Cancer utilizes biomolecular condensation mechanisms to promote cancer cell survival and proliferation. Recent studies are revealing how intrinsically disordered regions (IDR) contribute to onco-condensate formation during cancer. In this issue, Xu *et al.* review the mechanisms by which onco-condensates are established and organized to promote oncogenesis. Image courtesy of gettyimages /Mandy Disher Photography.

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Trends in Cancer

Review

Onco-condensates: formation, multi-component organization, and biological functions

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Numerous cellular processes occur in the context of condensates, a type of large, membrane-less biomolecular assembly generated through phase separation. These condensates function as a hub of diversified cellular events by concentrating the required components. Cancer frequently coopts biomolecular condensation mechanisms to promote survival and/or proliferation. Onco-condensates, which refer to those that have causal roles or are critically involved in tumorigenicity, operate to abnormally elevate biological output of a proliferative process, or to suppress a tumor-suppressive pathway, thereby promoting oncogenesis. Here, we summarize advances regarding how multi-component onco-condensates are established and organized to promote oncogenesis, with those related to chromatin and transcription deregulation used as showcases. A better understanding should enable development of new means of targeting onco-condensates as potential therapeutics.

Highlights

Oncogenic condensates, often produced due to recurrent mutations in cancers, can be causal for tumorigenesis.

Onco-condensate formation is often induced due to acquisition of intrinsically disordered regions or genetic alteration that increases actual concentration or decreases the threshold concentration required for liquid–liquid phase separation.

Onco-condensates can supercharge proliferation-related signaling or coopt stress-related condensation mechanisms to promote cellular survival, or can inhibit a tumor-suppressive pathway, leading to oncogenesis.

Targeting oncogenic condensate holds promise for more effective therapeutics.

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Introduction to biomolecular condensation and phase separation

In addition to membrane-bound organelles, the eukaryotic cell contains various membrane-less compartments, such as the nucleolus, Cajal bodies, and nuclear speckles in the nucleus, as well as P-bodies and stress granules in the cytosol [1–4]. Many of these membrane-less compartments are established through phase transition, such as liquid–liquid phase separation (LLPS; Box 1). Initially, Brangwynne *et al.* demonstrated that **P granules** (see Glossary) in the germ cell of *Caenorhabditis elegans* are essentially liquid droplets exhibiting liquid-like behaviors, such as fusion, dripping, rounding off, and wetting due to surface tension; importantly, these P granules are generated through LLPS of polarity proteins and associated RNAs [5]. Since then, LLPS has been shown to be a fundamental mechanism in forming numerous membrane-less assemblies, often termed '**biomolecular condensates**' [6]. Establishment of specialized condensates not only allows spatiotemporal organization of diverse biological processes, but also enhances functional efficiency by concentrating the required components within compartments.

Biomolecular condensation has been linked to a wide range of biological processes, including transcription, DNA damage response and repair, RNA biogenesis and processing, signal transduction, metabolism, and immunity, among others [6–10]. Meanwhile, oncogenesis is increasingly appreciated to be associated with biomolecular condensation. In this review, we focus on those cancer-related condensates, or onco-condensates (Box 2). Herein, we discuss general principles and recent progress concerning onco-condensate formation and function. We favor a view that targeting oncogenic condensates holds great promise for more effective therapeutics.

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Box 1. Principles governing phase transition and biomolecular condensation

Formation of large biomolecular assemblies, or condensates, involves phase separation, a thermodynamic process that mediates partitioning of biomolecules due to their weak, multivalent interactions [5,6,10,47,56,83–92]. For instance, a solution of macromolecules, such as protein, RNA, DNA, and metabolite with a polymer feature, can demix and undergo LLPS by spontaneously condensing macromolecules into a dense phase that resembles liquid-like droplets, in addition to a diluted bulk phase [10,87]. LLPS depends on molecular multivalency of the biomolecules, their concentration (which needs to reach a so-called 'threshold concentration'), and various environmental conditions, such as temperature, pH, cosolute, salt type, and concentration [4,93,94]. For example, filaggrin, the gigantic cytosolic protein that contains several histidine-rich IDRs, assembles keratohyalin granules (KGs) through LLPS in basal progenitors and late-granular cells of the skin; such KGs then undergo a phase transition change near the skin surface and are dissolved to drive squame formation, due to a sudden shift in intracellular pH (from ~7.4 to <6.5, which alters the physiochemical property of pH-sensitive histidine residues in filaggrin) [95].

Take LLPS of protein for example. LLPS can be established due to heterogeneous multivalent interactions between the protein containing tandem repeats of a structurally defined domain and its ligand, as exemplified by SRC homology 3 (SH3) and the SH3 ligand, proline-rich motifs (PRMs) [96]. As well as structurally defined domains, IDRs frequently drive LLPS. IDRs lack a defined 3D structure, and their sequences exhibit notable enrichment or patterns of specific amino acids, such as clustered hydrophilic residues [e.g., lysine (K) and glutamic acid (E) in the oppositely charged residue blocks [20]] or tandemly dispersed motif repeats, such as phenylalanine-glycine (FG) repeats and arginine-glycine-glycine (RGG) motifs. Via multivalent interactions (hydrophobic stacking, pi–pi, electrostatic, or pi–cation interactions), IDRs self-associate, driving LLPS formation, as demonstrated by comprehensively studied RNA-binding proteins, such as FUS and EWS [11,12]. Furthermore, the protein conformational state contributes significantly to LLPS, by exposing the buried interfaces for multivalent interactions, as implicated in the disease-related mutants of SHP2 [40] and ENL [41].

Multivalent interactions drive condensate formation while compartmentalizing the functional components simultaneously [20,38,41]. Furthermore, additional partners (i.e., the client) can be passively recruited into condensates established by the scaffold in scaffold–client model [84,97].

