REVIEW
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Liquid-liquid phase separation in the nucleus: pathogenesis and therapy

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Author contributions

All authors contributed substantially to the scientific process and writing of the manuscript. Zi-Ang Ma, Zhong-Yong Zhang, Xiu-Min Gao and Duo Wang designed the structure and wrote the first draft of the manuscript. Ya-Mei Gao, Wei Wang and Tong-Jin Liu drew complied Figures, and they have compiled references. Fei-Tian Min, Huan-Tian Cui, and Shu-Quan Lv supervised and revised the final version of the manuscript. All authors approved the final version of the manuscript submitted.

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Abbreviations

LLPS, liquid-liquid phase separation; IDRs, intrinsically disordered regions; CTD, carboxy-terminal domain; Pol II, polymerase II; NELF, negative elongation factor; P-TEFb, positive transcription elongation factor b; CDK9, cyclin-dependent kinase 9; FOXK1, forkhead box protein K1; TECs, tubular epithelial cells; BRD4, bromodomain-containing protein 4; NTD, N-terminal domain; HDAC6, histone deacetylase 6; CKD, chronic kidney disease; AS, atherosclerosis; CVDs, cardiovascular diseases; HER2, human epidermal growth factor receptor 2; p-HDAC6, phosphorylated histone deacetylase 6; AAV, adeno-associated virus; DIC, differential interference contrast.

Citation

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Abstract

Intracellular liquid-liquid phase separation (LLPS) represents a pivotal biological process for the formation of cellular compartments, which involves the formation of droplet-like condensates through the hydrophobic and hydrophilic interactions of biomolecules. LLPS plays a crucial role in normal cellular physiological activities, and it is also intimately linked to the pathological mechanisms of various diseases. This review summarizes the significant role of LLPS in regulating transcriptional mechanisms within the nucleus, with a focus on the mechanisms by which LLPS modulates gene transcription. Additionally, we highlight the roles of key proteins involved in LLPS, such as FOXK1, BRD4, Tau protein, and HDAC6, in the context of disease. We delve into the relationships between LLPS and diseases such as chronic kidney disease, atherosclerosis, Alzheimer's disease, and triple-negative breast cancer, revealing the regulatory mechanisms of LLPS in disease onset and progression. Furthermore, we discuss the potential of pharmacological interventions targeting LLPS as novel therapeutic approaches, encompassing strategies such as gene therapy, traditional Chinese medicine monomers, and small-molecule inhibitors. In the future, a deeper understanding of the LLPS mechanism will continue to propel its application in disease prevention and treatment, providing robust support for the development of novel therapeutic strategies.

Keywords: liquid-liquid phase separation; nuclear condensates; transcription regulation

Background

Numerous biomolecules exist within cells and require the formation of distinct compartments to perform their biological functions. These compartments play a crucial role in maintaining the organization and efficiency of complex biochemical reactions [1]. How do these compartments form and function within cells? One biological process, liquid-liquid phase separation (LLPS), plays a significant role in this context. A phase refers to a distinct and homogeneous form of matter (i.e., a specific solid, liquid, or gas). Phase separation involves the emergence of two distinct phases from a single homogeneous mixture, with the most common type being the separation between two immiscible liquids, such as oil and water [1]. In cells, LLPS is a reversible, spontaneous biological process whereby biomolecules with both hydrophobic and hydrophilic regions can separate a homogeneous solution into two or more coexisting phases through mutual attraction, forming a dilute hydrophilic phase and a dense hydrophobic phase [2, 3]. Specifically, this process involves the concentration of biological macromolecules such as proteins and nucleic acids into droplet-like condensates, encapsulating some molecules within the droplets while excluding others [4, 5]. This process is increasingly recognized by the scientific community as a key mechanism underlying the normal function of certain proteins and the formation of membraneless organelles [6].

Notably, LLPS not only plays a pivotal role in normal cellular physiology but also has significant implications in pathological conditions. Under physiological conditions, LLPS can respond to various external stimuli in living cells and maintain normal cellular functions by regulating transcription [7]. However, excessive or abnormal external stimuli can lead to abnormal regulation of transcription within LLPS condensates, triggering a range of diseases [8]. Importantly, LLPS abnormalities are closely linked to the pathogenesis of multiple human diseases, making it a research hotspot, including neurodegenerative diseases, metabolic diseases, cancer, and aging-related diseases [9]. The condensates formed through LLPS can gradually mature over time, transitioning from liquid to solid, a process known as droplet aging. These dynamic droplets can become persistent disease foci within cells, exacerbating disease progression [10]. In summary, the unique functions of LLPS within the nucleus make it a core mechanism for transcriptional regulation, profoundly influencing cellular fate and disease development.

Despite the growing research on LLPS, we are beginning to understand its crucial role in physiology and disease. This underscores the need for an updated overview of recent advances in this field. This article briefly reviews the development of the LLPS concept. We focus on the mechanisms by which LLPS regulates transcription within the nucleus and the potential for drug interventions targeting LLPS to influence gene expression, aiming to provide new directions and perspectives for disease prevention and treatment.

