-Sucrose Non-fermentable (SWI/SNF) chromatin-remodeling complex, also known as BAF (BRG1-associated factors), can be recruited into FET fusion condensates. This process is likely to occur via heterotypic interactions among prion-like domains commonly present in both FET fusions and components of the Switch-Sucrose Non-fermentable (SWI/SNF) (BAF) complex, resulting in cancer-specific chromatin alterations favorable for gene activation [45–47].

Other nuclear phase separating fusion proteins in cancer

Several other fusions have been described that also act via phase separation-driven changes to 3D chromatin organization, albeit by mechanisms distinct from the one described above (Table 1). Rosencrance et al. found that BRD4-NUT, a BRD4 fusion oncoprotein found in midline carcinoma, alters 3D chromatin at the compartment scale in a manner that is consistent with phase separation [48**]. In this case, the fusion protein BRD4-NUT produces changes observed at a larger scale, resulting in the formation of aberrant chromatin subcompartments referred to as subcompartments ‘M’. These subcompartments ‘M’ encompass broad stretches of active chromatin that exhibit heightened interactions both within and between different chromosomes. These modifications in interaction occur due to the ability of BRD4-NUT to drive massive changes in histone acetylation. The BRD4 portion of the fusion protein binds acetylated histones, while the NUT portion recruits the p300 histone acetyltransferase, driving a proposed feed-forward loop of histone acetylation which results in exceptionally broad linear (100 kb to 2 Mb) hyperacetylated chromatin areas called ‘megadomains’ [49]. These megadomains interact with one another even between chromosomes, are associated with increased transcription of Myc and other important oncogenes for NUT-carcinomas, and appear as large nuclear puncta [48]. Although no experiments were performed to directly test for BRD4-NUT’s LLPS capabilities, previous studies have shown that it is clearly present in nuclear puncta, consistent with an LLPS-driven mechanism [49,50] and evidence for phase separation of the short isoform of BRD4 has been reported [51]; also, LLPS of the acetylated chromatin in combination with the multibromodomain of BRD4 as it has been previously proposed [24]. Interestingly, a subclass of NUP98 fusions

Table 1

Other nuclear phase-separating fusion proteins in cancer.

Fusion proteins	Type of cancer	Evidence for phase separation					Alters 3D chromatin
		Condensates observed	General[a]	FRAP	1,6-Hexanediol disruption	IDR mutations	
BRD4-NUT	Squamous cell carcinoma, midline carcinoma	Yes [48–50]	–	–	–	–	Yes [48]
SS18-SSX1/2	Synovial sarcoma	Yes [53,55]	Yes [53]	Yes [53]	Yes [53]	Yes [53]	–

A list of phase-separating fusion proteins frequently found in cancer that combine an IDR/chromatin-associating-containing proteins and a fusion partner that alters the distribution and/or role of the first component. FRAP: fluorescence recovery after photobleaching.
[a] *In vitro/in vivo* assays for coalescence, fusion/fission, concentration dependency.

that carry the plant homeodomain (PHD), a H3K4me2/3 reader motif, were shown to exhibit comparable large nuclear condensates [52]. Moreover, similar to the BRD4-NUT fusion, we have observed that condensates of NUP98-PHD fusions are enriched for H3K4me2/3, to which the PHD domain of the fusion binds, as well as MLL1, an enzyme that catalyzes H3K4 methylations (unpublished). This suggests that a similar feed-forward loop mechanism could underlie the formation of broad domains of inter/intrachromosomal interactions in leukemia cells.

Similarly, a fusion protein typically found in synovial sarcoma between the Switch-Sucrose Non-fermentable (SWI/SNF) (BAF) complex member SS18 and one of several SSX genes is another example of a fusion oncoprotein containing an unstructured region that can associate with specific chromatin areas and alter gene transcription. SS18 contains an IDR-rich C-terminal and has been shown to mediate the BAF complex assembly via LLPS [53]. When SSX is fused to SS18, this fusion evicts another member of the BAF complex and retargets BAF from enhancer regions to broad polycomb domains in the chromatin. This new BAF complex occupancy opposes PRC2-mediated chromatin repression and results in activation of bivalent genes [54]. The SS18-SSX fusion forms multiple large nuclear condensates and exhibits dense and broad binding patterns at target loci, reflecting typical characteristics of phase-separated molecules [55]. Despite the broadly known capacity of these fusion proteins to phase separate and cause gene misregulation, their potential roles in inducing alterations in the 3D chromatin structure have not yet been fully explored. Conversely, additional research needs to be performed to uncover whether other fusion oncoproteins that alter 3D chromatin structure do so via an LLPS mechanism. One such fusion is PAX3-FOXO1, a recurrent mutation found in rhabdomyosarcoma that is associated with altered 3D chromatin structure and has the capacity to form *de novo* super enhancers and recruit BRD4 and other master TF [56,57].

