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Liquid-liquid phase separation drives cellular function and dysfunction in cancer

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Abstract

Cancer is a disease of cells gone off the rails, of biochemical pathways that have escaped the regulatory bounds that define normal homeostatic balance. This balance is maintained through precise spatiotemporal regulation, and the formation of biomolecular condensates via liquid-liquid phase separation (LLPS) has recently emerged as a widespread mechanism underlying the spatiotemporal coordination of biological activities in cells. Biomolecular condensates are widely observed to directly regulate key cellular processes involved in cancer cell pathology, and the dysregulation of LLPS is increasingly implicated as a previously hidden driver of oncogenic activity. Here, we offer our perspective on how LLPS shapes the biochemical landscape of cancer cells.

INTRODUCTION

Cellular processes are tightly coordinated in both space and time to ensure proper biological function. Disrupting this precise spatiotemporal regulation can have devastating pathological consequences, as exemplified by the broad dysregulation of basic cellular processes in cancer, including transcription, genomic integrity, quality control pathways, and intracellular signaling[1–6], which underlies the acquisition of disease hallmarks such as uncontrolled cell growth and proliferation, increased cell survival, and metabolic reprogramming[7–9]. Unraveling the mechanisms of spatiotemporal coordination in biological pathways can therefore shed light on the molecular basis of pathway disruption in cancers.

Spatiotemporal control is often achieved by sequestering biological reactions within discrete subcellular compartments, such as organelles, with distinct biochemical environments. Increasingly, subcellular compartmentation is being found to arise through higher-order molecular assemblies, variously known as membraneless organelles, droplets, puncta, granules, and of late, biomolecular condensates, formed via liquid-liquid phase separation

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COMPETING INTERESTS

The authors declare they have no competing interests.

(LLPS)[10]. Biomolecular condensates lack the lipid membranes of classic intracellular organelles, as well as the fixed stoichiometries of more traditional macromolecular complexes, instead being defined by weak, multivalent interactions between biological polymers (e.g., proteins, RNA, DNA), which coalesce into stable, micron-sized bodies with distinct compositions from the cellular milieu[10,11]. The past decade has revealed a pervasive role for biomolecular condensates in organizing myriad biochemical pathways throughout the cell, upending established views of cellular compartmentation via membrane bound organelles. Recent evidence has also begun to highlight the particular importance of biomolecular condensates in not only controlling cancer-related processes but also in directly promoting oncogenic dysregulation[12–16], thus helping to reconcile conflicting observations and longstanding mysteries in the field while also opening up new avenues for investigating disease pathology and treatment.

Here, we examine how compartmentation via LLPS defines the biochemical landscape of cancer cells. We first summarize the thermodynamic and molecular principles underlying LLPS and the formation of biomolecular condensates. Then, we take a detailed look at several key cellular processes that are critical to cancer development, focusing specifically on the role of LLPS in controlling nuclear function, regulating cellular quality control, and organizing biochemical networks, followed by a discussion of recent studies highlighting the emerging view of dysregulated LLPS as a cryptic driver of cancer pathology. Finally, we look ahead to future strategies for investigating the molecular function and therapeutic potential of LLPS in cancer.

PHASE SEPARATION AT A GLANCE

LLPS describes the spontaneous de-mixing of a homogeneous solution into two or more distinct phases. Phase separation occurs when interactions among groups of like molecules overcome the tendency to remain disordered in solution (i.e., entropy)[11,17], causing these molecules to become enriched in the de-mixed (i.e., condensed) phase and depleted from the bulk (i.e., diffuse) solution (Figure 1a). Surface tension causes the condensed phase to adopt a spherical shape and form droplets within the diffuse phase. Molecular interactions also shift the free-energy landscape such that, although individual molecules freely diffuse between phases, there is no chemical potential difference across the phase boundary, and thus no net diffusive flux, despite a dramatic concentration gradient. Hence, the two phases dynamically exchange material over short timescales (e.g., seconds) (Figure 1b) while stably co-existing over long timescales (e.g., minutes or hours).

First suspected over a century ago[18], the biological significance of LLPS is evidenced by the many membraneless structures found in cells[10]: these spherical bodies rapidly form and dissolve, can fuse and be deformed by shear flow, and exhibit dynamic internal organization (Figure 1b), as first revealed through elegant studies of *Caenorhabditis elegans* germline granules[19]. Biomolecular LLPS is driven by multivalency, or the ability to engage in multiple weak interactions that rapidly form, break, and reform[10,17]. Proteins achieve multivalency via tandem binding modules, as well as through repetitive motifs, often containing aromatic or charged residues, within stretches of low sequence complexity (Figure 1c). These interactor domains, called stickers, are typically interspersed between

unfolded loops or within intrinsically disordered regions (IDRs), called spacers[17,20] (Figure 1c). Nucleic acids often exhibit a similar multivalent sticker-and-spacer configuration and can thus also phase separate in the presence or absence of proteins. Together, these features promote liquid-like behavior by extending the range over which molecules interact, preventing them from becoming too densely packed and incapable of dynamic rearrangement[11].

Multivalency gives rise to oligomeric complexes, whose growth decreases solubility while simultaneously promoting additional interactions[10]. This positive-feedback loop drives the switch-like phase transition that occurs at the critical threshold concentration (Figure 1d). Considering this exquisite concentration dependence, one way that cells can regulate condensate formation is thus by controlling the concentration (e.g., expression) of constituent molecules. These are loosely characterized as scaffolds, which are obligatory for condensate formation, and clients, which bind scaffolds and make up the bulk of the condensate but are largely dispensable for LLPS[10,21] (Figure 1e). Cells can further regulate droplet composition by altering the relative stoichiometry and valency of scaffolds and clients. The physical properties of condensates can also vary, as condensates are not exclusively liquid-like. In fact, as the interaction density increases, particularly among IDR-rich constituents, condensates can “mature” from liquid-like to gel- or solid-like materials[10]. Notably, many of these properties can be modulated via post-translational (or post-transcriptional, in the case of RNA) modifications, which can add or remove interaction sites or alter surface charges, thus altering valency, solubility, and condensate dynamics[10].

The ability of biomolecular condensates to concentrate specific molecules within stable, defined structures whose dynamic, liquid-like interiors are conducive of chemical reactions lends itself to the formation of biochemically distinct subcompartments. Indeed, biomolecular condensates are implicated in the spatiotemporal regulation of myriad cellular functions. Below, we highlight several key processes regulated by LLPS that also figure prominently in the pathophysiology of cancer cells.

NUCLEAR FUNCTION

Eukaryotic nuclei comprise a multitude of distinct microenvironments essential for the spatiotemporal regulation of nuclear processes. Visible disruption of this complex architecture due to the sweeping dysregulation of nuclear function is a prominent feature of cellular transformation and is frequently exploited in cancer diagnosis[22]. LLPS is already known to control the formation of constitutive nuclear substructures including nucleoli, speckles, Cajal bodies, promyelocytic leukemia (PML) bodies[23,24], the latter playing a prominent role in cancer[25–27], and mounting evidence reveals that LLPS is the primary driver of nuclear subcompartmentation[23]. Indeed, biomolecular condensates are increasingly found to play an integral role in diverse nuclear processes, such as gene expression[28–31], DNA repair[32,33], and chromatin organization[34–36].

Regulation of gene expression

Activation of oncogenic transcriptional programs causes myriad changes in gene expression, often leading cancer cells to exhibit “transcriptional addiction”[4] via the actions of so-called

super-enhancers (SEs). These clusters of enhancers were initially identified by their extreme enrichment of transcriptional regulators such as bromodomain-containing protein 4 (BRD4) and the Mediator complex and are essential for maintaining cell-lineage-specific gene expression[5]. Numerous oncogenes also acquire SEs during tumorigenesis[37], likely marking a critical step in oncogene activation. LLPS is predicted to underlie SE function[38] (Figure 2a), and indeed, both BRD4 and MED1, a core Mediator subunit, were shown to phase separate via their IDRs upon in vitro reconstitution and to form liquid-like condensates at SE sites in cell nuclei[28,29]. BRD4 phase separation also requires its tandem bromodomains[39], which increase valency through interactions with acetylated histones. Recent evidence also implicates the BRD4 short isoform (BRD4S) in driving BRD4 LLPS and influencing BRD4 function in gene transcription, as BRD4 puncta size mirrors BRD4S expression in cancer cells and changing BRD4S levels alone impacts puncta formation and gene transcription[39]. SEs are also highly enriched in enhancer RNAs (eRNAs) (Figure 2a), a class of long-noncoding RNA (lncRNA) transcribed from active enhancers and implicated in enhancer function[40]. RNA-protein interactions promote LLPS by increasing interaction valency[41], and although their precise mechanism remains unclear, eRNAs have been implicated in SE phase separation[40]. Indeed, eRNA-containing ribonucleoprotein condensates were recently shown to drive rapid ligand-induced SE assembly and activation by estrogen receptor α in breast cancer[42]. Finally, the nucleated assembly of transcriptional regulators via LLPS has been implicated in positive feedback-mediated amplification of transcriptional activity[43] (Figure 2a). LLPS thus seem to play an essential role in promoting robust oncogene activation and expression to establish and maintain cancer cell identity.

