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Phase Separation in Kidney Diseases: Autosomal Dominant Polycystic Kidney Disease and Beyond

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Abstract
Background: The formation of biomolecular condensates via phase separation has emerged as a fundamental principle underlying the spatiotemporal coordination of biological activities in cells. Aberrant biomolecular condensates often directly regulate key cellular process involved in the pathogenesis of human diseases, including kidney diseases. ***Summary:*** In this review, we summarize the physiological roles of phase separation and methodologies for phase separation studies. Taking autosomal dominant polycystic kidney disease as an example, we discuss recent advances toward elucidating the multiple mechanisms involved in kidney pathology arising from aberrant phase separation. We suggest that dysregulation of phase separation contributes to the pathogenesis of other important kidney diseases, including kidney injury and fibrosis. ***Key Messages:*** Phase separation provides a useful new concept to understand the mechanisms underlying kidney disease development. Targeting aberrant phase-separated condensates offers new therapeutic avenues for combating kidney diseases.

Introduction

The correct organization of biomacromolecules (such as proteins and nucleic acids) within cells ensures the precise regulation of biological reactions involved in various cellular processes. It has long been known that biomacromolecules can be encapsulated by bilayer phospholipid in membrane-bound organelles, such as the nucleus, mitochondria, endoplasmic reticulum, and Golgi apparatus, whose perturbation causes various diseases. Biomacromolecules have also been seen concentrated in various non-membrane-bound compartments, such as the P granules, nucleoli, postsynaptic densities (PSDs) in synapses, stress granules (SGs), Cajal bodies, and protein spots at DNA damage repair sites. However, the assembly mechanisms of these membraneless compartments have

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long been elusive. Recent studies indicate that most membraneless compartments (also called biomolecular condensates) are formed by a process termed liquid-liquid phase separation (LLPS). LLPS in biology refers to the phenomenon that biomacromolecules spontaneously demix from the bulk environment and separate into two distinct phases with different concentrations, one condensed phase and one dilute phase [1]. Formation of multivalent and low-affinity interactions between various protein and/or nucleic acid components, such as hydrophobic interactions and electrostatic interactions, is the molecular basis for the occurrence of LLPS in cells [2]. When the content of these components exceeds some critical concentration, the molecules spontaneously coalesce to form a new liquid phase. Multivalent proteins, which contain multiple interacting domains or repeated motifs, or intrinsically disordered regions (IDRs), tend to undergo LLPS [3]. In addition, RNA molecules can also undergo phase separation independently or can regulate the phase separation of proteins [4].

LLPS-driven biomolecular condensates may affect cellular processes through several mechanisms. First, forming condensates increases the local concentration of enzymes, substrates, and interaction partners, leading to increased rates of biochemical reactions [5, 6]. Second, the condensates can promote reactions by excluding inhibitors [6]. Third, some condensates mediate molecular transport and protein sorting [7, 8]. Finally, condensates can sequester key components to limit certain reactions [9]. In many diseases, especially in cancer, neurodegenerative disorders, and inflammatory diseases, a connection between abnormal LLPS and disease progression has been reported, but the relationship between defective LLPS and kidney diseases has just begun to be revealed.

In this review, we first summarize current methods for the analysis of phase separation. Second, we elucidate the physiological roles of phase separation in regulating cellular processes. Third, we survey recent studies reporting aberrant phase separation in kidney diseases, using autosomal dominant polycystic kidney disease (ADPKD) as an example. Finally, we provide a future perspective on studying phase separation in other kidney diseases, including kidney injury and fibrosis, and propose potential therapeutic strategies targeting phase separation for kidney disease treatment.

Methods to Investigate the LLPS Phenomenon

Various experimental methods and techniques have been used to investigate LLPS of biomacromolecules [10] (shown in Fig. 1). First, LLPS condensates derived from in vitro purified or endogenously expressed macromolecules can be morphologically detected by microscopy. A classic example is the use of standard light microscopy to monitor the liquid-like behavior of LLPS condensates, such as droplet formation and fusion. Confocal microscopy and super-resolution imaging can provide more detailed information about LLPS-driven biomolecular condensates [11]. LLPS condensates can also be visualized by electron microscopy in a label-free manner [12].

Second, the material properties and dynamics of LLPS condensates can be explored by fluorescence recovery after photobleaching (FRAP) [13]. FRAP is widely used to demonstrate the liquid-like property of LLPS condensates. The macromolecules under study (mostly proteins) are first fused with fluorescent labels, and the speed of FRAP is used for measuring both diffusion within LLPS condensates and molecular exchange with the surrounding environment.