Principles concerning onco-condensate formation

Intensive research over the past decades has gained important insights into general principles governing biomolecular condensation during normal cellular processes. Simply put, biomolecular condensates can be established via a variety of multivalent homotypic and/or heterotypic interactions between protein and protein (such as mediator and transcriptional activator), DNA and protein (such as chromatin and reader), and RNA and protein (such as long noncoding RNA and RNA-binding protein), among others. Aberrantly formed condensates are frequently detected in cancer, with some causally linked to tumorigenesis. Generation of onco-condensates can roughly be categorized into the five scenarios detailed below. Condensation occurs only when a critical concentration is reached (Box 1); accordingly, decreasing critical concentration and/or increasing actual concentration are common mechanisms underlying aberrant condensation under different scenarios. For example, acquisition of **intrinsically disordered regions (IDRs)** or change in protein conformation may decrease the critical concentration required for oncoprotein LLPS; likewise, oncogene overexpression and amplification, or enhanced oncoprotein stability due to mutation, can elevate the protein level above the critical concentration

Box 2. Onco-condensates and oncogenic condensates

Cancerous transformation and malignant progression are intimately associated with, and sometimes causally linked to, deregulated biomolecular condensation. The term 'onco-condensate' was initially coined by Tanja Mittag and Aseem Ansari to specifically refer to liquid droplets formed by cancer-specific oncoproteins, such as NUP98-HOXA9 [98] (a chimeric protein produced only in cancer cells due to aberrant cancer-specific chromosomal translocation) [16]. To make this review more accessible to the readers, we use 'onco-condensates' to refer to any type of condensate, formed by proteins, nucleic acids, metabolites, or their complexes, that contribute significantly to oncogenic transformation of a cell. However, in addition to those onco-condensates formed from proteins and nucleic acids, other biomolecules with a polymer feature can also establish onco-condensates. For instance, Liu *et al.* recently showed LLPS of accumulated glycogen, a carbohydrate polymer, in liver cancer [66]. Onco-condensates can supercharge oncogenic signaling or suppress a tumor-suppressive pathway.

In addition, oncogenic condensates specifically refer to those that have a causal role in either cancer initiation or its malignant progression. Thus, targeting oncogenic condensates represents a potential therapeutic approach.

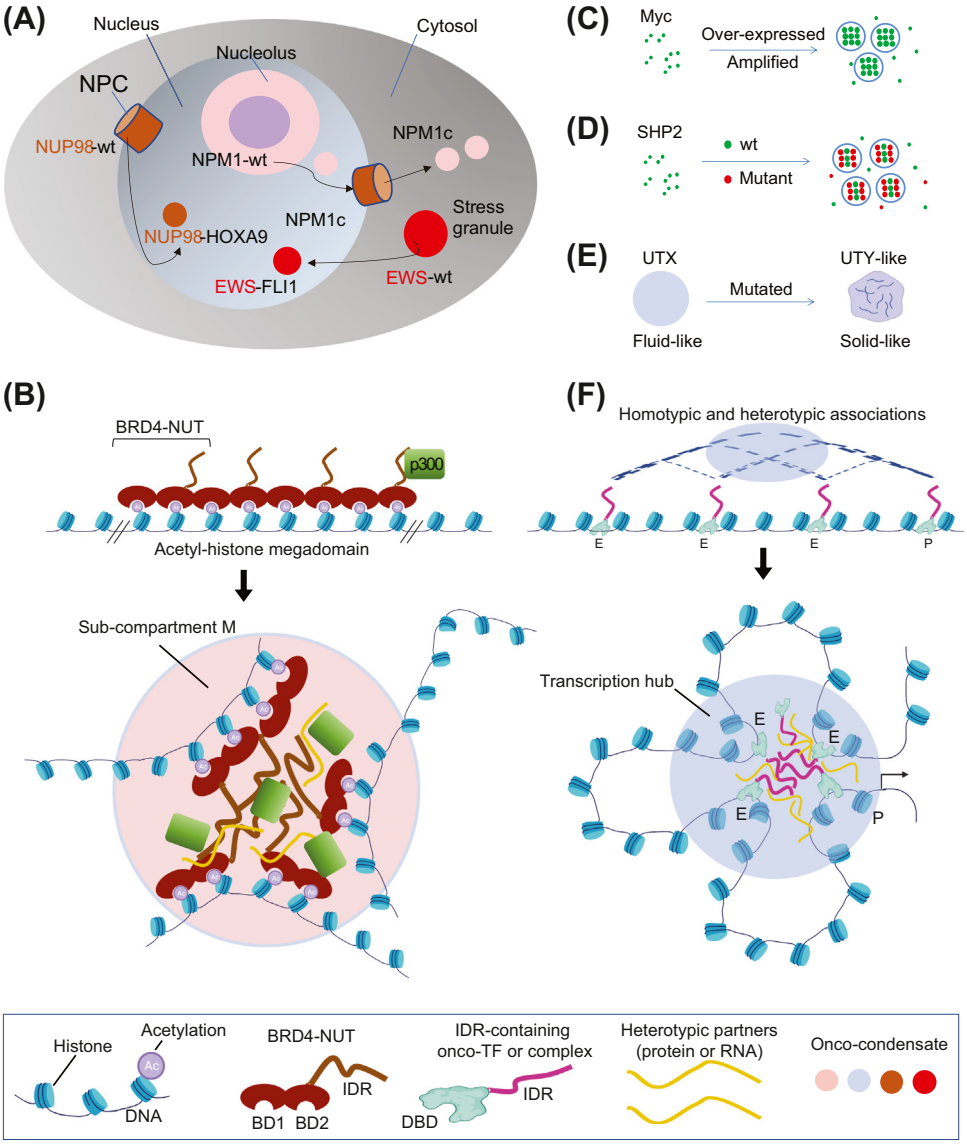


Figure 1. Formation and function of onco-condensates, with special emphasis on those leading to aberrant chromatin organization and/or gene-expression dysregulation. (A) Schematic of onco-condensates of NUP98-HOXA9 and EWS-FLI, which hijack intrinsically disordered regions (IDRs) in proteins normally confined to the nuclear pore complex [NPC, which contains wild-type (wt) NUP98] or stress granules (which contain wt EWS), and condensates of mutant NPM1c (NPM1c) that translocates from the nucleolus (which contain wt NPM1) to the nucleoplasm and then the cytosol. (B) Via liquid–liquid phase separation (LLPS), BRD4-NUT establishes transcriptional superhubs, termed megadomains and subcompartment M. Top: the NUT segment recruits p300 to induce histone acetylation, which is then bound by bromodomains (BDs) in BRD4-NUT to form a feedforward loop. Bottom: BD-acetylation multivalency, together with IDR-mediated self-association and heterotypic interactions, mediates condensation of BRD4-NUT and partners. (C–E) Schemes illustrating LLPS induced by oncogene overexpression [Myc (C)] or mutation-associated structural change [SHP2 (D)]; additionally, cancer-associated missense mutations recurrently target IDR of the tumor suppressor UTX, which converts the liquid-like material state of UTX condensate into a solid-like one (E). (F) Oncofusions, such as NUP98-HOXA9 and EWS-FLI, gain LLPS-inducing IDRs, which mediate homotypic and heterotypic interactions with (co)activators for establishing transcriptional onco-condensates; concurrently, **chromatin loops** are formed at proto-oncogene promoters and enhancers due to oncofusion LLPS.