Aspects of this model have recently been challenged by data suggesting that SE proteins (e.g., MED1, BRD4) are not required for enhancer-promoter interactions[44–46], leading some to completely dismiss the role of LLPS in transcriptional regulation[47]. However, these studies do not rule out a specific role for LLPS in transcriptional activation, and in fact observed that loss of SE proteins disrupted transcription, particularly of lineage-specific genes[44–46]. Thus, although condensates may not drive all aspects of SE function, LLPS may still play a key role.

The transcriptional co-activator Yes-associated protein (YAP) and its paralogue transcriptional co-activator with PDZ-binding motif (TAZ) are another set of key players linked to cancer cell transcriptional addiction via SEs[48]. YAP and TAZ are widely activated in cancer cells[49], driving proliferation, survival, and invasiveness, as well as stem-like properties. Both YAP and TAZ form condensates in vitro and in cell nuclei through their protein-interaction domains[31,50] and extensive intrinsic disorder[30,50]. YAP/TAZ condensates associate with transcriptionally permissive chromatin regions, recruit transcriptional components such as RNA polymerase II (Pol II), TEA-domain-family transcription factors, BRD4, and MED1, and even co-localize with nascent mRNAs[30,31,50], demonstrating that YAP/TAZ condensates represent transcriptionally active SE sites. Disrupting YAP/TAZ condensates abolishes downstream gene expression, further confirming the importance of LLPS in YAP/TAZ transcriptional regulation[30,31,50]. Curiously, YAP and TAZ phase separate under distinct conditions. Nuclear TAZ condensates appear to form constitutively in proliferating cells and were in fact shown to be elevated in cancer versus healthy tissues[31]. YAP condensates fail to form under similar conditions but are induced by external

stimuli such as hyperosmotic stress[30] and interferon (IFN)-γ[50]. Despite their considerable homology, YAP and TAZ exhibit non-redundant functions, likely mediated by differential interactions with binding partners[51]. Differences in YAP/TAZ phase separation may further highlight distinct roles in regulating cancer proliferation versus adaptation and survival. Indeed, IFN-γ-induced YAP phase separation confers resistance to α-PD1 immunotherapy in tumor cells, which are sensitized by YAP condensate disruption[50].

DNA damage and repair

Aberrant cell cycle regulation triggered by oncogene activation places cancer cells in a constant state of replicative stress, leading to the frequent generation of DNA double-strand breaks (DSBs)[52] (Figure 2b). DSBs are a major source of genome instability in cancer[1], resulting in persistent activation of the DNA damage response (DDR) pathway[52,53]. An early step in the DDR is the deposition of poly(ADP-ribose) (PAR) polymers at DSB sites by poly(ADP-ribose) polymerase 1, which has been shown to trigger LLPS of intrinsically disordered FET proteins (fused in sarcoma [FUS]/Ewing sarcoma/TATA box-binding protein-associated factor 15) at DSBs[32] (Figure 2b). Another key factor in the DDR is p53-binding protein 1 (53BP1), which localizes at nuclear foci corresponding to DSB sites[53]. Nuclear 53BP1 foci were recently shown to exhibit properties of biomolecular condensates, including liquid-like internal dynamics and coalescence of individual foci[33,54], the latter being consistent with the clustering of DSBs, which may aid chromosomal translocations[55] (Figure 2b). Interestingly, FET protein recruitment to PAR chains is mediated by their Arg/Gly-rich (RGG) LC domains[32,56], which also participate in RNA binding, and PAR chains appear to compete with mRNAs for FUS binding[56]. Although FET protein RNA binding has not been directly implicated in LLPS at DSBs, RNA does play a role in forming DSB foci. Specifically, DSBs actively recruit Pol II, MED1, and other factors to transcribe damage-induced lncRNAs, which were recently shown to facilitate 53BP1 condensate formation[54]. Ironically, transcription itself can induce DNA breaks[57] (Figure 2b), and the need for cancer cells to balance repairing DNA damage with feeding transcriptional addiction likely places tremendous strain on their transcriptional apparatus. Further insights into how LLPS regulates DSB repair may thus reveal opportunities to therapeutically exploit this delicate balance.

Chromatin organization

The assembly of eukaryotic nuclear DNA into chromatin is essential for genome function and regulation. Recent work has revealed that nucleosome/DNA arrays, the fundamental unit of chromatin, are intrinsically capable of undergoing LLPS[34]. Condensate formation depended on intrinsically disordered histone tails and was also sensitive to post-translational modifications, being dispersed by histone acetylation, a typical marker of transcriptionally active, de-condensed chromatin. Strikingly, the addition of multi-bromodomain-containing proteins reformed droplets that were immiscible with un-acetylated chromatin condensates, suggesting that LLPS spontaneously organizes chromatin into distinct physical and functional domains[34]. Chromatin LLPS was further increased by addition of the linker histone H1, which caused condensates to become more dense and less internally dynamic, consistent with the role of H1 in heterochromatin formation[34]. Heterochromatin plays major roles in gene silencing and genome stability, and altered heterochromatin is a frequent

hallmark of disrupted chromatin organization in cancer[22]. Another major component, heterochromatin protein 1 α (HP1α), has also been shown to undergo LLPS in vitro and in cells[35,36,58]. LLPS of human HP1α required DNA binding or phosphorylation of its unstructured N-terminus, either of which promoted multivalency through its disordered hinge domain[35]. HP1α was also recently shown to promote nucleosome LLPS through conformational rearrangements that expose buried histone tails, thereby increasing overall disorder[36]. Notably, peptides can be identified to bind the interface between HP1α dimers to either increase or decrease LLPS[35], hinting at a potential novel strategy of targeting HP1α LLPS to rescue chromatin alterations in cancers where HP1α expression is upregulated[59]. However, such enthusiasm is tempered by recent work showing little HP1α LLPS in cells and a largely dispensable role for HP1α maintaining heterochromatin[60], highlighting the need for further investigation.

CELLULAR QUALITY CONTROL

Effective quality control mechanisms are essential for maintaining cellular homeostasis[61]. These pathways rapidly clear damage to proteins and/or organelles incurred through changes in growth, metabolism, or adaptations to environmental stress. Two major quality control processes in cells, the ubiquitin-proteasome system and autophagy, are highly interconnected pathways that also play important roles in cancer[6]. These and other adaptive pathways are often exploited by cancer cells for survival[62–64]. Recent studies have begun to uncover evidence strongly linking LLPS to proteasomal degradation and autophagy, deepening our understanding of their pathophysiological mechanisms and potentially illuminating new avenues for therapeutic targeting.

Proteasomal degradation

Proteins bound for the ubiquitin-proteasome system are typically selected and targeted via a series of adaptor proteins and enzymes that ultimately catalyze the addition of poly-ubiquitin chains, marking the target protein for degradation[61] (Figure 3a). One such adaptor protein, speckle-type POZ protein (SPOP) is a known tumor suppressor that regulates the degradation of numerous oncoproteins[65]. While SPOP is known to associate with existing nuclear condensates, SPOP has also been shown to undergo LLPS itself[66]. SPOP was observed to form phase-separated condensates in conjunction with its targeted protein death-domain-associated protein (DAXX), both in vitro and in cell nuclei. Phase separation was mediated both by SPOP oligomerization through its self-interaction domains and by multivalent interaction with the DAXX C-terminal IDR (Figure 3a). Co-condensation of SPOP and DAXX also triggered DAXX polyubiquitination, which was disrupted by mutants lacking LLPS capability, indicating that SPOP utilizes LLPS for efficient substrate targeting[66]. More recent work suggests that the proteasome is also capable of undergoing LLPS, as evidenced by the formation of proteasome-containing biomolecular condensates in the nuclei of various cell lines under acute hyperosmotic stress[67]. Proteasomal condensates were found to be sites of active proteolysis and to form through multivalent interactions between polyubiquitinated substrates and ubiquitin-binding domains in the proteasomal shuttle protein RAD23B[67]. Although it remains to be seen whether proteasome condensates can be triggered by other stress conditions, cancer cells are known to exhibit severe

proteotoxic stress[68] and to upregulate proteasomal activity for survival[69]. Further insights into LLPS within this pathway may therefore yield strategies to augment the growing use of proteasome inhibitors for cancer treatment[63].

Autophagy

The autophagy pathway channels aberrant proteins and damaged organelles (e.g., mitochondria, endoplasmic reticulum) to the lysosome for degradation and recycling[61]. During autophagy, poly-ubiquitinated cellular components are bound and sequestered by adaptor proteins such as p62, followed by engulfment within double-membrane structures known as autophagosomes (Figure 3b). Autophagosome formation is triggered by the activation of a protein complex containing unc51-like kinase 1/2 (ULK1/2), autophagy-related protein 13 (ATG13), and 200 kD focal adhesion kinase family interacting protein (FIP200). Recently, the yeast counterparts of these proteins, Atg1, Atg13, and Atg17, were found to assemble into a dynamic liquid-like condensate both in vitro and in living yeast cells[70] (Figure 3b). Condensate formation was primarily scaffolded by interactions between IDRs in Atg13 and specific sites in Atg17 dimers. Atg13 also mediated condensate localization to the yeast vacuole membrane[70]. ULK1/2 and ATG13 also both contain IDRs and analogously seed autophagosome formation on the ER surface[71,72]. It thus seems plausible to speculate that ULK/ATG13/FIP200 LLPS occurs in higher eukaryotes. Meanwhile, p62 has previously been shown to form poly-ubiquitin-containing cytosolic inclusions in cells, which were recently revealed to be liquid-like biomolecular condensates[73]. LLPS was triggered by poly-ubiquitinated proteins, with longer poly-ubiquitin chains more effectively driving condensation, and also required p62 oligomerization (Figure 3b). In cells, p62 bodies were observed to recruit the autophagosome receptor LC3 and could in fact be engulfed by autophagosomes[73], suggesting that p62 LLPS plays an active role in cargo trafficking.