Biological development of LLPS

As early as 1899, Wilson observed that different liquids within cells could form suspended emulsion droplets and first proposed the existence of droplets within cells, likening the cytoplasm to a mixture of different chemical suspension droplets. By 2009, Brangwynne [11] made a significant discovery: in the single-cell embryos of *Caenorhabditis elegans*, the membraneless organelle P granules exhibited rapid dissolution and condensation, similar to liquids. This finding suggested that the localization mechanism of P granules, and potentially other ribonucleoprotein complexes like P bodies, Cajal bodies, and stress granules, is a ubiquitous phenomenon centered on the mutual aggregation of weakly “sticky” molecules.Subsequent studies have supported Brangwynne’s observations, revealing that various membraneless organelles in cells are formed through LLPS of biological macromolecules. Examples include nucleoli, Cajal bodies, and PML nuclear bodies within the nucleus [11, 12].

Role of multivalency and intrinsically disordered regions (IDRs)

In 2012, Pilong Li [13] discovered that multivalent macromolecules (including multi-domain proteins and RNA, where multivalency refers to the ability to establish multiple interactions with other molecules [14]) rapidly transition from small complexes to large polymer assemblies as their concentration increases during interaction, indicating that multivalency is a key promoter of LLPS [15]. In 2015, research found that proteins containing IDRs can drive the formation of LLPS in vitro under conditions of local high concentration [13]. These IDRs spontaneously form LLPS droplets through weak electrostatic and hydrophobic homotypic and heterotypic protein-protein interactions, exhibiting various properties similar to stress granules in vivo [16–18]. IDRs typically cannot form stable tertiary structures and often contain biased amino acid distributions, with a smaller proportion of hydrophobic residues and a larger proportion of hydrophilic and charged residues. These unique characteristics are significant for IDR-mediated LLPS [7, 19]. Specifically, IDRs form highly dynamic condensates through multivalent interactions, such as π-π interactions, charge-charge interactions, and cation-π interactions among proteins. The strength and type of these interactions can be modulated by altering environmental conditions (e.g., salt concentration, temperature, pH value) or through post-translational modifications of proteins (e.g., phosphorylation, ubiquitination) [20]. This modulation subsequently influences the formation, stability, and function of the condensates. Multivalent interactions, therefore, constitute the foundation for the formation and regulation of biomolecular condensates [20].

RNA molecular interactions and chromatin organization

In 2016, it was reported that precise control over the intracellular localization of ribonucleoprotein particles was achieved by regulating the competitive mechanism between RNA molecules, influencing the formation of membraneless organelles driven by multivalent RNA-RNA, RNA-protein, and protein-protein interactions [21]. Moreover, RNA is not merely a constituent of biomolecular condensates; it also plays a crucial role in regulating their properties and functions through its unique sequence and structural features. For instance, long non-coding RNA can interact with proteins within condensates to either enhance their fluidity or inhibit the recruitment of other molecules. Furthermore, RNA can act as a scaffolding molecule, facilitating the phase separation of specific proteins and the subsequent formation of condensates [22]. Remarkably, RNA can also serve as a scaffolding molecule, facilitating the phase separation of specific proteins and subsequently promoting the formation of condensates. This demonstrates the possibility of precisely controlling the structure and function of intracellular biomolecules by modulating RNA molecular interactions [23]. In recent years, Gibson [24] found that the LLPS mechanism promotes the formation of functionally distinct yet physically interconnected chromatin regions within the nucleus, highlighting the intrinsic property of chromatin polymers to undergo LLPS. This further implies that the LLPS mechanism could offer an optimal transcriptional milieu for open chromatin regions.

Pathological implications of LLPS

As research on the LLPS biological process deepens, Polymenidou suggest that protein concentration is an important driver of LLPS and aggregation. Under disease conditions, protein expression and degradation processes are disrupted, leading to abnormal increases in protein concentration [25]. Proteins originally involved in normal cellular physiological functions, such as α-synuclein, FUS, tau, and TDP-43, gradually form dynamic droplets through LLPS as their concentrations rise. However, these dynamic droplets may lose their dynamic properties and transform into relatively stable, non-dissipating fibrous protein aggregates after undergoing a maturation process over time. Based on this, researchers have proposed a hypothesis that under pathological conditions, the dynamic droplets formed through LLPS by elevated biomolecular concentrations may lose their dynamic properties and transform into

relatively stable, immobile protein aggregates after undergoing a maturation process, leading to chronic and intractable diseases [26].

In summary, although LLPS plays an important physiological role in the assembly and regulation of intracellular biomolecules, abnormal LLPS processes, particularly within the nucleus, have been found to be closely related to the pathogenesis of various diseases. In some diseases, proteins originally involved in normal cellular physiological functions form condensates through LLPS, leading to chronic and intractable diseases. In recent years, groundbreaking research findings in the field of LLPS have emerged, and its potential application value in drug development has received unprecedented attention. It is believed that a deeper understanding of the LLPS mechanism may open new pathways for drug development and potentially rewrite the traditional rules of drug discovery [26].