Third, the function of LLPS condensates can also be explored by measuring cellular responses after disrupting LLPS using chemical agents (for example, 1,6-hexanediol) or generating amino acid mutations in macromolecules that are responsible for establishing LLPS condensates. In addition, several optogenetic approaches have been developed to spatiotemporally manipulate LLPS. For example, the "optoDroplet" system uses an *Arabidopsis thaliana* photoreceptor protein, Cry2, which forms oligomers following blue light activation, to tag target proteins [14]. The LLPS condensates derived from Cry2-tagged proteins can then be manipulated by blue light treatment. The optoDroplet system is particularly useful for studying the role of LLPS condensates in regulating cellular processes in vivo. More recently, the "CasDrop" system was developed to induce LLPS condensate formation at specific genomic sites [15]. This CasDrop system combines genome-targeting ability and optogenetic controllability, which can be used to study the roles of LLPS condensates in regulating gene expression.

Fourth, the composition of LLPS condensates can be explored by proximity-labeling techniques coupled with mass spectrometry analyses. For example, Markmiller et al. [16] used ascorbate peroxidase) proximity labeling and mass spectrometry to study the compositional diversity of SGs in different cell types. They identified ~150 previously unknown human SG components and demonstrated aberrant composition and subcellular distribution of SG components in cells from amyotrophic lateral sclerosis (ALS) patients [16]. Lu et al. [17] used isotope labeling mass spectrometry to identify the