Autophagy is important for cell survival not only as a quality control pathway but also as a source for raw materials for metabolic pathways during nutrient-deficient conditions. Thus, autophagy has a complex relationship to tumorigenesis, being implicated in tumor suppression through the elimination of pre-cancerous cells while also being essential for the survival of more advanced tumors by sustaining tumor growth[74,75]. Mutations in autophagy components, including ULK1/2, have been detected in various cancers[74,76], though their role in driving tumorigenesis is debated[77]. Nevertheless, autophagy is emerging as a promising target for cancer therapy[75], and further investigation into the links between LLPS and autophagy in cancer may yet reveal important new insights.

BIOCHEMICAL PATHWAY ORGANIZATION

Subcellular compartmentation through the assembly of higher-order molecular complexes is critical for organizing biochemical pathways within cells[78], whether it be to channel metabolic flux or maintain the specificity of intracellular signaling. The accelerating pace of discovery over the last 5 years has unequivocally demonstrated that molecular assembly via LLPS is a major force governing the spatiotemporal organization of biochemical pathways.

Condensates regulate metabolic flux

Cancer cells are typified by dysregulated metabolism. Famously, the Warburg effect was coined almost a century ago to describe the reliance of tumors on glycolysis rather than aerobic respiration to metabolize glucose[9]. Rather than being a mere symptom of energetic imbalance, the Warburg effect is now understood as a hallmark of the extensive metabolic reprogramming that shifts biosynthetic pathways to meet the demands of uncontrolled proliferation[9]. For example, increases in glycolysis drive metabolic flux through the pentose phosphate and de novo serine synthesis pathways, both of which are upregulated in cancers and function to supply critical metabolites required to support tumor cell proliferation and survival[79,80]. These pathways are also both linked to the formation of the glucosome, a multi-enzyme metabolic complex (i.e., metabolon[81]) that comprises four rate-limiting cytosolic enzymes involved in glycolysis and gluconeogenesis[82]. Notably, glucosome size has been found to increase as a function of flux through the pentose phosphate and serine synthesis pathways, with cancer cells exhibiting higher numbers of large glucosome puncta compared with normal cells, suggesting a link to cancer metabolism[82].

Another well-studied cytosolic metabolon, the purinosome, encompasses all of the enzymes responsible for catalyzing de novo purine biosynthesis[83]. Itself an offshoot of the pentose phosphate pathway, de novo purine biosynthesis is also upregulated in cancers and has been implicated in therapeutic resistance[84] and metastasis[85]. Purinosomes rapidly form in cells upon purine depletion[83], and upregulated purinosome formation was recently observed in highly metastatic breast cancer cells. Emerging evidence also suggests that purinosomes can be induced by hypoxia[86], a hallmark of the tumor microenvironment.

The formation of metabolons is thought to enhance the specificity and efficiency of metabolic reactions by insulating metabolic intermediates from the bulk milieu and allowing them to pass directly between enzymes that catalyze sequential reaction steps (i.e., substrate channeling)[81], as was elegantly demonstrated for purinosomes using mass spectrometry imaging[87]. Both glucosomes and purinosomes appear as roughly circular cytoplasmic puncta that exhibit characteristic liquid-like internal dynamics of biomolecular condensates in cells[82,88], though additional mechanistic factors (e.g., concentration dependence, sources of multivalency, scaffold-client relationships) will need to be resolved before these structures can be regarded as examples of LLPS. Nevertheless, these liquid-like metabolic assemblies are emerging as potentially central factors in metabolic rewiring and key targets for deeper investigation.

Phase separation in intracellular signaling

Intracellular signaling pathways form a critical regulatory network governing cellular behavior and homeostasis, the dysregulation of which underlies multiple facets of cancer pathology[3]. The molecular elements that promote LLPS are common features of signaling proteins[89], and indeed, a growing body of evidence argues compellingly for biomolecular condensates as pervasive organizers of the signaling machinery. Through these studies, we are also beginning to unravel the direct functional role of LLPS in shaping biochemical dynamics.

Phase separation controls cytosolic DNA sensing and immune signaling—The presence of cytoplasmic DNA is an acute danger signal that triggers innate immunity, cellular senescence, and cell death[90]. These responses are mediated by pattern recognition receptors such as cyclic 2',3' guanosine monophosphate-adenosine monophosphate (cGAMP) synthase (cGAS), which becomes activated upon dsDNA binding to synthesize cGAMP from ATP and GTP. cGAMP binds the ER transmembrane receptor stimulator of interferon (IFN) genes (STING), which then traffics to the Golgi apparatus and activates TBK1 and IKK, triggering type I IFN and proinflammatory cytokine production[90].

dsDNA binding by cGAS was recently shown to induce LLPS of cGAS:DNA biomolecular condensates, both in vitro and in living cells[91]. cGAS binds DNA in a sequence-independent manner through multivalent interactions mediated by its catalytic core and its positively charged, disordered N-terminal domain (Figure 4b). cGAS phase separation not only requires its disordered N-terminus but is also enhanced by longer dsDNA, underscoring the critical role of multivalency in condensate formation. Importantly, cGAS condensates are catalytically active, and their disruption drastically impairs cellular cGAMP production[91], directly implicating LLPS in pathway function. cGAMP production may be further enhanced through the exclusion of a negative regulator, the exonuclease TREX1, which degrades cytosolic dsDNA and thus antagonizes cGAS activation. In vitro, TREX1 phase separates to form a shell surrounding cGAS:DNA condensates, thereby restricting its activity and prolonging cGAMP production[92]. LLPS thus produces a discrete biochemical compartment for efficient DNA sensing and switch-like pathway activation[91].

Cytoplasmic DNA is frequently present in cancer cells, in the form of cytoplasmic chromatin fragments and micronuclei generated via DNA damage and other nuclear aberrations, and activation of the cGAS-STING pathway is thought to play a tumor-suppressive role by stimulating senescence and immune-mediated clearance of pre-cancerous cells[90]. Indeed, many cancers show reduced or loss of cGAS and/or STING expression. cGAS-STING signaling has also been linked to antitumor immunity through host cell uptake of tumor DNA[93] and tumor-produced cGAMP[94], which triggers host-cell STING activation and immune responses. cGAS-STING activation thus represents an attractive therapeutic target and in fact contributes to the efficacy of established therapies[90]. However, emerging evidence that cGAS-STING proinflammatory signaling promotes tumorigencity[95] represents a potential complication, as does the recent discovery of LLPS by STING to form ER-incorporating spherical condensates that buffer cGAMP and dampen immune responses[96] (Figure 4b). Unravelling the dual role LLPS plays in tuning cGAS-STING signaling may thus prove essential for effectively targeting this pathway in cancer.

cAMP/PKA signaling compartmentation by phase separation—The ubiquitous intracellular messenger cyclic 3'5' adenosine monophosphate (cAMP) is involved in regulating a diverse array of cellular processes, including gene expression, growth, proliferation, and migration[97]. cAMP is typically produced in response to G protein-coupled receptor-induced activation of adenylyl cyclases via the Gαs heterotrimeric G protein subunit. *GNAS*, which encodes Gαs, is the most frequently mutated G protein gene in cancer, with key mutational hotspots linked to constitutive Gαs activation[98], implicating

hyperactive cAMP production in oncogenesis. cAMP signaling is primarily mediated by the cAMP-dependent protein kinase (PKA), a tetrameric holoenzyme composed of a regulatory (R) subunit dimer bound to a pair of catalytic (C) subunits. Of the various R subunit isoforms (RIα, RIβ, RIIα, RIIβ), only RIα is ubiquitously expressed, and RIα loss through various mechanisms is linked to tumorigenesis[99,100]. RIα has recently been shown to undergo LLPS to form biomolecular condensates in vitro and at endogenous expression levels in cells[101] (Figure 4c). LLPS of RIα requires its dimerization domain and a disordered linker region and is promoted by cAMP binding, which is thought to induce a conformational change in the holoenzyme that exposes the disordered linker[101].

Functionally, RIα condensates are directly involved in the spatial control of cAMP signaling. The compartmentation of cAMP elevation within discrete subcellular microdomains is essential for encoding specificity in the regulation of downstream targets. Indeed, the idea of compartmentalized signaling was first articulated with respect to the cAMP pathway[102]. The classic model of cAMP signaling has focused on cAMP-degrading phosphodiesterases (PDEs) as the major drivers of compartmentation through the formation of sinks that consume cAMP and limit its subcellular mobility[103]. However, restricted diffusion through cAMP buffering is emerging as another critical mechanism underling compartmentation[104]. RIα condensates are strongly implicated as the source of cAMP buffering, as genetically encoded biosensors targeted directly to endogenous RIα in cells report saturating levels of cAMP inside droplets, which was also confirmed through in vitro droplet reconstitution using purified RIα and a fluorescent cAMP analogue[101]. In fact, RIα LLPS was shown to be required for effective cAMP compartmentation by PDEs, with loss of phase separation disrupting PDE-mediated cAMP sinks (Figure 4c). Notably, disrupting RIα condensates phenocopies the ability of RIα knock-out to induce cell proliferation and anchorage-independent growth, two markers of cell transformation[101]. Thus, it may be time to reconsider the role of cAMP and RIα in cancer through the lens of phase separation.