Glossary

Amyloid (A)-bodies: refer to nuclear condensates that are enriched in heterogeneous proteins exhibiting amyloidogenic biophysical properties.

Acute myeloid leukemia (AML): type of blood cancer that exhibits arrested differentiation and aggressive proliferation of hematopoietic stem and progenitor cells.

Balbiani body: type of non-membrane solid-like structure comprising RNA, mitochondria, and other organelles that is found adjacent to the nucleus of primary oocytes.

Biomolecular condensates: class of membrane-less cellular organelles and compartments established often by virtue of phase separation; function to concentrate biomolecules, such as proteins and nucleic acids

Chromatin loop: refers to focal enrichment in frequency of contacts between a pair of genomic loci, often mapped out based on high-throughput chromosome conformation capture (Hi-C) and derivative techniques, such as micro-C.

Hotspot mutations: nucleotide positions showing a particularly high frequency of mutation that are recurrently detected among patients.

Hydrogel: 3D network of hydrophilic polymers with interpenetrating solid and aqueous phases.

Intrinsically disordered regions (IDRs): unstructured protein sequences that do not form stable 3D structures.

NUT midline carcinoma: also known as NUT carcinoma; refers to a type of rare cancer that grows from the squamous cells in the body (such as the skin and lining of organs such as the lung and stomach); usually found in the head, neck, and lung.

Oncofusion: fusion genes and/or encoded chimeric proteins produced in cancer cells due to aberrant chromosomal translocation.

P granules: class of perinuclear RNA granules in the germline of *C. elegans*.

Superenhancers: clusters of putative enhancers carrying exceedingly abundant levels of gene activation-related histone marks (e.g., H3K27ac) and binding of transcription factor and coactivators (e.g., BRD4)

Transcriptional superhubs: biomolecular assemblies exhibiting robust transcription that are enriched for *cis*-regulatory DNA elements, transcription factors and coactivators, and RNA polymerase II.

required for LLPS. Herein, we focus the following sections more on the features of genetic aberration [such as gain-of-function (GOF) or protein conformational alteration].

Ubiquitously transcribed tetratricopeptide repeat, X chromosome (UTX): also known as lysine-specific demethylase 6A (KDM6A); a demethylase specific to methylation of histone H3 lysine 27, which escapes from X-inactivation and can act as a tumor suppressor.

Gain or alteration of IDRs in oncogenic factors

First, IDRs can mediate weak multivalent interactions, thereby inducing phase separation (Box 1), as observed with RNA-binding proteins, such as FUS and EWS [11,12]. Classic examples of this scenario are aberrant chromosomal translocation events [13], which produce cancer-specific oncoproteins by fusing a transcription factor (TF) with a phase separation-inducing IDR of another unrelated protein, such as the more comprehensively studied EWS-FLI [14,15] and NUP98-HOXA9 [16–18] (Figure 1A). Normally, wild-type EWS and NUP98 are confined within their respective phase-separated cellular bodies or organelles, such as the nuclear pore complex. Through IDR GOF, cancer-specific TF-IDR chimeras exhibit either newly acquired or dramatically enhanced LLPS capability compared with wild-type TF [14–18]. Initially, phase separation of these chimeric fusions was described as being driven by IDR homotypic interactions; however, it has become increasingly clear that co-partitioning of interacting co-activators into the formed condensates is biologically important [15–20]. Indeed, these fusion oncoproteins phase separate via a blend of homotypic and heterotypic interactions, with the latter involving those interactions between the IDR and co-activators and between the DNA/chromatin-binding domain and DNA/chromatin, thereby establishing a functional hub that contains various functional components. Predominance of homotypic interactions in fact appears to suppress activity [21]. Such a theme can be extended to many other chromosomal translocations that target other protein classes known to be involved in tumorigenesis [13], which include transcriptional coactivators (such as YAP fusion [22]), chromatin readers, modifiers, or remodelers (such as NUP98-KDM5A [17], NUP98-NSD1 [19], and SS18-SSX [23]), and kinases or signal transduction proteins (such as EML4-ALK [24]). Likewise, disease-related trinucleotide expansion produces aberrant RNAs [such as those with expanded cytosine–adenine–guanine (CAG) triplets] and aberrant proteins harboring simple repeats [such as polyglutamine (PolyQ), encoded by CAG triplets], both of which undergo phase separation [25,26].

In addition to acquisition of potent LLPS-inducing IDRs by **oncofusion**, damaging mutations, such as frameshift and deletion, often target IDRs of tumor suppressors, leading to loss-of-function (LOF) of IDR and, thus, defects in LLPS and tumor-suppressive activities. For instance, the IDR of **ubiquitously transcribed tetratricopeptide repeat, X chromosome (UTX),** a tumor suppressor, was reported to be recurrently targeted by cancer-associated LOF mutations, which resulted in suppressed UTX LLPS [27].