Mechanisms of LLPS in regulating gene transcription

Transcription is an indispensable part of gene expression. Although transcription is tightly regulated by various factors, abnormal regulation can lead to disease [9, 27]. Under normal conditions, multiple transcription factors can form phase-separated condensates with the coactivator complex mediator [28]. The mediator stabilizes the pre-initiation complex and promotes transcription by recruiting the intrinsically disordered carboxy-terminal domain (CTD) of RNA polymerase II (Pol II), thereby initiating transcription of target genes [28]. Transcription primarily involves three steps: initiation, elongation, and termination [9]. After transcription initiation, Pol II does not immediately enter the elongation phase but pauses at a region approximately 50 bp downstream of the transcription start site, a phenomenon known as promoter-proximal pausing. LLPS-mediated Pol II within condensates accelerates the transcription of mRNA and non-coding RNA, while negative elongation factor (NELF) and positive transcription elongation factor b (P-TEFb) regulate the transition from transcription initiation to elongation within LLPS [29, 30]. These factors carry IDRs and can spontaneously form nuclear condensate structures. Pol II co-aggregates tightly with transcription factors and coactivators carrying IDRs through LLPS, forming active centers for transcriptional regulation [28]. NELF, as a transcription elongation inhibitor, exerts its function by stabilizing Pol II in a stalled state, while P-TEFb, a complex composed of the catalytic subunit cyclin-dependent kinase 9 (CDK9) and the regulatory subunit cyclin T1 (CCNT1), can interact multivalently with the disordered CTD of Pol II through the histidine-rich domain in CCNT1 to form condensates [30, 31]. Through CDK9 phosphorylation activity, P-TEFb can phosphorylate NELF and the CTD of Pol II, promoting the separation of P-TEFb from stalled Pol II and its entry into condensates. Phosphorylated Pol II CTD interacts with transcription elongation and splicing factors to form elongation condensates that promote transcription. This process facilitates the dissociation of NELF from Pol II and the phosphorylation of Pol II itself, thereby driving transcription into the elongation phase. Furthermore, various transcription factors can form phase-separated condensates with the coactivator mediator complex, further regulating transcription [27, 28] (Figure 1). These factors synergistically ensure precise control of gene expression through the LLPS mechanism.

Several proteins involved in LLPS currently identified

Over the past few decades, genetics, biochemistry, molecular biology, and genomics research have identified many factors that play crucial roles in regulating transcription, such as transcription factors, transcription coactivators, and enhancers [32–34]. Among them, transcription factors and coactivators have been the focus of research in recent years, further regulating biological functions through mechanisms such as epigenetic modifications and metabolic reprogramming via LLPS [35].

Transcription factor forkhead box protein k1

Forkhead box protein K1 (FOXK1), a transcription factor belonging to the FOX family, has multiple functions [36, 37]. Members of the FOX family play important roles in organ fibrosis. As a member of the FOX family, FOXK1 may promote organ fibrosis by positively regulating aerobic glycolysis. Knockdown of FOXK1 reduces glycolytic intermediates without significantly affecting mitochondrial oxidative metabolism [38]. It has been reported that FOXK1 mediates aerobic glycolysis in adipocytes by regulating glycolysis-related enzymes hexokinase-II and pyruvate kinase M2 [39]. Zhang found that under the influence of TGF-β, FOXK1 enters the nuclei of renal tubular epithelial cells (TECs) and regulates transcription within the nucleus through LLPS, initiating metabolic reprogramming that exacerbates renal fibrosis [40].

Bromodomain-containing protein 4

Bromodomain-containing protein 4 (BRD4), a member of the BET family of epigenetic regulatory proteins, is widely expressed in cells and participates in regulating biological functions such as gene expression, cell proliferation, cell differentiation, and cell apoptosis, involving the development of various diseases [41]. As the most important member of the BET family, BRD4 has attracted widespread attention from researchers [41, 42]. BRD4 forms condensates within the nucleus through LLPS, thereby regulating transcription [43]. Specifically, BRD4 and mediator complex 1, as important components of super-enhancers, recognize and bind acetylated histones through their bromodomains to form the BRD4-PTEFb complex. The BRD4-PTEFb complex then phosphorylates serine 2 and serine 5 residues of the Pol II CTD through its kinase activity, releasing stalled Pol II and driving transcription into the elongation phase [44, 45].

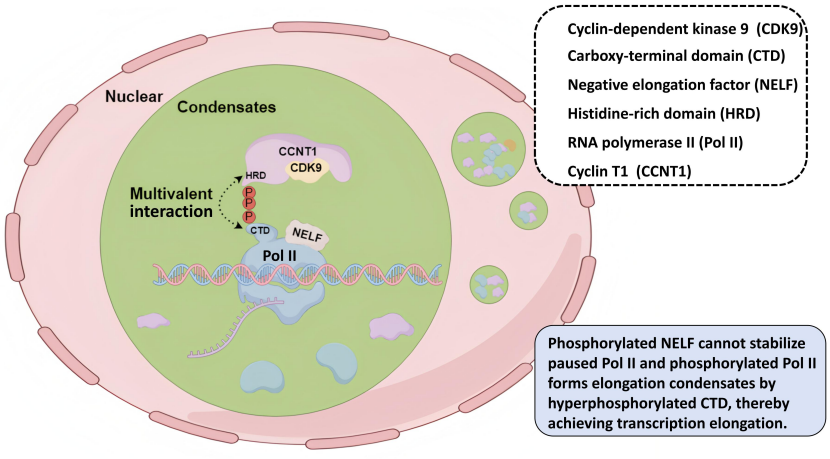


Figure 1 The process of LLPS within the nucleus in regulating gene transcription. LLPS, liquid-liquid phase separation.

Tau protein

Tau protein, a microtubule-associated protein with four structural domains—the N-terminal domain (NTD), proline-rich domain, microtubule-binding domain, and CTD—primarily functions in microtubule assembly and stabilization, thereby maintaining cytoskeletal structure and promoting cellular biological activities. Different regions of Tau protein play diverse regulatory roles in its functions [46, 47]. CTD directly promotes LLPS of Tau protein through electrostatic interactions with negatively charged molecules, while NTD may indirectly influence LLPS formation through interactions with CTD [48]. Wang [49] observed in Alzheimer's disease (AD) that the main component of neurofibrillary tangles is aggregated Tau protein. A study found that the presence of NTD within Tau can induce LLPS, transforming Tau protein from a liquid state to an insoluble solid and leading to pathological aggregation, affecting its assembly with microtubules and causing tauopathy [50]. Tau protein can form condensates within the nucleus through LLPS, regulating the transcription of specific gene [51].