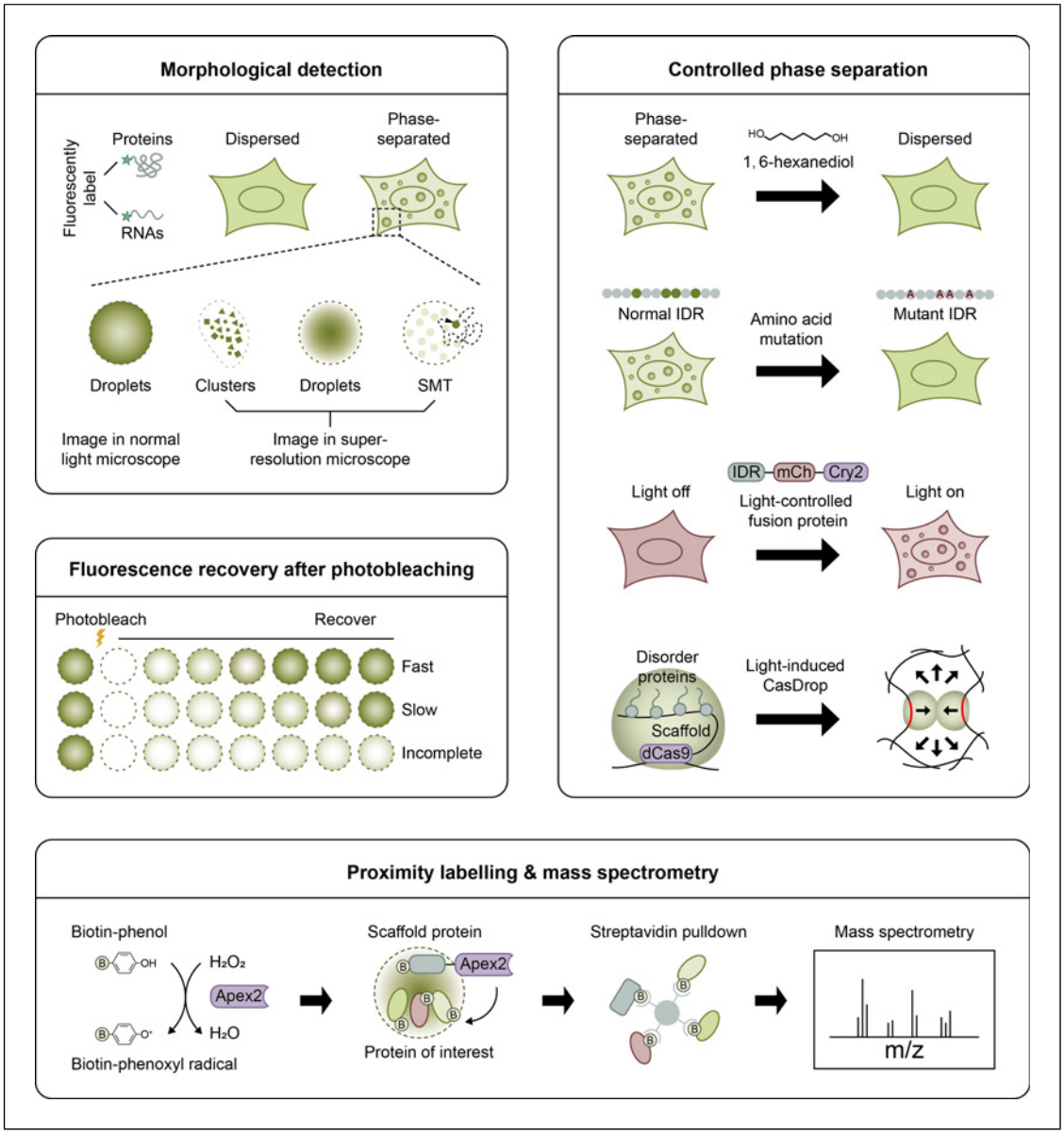

Fig. 1. Summary of experimental methods and techniques to studying phase-separated condensates. First, LLPS condensates can be morphologically detected by light microscope or super-resolution microscope. Second, the material properties and dynamics of LLPS condensates can be explored by fluorescence recovery after photobleaching (FRAP). The liquid-like nature of LLPS condensates allows for dynamic exchange of molecule, as exemplified by FRAP that demonstrates fast fluorescence recovery. However, macromolecules within a gel/solid-like phase exhibit slow or incomplete FRAP recovery. Third, the function of LLPS condensates can also be explored by measuring cellular responses after disrupting LLPS using chemical agents (for example, 1,6-hexanediol) or generating amino acid mutations in macromolecules that are responsible for establishing LLPS condensates. In addition, several optogenetic approaches, such as "optoDroplet" and "CasDrop" systems, have been developed to spatiotemporally manipulate LLPS. Fourth, the composition of LLPS condensates can be explored by proximity-labeling techniques coupled with mass spectrometry analyses.

interacting partners of phase-separated cytoplasmic TAR DNA-binding protein of 43 kDa (TDP-43). They found that HSPB1 partitioned into TDP-43 droplets and inhibited TDP-43 assembly into fibrils, which is essential for dissolving the stress-induced TDP-43 droplets [17].

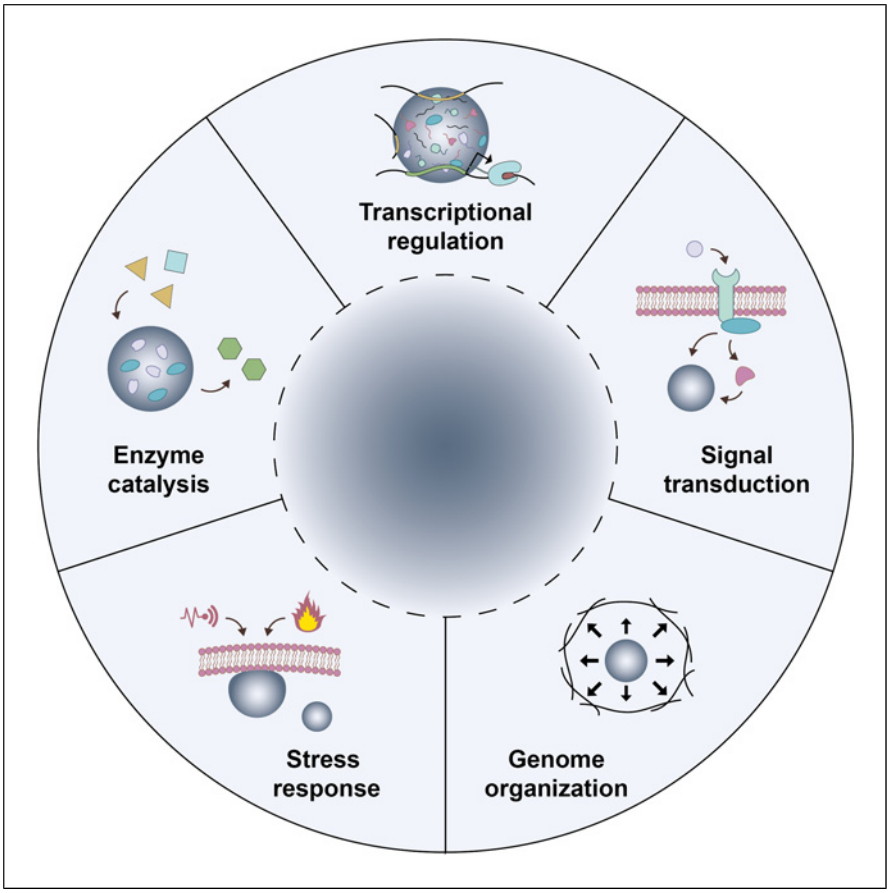

Fig. 2. Physiological roles of intracellular LLPS condensates. LLPS-mediated condensates can concentrate enzymes and their substrates, thus accelerating catalytic reactions. LLPS condensates regulate gene expression by concentrating transcription regulators. LLPS is an intrinsic feature of chromatin, and the formation of chromatin condensates is affected by numerous factors, such as modifications of histone tails. Sequestration of biomacromolecules into LLPS condensates can coordinate cellular responses to stress. LLPS has been recognized as a potentially ubiquitous framework for organizing diverse signaling pathways.