Aberration of protein module LLPS due to oncogenic alterations

Second, LLPS can be established by proteins carrying tandem repeats of structurally defined modules and their ligands (Box 1). Previously, Gibson *et al.* demonstrated that an engineered histone reader carrying five tandemly repeated bromodomains of BRD4, together with the acetylated nucleosomal array, undergoes LLPS *in vitro*, and these droplets do not mix with those formed by unmodified chromatin alone, indicating that formation of distinctive phase-separated chromatin underlies genomic organization [28]. Aberrant oncogenic fusion characterizing lethal **NUT midline carcinoma**, BRD4-NUT, essentially hijacks the above LLPS mechanism to create **transcriptional superhubs**, termed 'megadomains' [29–31] and subcompartment M [32]. BRD4-NUT fuses the entire short isoform of BRD4, BRD4S, to NUT, a protein that contains multiple transactivation domains (TADs) to recruit p300 and allosterically activate it for histone acetylation [29–31,33,34]. Thus, BRD4-NUT establishes a feedforward loop involving repeated cycles of histone acetylation writing and reading, which generates megadomains that harbor exceedingly abundant levels of histone acetylation over a long chromatin region (Figure 1B,

top; up to 1–2 Mb in size) [29–31]. In the nucleus, BRD4-NUT forms liquid onco-condensates that concentrate p300 and acetylated chromatin (Figure 1B, bottom) [29–31]. Targets of BRD4-NUT include prominent oncogenes, such as c-MYC and SOX2. At the 3D chromatin level, megadomains from either the same or different chromosomes form spatially confined compartments termed 'subcompartment M' [32]. A recent study showed that bromodomain-dependent multivalent interaction with histone acetylation is sufficient to form BRD4-NUT onco-condensates in an ectopic expression system [33], analogous to LLPS observed *in vitro* [28]. Other studies additionally found that IDR within BRD4S contributes partially to LLPS *in vivo* [35], and that NUT is intrinsically disordered and forms droplets, either alone or together with p300 [34]. Thus, BRD4-NUT can use multiple LLPS mechanisms, including multivalent associations dependent on reader-acetylation multivalency and IDRs. A few relatively less studied fusions in NUT midline carcinomas, such as BRD3-NUT, NSD3-NUT, and ZNF532–NUT, most likely act in a fashion similar to BRD4-NUT, because BRD3 and BDR4 are homologous acetylation readers while BDR4, NSD3, and ZNF532 are interacting proteins in the same complex [36].

Conversely, protein module LLPS can be suppressed in the case of tumor repressors. For example, SPOP, an E3 ligase adaptor protein that forms oligomers with substrates, undergoes LLPS leading to degradation of substrate oncoproteins, such as androgen receptor (AR) and estrogen receptor (ER) [37,38]. As an early tumorigenic event in prostate and breast cancers, SPOP mutation often targets its substrate-binding meprin and TRAF homology (MATH) domain, which prevents SPOP from condensing with substrates and, thus, diminishes its enzymatic and tumor-suppressive activities [38].

Oncogenic mutation alters protein conformation to promote condensation

As mentioned above, enhanced oncoprotein LLPS is also caused by increasing actual concentration due to overexpression of oncogenes, such as Myc [39] (Figure 1C). In other cases, LLPS may occur due to mutation-induced protein conformational changes that decrease the critical concentration, as exemplified by **hotspot mutation** of SHP2 in blood malignancy [40] and ENL in Wilms' tumor [41,42].

SHP2 (Figure 1D), a nonreceptor protein tyrosine phosphatase (PTP), is an activator of RAS-MAP kinase signaling. Compared with wild-type SHP2 (SHP2-wt), cancer-associated SHP2 hotspot mutants all exhibit enhanced LLPS [40]. SHP2-wt undergoes LLPS *in vitro* but requires a critical concentration higher than its endogenous level, indicative of tightly regulated LLPS in cells; meanwhile, the critical concentration for LLPS of mutant SHP2 is close to its physiological level [40]. Enhanced LLPS seen with mutant SHP2 is due to conformational transition, in which the mutation disrupts intramolecular interaction between the N-terminal SH domain and C-terminal PTP domain of SHP2; the resulting unmasked PTP domain, which contains both negatively and positively charged patches, mediates LLPS via multivalent electrostatic interactions [40]. Interestingly, compared with SHP2-wt, different disease-related SHP2 mutants may have elevated or decreased PTP activity [40]. For the latter LOF mutant, the conundrum can be explained by the fact that onco-condensates formed by mutant SHP2 further recruit and concentrate SHP2-wt, leading to its hyperactivation and downstream signaling [40].

Recent analyses of the mutant ENL by Song *et al.* also indicate the critical involvement of conformational change in enhancing LLPS [41]. ENL contains multiple interfaces for either self-association (via its central IDRs, which harbor oppositely charged regions) or interaction with acetylated histones (via its N-terminal YEATS domain) and partner proteins (via a C-terminal AHD domain that binds DOT1L and cofactor) [41,43]. In Wilms' tumor, small mutations of ENL alter its structural conformation to enhance LLPS, whereas reversing such a conformational

change via a 'correction' mutation strategy abrogated LLPS [41]. Systematic mutagenesis further showed essential roles for all of the aforementioned interaction interfaces in mediating LLPS of mutant ENL [41]. Together, these observations demonstrate a coordinated process wherein various structural modules and IDR-mediated multivalent interactions organize a functional hub with required components, while driving onco-condensate formation.

Oncogenic aberration causes condensate mis-localization

Fourth, cellular location of condensate is critical for its function. Therefore, cancer-associated mutations may cause mislocalization of condensate, leading to deregulation of the related cellular process. Nucleophosmin (*NPM1*; Figure 1A) encodes a multifunctional nucleolar protein and is among the most frequently mutated genes in patients with **acute myeloid leukemia (AML)** [44]. Normally, wild-type NPM1 (NPM1-wt) is retained primarily in the nucleolus owing to a C-terminal nucleoli localization signal (NoLS). In AML, a four base-pair insertion in the last exon of NPM1 produces a truncated NPM1 mutant, termed 'NPM1c', which loses the NoLS; as a result, NPM1c exhibits a predominantly cytoplasmic localization, in addition to nucleoplasmic retention [44,45]. In their respective compartments, both NPM1-wt and NPM1c undergo LLPS due to multivalent interactions with specific proteins (via charged IDRs) and with RNA (via a C-terminal nucleic acid-binding domain) [45,46]. However, functions of NPM1-wt and NPM1c condensates differ due to different cellular contexts. NPM1c condensates are much smaller than those of NPM1-wt (with a median diameter of the former around 195 nm compared with ~1–5 μm of the nucleolus) [45]. While NPM1-wt LLPS contributes to nucleolus formation [46], nuclear condensates of NPM1c recruit and concentrate the RNA polymerase II (Pol II) and active transcription apparatus to form a transcriptional hub at target genes [45].