CONDENSATES IN THE DRIVER'S SEAT

Alongside work implicating LLPS in basic cellular processes that are intimately connected to cancer biology, recent studies are increasingly affirming a direct link between LLPS and tumorigenesis. In particular, prominent nuclear condensates such as PML bodies are being shown to play an expansive role in tumorigenesis[25,26,105]. A striking example comes from the study of paraspeckles[105,106], which have been shown to form upon activation of p53, a critical tumor suppressor that directs multiple pathways, including cell cycle regulation, DNA repair, and cell death[107,108]. p53 induces paraspeckles by directly upregulating the scaffold lncRNA nuclear-enriched abundant transcript 1 (NEAT1) in response to various oncogenic stimuli[105,106,109]. Knocking out NEAT1 not only disrupts p53-induced paraspeckle formation but also prevents the formation of chemically induced skin tumors in mice, indicating that paraspeckles are required for tumorigenesis[105]. Cells lacking NEAT1 expression, and thus paraspeckles, were found to show elevated levels of replicative stress, DNA damage, and apoptosis compared with control cells in response to chemical carcinogens. p53 accumulation was also markedly prolonged in the absence of NEAT1[105], suggesting that paraspeckle formation triggers a negative-feedback loop to

limit DNA damaged and attenuate p53 signaling, allowing precancerous cells to survive and accumulate additional mutations (e.g., loss of p53) to drive cancer progression.

LLPS of the nuclear-localized A-kinase anchoring protein (AKAP) AKAP95, which regulates RNA splicing[110], was also recently shown to directly promote tumorigenesis[111]. AKAP95 is overexpressed in a broad spectrum of cancers[111–113], including ovarian, rectal, and breast cancers, and was shown to be required for in vitro proliferation and anchorage-independent growth, as well as in vivo tumor formation, by cancer cells[111]. Knocking down AKAP95 expression in cancer cells leads to the downregulation of pro-tumorigenic gene expression pathways (e.g., cell proliferation, DNA damage repair) and upregulation of tumor-suppressive pathways (e.g., senescence, apoptosis)[111], suggesting that AKAP95 plays a similar role to that of paraspeckles in enabling survival and adaptation to oncogene-induced stress. The pro-tumorigenic effects of AKAP95 largely stem from its role in controlling the alternative splicing of multiple cancer-associated gene transcripts, such as *CCNA2* and *SMAD6*, which was found to depend on AKAP95 phase separation[111]. AKAP95 LLPS behavior is mediated primarily by a core disordered region (residues 101–210) that is sufficient to induce droplet formation in vitro, whereas deleting this entire region or mutating key residues blocks both in vitro droplet formation and the appearance of AKAP95 nuclear puncta[111]. Importantly, these LLPS-disrupting mutants lacked RNA splicing activity, despite successfully interacting with key binding partners, and failed to rescue the defects in gene expression, RNA splicing, and tumorigenesis observed in AKAP95-knockout cells[111].

Cancers also rely on mutations that disrupt the normal functions of key gene products, either via loss (e.g., inactivation, silencing, deletion) or gain (e.g., amplification, hyperactivation) of function. Yet the mechanistic link between specific cancer-related mutations and their pathological effects is not always clear. For example, the protein tyrosine phosphatase (PTP) SHP2, a major positive regulator of Ras activation, is frequently mutated in cancers and is an emerging therapeutic target[114,115]. Curiously, mutations that increase and decrease SHP2 phosphatase activity both increase cancer risk[116], suggesting that altered catalytic activity is not sufficient to drive disease pathology and raising questions about the actual molecular mechanism. New work is revealing that these and other seemingly cryptic mutations achieve their pathological effects by dysregulating LLPS. Indeed, several disease-associated SHP2 mutants were recently shown to undergo LLPS in vitro and in cells[117] (Figure 5a). SHP2 LLPS is driven by charged residues within the PTP domain, which become exposed when SHP2 adopts its open, active conformation, and allosteric modulators that lock SHP2 in its open and closed states can promote and disrupt LLPS, respectively[117]. The open conformation is also prevalent in disease mutants, regardless of their catalytic activity, suggesting that SHP2 mutants gain LLPS ability through their altered conformations. Importantly, both activating and inactivating SHP2 mutants were found to promote ERK pathway hyperactivation in cells, which required their ability to form condensates[117]. Yet whereas the intrinsic activity of condensates formed by SHP2-activating mutants was sufficient to induce signaling, condensates formed by SHP2-inactivating mutants were found to acquire catalytic activity by recruiting and activating WT SHP2[117] (Figure 5a). Thus, SHP2 disease-causing mutants gain the capacity for constitutive molecular assembly via

LLPS, allowing them to induce pathological signaling independent of their intrinsic catalytic activity.

Many cancers are specifically driven by chimeric proteins generated by chromosomal rearrangments[118–122], and gain of LLPS ability is increasingly being found to underlie the pathology of these oncogenic fusions. For example, multiple fusion oncoproteins contain intact kinase domains from receptor tyrosine kinases (RTKs) but lack transmembrane domains for canonical RTK signaling[118], leaving their tumorigenic mechanism unclear. Recently, several variants of the echinoderm microtubule-associated protein-like 4/anaplastic lymphoma kinase (EML4-ALK) fusion found in lung cancers[118,123] were shown to form biomolecular condensates, both in vitro and in vivo, spanning a range of liquid- to gel-like properties depending on their precise fusion architecture[124,125]. Phase separation of EML4-ALK variants appears largely mediated by oligomerization via EML4-derived self-interaction domains, as removing these domains or mutating key residues abolished condensates[124,125]. EML4-ALK condensates were also shown to recruit a number of Ras-activating factors, such as SOS1 and SHP2, notably triggering their dramatic redistribution from the cytosol into condensates[124]. Meanwhile, negative regulators of Ras activity, such as GTPase activating proteins, were excluded from EML4-ALK condensates, similar to the behavior of other Ras-activating condensates[126]. Disrupting the formation of EML4-ALK condensates blocked the hyperactivation of Ras/ERK signaling, which was found to specifically occur through a cytosolic rather than membrane-associated pool of Ras[124]. Given that similar RTK fusions typically contain oligomerization domains such as coiled-coils[118], gain of LLPS may be a general mechanism for hyperactive signaling by these oncogenic chimeras, and indeed, similar behavior was observed with a CCDC6-RET fusion kinase[124].

Aside from altering signaling, many oncogenic fusions incorporate elements of transcription factors or other DNA-binding proteins and are directly associated with profound changes in gene regulation[119–121,127], yet how these effects are accomplished is unknown. Ewing sarcoma, for instance, is typically driven by fusion of the N-terminus of Ewing sarcoma RNA-binding 1 (EWSR1) and the DNA-binding domain of the E-Twenty Six (ETS) transcription factor Friend leukemia integration 1 (FLI1)[120,128,129] (Figure 5b). The resulting EWS-FLI1 chimera binds GGAA microsatellite repeats to induce de novo activation of oncogenic enhancers via chromatin remodeling, while inactivating canonical ETS sites[130,131]. Both EWS-FLI1 and native FLI1 recognize the GGAA core ETS consensus sequence[130,131], but only EWS-FLI1 binds GGAA repeats[120], suggesting that DNA-binding *per se* is not responsible for tumorigenic activity. Similarly, EWS-FLI1 recruits the BRG1/BRM-associated factor (BAF) chromatin remodeling complex to GGAA repeats, yet both EWSR1 and FLI1 interacted with the BAF complex on their own, which was not sufficient to reproduce the actions of the EWS-FLI1 fusion[120]. Instead, the novel functions of EWS-FLI1 were shown to stem from its ability to undergo LLPS and form liquid-like nuclear condensates. The N-terminus of EWS-FLI1 corresponds to the N-terminal IDR of EWSR1, and IDR-mediated phase separation was found to be required for the tumorigenic activities of EWS-FLI1[120]. Specifically, mutating key aromatic residues to disrupt LLPS abolished BAF recruitment to GGAA repeats, chromatin remodeling, and enhancer activation by

EWS-FLI1, whereas a minimal repeat derived from the EWSR1 IDR was sufficient to recapitulate these effects when fused to the FLI1 DNA-binding domain[120].

Recent work on the oncogenic nucleoporin 98/homeobox A9 (NUP98-HOXA9) chimera further demonstrates the role of acquired LLPS as a key molecular alternation driving tumorigenicity[121]. This chimeric protein found in leukemia combines the NUP98 IDR, containing the characteristic phenylalanine/glycine (FG) repeats[132], with a DNA-binding homeodomain and was shown to form liquid-like condensates in vitro and in cell nuclei[121]. NUP98-HOXA9 was found to exhibit a dense, super-enhancer-like DNA binding pattern and to drive aberrant chromatin looping within genomic regions located near leukemia-associated genes; however, an NUP98-HOXA9 mutant in which FG-repeat phenylalanines were changed to serines, which abolished LLPS, showed dramatically reduced DNA binding and chromatin looping behavior and also lacked tumorigenic activity[121]. Notably, a similar disruption was observed using NUP98-HOXA9 mutants whose FG repeats were below a threshold number, illustrating a direct link between multivalency and tumorigenicity.