Physiological Role of LLPS in Cell Biology

Recent studies suggest that LLPS is involved in many cellular processes, such as enzyme catalysis, transcription regulation, genomic organization, stress responses, and signal transduction (shown in Fig. 2).

Regulation of Enzyme Catalysis via LLPS

LLPS-mediated condensates can concentrate enzymes and their substrates, thus accelerating catalytic reactions. For example, Jin et al. reported that hypoxia induces concentration of glycolytic enzymes into membraneless condensates termed glycolytic bodies. They showed that the presence of glycolytic bodies enhanced the rate of glycolysis and correlated with increased glucose consumption and cell survival [18]. Sheu-Gruttadauria et al. [19] demonstrated that Argonaute2 and TNRC6B, two components of the human miRNA-induced silencing complex, condensed into phase-separated droplets to accelerate deadenylation by sequestering target RNAs from the surrounding solution. Similarly, FMRP and CAPRIN1, two interacting translational regulators, were found to phase separate with target RNA. Additionally, the phosphorylation pattern of FMRP-CAPRIN1 tunes rates of deadenylation and translation [20]. In addition to increasing the enzymatic activity, LLPS can also expand kinase specificity. Sang et al. [21] generated a synthetic condensate system based on multivalent interactions between ten repeats of small ubiquitin-like modifier (SUMO) domains (SUMO10) and six repeats of a SUMO-interacting motif, which readily undergo LLPS when mixed. They later added the kinases and substrates to these synthetic LLPS condensates. The first tested kinase and substrate pair were the human mitogen-activated protein kinase 3 (also called ERK1) and one of its substrates, ELK1. They observed an accelerated phosphorylation reaction in these LLPS condensates both in vitro and in vivo. They also found that kinases in these synthetic condensates phosphorylated unexpected peptides, suggesting that LLPS can expand the substrate specificity.

Transcription Regulation via LLPS

Transcription is the first step of gene expression and determines which proteins are produced and at what speed.

RNA polymerase II (Pol II), transcription factors, and coactivators are the key components of the transcription machinery. It is known that proteins containing IDRs tend to undergo LLPS when they reach a threshold concentration, and mutations in IDRs are often related to various diseases [22]. IDRs are frequently found in these transcription determinants. For example, cyclin T1, a subunit of the positive transcription elongation factor b (P-TEFb), contains an IDR domain, which promotes the formation of LLPS droplets in vitro and the localization of P-TEFb to nuclear speckles [23]. More interestingly, the cyclin T1 condensates can also bring the Pol II C-terminal domain into its condensate environment to ensure hyperphosphorylation of C-terminal domain and efficient transcription elongation [23]. Transcription regulators involved in enhancer activation, such as BRD4 and MED1, also contain IDRs. They were both reported to form LLPS condensates at super-enhancer sites, disruption of which abolished gene activation [24]. The transcriptional coactivators yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ), critical for driving cell growth, also form LLPS condensates both in vitro and in vivo [25, 26]. YAP and TAZ condensates recruit transcriptional components such as Pol II, BRD4, and MED1. Disrupting YAP or TAZ condensates abolishes the expression of their target genes, further confirming the importance of LLPS in YAP- and TAZ-mediated transcription activation [25, 26].

Genome Organization via LLPS

The organization of chromatin into open versus compact compartments is critical for fine-tuning gene regulation. The nucleosome, which comprises an octameric histone subunit wrapped by 146 bp DNA, is the minimal functional unit of chromatin. Recent in vitro nucleosome reconstitution assays have suggested that LLPS is an intrinsic feature of chromatin [27]. Numerous factors affect the formation of chromatin condensates. Acetylation of histone tails dissolves chromatin condensates, accelerating transcription activation. In contrast, adding the linker histone H1 reduces the condensate dynamics, enhancing chromatin phase separation [27]. Constitutive heterochromatin, an important component of eukaryotic genomes, has essential roles in genome stability, nuclear architecture, and silencing of transposon and gene expression [28]. Heterochromatin is epigenetically marked by trimethylation of histone H3 at lysine 9. Heterochromatin protein 1, the trimethylation of histone H3 at lysine 9 reader protein, is proposed to undergo LLPS and promote phase separation of heterochromatin [28–30].