Histone deacetylase 6

Histone deacetylase 6 (HDAC6), an enzyme with multiple cellular functions belonging to the class II histone deacetylase family, plays crucial roles in both the cytoplasm and the nucleus in regulating chromatin structure and gene expression [52]. HDAC6 has two catalytic domains and multiple functional domains, including zinc finger domains and ubiquitin-binding domains, enabling it to interact with various substrates simultaneously and perform multiple cellular functions. The localization of HDAC6 within cells is dynamic, as it shuttles between the nucleus and cytoplasm in response to different cellular signals and stress conditions. HDAC6 is phosphorylated in the cytoplasm, which is a critical step for its entry into the nucleus [52]. Phosphorylated HDAC6 can form LLPS condensates within the nucleus, which further regulate chromatin structure and gene transcription [53].

The relationship between LLPS and disease

In recent years, LLPS, as an emerging molecular biological phenomenon, has gradually revealed its crucial role in regulating gene expression and disease development within the nucleus. LLPS not only participates in normal cellular physiological functions but also, when abnormal, can lead to a range of diseases. The following sections discuss in detail the regulatory mechanisms and pathological roles of LLPS in chronic kidney disease (CKD), atherosclerosis (AS), AD, and triple-negative breast cancer (TNBC) (Figure 2).

CKD

CKD is characterized by a prolonged course and poor prognosis. Regardless of its etiology, the progression of CKD ultimately leads to end-stage renal disease and the closely associated pathological changes of renal fibrosis [54]. Renal fibrosis is marked by myofibroblast proliferation, extracellular matrix deposition, and loss of glomerular, tubular, and interstitial capillary integrity [54]. During the progression of CKD, TECs play a pivotal role in the regulatory network promoting renal fibrosis [54]. TECs undergo an energy-related metabolic shift from fatty acid oxidation to glycolysis [40]. Wang [40, 54] revealed that FOXK1, an emerging and crucial regulator of glycolysis, is expressed in TECs during renal fibrosis and exerts significant pro-fibrotic and metabolic reprogramming effects. Upon stimulation with TGF-β1, FOXK1 undergoes nuclear translocation and forms condensates through the LLPS mechanism. Simultaneously, Pol II and target gene elements are sequestered within these droplets, forming membraneless compartments that enable FOXK1 to efficiently exert its transcriptional activity. This promotes the expression of a series of glycolysis-related genes, including Slc2a2, Hk1, Fbp1, Aldoa, Bpgm, and Ldhc, shifting the energy supply in TECs from fatty acid oxidation to glycolysis, thereby exacerbating renal fibrosis in the progression of CKD [40].

AS

As a potentially life-threatening pathological process underlying cardiovascular diseases (CVDs), the incidence of AS continues to rise globally, posing a costly public health burden Dyslipidemia [55], particularly macrophage-associated lipid metabolic disorders, is a key contributor to the pathogenesis of AS [44]. Imbalances in lipid catabolism and the retention of lipid-laden macrophages in susceptible regions of arterial walls trigger inflammatory responses, collectively contributing to the primary pathogenesis of AS [56]. In the early stages of the disease, modified lipoproteins are sequestered in the subendothelial space and are typically phagocytosed by macrophages, which balance this through reverse cholesterol transport mechanisms. However, excess lipids force macrophages to transform into foam cells, thereby promoting the formation of atherosclerotic lesions [57].

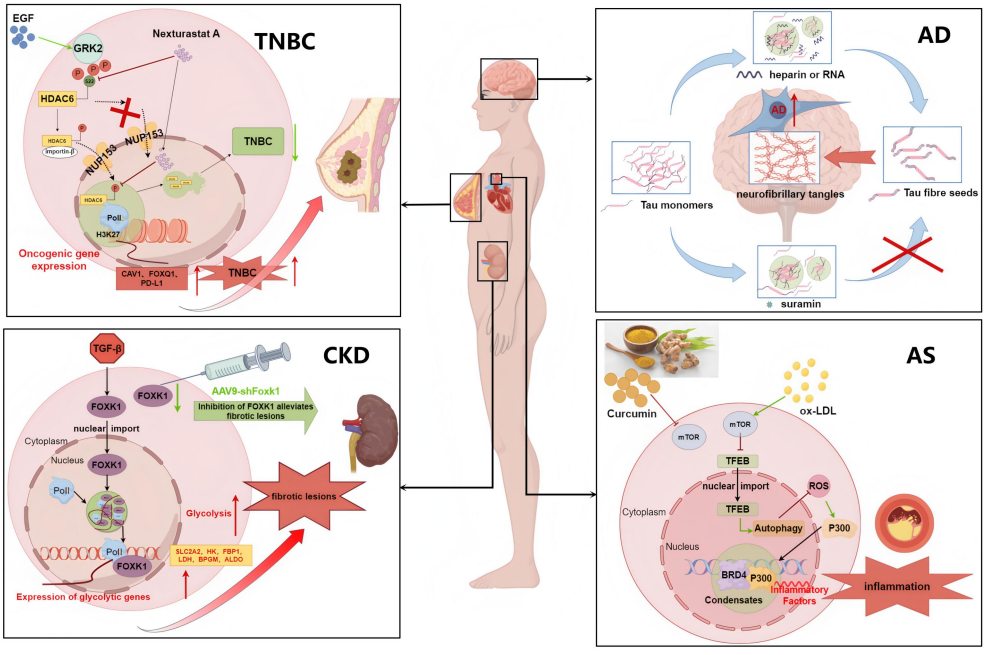


Figure 2 The regulation of transcription by LLPS within the nucleus contributes to disease progression and related drug interventions. AS, atherosclerosis; TNBC, triple-negative breast cancer; AD, Alzheimer's disease; CKD, chronic kidney disease.