The loss of LLPS can likewise account for the tumorigenic effects of cancer-driving mutations with no clear mechanistic explanation. Fibrolamellar carcinoma (FLC), for instance, is a rare and lethal form of liver cancer that predominantly strikes in the young that is almost universally driven by a single mutation: an ~400 kilobase heterozygous deletion in chromosome 19 joins the 1st exon of *DNAJB1*, encoding the J domain of an Hsp40-like molecular chaperone, with exon 2 of *PRKACA*, which encodes the α isoform of PKA-C (PKA-Cα)[133,134] (Figure 5c). The resulting fusion is transcribed from the *DNAJB1* promoter, resulting in ~10-fold higher accumulation of DNAJB1-PKA-Cα versus WT PKA-Cα[133,135]. Yet overexpressing PKA-Cα fails to recapitulate FLC tumorigenesis[134]. DNAJB1-PKA-Cα also differs little from WT PKA-Cα with respect to R-subunit binding, cAMP-induced activation, or intrinsic catalytic activity[133,136]. Instead, DNAJB1-PKA-Cα was found to completely abolish the LLPS behavior of RIα, both when reconstituted using purified proteins and when expressed in cells[101], resulting in the total collapse of PDE-mediated cAMP compartmentation. This effect is identical to that of RIα deletion, and indeed, a small number of FLC cases have been linked to the loss of RIα protein expression, rather than acquisition of DNAJB1-PKA-Cα[137]. Thus, although the principal genetic alteration in FLC is PKA-Cα fusion and overexpression, the actual molecular defect appears to be loss of RIα LLPS. Similar PKA-C chimeras are recurrent in intraductal oncocytic papillary neoplasms[138], and while their effect on RIα LLPS remains unknown, it is tempting to speculate on a general role for loss of LLPS-mediated cAMP compartmentation in tumorigenesis.

Another example of defective LLPS driving tumorigenesis comes from work on ubiquitously transcribed tetratricopeptide repeat (TPR) on chromosome X (UTX), a histone demethylase that plays an important role in developmental gene regulation and is also a major tumor suppressor[139–141]. Although *UTX* is mutated in several cancers[142], mutations affecting catalytic activity are uncommon, and UTX demethylase activity is often dispensable for developmental regulation and tumor suppression [139–141]. Instead, *UTX* mutations typically produce truncated proteins[139,142]. A recent study identified a central IDR in UTX spanning residues 549–848 that drives UTX phase separation in vitro and

in cell nuclei and is almost completely deleted in the most frequent *UTX* mutant, which is truncated at residue 555[139]. The formation of UTX condensates was required for its tumor-suppressive function in cancer cell lines and in vivo, which was abolished by either deleting the IDR or mutating residues enriched in this region and preserved by mutants containing an unrelated IDR, as well as catalytically dead UTX[139]. LLPS-deficient UTX mutants showed reduced chromatin binding, which also coincided with global changes in histone methylation and dysregulated gene expression. The UTX N-terminal TPR domain was shown to recruit other histone-modifying enzymes, such as multiprotein mixed-lineage leukemia 4 (MLL4) and p300, which also form condensates[143,144], suggesting a potential scaffolding role for UTX in the co-condensation of chromatin-modifiers at key genomic sites[139] (Figure 5d).

Considering the tight link between biomolecular condensates and multiple fundamental processes that both protect against cancer and drive its progression, it is only to be expected that the dysregulation of LLPS directly contributes to cancer pathology. Nevertheless, it is becoming increasingly clear that LLPS behavior is a critical aspect of protein function that, if aberrantly gained or lost, can fundamentally alter cellular homeostasis and drive tumorigenesis. These examples highlight the need to pay careful attention to LLPS when attempting to decipher the molecular mechanisms underlying seemingly cryptic pathological mutations.

CONCLUDING REMARKS

A clear picture has emerged that places LLPS in direct control of multiple processes that define the hallmarks of cancer. Yet too often, the precise functional link between condensates and cancer cell pathophysiology remains elusive, owing to an incomplete understanding of how condensates actually shape biochemical reactions. Bridging this gap requires expanding the toolkit for studying biomolecular condensates (see Box 1). Several recent studies devised approaches combining in vitro reconstitution of biomolecular condensates with enzyme activity assays[117,124,145], while a recent strategy for targeting genetically encoded fluorescent biosensors to endogenously expressed proteins[101,146] proved invaluable for dissecting in situ biochemical regulation by native condensates in living cells[101]. Tools to manipulate LLPS in cells are similarly essential for unraveling the functional impact of condensates. Although recent approaches have enabled light-controlled induction of LLPS by heterologously expressed proteins[147,148], strategies to selectively perturb LLPS by specific, endogenously expressed proteins remain lacking. Continued innovations along these lines are eagerly anticipated to achieve greater insights into condensate function.

Deeper investigation into the molecular function of condensates enabled by these new technologies will ultimately intersect with and inform budding efforts to therapeutically target dysregulated LLPS. One potential strategy for effective targeting will be to develop drugs tailored towards specific condensates, and indeed, several small molecules that disrupt LLPS by specific proteins have already been identified[149–152], which may enable selective targeting of aberrant gain of LLPS without the deleterious effects of broader-acting chemotherapies. Nevertheless, the effectiveness of these compounds, and whether

their design principles are readily generalized, remains to be determined. Rescuing the loss of LLPS may also be possible, as illustrated by the case of PML bodies, which are disrupted in acute promyelocytic leukemia by fusion of the PML protein to retinoic acid receptor-α (RARα). LLPS can be recovered by treating with all-trans retinoic acid and inducing degradation of the PML-RARα fusion, leading to complete recovery[27]. Other condensate-disrupting oncogenic fusions may be similarly druggable, potentially via proteolysis-targeting chimeras[153] or RNA therapeutics[154,155]; however, this has yet to be demonstrated. Successful treatments may also emerge from efforts to identify and target the molecular factors responsible for modulating condensate formation (e.g., through post-translational modification). Further insights may also come from studying how existing cancer drugs interact with condensates[156]. Of course, all of these efforts remain in their infancy. Regardless, we expect the study of LLPS to foster a deeper understanding of pathological mechanisms in cancer and unlock new therapeutic opportunities.

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BOX 1

TOOLS FOR STUDYING BIOMOLECULAR CONDENSATES

Various strategies have been used to investigate biomolecular condensates[157], with new techniques continually emerging and gaining wider use. The chosen technique depends primarily on the question(s) being explored.

Formation and Composition

Observing droplet formation by purified proteins can yield key insights into condensate formation, including detailed phase behavior, as well as composition, although purifying multiple candidate proteins is labor-intensive. Alternatively, condensates can be reconstituted with cell lysates, or directly in live cells, expressing fluorescently tagged proteins. Endogenous tagging via CRISPR/Cas gene-editing[29,158,159] can help avoid overexpression[117], especially combined with split-fluorescent protein tagging[101,124,160]. Proximity-labeling techniques[161] (e.g., APEX, BioID) are also increasingly facilitating unbiased explorations of condensate composition[96,162,163]. Similarly, individual RNA/DNA species can be synthesized for in vitro reconstitution studies, while advanced aptamer-based labeling[164] and APEX-seq[165] promise to enable broader profiling of nucleic acid-containing condensates.

Physical Characteristics

Microscopy is widely used to investigate the physical properties of biomolecular condensates. A classic example involves imaging condensate fusion via standard light microscopy to monitor liquid-like behavior. More sophisticated techniques, such as fluorescence recovery after photobleaching (FRAP)[166] and fluorescence correlation spectroscopy, are typically used to obtain greater detail, with FRAP being particularly useful for measuring diffusion within condensates and molecular exchange with the bulk phase. Atomic force microscopy is also being taken up to probe not only condensate fluidity[167] but also structure[70,168], while other recent studies have adopted various superresolution[169,170] imaging approaches[29,30,124,171], as well as electron microscopy[96,162,172], to extract information on condensate structure.

Function

Disrupting LLPS using mutations or chemical agents (e.g., 1,6-hexanediol[173]) and measuring cellular responses is often used to infer the function of biomolecular condensates, and optogenetic approaches to spatiotemporally manipulate LLPS[147,148] may further elevate these approaches. Meanwhile, strategies based on the enrichment of fluorescently labeled enzyme substrates[117] or binding partners[145] by condensates reconstituted in solution or on model membranes have recently been used to directly monitor biochemical reactions within condensates, as have biochemical pull-downs from cell lysates[124]. Genetically encoded biosensors are also being used to probe condensate function[101,167], and a new suite of fluorescent sensors targeted to endogenously expressed proteins[146] shows promise as a generalizable platform for illuminating condensate biochemical dynamics in situ.