Stress Response via LLPS

Cells adapt to stress conditions by adjusting their metabolism, physiology, and architecture [31]. Accumulating evidence suggests that the process of phase separation is adopted by cells to facilitate adaptation to stress [31]. SGs, the cytosolic biomolecular condensates that contain translation-halted mRNAs and various proteins, are known to play critical roles in stress responses [32]. Yang et al. [33] revealed that SGs assemble through RNA-dependent LLPS arising from a G3BP1-centered protein-RNA interaction network. In addition, Riback et al. [34] showed that poly(A)-binding protein, a marker of SGs, phase-separated and formed hydrogels upon exposure to stress conditions. Poly(A)-binding protein phase separation was essential for organism fitness during prolonged stress [34]. In stressed conditions, a canonical yeast prion protein and translation termination factor, Sup35, formed protective gels via pH-regulated phase separation, which promoted yeast cell survival by preventing the damage of Sup35 translation factor [35]. Ded1p, an essential translation initiation factor, was reported to undergo heat-induced phase separation, which selectively stimulated translation of stress mRNAs while repressing translation of housekeeping mRNAs to promote cell survival under heat stress [36].

LLPS and Intracellular Signaling

Cells activate diverse intracellular signaling pathways to channel environmental information into corresponding functional outcomes. Recently, LLPS has been recognized as a potentially ubiquitous framework for organizing diverse signaling pathways [37], such as immune signaling, synaptic signaling, the Wnt pathway, and the Hippo pathway.

The cyclic GMP-AMP synthase (cGAS) pathway plays critical roles in innate immunity and inflammation [38]. cGAS senses cytosolic double-stranded DNA, arising from pathogen infection or damaged host DNA, both of which elicit strong inflammatory responses. DNA binding to cGAS initiates the synthesis of 2'3'-cGMP-AMP, which ultimately activates immune gene expression [38, 39]. LLPS of cGAS can be induced by sensing double-stranded DNA above a threshold of about 30 nM [5]. cGAS enzymatic activity increases exponentially in cGAS-DNA condensates, leading to robust synthesis of 2'3'-cGMP-AMP and thus activating innate immune signaling [5]. Interestingly, cGAS-DNA condensates can also limit the activity of the cytosolic exonuclease TREX1, restricting DNA degradation and enhancing cytosolic DNA sensing [40]. LLPS has also been implicated in adaptive immune responses, such as in T-cell receptor