Foam cells not only secrete pro-inflammatory cytokines, leading to an inflammatory state within plaques and impairing phagocytic clearance, but also exacerbate the formation of vulnerable late-stage lesions characterized by necrotic lipid cores [57]. These cores, if ruptured, can trigger severe clinical complications. Notably, LLPS-formed condensates in macrophages provide a highly dynamic interaction platform for transcription factors and coactivators, thereby finely regulating the expression of inflammatory genes. Li demonstrated that LLPS in macrophages participates in the pathogenesis of AS [44]. Oxidized low-density lipoprotein inhibits the cytoplasmic-nuclear translocation of TFEB, subsequently suppressing the activation of lysosomal genes and leading to autophagy deficiency [43, 56, 58]. Consequently, autophagy deficiency elevates reactive oxygen species levels and histone acetyltransferase P300 activity. P300, through LLPS, synergizes with the downstream histone acetylation reader BRD4 to achieve transcriptional activation [59]. As a transcriptional activator, P300 recruits transcription factors to the promoter regions of inflammatory genes, thereby promoting the transcription of inflammatory factors [60–62]. Clearly, LLPS plays a pivotal role in oxidized low-density lipoprotein-induced autophagy deficiency and inflammation, thereby contributing to the progression of AS.

AD

AD is a neurodegenerative disease that severely impairs patients' quality of life. The core and primary pathological feature of AD is the abnormal aggregation of proteins such as tau, forming neurofibrillary tangles, which are crucial factors in disease progression [63]. Tau, due to its lack of a fixed folding structure and specific charge pattern, can form weak multivalent interactions and is prone to LLPS [64]. Within the nucleus, the C-terminal region of tau is rich in positive charges, enabling it to strongly interact electrostatically with negatively charged polyanions. Under pathological conditions, when tau concentration increases or reaches a specific threshold with polyanions, tau molecules begin to aggregate through these electrostatic interactions, forming condensates within the nucleus through LLPS [65, 66]. However, over time and due to changes in nuclear environmental conditions (such as fluctuations in pH and ionic strength), tau condensates may gradually lose their dynamic equilibrium, transitioning into more stable and dense pathological aggregates [48]. During this process, specific sites in tau, particularly those in its repeat domain, may play crucial roles. These sites not only participate in tau-tau interactions but may also stabilize the structure of pathological aggregates by forming secondary structures such as β-sheets [67]. These pathological tau condensates not only persistently interfere with gene expression but may also exacerbate the pathological progression of neurodegenerative diseases like AD through their neurotoxicity [68]. Therefore, the formation of tau condensates through electrostatic interactions between the C-terminal region of tau and polyanions, and their subsequent transition into pathological aggregates, represent a crucial link between abnormal tau aggregation and the pathogenesis of AD.

TNBC

Breast cancer is a malignant tumor originating from breast tissue, typically occurring in the ducts or lobules of the breast and can be in situ (non-invasive) or invasive [69]. It is mainly classified into luminal A, luminal B, human epidermal growth factor receptor 2 (HER2)-overexpressing, and TNBC subtypes. TNBC, characterized by its high heterogeneity, aggressiveness, poor prognosis, and lack of effective treatment strategies, poses significant challenges for patients [70]. Due to the absence of estrogen receptor, progesterone receptor, and HER2, traditional endocrine therapy or chemotherapy is ineffective against this tumor type [71]. Zhao [53] discovered that pathological LLPS plays a crucial role in the pathogenesis of TNBC. Phosphorylated histone deacetylase 6 (p-HDAC6) forms LLPS condensates within the nuclei of TNBC cells, which are vital for establishing abnormal chromatin structures. Specifically, epidermal growth factor induces G protein-coupled receptor kinase 2-mediated phosphorylation of HDAC6 at the S22 site of its N-terminal IDR. This phosphorylation enables the interaction between p-HDAC6 and the nuclear transport protein importin-β, promoting the nuclear import of HDAC6 through the nuclear pore complex. Nuclear pore protein NUP153 plays a crucial role in this process. Once in the nucleus, p-HDAC6 forms condensates through LLPS, further interacting with chromatin to remodel its structure, influencing chromatin accessibility, histone acetylation status, and Pol II elongation activity. These changes subsequently regulate the transcriptional profile, promoting the expression of genes associated with tumorigenesis and development, such as CAV1, FOXQ1, and PD-L1, which are aberrantly expressed in various cancers and are closely related to cell proliferation, migration, invasion, tumorigenesis, development, metastasis, and immune escape.

Drug modulation and intervention of phase separation

With the deepening of research on LLPS, drug modulation and intervention of phase separation have emerged as novel therapeutic approaches for related diseases. This discovery provides new avenues for the development of specific drugs targeting LLPS, potentially offering new solutions for the treatment of these diseases (Figure 2).