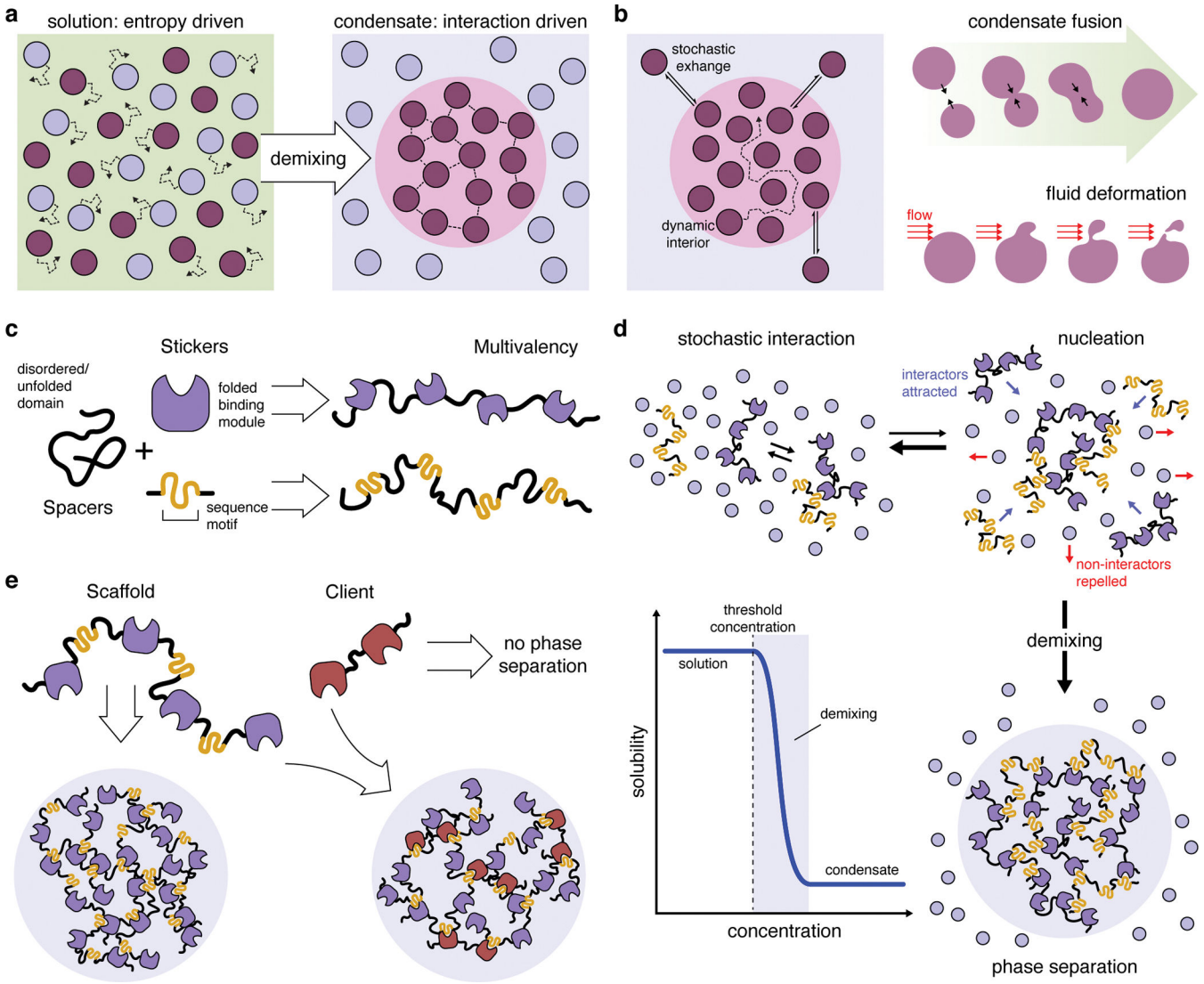

Figure 1. Basic principles of liquid-liquid phase separation and biomolecular condensates.

(a) Whereas entropy typically drives molecules to become dispersed in solution, mutual interactions among a subset of molecules can shift the free-energy landscape to favor de-mixing and drive the formation of a separate condensed phase. (b) Biomolecular condensates display liquid-like properties, such as highly dynamic interiors capable of rapid rearrangement and stochastic exchange with the bulk solution, as well as dynamic fusion, deformation, and fission events. (c) Phase separation of biomolecules is driven my multivalent binding interactions. These molecules often exhibit a sticker-and-spacer configuration, in which multiple folded binding modules and/or short interaction sequence (stickers) are separated by unfolded or disordered regions (spacers), thus achieving multivalency while promoting liquid-like behaviors. (d) At low concentrations, molecules capable of phase-separation interact randomly but remain in solution. Increasing the concentration of these molecules promotes nucleation of small complexes, which begin to exclude the surrounding solution. Each new interactor contributes binding sites that attract more interactors. This positive feedback loop results in switch-like de-mixing and

phase separation (blue curve) once a critical threshold concentration is reached. (e) Phase-separating biomolecules are often categorized as scaffolds, which are both necessary and sufficient for phase separation, and clients, which can selectively partition into scaffold-containing condensates but cannot independently phase separate.

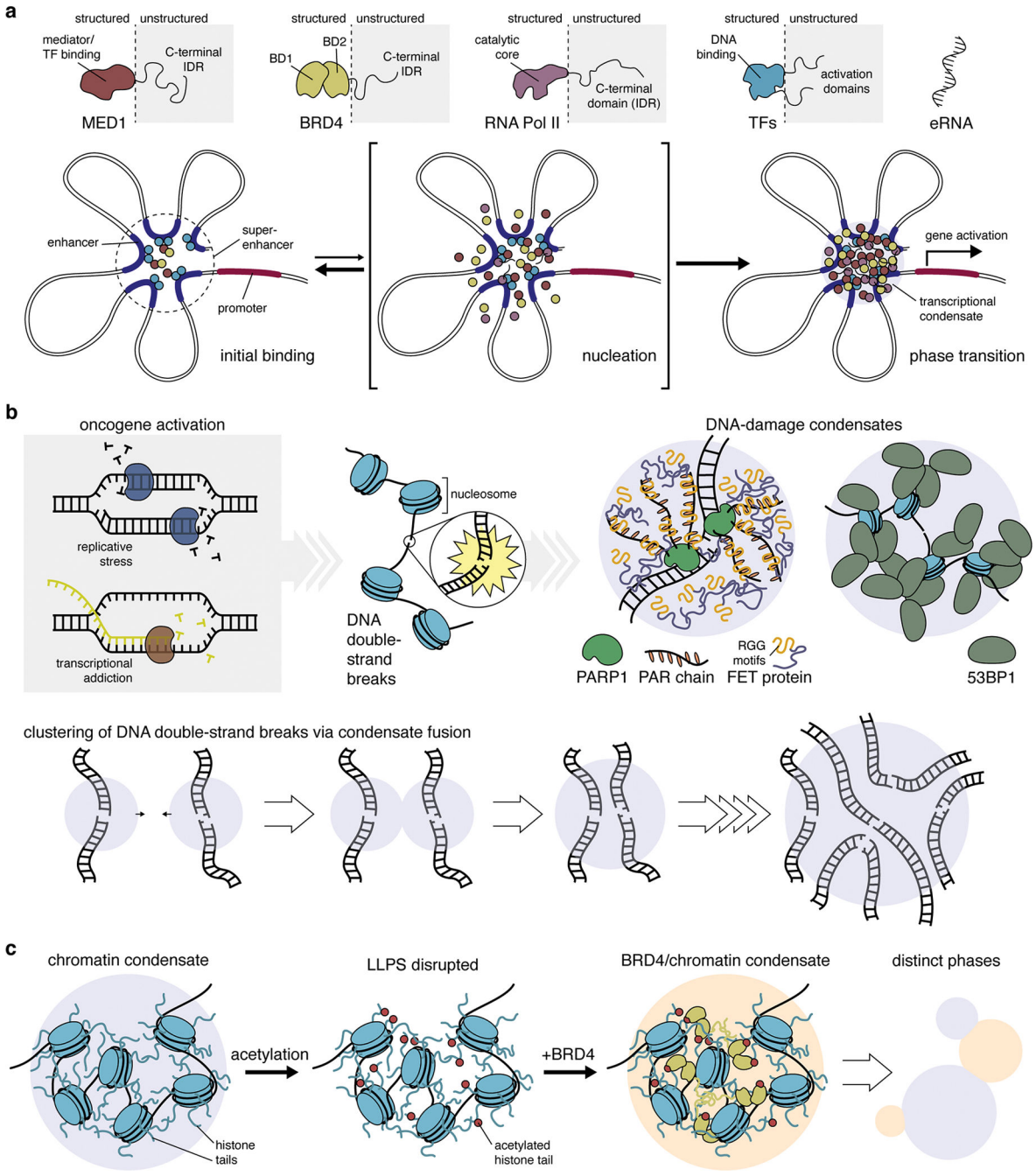

Figure 2. The regulation of nuclear function by liquid-liquid phase separation.