Oncogenic mutation can alter the material property of a condensate to promote oncogenesis

Besides the liquid droplet, the material property of condensates can be gel-like or solid-like [47] (Box 3). Gel/solid-like phases are less dynamic, significantly decreasing the functional output of condensates. For example, multicomponent condensates formed by ER-alpha in breast cancer cells progressively harden and transition from a metastable liquid state to a solid-like one during prolonged hormone treatment, indicative of a mechanism underlying differential effects seen with acute versus chronic stimulation [48]. Shi *et al.* recently compared condensates formed by the UTX IDR (UTX^{IDR}) with those by the IDR of UTY, a male-specific homolog of UTX but a weaker tumor suppressor relative to UTX [27]. Compared with UTX^{IDR}, UTY^{IDR} is more enriched with LLPS-promoting sequences, such as aromatic residues and oppositely charged blocks, and, consistently, UTY^{IDR} demonstrated a stronger phase-separation capability [27]. Unlike liquid-like condensates of UTX^{IDR}, those of UTY^{IDR} were less fluid and solid-like [27]. Certain cancer-

Box 3. The material properties of phase-separated assemblies

The material property of a condensate is defined by multiple factors, such as the organization of involved molecules and the timescale of making and breaking of interactions among molecules [90,99,100]. Condensates can exhibit a liquid-like property, such as that seen with stress granules, P granules, and nucleoli [76,90]. However, some behave more as a **hydrogel**-like assembly, such as the centrosome and RNA expansion repeats, or as a solid phase, such as **Balbiani body** and **amyloid (A)-bodies** [76,90]. When a cell faces environmental perturbations, such as those of pH, temperature, and salt concentration, the material property and organization of cellular condensates may transition through a continuum of states [90,99,100]. For instance, the phase-separated droplets formed by IDR-containing proteins can initially be fluid, but with time they become less dynamic and behave more as a solid phase. Alternatively, misfolding proteins can directly assemble into the aggregated oligomers and protofibrils, which can subsequently convert to amyloid-like fibrils, as comprehensively reviewed in [90]. This process is called maturation or hardening [101,102]. It is proposed that hardening can shut down the biochemical reactions, thereby contributing to pathogenesis [102,103]. Measurement of the solid-like state in the literature often relies on slow signal recovery by fluorescence recovery after photobleaching (FRAP), which does not necessarily indicate that a material is solid. Recent studies also point to the changing viscoelastic properties of liquid condensates over time, which behave as Maxwell fluids or Maxwell materials [104,105].

related mutations at UTX^{IDR} produced condensates that resembled the viscous UTY^{IDR} condensates (Figure 1E) [27]. UTX/UTY represents a significant contributing factor for higher tumor susceptibility seen in men than in women [49]. Different material properties of UTX and UTY condensates may provide a molecular explanation. In support, replacing UTX^{IDR} with UTY^{IDR} led to significantly decreased tumor-suppressive activity [27]. Thus, appropriate material state of condensates can significantly influence oncogenicity.

Functionality of onco-condensates

Next, we showcase some better-studied onco-condensates to illustrate their general functions. We favor a view that deregulated biomolecular condensation represents one of the central oncogenic mechanisms.

Onco-condensates enhance the biological output of cellular proliferation signaling, leading to oncogenesis

Normal cells rely on tightly controlled mitotic signaling, such as that related to signal transduction, gene expression, chromatin modification, and cell metabolism, to replicate themselves while preventing cancerous transformation. Frequently, cancer hijacks such a normal process and overactivates it through aberrant LLPS. Take transcriptional onco-condensates for example (Figure 1F): EWS-FLI, a hallmark mutation of Ewing's sarcoma, aberrantly fuses the DNA-binding domain (DBD) of FLI to the IDR of EWS and forms nuclear condensates [14,15]. Likewise, the IDR of NUP98 is recurrently fused to DBD of TFs or a chromatin-binding domain of chromatin regulators in blood cancers, leading to condensation of oncofusions, such as NUP98-HOXA9, NUP98-KDM5A, and NUP98-NSD1 [16–19]. Using a set of IDR deletion, mutagenesis, and/or swapping strategies [14,16,18], researchers demonstrated that IDR-mediated LLPS is essential for enhancing genomic targeting or establishing new target sites of oncofusions, is required for inducing oncogenic gene-expression programs, and is essential for tumorigenicity [14,16,18]. IDR deletion, a strategy frequently used in the literature, does not necessarily point to an involvement of LLPS because IDRs may have additional LLPS-independent functions (such as the formation of soluble complexes). Thus, it is crucial to use approaches that can causally connect IDR-mediated LLPS *per se* to the studied biological process. Ahn *et al.* further dissected the role for IDR in reorganization of 3D chromatin structure, and found that NUP98-HOXA9 LLPS promotes formation of CTCF-independent loops between its cognate binding sites at proto-oncogenes (such as NUP98-HOXA9-bound enhancers and promoters at *PBX3* and *HOX* gene clusters) [16]; similarly, EWS-FLI

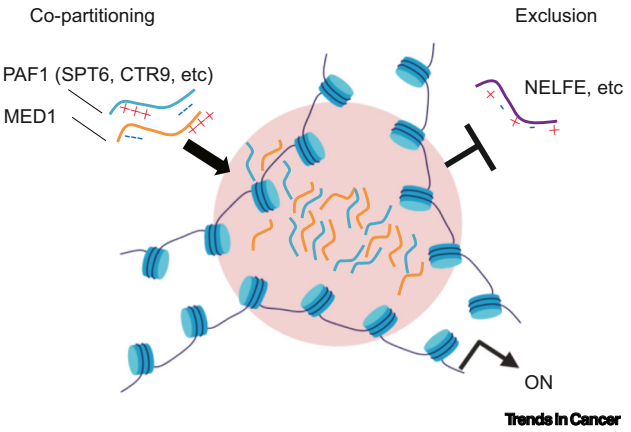


Figure 2. Selective recruitment and exclusion by the MED1 condensate. The existence of alternating blocks of oppositely charged amino acids within intrinsically disordered regions (IDRs) of MED1 and positive regulators of transcription (e.g., PAF1 complex subunits, SPT6 and CTR9) permits selective recruitment and co-partitioning into MED1 condensates; meanwhile, negative regulators of transcription (such as NELFE) harbor IDRs lacking such a 'blocky' feature and are excluded from the condensates, thus allowing robust transcription.

also establishes new DNA loops, with their anchors bound by EWS-FLI and not CTCF [50]. Aside from IDR gain in oncoproteins, relatively smaller mutations, such as Wilms' tumor-associated amino acid insertion and deletion in ENL as discussed above [41,42], also can profoundly enhance LLPS, thereby elevating the associated transcriptional output.