Gene therapy, as one of the cutting-edge fields of biomedical science, offers new pathways for precision medicine in human genetic diseases. Adeno-associated virus (AAV) is widely used in gene therapy due to its high safety, stability, tissue targeting, and low immunogenicity [72]. By replacing the sequence between the ITRs at both ends of the AAV genome with gene therapy elements, a gene expression cassette plasmid can be created. The *REP* and *CAP* genes in the genome are then cloned into new eukaryotic expression vectors as capsid plasmids, which are co-transfected with helper virus expression plasmids into eukaryotic cells to self-assemble into new recombinant AAVs carrying the target gene sequence. The unique structure and plasmid packaging of AAV underpin its use as a viral vector [73]. Wang [40] used AAV as a vector to deliver short hairpin RNA (shRNA) targeting FOXK1, specifically knocking down its expression. Experimental results showed that treatment with AAV9-shFoxk1 significantly reduced FOXK1 expression levels in TECs, decreasing the formation of FOXK1 condensates and downregulating the expression of glycolysis-related genes, including Slc2a2, Hk1, Fbp1, Aldoa, Bpgm, and Ldhc. These changes alleviated TEC damage, manifested as improvements in cell morphology and function, as well as reduced extracellular matrix deposition. In animal models, significant therapeutic effects were also observed following subcapsular renal injection of AAV9-shFoxk1. Compared to controls, mice models of unilateral ureteral obstruction and ischemia-reperfusion injury treated with AAV9-shFoxk1 exhibited reduced renal fibrosis, as evidenced by lower tubular injury scores, decreased interstitial fibrosis percentages, and downregulated expression of fibrosis markers such as fibronectin and α-smooth muscle actin. Additionally, glycolytic activity and the expression of related transcripts were effectively inhibited.

Natural product monomers can be isolated, purified, and structurally identified through modern drug development technologies, facilitating a deeper understanding of their pharmacological activities, toxicological properties, and relationships with diseases, laying the foundation for further drug development and clinical application. Curcumin is currently the only known natural inhibitor of P300 with both in vitro and in vivo specificity and cell permeability. Its specific binding to P300/CBP induces conformational changes, reducing the binding efficiency of histone H3, H4, and acetyl-CoA [74]. A curcumin analog has also been reported to directly bind and activate TFEB, inducing autophagy independently of mTOR, thereby treating neurodegenerative diseases [75]. Li [43] found that curcumin-enhanced autophagy improved inflammation in AS through the TFEB/P300/BRD4 axis. Curcumin promoted TFEB nuclear translocation by inhibiting mTOR, optimizing lipid catabolism, and significantly restoring autophagy in foam cells. Increased autophagy inhibited the effect of reactive oxygen species on P300 activity, reducing the synergistic action between P300 and the downstream

histone acetylation reader BRD4 within LLPS-formed condensates in foam cell nuclei, thereby lowering the transcription of inflammatory factors IL-1b, IL-6, IL-8, TNF-α, and MCP-1, achieving the goal of treating AS.

Small molecule inhibitors are organic compounds with a molecular weight of less than 1000 Da that can directly target enzyme proteins, compete with substrates, alter protein structures, reduce protein activity, or block biochemical reactions [76]. Small molecule inhibitors are commonly found in various clinically used drugs, particularly for cancer treatment. Bergman and his research team, in their quest for selective HDAC6 inhibitors, synthesized a series of urea-based branched linker compounds and identified Nexturastat A through screening [77]. Nexturastat A exhibits low nanomolar inhibitory activity against HDAC6 and approximately 600-fold selectivity over other HDAC isozymes (such as HDAC1). Zhao [78], focusing on TNBC, utilized BT-549 and MDA-MB231 cell models to quantify the number of nuclear condensates and found that the selective HDAC6 inhibitor Nexturastat A effectively inhibited the formation of phosphorylated HDAC6 condensates, whereas another HDAC inhibitor, HPOB, had no such effect. This discovery revealed the potential of Nexturastat A in treating TNBC through the LLPS process. Specifically, within TNBC cell nuclei, p-HDAC6 is significantly enriched and promotes the formation of LLPS condensates through its IDR N-terminal domain and phosphorylated residues. These condensates are primarily localized in transcriptionally active chromatin regions, co-localizing with active transcription markers such as Pol II-S5P and acetylated histone H3K27. Nexturastat A blocks the interaction between S22-phosphorylated HDAC6 and importin-β, preventing HDAC6 nuclear import. It also disrupts the structure of p-HDAC6 by interfering with its interaction with 14-3-3θ, releasing p-HDAC6 molecules from the condensed phase into the diluted phase, where they are subsequently dephosphorylated by PP1 (a phosphatase that interacts with HDAC6). The disruption of p-HDAC6 condensates remodels chromatin structure, influencing transcription factor binding and gene expression, involving epigenetic modifications such as chromatin accessibility and histone acetylation. In summary, Nexturastat A inhibits the formation of p-HDAC6 LLPS condensates, regulates chromatin structure and transcription, ultimately suppressing TNBC cell proliferation and invasion, demonstrating the potential anticancer therapeutic value of small molecule inhibitors.