(a) Many transcriptional regulators, such as mediator complex subunit 1 (MED1), bromodomain-containing protein 4 (BRD4), RNA polymerase II (RNA Pol II), and various transcription factors (TFs) feature large intrinsically disordered regions (IDRs) that allow them to undergo phase separation and form biomolecular condensates at super-enhancer sites. Initial binding of TFs and other regulatory proteins at neighboring enhancer sites triggers the nucleation of additional factors via multivalent interactions mediated by their IDRs. This short-lived intermediate rapidly gives rise to a phase transition, yielding a transcriptional condensate that drives gene activation. Thus, LLPS is thought to play a

key role in the robust, switch-like regulation of cell-identity genes by super-enhancers. (b) High levels of replicative stress and excessive transcription induced by oncogene activation cause the accumulation of genome damage, including DNA double-strand breaks (DSBs). The binding of poly-(ADP-ribose) polymerase 1 (PARP1) and synthesis of PAR chains at DSB sites leads to the recruitment of disordered FET-family proteins via their RGG repeats, resulting in the formation of phase-separated condensates. Similarly, P53 binding protein 1 (53BP1) binds nucleosome proteins near DSB sites, resulting in the formation of phase-separated condensates via 53BP1 oligomerization. Notably, 53BP1 condensates have been observed to fuse, suggesting a possible mechanism of the clustering of DSBs in cells (lower panel). (c) Chromatin undergoes LLPS as a result of multivalent interactions driven by intrinsically disordered histone tails. LLPS of chromatin can be modulated by post-translational modification of histone tails, such as acetylation, which disrupts condensate formation. The addition of BRD4, which binds acetylated histone tails, restores LLPS of acetylated chromatin. The resulting condensates cannot mix with unmodified chromatin condensates, yielding two distinct phases, similar to distinct chromatin domains in the nucleus.

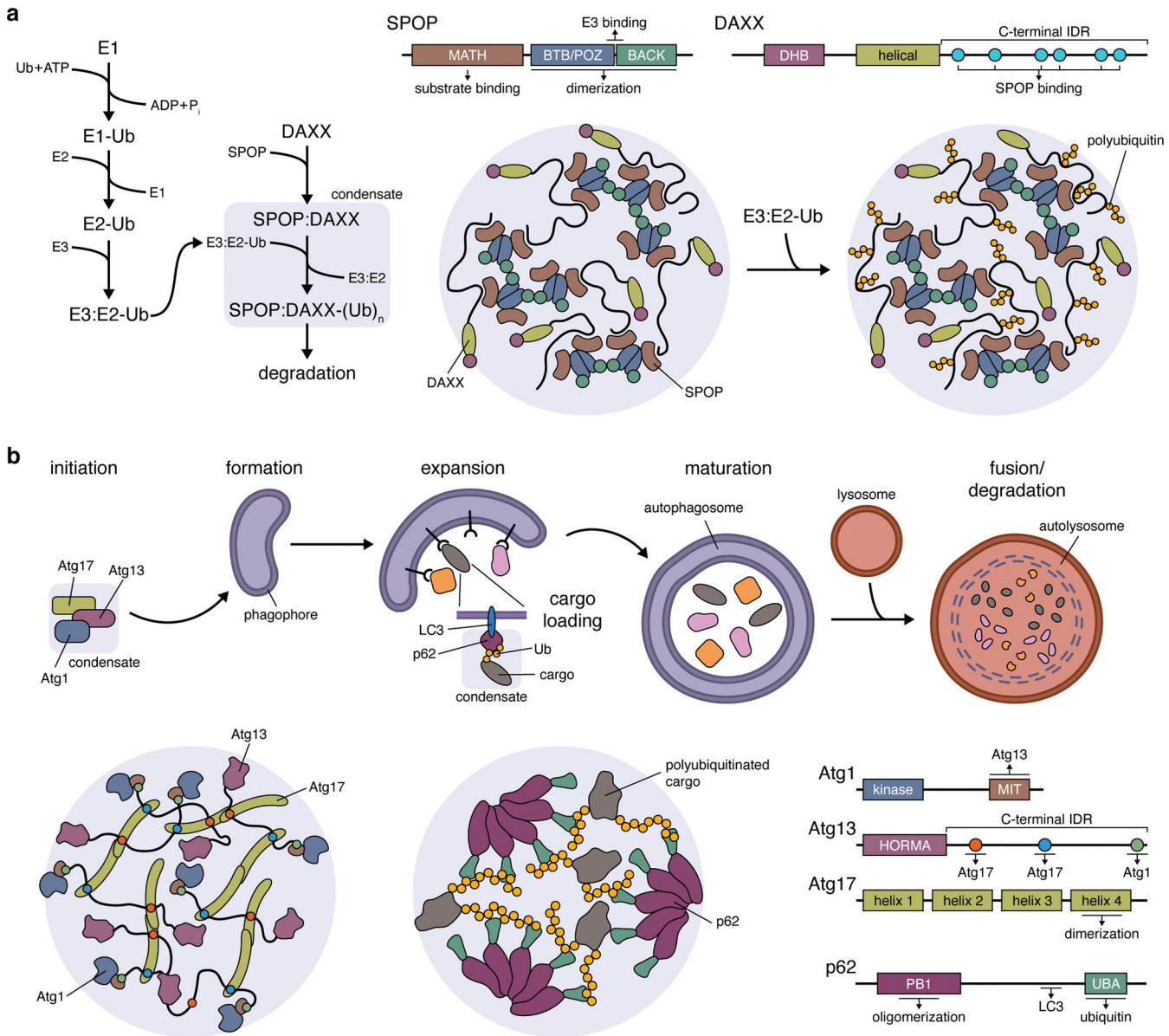

Figure 3. The role of liquid-liquid phase separation in cellular quality control.

(a) The formation of biomolecular condensates between speckle-type POZ protein (SPOP) and its target death-domain-associated protein (DAXX). Left: Pathway schematic highlighting the role of SPOP:DAXX condensates in facilitating DAXX polyubiquitination. Right: LLPS of SPOP and DAXX is driven by the formation of SPOP dimers through its BR-C/ttk/bab (BTB)/Pox virus and zinc finger (POZ) domain, interactions between SPOP dimers mediated by their BTB and C-terminal Kelch (BACK) domains, as well as interactions between the long, C-terminal IDR of DAXX with multiple copies of the N-terminal meprin and TRAF homology (MATH) domain of SPOP. The BTB/POZ-BACK domain junction in SPOP also binds E3 ubiquitin ligases, recruiting them into condensates (omitted from cartoon) to promote DAXX polyubiquitination. Ub, ubiquitin; $(Ub)_n$, polyubiquitin; E1, Ub-activating enzyme; E2, Ub-conjugating enzyme; E3, Ub-ligase.

(b) Top: Simplified schematic of the autophagy pathway, highlighting the involvement of Atg1, Atg13, and Atg17 condensates in autophagy initiation and the role of p62 condensates in cargo loading during phagophore expansion. Bottom: Atg1:Atg13:Atg17 condensates (left panel) are scaffolded by Atg13 and Atg17, with the C-terminal IDR of Atg13 containing a pair of binding sites that crosslink Atg17 dimers. Atg1 is recruited as a client protein via its C-terminal microtubule-interacting and transport (MIT) domain, which binds Atg13. Bottom: LLPS of p62 condensates (middle panel) requires the formation of p62 oligomers, mediated by an N-terminal Phox and Bem1p (PB1) domain, whose C-terminal ubiquitin-associated (UBA) domains then participate in multivalent interactions with the ubiquitin chains on polyubiquitinated substrate proteins.

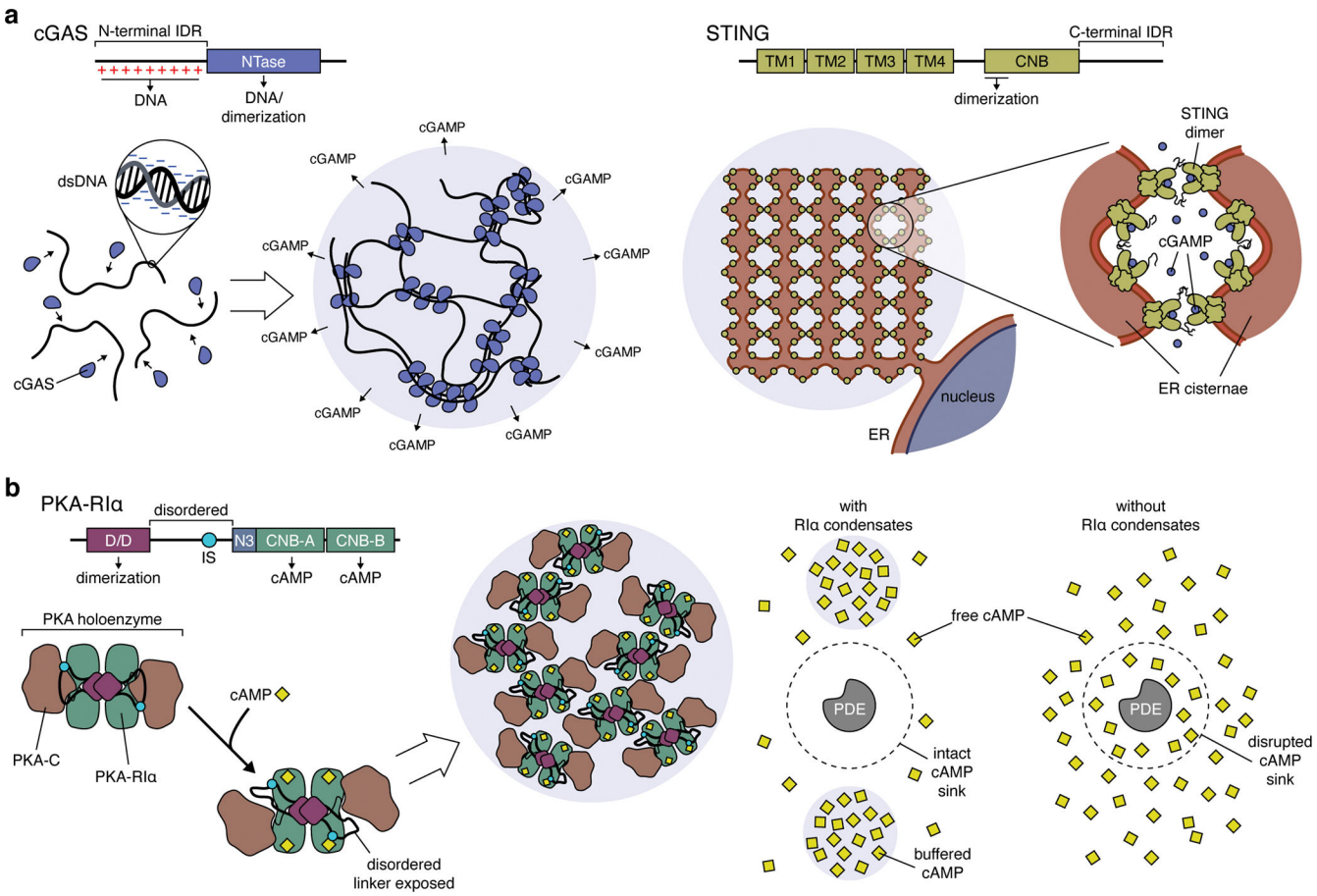

Figure 4. Liquid-liquid phase separation shapes signaling pathway function.