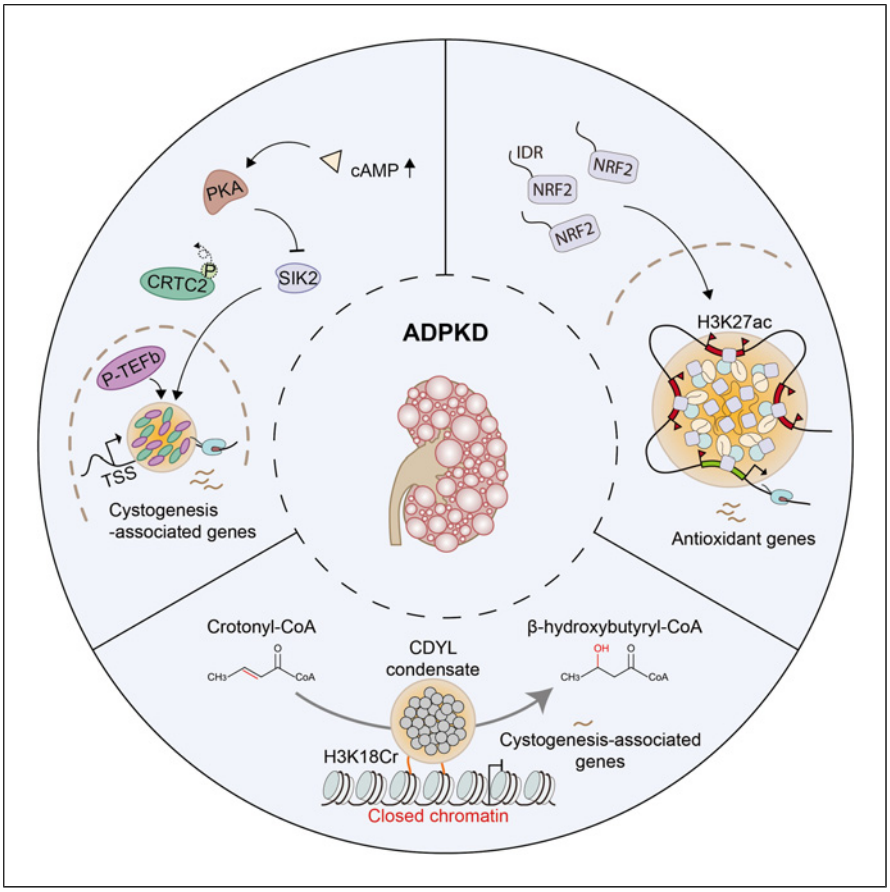

Fig. 3. Phase separation in the pathogenesis of ADPKD. NRF2, a key component of antioxidant pathways, forms nuclear condensates with the mediator complex to activate antioxidant genes. Defective NRF2 is associated with ADPKD progression, and activating NRF2 can ameliorate ADPKD. Aberrant activation of cAMP pathway plays central roles in ADPKD pathogenesis. CRTC2, a cAMP-responsive transcription coactivator, forms LLPS condensates with the P-TEFb complex following cAMP activation and activates transcription of cystogenesis-associated genes. CDYL, a nuclear crotonyl-CoA hydratase, forms condensates to reduce histone Kcr and suppresses the cystogenesis-associated genes. Defective CDYL in ADPKD promotes cystogenesis, and increasing CDYL expression attenuates ADPKD progression.

signaling. Upon T-cell receptor activation, LAT is phosphorylated and acts as the core scaffold to recruit Grb2 and Sos1 to form phase-separated clusters on the plasma membrane, promoting signaling outputs [6].

LLPS participates in the modulation of synaptic signaling. PSD, a protein-enriched cell compartment beneath the postsynaptic membrane, plays key roles in synaptic plasticity in the nervous system. Binding of multiple copies of PSD-95 with SynGAP, one of the most abundant PSD proteins, induces phase separation of the SynGAP/PSD-95 complex, which regulates synaptic plasticity [41].

Accumulating evidence indicates that LLPS is involved in multiple steps of the Wnt/β-catenin signaling pathway. In the absence of Wnt ligands, β-catenin is continuously degraded by a destruction complex consisting of AXIN, adenomatous polyposis coli (APC), and glycogen synthase kinase 3b (GSK3β) [42]. A recent study revealed that the AXIN condensates function as a scaffold to recruit APC and GSK3β, inducing the assembly of the destruction complex. Cytosolic β-catenin proteins are then recruited to this phase-separated complex through interactions with APC, leading to their phosphorylation by GSK3β and degradation [43]. In the presence of Wnt ligands, the destruction complex is disrupted. The unphosphorylated β-catenin proteins then accumulate and are transported into the nucleus to activate Wnt target genes [44]. Following its nuclear translocation, β-catenin uses its IDRs to co-phase-separate with master TFs and mediator at super-enhancers of its target genes [45].

The Hippo pathway is an evolutionarily conserved pathway that regulates cell growth, organ size, and tissue homeostasis and regeneration. YAP and TAZ are two core components of the Hippo pathway that control transcriptional programs involved in cell growth, survival, stemness, mobility, and differentiation [46]. YAP was found to undergo LLPS under osmotic stress, compartmentalizing the transcription factor TEAD1 and YAP-related coactivators, including TAZ, leading to the induction of YAP target genes [25]. TAZ can also activate transcription by forming LLPS condensates to compartmentalize its DNA-binding cofactor TEAD4 with coactivators MED1 and BRD4, as well as the transcription elongation factor CDK9 [26].

Aberrant LLPS in Diseases

LLPS-mediated condensate assembly is tightly regulated within cells, and the dysregulation of condensate formation, properties, and dissolution may lead to protein misfolding and aggregation, which are often considered as the cause of aging-associated disorders, such as neurodegenerative diseases [47]. The aggregation of cytotoxic proteins in neural cells is the main characteristic of many neurodegenerative disorders, such as Alzheimer's disease, frontotemporal dementia, and ALS. Recent studies suggest that aberrant phase separation is associated with the pathogenesis of these devastating neurodegenerative disorders. Most of the aggregate-forming proteins associated with these disorders undergo LLPS. Examples include the microtubule-associated protein Tau in Alzheimer's disease [48], fused in sarcoma, and the RNA-binding protein TDP-43 in ALS and frontotemporal dementia [49, 50]. In addition, disease-causing mutations of these proteins often affect the properties of LLPS droplets, such as inducing a liquid-to-gel or liquid-to-solid transition [51, 52].