Multivalent interactions drive condensate formation while compartmentalizing the functional components at the same time. A functional condensate requires concerted actions of functional partners while excluding those unwanted ones (e.g., negative regulators or inhibitors). However, whether and how the IDR-mediated interactions can compartmentalize specific sets of functional partners are largely unclear. Lyons *et al.* recently took an unbiased approach and studied condensation of the IDR of MED1, a transcriptional coactivator [20]. $MED1^{IDR}$ (Figure 2) selectively recruits and co-mixes with positive regulators of transcription while excluding negative regulators [20]. Furthermore, the authors elegantly showed that existence of alternating blocks of oppositely charged amino acids is a key feature of those co-mixed IDRs, which operate to establish a functional multicomponent hub [20]. Disrupting such patterning of oppositely charged blocks in the IDRs of coactivators led to defective transcriptional induction [20]. Such a co-partitioning principle is also used by onco-condensates. Wang *et al.* assessed how NPM1c condensates (Figure 1A) promote AML oncogenesis [45]. Direct binding of NPM1c, and not NPM1-wt, at AML-related proto-oncogenes is essential for their activation [45]. Furthermore, heterotypic interactions between IDRs of NPM1c and RNA Pol II result in co-recruitment and concentration of the latter and associated apparatus (such as ENL and MLL/Menin) into condensates [45]. What is interesting is that NPM1c also binds and recruits HDAC1, a transcriptional corepressor, to the same onco-condensates [45]. Detailed analysis showed that NPM1c onco-condensates in fact keep HDAC1 in check, due to those recruited coactivators that antagonize the deacetylase activity of HDACs [45]. In the absence of NPM1c binding, AML proto-oncogenes are readily shut down by HDACs and corepressors. Moreover, the gene activation effect of NPM1c is cell type specific: when introduced to hematopoietic cell models representing a different developmental stage, NPM1c maintains high expression of a lineage-specific gene set distinct from that seen in NPM1c-positive AML. Thus, NPM1c functions to maintain and amplify pre-existing transcription programs (such as developmentally regulated HOX and MEIS1) rather than initiating *de novo* activation [45]. Such a notion is also applicable to oncogenic chromatin readers, such as NUP98-KDM5A and NUP98-PHF23 [51], which read specific chromatin states of AML-originating cells. The mechanism underlying the unique targeting of NPM1c to AML oncogenes is not entirely clear, but its association with MLL/menin, ENL, and cofactors may provide a degree of targeting specificity, which awaits further study.

Altogether, transcriptional onco-condensates coordinate a range of gene activation-related processes, which include: (i) recruiting and concentrating (co)activators and RNA PoL II while excluding and/or suppressing negative regulators; (ii) enhancing genomic binding of (co)activators and partners; (iii) elevating gene activation-related chromatin modifications (such as exceedingly abundant H3K27ac, a characteristic of **superenhancers**); and (iv) forming 3D looping between oncogene promoters and enhancer. Observed effects of transcriptional onco-condensates are in agreement with a previously proposed phase-separation model, in which master TFs and chromatin modulators form condensates leading to establishment of superenhancers and active transcription in spatially confined compartments [48,52–58]. Clearly, transcriptional onco-condensates are merely one type of many onco-condensates aberrantly formed in cancers. Same scenarios of overactivation and supercharged signaling can be applied to those related to signal transduction, metabolism, and other mitotic events, which are not discussed here.

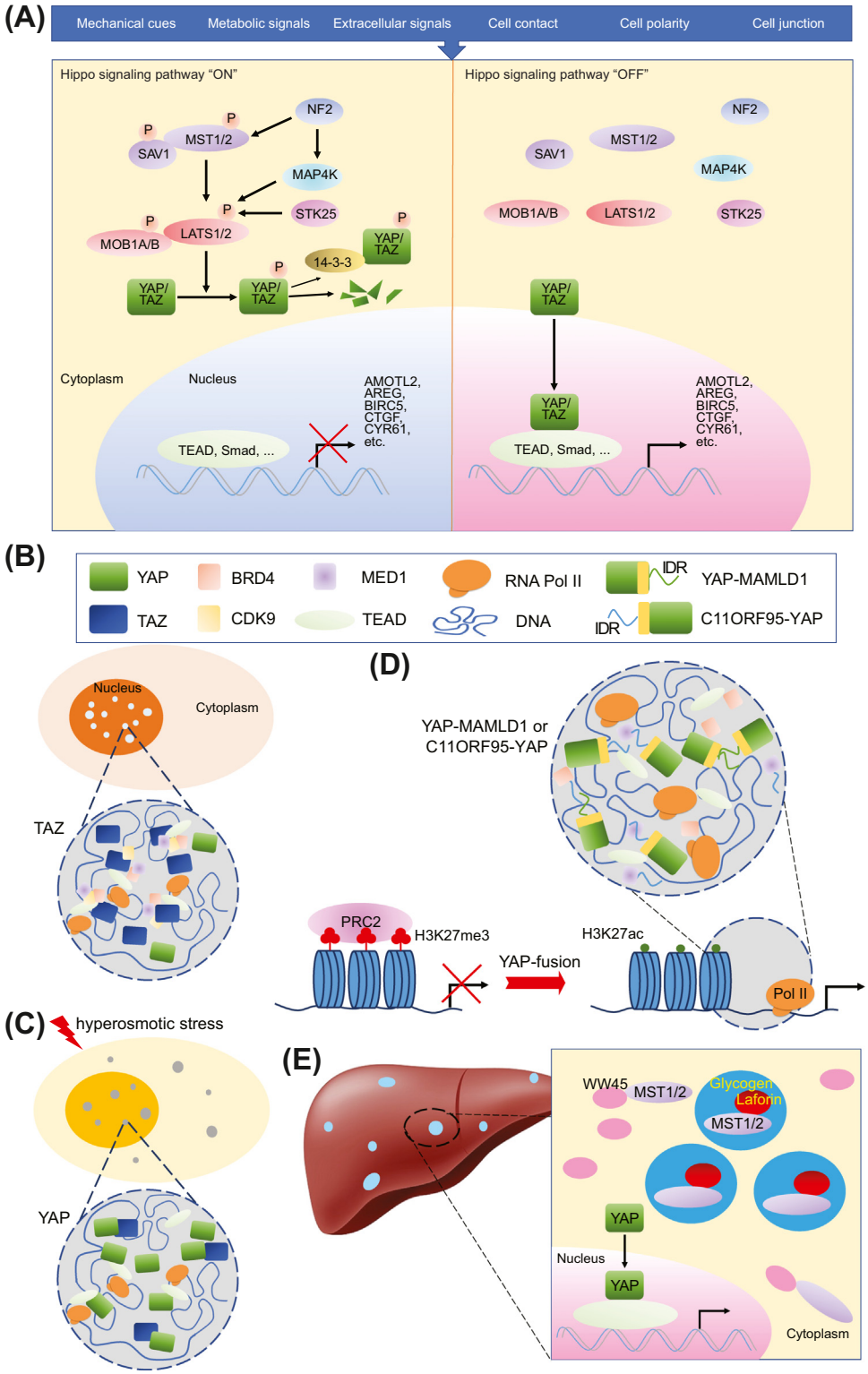
Cancer frequently coopts stress-related condensation mechanisms to enhance cell survival and/or promote cellular proliferation