(a) Left: cGAS binds double-stranded DNA (dsDNA) via its positively charged N-terminal IDR and its C-terminal nucleotidyltransferase (NTase) domain, which also mediates cGAS dimerization. These multivalent interactions rapidly nucleate the formation of cGAS:dsDNA condensates, dramatically lowering the concentration threshold for cGAS activation and leading to ultrasensitive dsDNA detection and cGAMP production. Right: However, excess cGAMP production has been shown to trigger LLPS of the ER-resident cGAMP receptor STING, which requires interactions mediated by the C-terminal IDRs in STING dimers and forms condensates containing intricately folded membrane networks. Because LLPS requires cGAMP-bound STING, these condensates may function to buffer excess cGAMP and regulate immune signaling. (b) Left: The formation of PKA-RIα biomolecular condensates requires the docking and dimerization (D/D) domain and a disordered linker region containing an inhibitory site (IS) that binds the PKA-C subunit. The binding of cAMP to RIα triggers a conformational rearrangement in the PKA holoenzyme that exposes the disordered linker to help drive LLPS, though the precise molecular mechanism remains unclear. Right: RIα condensates dynamically buffer cAMP levels, allowing PDEs to efficiently degrade free cAMP and act as cAMP sinks. In the absence of RIα condensates, PDEs catalytic activity is overwhelmed, resulting in the collapse of cAMP sinks and the breakdown of compartmentalized cAMP signaling.

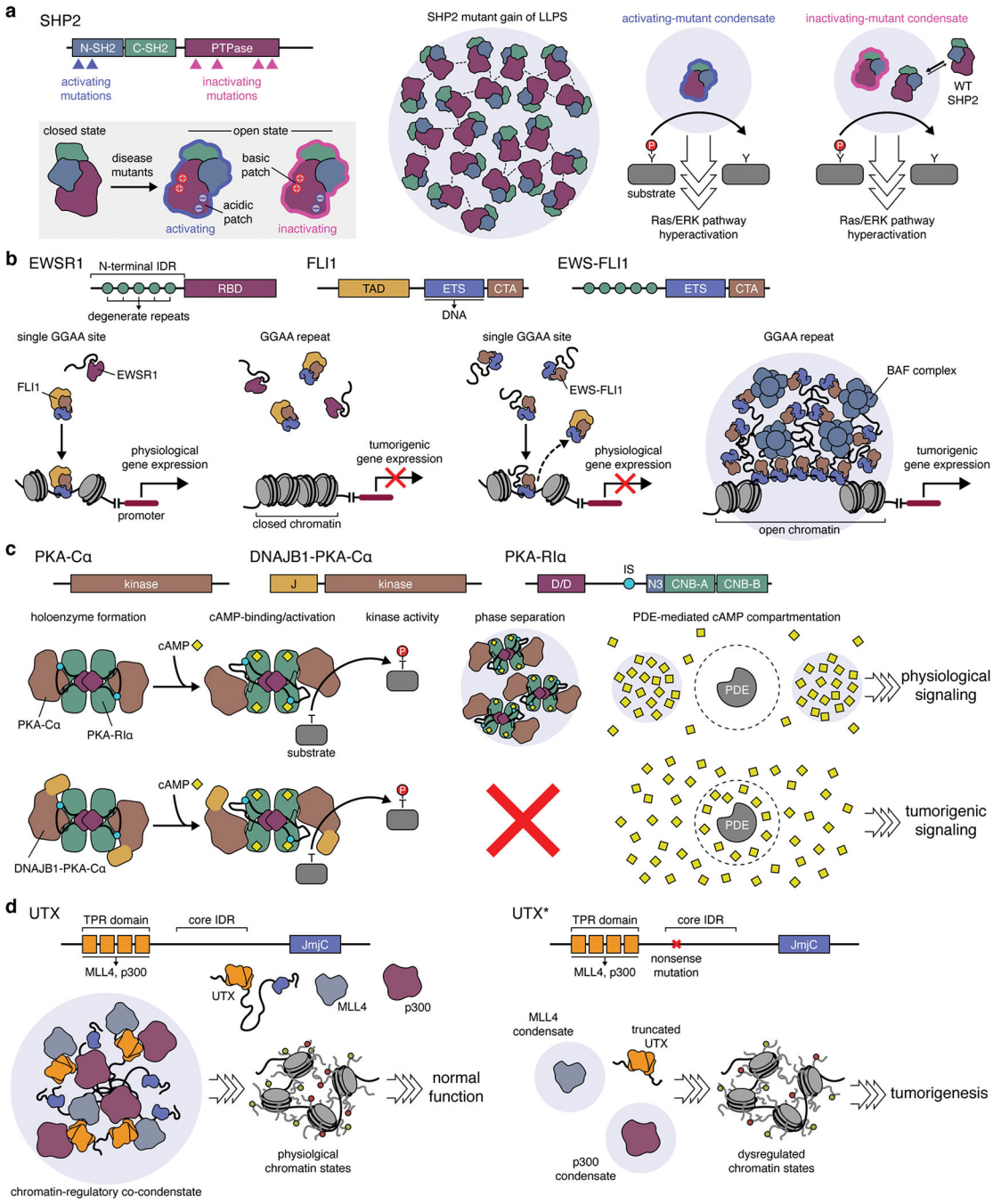

Figure 5. Dysregulation of liquid-liquid phase separation as a tumorigenic driver.

(a) Gain of LLPS ability by disease-causing SHP2 mutants. Both activating and inactivating mutations in SHP2 are able to trigger oncogenic signaling by altering SHP2 conformation to favor the open state. This conformation exposes a patch of positively charged, basic residues on the surface of the SHP2 protein tyrosine phosphatase (PTPase) domain, which can then form electrostatic interactions with negatively charged acidic residues elsewhere on the protein surface, thus driving LLPS by these mutants. Condensates formed by activating SHP2 mutants induce Ras/ERK pathway hyperactivation through their intrinsically high

PTPase activity. By contrast, while condensates formed by inactivating SHP2 mutants gain PTPase activity by recruiting and activating wild-type (WT) SHP2 to trigger hyperactive signaling. (b) Gain of LLPS by EWS-FLI1 oncogenic fusion. In Ewing sarcoma, the N-terminal IDR of the EWSR1 becomes fused to the C-terminal DNA-binding domain of FLI1. Left: Native FLI1 binds conserved enhancer sites containing a single GGAA consensus sequence to control physiological gene expression but is unable to bind GGAA microsatellite repeats. Right: The EWS-FLI1 fusion disrupts the binding of native FLI1 to enhancers and also gains the ability to undergo LLPS via its IDR. EWS-FLI1 condensates tightly bind GGAA microsatellites while also strongly recruiting the BAF chromatin remodeling complex, producing open chromatin sites that act as super-enhancers for pro-tumor gene expression. (c) Pathological loss of LLPS by an oncogenic fusion. In the DNAJB1-PKA-Cα chimera, an N-terminal portion of DNAJB1 encompassing the J-domain (J) replaces the native N-terminus of WT PKA-Cα. WT PKA-Cα and DNAJB1-PKA-Cα behave similarly in forming holoenzymes with PKA-R subunits (e.g., RIα), in undergoing cAMP-induced activation, and in phosphorylating substrates. However, DNAJB1-PKA-Cα completely abolishes RIα LLPS, which disrupts the compartmentation of cAMP signaling by PDEs, leading to tumorigenic signaling. (d) Loss of LLPS by UTX mutants disrupts chromatin regulation. Left: In healthy cells, histone demethylase UTX undergoes LLPS mediated by a core IDR between the tetratricopeptide repeat (TPR) and Jumonji C (JmjC) domains. The TPR domain of UTX recruits the MLL4 histone methyl transferase and the p300 histone acetyl transferase to form chromatin-regulating co-condensates that maintain physiological chromatin states. Right: UTX is frequently mutated in cancers, with the most frequent alteration being a nonsense mutation near the start of the core IDR. These mutant UTX variants (UTX*) cannot phase separate with MLL4 and p300, leading to dysregulated chromatin states and tumorigenesis.