Aberrant phase separation also provides a new framework to understand the mechanistic link between specific cancer mutations and their oncogenic effects. For example, cancer mutations in the protein tyrosine phosphatase SHP2, a major regulator of RAS activation, were linked to specific phase separation defects [53]. SHP2 mutants were found to promote MAPK hyperactivation, which required their ability to form LLPS condensates [53]. Cancer mutations in the tumor suppressor SPOP, a substrate adaptor of the Cullin3-RING ubiquitin ligase, were found to disrupt SPOP phase separation and SPOP substrate interactions, leading to intracellular accumulation of oncogenic SPOP substrate proteins that then promote cell growth [54]. Many cancer-driven chimeric proteins also promote oncogenesis by gaining LLPS ability. For instance, EML4-ALK fusion proteins, one of the most important oncogenic drivers of lung cancers, were found to form condensates via phase separation in various lung cancer cells [55]. Mutation of the EML4 IDR region significantly impairs the phase separation of EML4-ALK fusions and abolishes the activation of their downstream signaling pathways, leading to defective cancer transformation [55]. The leukemia-driven chimeric protein NUP98-HOXA9 has also been found to undergo LLPS in cancer cells [56]. NUP98-HOXA9 fusions establish oncogenic transcription factor condensates via LLPS, which enhances their genomic targeting and induces chromatin reorganization during tumorous transformation [56].

Phase Separation in the Pathogenesis of Autosomal Dominant Polycystic Kidney Disease

ADPKD is the most common monogenic hereditary kidney disease, manifested by numerous fluid-filled cysts in both kidneys, leading to progressively declining kidney function. Over half of ADPKD patients develop end-stage renal disease when they reach age 50. Mutations in either of two genes, encoding polycystin 1 (*PKD1*) or polycystin 2 (*PKD2*), are responsible for the initiation of cystogenesis in most ADPKD patients. Although the driver mutations in ADPKD have been recognized for over 20 years, the mechanisms of how defects in polycystin proteins lead to ADPKD progression remain largely unknown. Recent studies have provided new insights into understanding the pathogenesis of ADPKD from the perspective of phase separation (shown in Fig. 3).

Regulation of Redox Balance by LLPS in ADPKD

Mitochondrial dysfunction, which leads to an increase of oxidative stress, is frequently observed in cyst epithelial cells. However, the molecular mechanisms underlying the redox imbalance in cyst cells remain unclear. Lu et al. proposed that LLPS is involved in the regulation of redox balance in renal cyst cells. They found that NRF2, a key component of the antioxidant signaling pathway, is suppressed in ADPKD kidneys. Knocking out NRF2 accelerated cyst growth, whereas activation of NRF2 ameliorated cystogenesis in ADPKD mouse models. Purified NRF2 proteins displayed a high phase separation ability in vitro. NRF2-positive nuclear foci were detected in ADPKD cells, and the size and the number of these foci increased upon NRF2 activation by sulforaphane treatment. In addition, NRF2 interacted and co-condensated with the mediator complex, which binds gene enhancers and is frequently concentrated on super-enhancers. Super-enhancers are clusters of enhancers that recruit a high density of transcriptional regulators and drive robust expression of cell identity genes. Sulforaphane treatment increased the colocalization of NRF2 condensates and mediator condensates at the super-enhancers of NRF2 target genes, mostly cytoprotective genes, and that NRF2 was required for MED1 recruitment to these sites. Together, these observations suggest that promoting the LLPS of NRF2 may be a new therapeutic target for ADPKD treatment by improving redox balance.

Aberrant LLPS Interferes with the cAMP Signaling Pathway in ADPKD

It has long been known that an aberrantly elevated cyclic adenosine monophosphate (cAMP) signal is critical