Biomolecular condensation is intimately associated with cellular responses to stimulus (such as a ligand), insult (such as DNA damage), and environmental perturbation (such as heat and nutrient stress) [59]. For example, dehydrated cells, when challenged by hypertonicity, must recover their volume to survive. This process relies on the WNK kinase-mediated phosphorylation and regulation of the SLC12 cation chloride transporters to drive net ion influx [60]. Boyd-Shiwarski *et al.* recently demonstrated that, within seconds of cell exposure to hypertonicity, WNK1 kinases phase separate due to a molecular crowding effect and efficiently form condensates in a C-terminal IDR-dependent manner [60]. Subsequently, WNK1 and related pathway proteins are co-recruited and concentrated in these condensates and become activated, initiating a signal transduction cascade to restore cell volume [60].

While biomolecular condensation allows cells to respond appropriately and efficiently to insult and environmental change, cancer often coopts such mechanisms to enhance survival and growth. In support, a recent study demonstrated a cancer dependency on stress granules [61]. Take a stress-related Hippo signaling for example. Environmental cues, such as the mechanical force generated from cell–cell or cell–extracellular matrix contact, regulate activity of core Hippo kinases (MST1/2 and LATS1/2; Figure 3A), which in return coordinate the proliferation, apoptosis, migration, and functions of the cell [62,63]. Here, activation of Hippo signaling leads to cytosolic retention and subsequent degradation of YAP and its paralog TAZ, the two mechanotransducers downstream of Hippo (Figure 3A, left); conversely, Hippo signaling inhibition results in nuclear accumulation of YAP/TAZ, which then serves as coactivator of the TEAD family TFs for inducing transcription [62,63] (Figure 3A, right). Nuclear TAZ forms condensates in a coiled-coil (CC) domain-dependent manner, which compartmentalize TEAD4 and other active transcription machinery (BRD4, MED1, CDK9, and RNA Pol II) [64]. LATS1/2-mediated phosphorylation of TAZ enhances its cytoplasmic retention, thereby inhibiting the nuclear condensation of TAZ [64] (Figure 3B). Likewise, YAP also forms liquid condensates under hyperosmotic stress (Figure 3C), although a different protein region (TAD) has been shown to be involved in YAP LLPS [65]. The Hippo-YAP/TAZ axis functions to ensure appropriate execution of various physiological processes, such as organ development, growth control, and tissue injury and regeneration [62,63]. Recently, Hu *et al.* found that two clinically relevant YAP fusions found in ependymoma, YAP-MAMLD1 and C11ORF9-YAP (Figure 3D), formed nuclear condensates and were sufficient to drive ependymoma tumorigenesis when expressed in mouse cortical progenitor cells [22]. Here, IDRs within the YAP fusion partners (MAMLD1 and C11ORF9) were required for nuclear condensate formation and tumorigenesis, while forced nuclear translocation of wild-type or constitutively active YAP was insufficient to drive nuclear condensation or tumorigenesis. Condensates of YAP fusions recruit coactivators (BRD4 and MED1) and appear to repel PRC2, a transcriptional repressor, to activate targets [22]. Besides proto-oncogenes, such as Myc, YAP fusions also directly upregulated the expression of transcription coactivators (including BRD4 and MED1), which were then recruited to the YAP fusion-containing condensates in a feedforward loop [22]. Together, ependymoma coopts the YAP LLPS mechanism to enforce uncontrolled growth, leading to tumorigenicity.

Onco-condensates can suppress a tumor-suppressive pathway, promoting oncogenesis

Cancer-associated mutation can cause hardening of condensates formed by a tumor suppressor, thereby suppressing its activities. As mentioned above, the cancer-related mutant of UTX^{IDR} generates solid-like condensates that resemble those of UTY, a less potent tumor suppressor [27,49]. Alternatively, onco-condensates can actively sequester tumor suppressors. Lie *et al.* recently reported that, during liver cancer initiation, deficiency of glycogenolysis enzymes, such as



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glucose 6-phosphatase (G6PC) and liver glycogen phosphorylase (PYGL), leads to accumulation of glycogen, a glucose polymer and storage form that undergoes LLPS [66] (Figure 3E). These glycogen condensates recruit Laforin, a glycogen phosphatase carrying a carbohydrate-binding domain, which subsequently binds and concentrates cytosolic MST1/2 into the condensates [66]. Consequently, YAP is released due to condensate-mediated sequestration of MST1/2 [66]. Another sequestration example is amyloid-like aggregates of cancer-associated p53 mutants, which bind and sequester endogenous tumor suppressors, such as WT p53, p63, and p73 [67].