Suramin, a derivative of polysulfonated naphthylureas, is an anionic compound known for its efficacy against African trypanosomes and adult Onchocerca volvulus [79, 80]. Its insecticidal mechanism may involve the formation of stable complexes through the binding of its multiple anionic negative charges to cationic sites on the parasite's proteins. Additionally, it inhibits the parasite's glucose metabolism [81]. Betzel [48] discovered the regulatory role of suramin on the LLPS of Tau protein. Their experiments revealed that suramin, through electrostatic interactions between its negative charges and the positive charge regions of Tau protein, successfully induces LLPS of Tau protein, leading to the formation of Tau-suramin condensates. These condensates exhibit typical droplet-like morphologies under both light and electron microscopes, with their size increasing with suramin concentration. Furthermore, when suramin is added to Tau condensates induced by polyanionic polymers such as heparin or RNA, it competitively binds to Tau protein, causing the disassembly of existing condensates into smaller particles or monomers, thereby significantly reducing their potential to further convert into pathological Tau forms. These findings suggest that suramin not only induces LLPS of Tau protein but also effectively interferes with Tau condensates induced by other polyanionic polymers, collectively maintaining the dynamic equilibrium of Tau protein within condensates and preventing the formation of stable pathological structures. This offers new potential strategies for the treatment of neurodegenerative diseases such as AD.

In the pursuit of therapeutic strategies for diseases associated with LLPS mechanisms, it becomes clear that gene therapy, small-molecule inhibitors targeting LLPS, and treatments involving natural compounds each present distinct challenges and limitations. Specifically, gene therapy encounters difficulties related to immune system responses, the necessity for multiple administrations, and long-term safety concerns [82]. Small-molecule inhibitors face obstacles in target selection, as well as challenges in drug design and screening, alongside discrepancies between in vitro and in vivo efficacy. Natural compounds are limited by uncertainties surrounding their active ingredients, unstable pharmacodynamics, low bioavailability, safety concerns, and a lack of robust clinical trial data. Addressing these challenges calls for continued exploration and innovation from researchers, which is essential for overcoming these obstacles and advancing new ideas and methodologies in the treatment of diseases linked to LLPS mechanisms.

The crruent state of LLPS research and the advancements and applications of microscopy techniques

Currently, research on LLPS is primarily conducted within in vitro cellular environments, utilizing purified proteins and nucleic acids to authenticate the LLPS process. However, these simplified systems may not adequately replicate the intricate molecular interactions and physiological conditions found within actual cells. The study of LLPS in living cells poses significant challenges, owing to the complexity and dynamic characteristics of cellular structures, which complicate the accurate identification and quantification of the LLPS process [4].

LLPS-formed condensates exhibit a high degree of dynamicity, being prone to coalescence and decomposition under the influence of various external environmental factors, including temperature, pH, and ion concentration [4]. These factors can significantly impact the formation and stability of the condensates. Currently, 1,6-Hexanediol is employed as a frequent indicator in LLPS research due to its ability to selectively disrupt the barrier formed by FG nucleoporin gel. While hexanediol may interfere with numerous LLPS processes mediated by IDRs, certain interactions, such as π-π, cation-π, and electrostatic interactions, remain insensitive to its effects. Furthermore, cellular studies have demonstrated that hexanediol can modify membrane permeability, resulting in spurious artifacts that disrupt normal observations [83]. To address these limitations, future research must adopt more intricate experimental systems that closely approximate physiological conditions, combined with advanced imaging and quantification techniques, to enable more precise observation and interpretation of LLPS phenomena.

In the realm of LLPS research, microscopy techniques occupy a pivotal position. Differential interference contrast (DIC) microscopy, a non-invasive imaging modality, is extensively employed to observe the formation and characteristics of in vitro LLPS droplets. This technique has the capability to capture the dynamic transformations of proteins, such as Tau and RNA molecules, throughout the LLPS process. A notable strength of DIC microscopy is its capacity to image cells and samples possessing high refractive index gradients directly, without the need for fluorescent labeling. This attribute provides enhanced contrast and renders it exceptionally suitable for delineating the properties of LLPS droplets [84]. Nevertheless, DIC microscopy is not devoid of limitations, particularly its lack of specificity, which hinders its ability to detect small LLPS droplets within cellular contexts.

In comparison to DIC microscopy, wide-field fluorescence microscopy demonstrates superior performance in investigating the formation and properties of both in vivo and in vitro LLPS droplets. This technique facilitates the observation of protein changes, such as Tau, FXR1, and CRY2, during the LLPS process, and is characterized by its ease of implementation, low phototoxicity, and high acquisition rates [84]. Another microscopy technique of note is total internal reflection fluorescence microscopy, which possesses unique advantages in the analysis of cell membranes and the study of single molecules. TIRF microscopy facilitates the observation of proteins like the Escherichia coli single-stranded DNA-binding protein and P62/SQSTM1 during the LLPS process. It stands out with its high signal-to-noise ratio, single-molecule sensitivity, low phototoxicity,

and rapid acquisition rate. Moreover, TIRF microscopy can be easily adapted from widefield microscopes [85]. Nonetheless, its imaging depth is confined primarily to the sample surface vicinity, posing challenges in accurately characterizing and quantifying early LLPS droplets that may be smaller than the optical diffraction limit.

Advancements in confocal microscopy and super-resolution imaging techniques have ushered in clearer insights into biomolecular condensates. By integrating deep learning technology, confocal microscopy elevates spatial resolution to achieve super-resolution imaging. This method is systematically simple, demands low optical power, and offers deeper imaging capabilities than widefield imaging, showcasing significant potential in live-cell and in vivo imaging [86]. As a pivotal tool for LLPS studies, confocal microscopy is renowned for its exceptional signal-to-noise ratio and axial resolution. Despite slower acquisition speeds and higher phototoxicity, it excels in providing depth information and enabling three-dimensional reconstructions [86, 87].