Future directions: fusions, mechanisms, and interventions

These studies provide the first glimpse into a cancer-driving mechanism in which fusions between IDRs and chromatin-associated domains drive oncogenic transcription via alterations in the 3D chromatin structure. However, it is currently unclear how broad this mechanism is across various cancer subtypes and other diseases. One ongoing challenge will be to identify and characterize other cancer fusions that operate via alterations to chromatin interaction frequencies. Ever-expanding databases of disease-associated fusion proteins (e.g. chimerDB [58] and the Catalogue Of Somatic Mutations In Cancer (COSMIC) [59]), intrinsically disordered domains (e.g. MobiDB [60], DisProt [61], and IDEAL [62]), and phase-separating proteins (e.g. LLPSDB [63], PhaSePro [64], PhaSepDB [65]) provide rich resources to mine for such possibilities. Improving and assessing our ability to detect IDRs and phase-separation-competent regions should accelerate discovery further. Necci et al. [66] suggest that deep learning-based approaches show the most promise here, highlighting one way in which advances in artificial intelligence may contribute to this field moving forward.

Further mechanistic studies are required to better understand how these 3D structures are formed as well as their impact on transcription. While DNA loops formed via NUP98-HOXA9 are not anchored by CTCF [39], their dependence on cohesin and/or Adenosine triphosphate (ATP) is unclear. Another open question involves how the oncogenic condensates interact with other nuclear condensates and microenvironments. For example, it has been shown that RNA Pol II forms phase-separated condensates [12–14] and in some cases is recruited into condensates formed by fusion oncoproteins to activate gene transcription [41,48]. Further development of experimental approaches to simultaneously monitor and visualize multiple classes of nuclear condensates will enable a deeper understanding of the interplay between them.

It is important to note that several researchers have expressed concern regarding the widespread attribution of LLPS to explain IDR-driven nuclear structures [67–69]. The main concerns refer to studies employing *in vitro* assays or ectopic overexpression models, which might fail to fully reflect physiological conditions (i.e. local concentrations, microenvironment, and interaction partners) that are fundamental for the occurrence of LLPS. Moving forward, the use of approaches that involve targeting of endogenous IDR-containing proteins and single-molecule tracking might enable more precise mechanistic interrogations.

Finally, the discovery of 3D chromatin alterations produced by IDR-containing fusion oncoproteins as a cancer-driving mechanism may reveal new avenues for therapeutic intervention. As reviewed by Wheeler [70], such interventions may look quite different from the classical enzyme lock-and-key or structured binding site approaches, since they would take into account the physicochemical properties of nuclear condensates and drug molecules for their mechanism of action. For example, a recent study showed how the partitioning, concentration, and activity of some oncodrugs were highly influenced by the physicochemical properties of nuclear condensates [71]. Tailoring drugs for either inclusion or exclusion from specific nuclear condensates could potentially improve specificity and/or efficacy. Another study demonstrated that small molecule-

induced degradation of BRD4-NUT eliminates its effect on chromatin compartmentalization, pointing to the potential of altering 3D chromatin structure as a novel approach to treat disease [48].

Conclusions

Recent studies have illuminated how aberrant gene fusion events seen in cancer can create chimeric proteins capable of binding DNA, altering 3D chromatin structure, and driving oncogenic transcription. While only a handful of such chimeras have been characterized, the enrichment of fusion proteins and IDRs in tumors suggests that this is a much broader mechanism to promote oncogenesis. Future research is required to determine the extent to which this mechanism explains other cancer-related fusions, the mechanisms through which these 3D chromatin structures form and impact gene transcription, and how such structures can be targeted to improve therapeutic interventions.

Conflict of interest statement

Nothing declared.

Acknowledgements

We would like to acknowledge Erika Deoudes for illustrations and graphical design. This work was supported by National Institutes of Health (NIH) Grants (R01-CA215284 and R01-CA218600 to G.G.W.; R35-GM128645 to D.H.P.), and UNC Lineberger Stimulus Awards (to D.H.P.). IYQ is supported by a BrightFocus Foundation postdoctoral fellowship 911831. G.G.W. is a Leukemia & Lymphoma Society Scholar and an American Cancer Society Scholar.

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- •• of outstanding interest.

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