Concluding remarks and future perspectives

Cancer initiation and/or malignant progression is increasingly appreciated to be a process involving abnormal establishment or deregulation of biomolecular condensates. Onco-condensates in certain tumor subtypes are induced by those hallmark or frequent mutations, such as the aforementioned EWS-FLI, BRD4-NUT, and NPM1c. In this review, we discussed phase separation-based mechanisms underlying formation of multicomponent onco-condensates and their diversified functions. We focused mainly on onco-condensates related to chromatin/gene regulation and touched on others related to protein degradation and signal transduction; similar scenarios could be applied to biomolecular condensation related to stress response [61], tumor metabolism [68], DNA damage response (such as PARP1 [69] and 53BP1 [70]), tumor-associated RNAs (including long noncoding RNAs, such as cancer-associated NEAT1 [71], and various RNA species related to transcription [72] and DNA damage repair [73]), among others. In short, oncogenic condensates can either aberrantly supercharge tumorigenic signaling, often coopting stress-related condensation mechanisms to evade death, or can block a tumor-suppressive pathway.

Despite advances in understanding the establishment and functionalities of onco-condensates, outstanding questions remain (see Outstanding questions). Both membrane-bound organelles and membrane-less condensates regulate the tumorigenic processes, indicating potential crosstalk between these two types of compartment [74], which merits further study. In addition, the 'blocky' patterning of oppositely charged amino acids was shown to dictate co-partitioning of MED1 and coactivators into transcriptional hubs [20], and whether such an IDR sequence pattern controls multicomponent condensation in other contexts remains to be investigated. Furthermore, despite recent advances, the molecular principles driving multicomponent condensation under various biological contexts remain to be investigated. Once these are determined, it might be possible to develop condensate-based therapeutics, although how this could be

Figure 3. Cancer coopts liquid–liquid phase separation (LLPS) of YAP/TAZ, the two mechanotransducers, to attain uncontrolled growth. (A) Hippo signaling in mammals. Left: upon activation of canonical Hippo signaling, NF2 phosphorylates MST1/2 and MAP4K. MST1/2, MAP4K, or STK25 phosphorylates LATS1/2, which then phosphorylates cytoplasmic YAP/TAZ. Phosphorylated YAP/TAZ undergoes proteasomal degradation or binds 14-3-3, which leads to cytoplasmic sequestration. 'X' indicates inhibition. Right: when Hippo signaling is off, unphosphorylated YAP/TAZ translocates to the nucleus and binds to TEAD, Smad, or other transcription factors, resulting in activation of target genes, such as *AMOTL2*, *AREG*, *BIRC5*, *CTGF*, and *CYR61*. (B,C) Nuclear TAZ forms condensates (B). In response to hyperosmotic stress, YAP forms both cytoplasmic and nuclear condensates in a manner dependent on an intrinsically disordered TAD domain (C). Nuclear TAZ/YAP condensates compartmentalize the indicated active transcription machinery to promote transcription of TAZ/YAP-specific proliferation genes. (D) Through multivalent interactions mediated by acquired IDRs, YAP-MAMLD1 or C11ORF95-YAP forms nuclear condensates, which concentrate transcriptional (co-) activators and switch the PRC2-mediated H3K27me3 to gene-active H3K27ac at target oncogenes. (E) Deficiency of a glycogenolysis enzyme in premalignant liver cells leads to glucose storage, which boosts glycogen accumulation. Accumulated glycogen undergoes LLPS to inactivate Hippo signaling through sequestration of Laforin and associated MST1/2. As a result, YAP translocates to the nucleus, leading to activation of downstream oncotargets.

achieved is unclear (see below). Answers to these questions will deepen our understanding of tumorigenicity and aid in developing new treatment strategies.

Given that an onco-condensate represents a hub driving tumorigenic signaling, targeting condensates is an attractive therapeutic strategy [9,75,76]. First, development of more effective small molecules requires consideration of physicochemical properties of onco-condensates to achieve targeting and co-partitioning of small molecules. Indeed, Klein *et al.* uncovered that certain compounds, such as cisplatin, mitoxantrone, CDK7 inhibitor THZ1, and BRD4 inhibitor JQ1, have the ability to be concentrated to transcriptional condensates, which affects their pharmacological properties independently of molecular targets [77]. Drug resistance needs to be re-evaluated in the context of condensates [77,78]. Furthermore, it is theoretically possible to modulate or dissolve onco-condensates, thereby decreasing the associated tumorigenic signaling and/or releasing the sequestered tumor suppressor. In support, methylation of arginine within the Arg-Gly-Gly (RGG) motif-rich IDR of FUS negatively regulates condensation [79,80]. Equally possible is compound-induced condensates that exhibit antitumor effects. However, IDRs are common in the human proteome [81] and are often involved in both normal and pathogenic processes. It remains a major challenge to determine how to specifically disrupt cancer-causing condensates without affecting normal cellular processes. Yet, recent studies reported some early successes wherein a small molecule termed ET516 specifically disrupted AR condensates in castration-resistant prostate cancer [78], while another compound, 4,4′-dianilino-1,1′-binaphthyl-5,5′-disulfonic acid (bis-ANS), modulated LLPS of the IDR of TDP-43, a neurodegeneration-related protein [82]. We remain optimistic and look forward to the discovery of more drug candidates that specifically target oncogenic condensates in the years to come.

Outstanding questions

Signaling elicited from membrane-bound organelles and membrane-less condensates may interplay. How exactly the two compartments crosstalk during oncogenesis remains unclear.

A 'blocky' pattern of oppositely charged amino acids dictates co-partitioning of MED1 and co-activators into transcriptional condensates. Can this principle be generalized to other active transcription-related condensates?

Many IDRs lack oppositely charged amino acid blocks. Thus, what is the molecular principle governing multicomponent condensation driven by these IDRs?

How can we develop drugs that more specifically target cancer-causing condensates without affecting normal biological processes?

How can we design a feature of a small molecule that enhances its co-partitioning to specific pathogenic condensates?

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Declaration of interests

The authors have no interests to declare.

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