Super-resolution imaging techniques, including stimulated emission depletion microscopy, structured illumination microscopy, and single-molecule localization microscopy, have transcended the diffraction limit of light, achieving nanometer-scale resolution. These techniques are widely employed in the study of subcellular structures, organelles, and macromolecular complexes, offering fresh perspectives on the spatial distribution and interactions of biomolecules [88]. Notably, the advent of super-resolution microscopy techniques such as photoactivated localization microscopy and stochastic optical reconstruction microscopy has provided sophisticated means to explore the properties and early morphologies of LLPS droplets. With their high signal-to-noise ratio, single-molecule sensitivity, and superior spatial resolution, these techniques unveil details unobservable by traditional microscopes [89]. However, these technologies grapple with challenges like slower acquisition speeds, higher phototoxicity, and the necessity for image post-processing, thereby limiting their application scope.

In conclusion, when selecting methods for observing LLPS, it is imperative to comprehensively consider sample characteristics, research objectives, and the pros and cons of each technique. Researchers can flexibly apply or integrate multiple techniques based on specific needs to gain a comprehensive understanding of LLPS behavior.

Conclusion and future prospects

This review summarizes the development of LLPS in biology, particularly its crucial role in regulating transcription mechanisms within the nucleus. As a unique biophysical phenomenon, LLPS not only reveals new mechanisms of biomolecular assembly and regulation within cells but also provides new insights into understanding the pathological mechanisms of various diseases. Through LLPS, key proteins such as transcription factors and coactivators form condensates within the nucleus, finely regulating critical steps in gene transcription initiation and elongation, which are essential for maintaining normal cellular physiological functions. However, when the LLPS process is dysregulated, it can lead to the onset of a range of diseases, including CKD, AS, AD, and TNBC.

In exploring the association between nuclear LLPS and diseases, we find a significant knowledge gap regarding many common conditions. Current research indicates that condensates formed through LLPS may transform into pathogenic foci under disease states, exacerbating disease progression by affecting gene expression, chromatin structure, and cellular metabolism. For example, in chronic kidney disease, FOXK1 regulates the expression of glycolysis-related genes through LLPS, promoting renal fibrosis; in AS, LLPS-regulated inflammatory gene expression facilitates foam cell formation and inflammatory responses; in Alzheimer's disease, abnormal LLPS of Tau protein is closely related to the formation of neurofibrillary tangles; and in TNBC, condensates formed through LLPS of p-HDAC6 affect chromatin structure and gene transcription, promoting tumorigenesis and progression.

As research on LLPS deepens, drug-based modulation and intervention of LLPS have emerged as new avenues for treating related diseases. This review introduces various intervention strategies, including gene therapy, traditional Chinese medicine monomers, and small-molecule inhibitors, which effectively inhibit disease progression by targeting the LLPS process. For instance, using AAV to deliver short hairpin RNA specifically knocks down FOXK1 expression, significantly alleviating renal fibrosis; curcumin improves inflammation in AS through the TFEB/P300/BRD4 axis; and the selective HDAC6 inhibitor Nexturastat A regulates the proliferation and invasion of TNBC cells by inhibiting the formation of p-HDAC6 condensates. These studies aim to directly or indirectly prevent the formation of condensates to achieve therapeutic goals (Table 1).

Table 1 Overview of diseases, pathological mechanisms, LLPS relationships, and key transcriptional regulatory proteins

Disease type	Pathological mechanism and relationship with LLPS	Key proteins	References
CKD	Renal fibrosis is the fundamental pathological alteration observed in chronic kidney disease CKD. TECs occupy a central role in the progression of renal fibrosis. FOXK1 emerges as a vital regulator in this fibrotic process.	FOXK1	[40]
AS	The formation of foam cells in macrophages is a pivotal step in the development of AS. oxLDL inhibits the nuclear translocation of TFEB, resulting in impaired autophagy. This autophagy deficiency subsequently promotes inflammatory responses.	TFEB, P300, BRD4	[43]
AD	Neurofibrillary tangles, serving as the core pathological feature of AD, involve a crucial pathological process wherein tau protein undergoes abnormal aggregation through LLPS.	Tau protein	[48]
TNBC	HDAC6 is aberrantly phosphorylated in TNBC and forms LLPS condensates, which subsequently influence chromatin structure and gene expression.	HDAC6	[53]

LLPS, liquid-liquid phase separation; CKD, chronic kidney disease; AS, atherosclerosis; AD, Alzheimer's disease; CDK9, cyclin-dependent kinase 9; TNBC, triple-negative breast cancer; FOXK1, forkhead box protein K1; TECs, tubular epithelial cells; BRD4, bromodomain-containing protein 4; HDAC6, histone deacetylase 6.

Looking ahead, a deeper understanding of the LLPS mechanism will continue to drive its application in disease prevention and treatment. With the development of high-throughput sequencing, Li [90] have developed ACC-seq, a novel technology that can detect chromatin-bound condensates genome-wide. Utilizing the differences in DNA fragment accessibility under various condensate-regulating conditions by Tn5 transposase, ACC-seq identifies condensate-occupied regions, enabling the analysis of nuclear condensates. This technology can be broadly applied to studies on condensate formation mechanisms, screening of transcription regulatory factors, and the mechanisms underlying disease onset and progression, facilitating the precise localization of chromatin condensates and in-depth analysis of gene expression regulatory networks. Simultaneously, drug screening and optimization targeting the LLPS process will also become research hotspots, providing strong support for the development of new therapeutic strategies. Furthermore, interdisciplinary collaboration and cross-integration will bring new opportunities and challenges to the field of LLPS research, propelling our understanding of life sciences to new heights.

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