for the activation of cyst-related genes; it induces the proliferation of cyst epithelial cells and the secretion of cyst fluid, leading to progressive cyst growth. However, how ADPKD cells transfer the elevated cAMP signal into the nucleus to activate the cyst-related genes remains elusive. Sun et al. reported that the cAMP signal induces cyst-related genes by activating a cAMP-responsive transcription factor, cAMP response element-binding protein (CREB), as well as the general transcription regulator P-TEFb. They demonstrated that inhibition of either CREB or P-TEFb suppressed the expression of cyst-related genes, attenuating ADPKD progression. Recently, Mi et al. suggested that LLPS is one of the key mechanisms for organizing transcriptional events downstream of the cAMP signal in ADPKD cells. CREB-regulated transcription coactivator 2 (CRTC2), a cAMP-responsive transcription coactivator, formed droplet structures after nuclear translocation following cAMP activation. Amino acid composition analysis of CRTC2 revealed a predicted IDR in the C-terminal region. Purified CRTC2 protein displayed high phase separation ability in vitro. An array of arginine (R) residues located in the IDR of CRTC2 was found to drive LLPS of CRTC2 proteins both in vitro and in cells. In addition, nuclear CRTC2 interacted and co-condensated with the P-TEFb complex upon cAMP stimulation. The formation of CRTC2 condensates was observed in cyst-lining epithelial cells, but not in normal renal epithelial cells. Knockout of CRTC2 suppressed cyst growth in the ADPKD mice. Mechanistically, they demonstrated that CRTC2 condensates facilitated P-TEFb recruitment and the activation of cyst-related genes. Thus, they proposed that gaining abnormal LLPS condensates in diseased cells underlies the pathogenesis of ADPKD.

Epigenetic Dysregulation Mediated by LLPS in ADPKD

Accumulating evidence supports a critical role for epigenetic regulation in cyst-associated gene expression and the progression of ADPKD, although the molecular mechanisms specifying how the epigenetic regulators contribute to cystogenesis remain largely unknown. Dang et al. recently reported that a defect in CDYL, a chromodomain Y-like crotonyl-CoA hydratase, was involved in epigenetic dysregulation in ADPKD kidneys. Amino acid composition analysis of CDYL revealed a predicted IDR between residues 52 and 233. A high concentration of CDYL could form LLPS condensates, which increased the interaction with its substrate, crotonyl-CoA, the acyl group donor for histone lysine crotonylation (Kcr). CDYL expression was found to be suppressed in ADPKD kidneys, accompanied by an increase of histone Kcr on cyst-related genes. Increasing the expression of CDYL induced CDYL LLPS in cells, enhancing the hydrolysis of crotonyl-CoA, thus reducing histone Kcr, and ultimately inhibiting the expression of cyst-associated genes. They further demonstrated that in the ADPKD zebrafish model, overexpression of CDYL could slow cyst growth, but LLPS-defective mutants of CDYL failed to alleviate ADPKD progression. The genome-wide distribution of CDYL in cystic cells was mostly concentrated in transcription regulatory regions. Thus, the condensate assembly of CDYL could establish a domain-specific microenvironment, compartmentalizing crotonyl-CoA into a dense phase to enhance the crotonyl-CoA hydration reaction and reduce local histone Kcr. Subnuclear metabolic niches or microdomains may enable site-specific recruitment of enzymes to efficiently modulate metabolite levels, accurately modify chromatin, and control gene expression. Indeed, recent studies show that metabolic enzymes involved in the synthesis of acyl-CoA, such as ACSS2, ACLY, or a-KGDH, can localize in the nucleus and associate with chromatin to generate acyl-CoA at specific genomic loci. Unlike these acyl-CoA synthetases, CDYL mainly functions as a crotonyl-CoA hydratase to decrease acyl-CoA. Therefore, the locus-specific modulation of histone Kcr by CDYL through nuclear condensation provides new evidence supporting this subnuclear metabolic niche model for precisely regulating chromatin modifications and gene transcription.

Conclusions and Perspectives

Emerging evidence indicates that dysregulation of phase separation plays critical roles in the pathogenesis of ADPKD. LLPS participates in various cellular processes, and it has been predicted that more than 30% of the human proteome has the propensity to form LLPS condensates [57]. Thus, it is conceivable that abnormal phase separation also participates in the progression of other kidney diseases. The surrounding physical and chemical factors profoundly influence the assembly of phase-separated condensates. For example, biomolecular condensation is affected by the stiffness of the surrounding matrix. During the progression of kidney fibrosis, due to extensive extracellular matrix deposition by activated myofibroblasts, the stiffness of the matrix increases, which may alter condensate assembly. Furthermore, kidneys are central to the control of pH homeostasis. Chronic kidney disease patients are typically exposed to chronic metabolic acidosis. The altered acid-base balance may create an environment affecting biomolecular condensation [58, 59]. In addition, hyperosmotic stress reduces cell size drastically and increases molecular crowding, which

affect the assembly of condensates [60, 61]. The kidney also plays critical roles in regulation of extracellular fluid volume and osmolarity. Phase separation may occur in specific segments of the kidney that have a surrounding hypertonic environment. Aberrant condensates may form in response to the alterations of osmolarity in disease conditions.

Currently, developing small molecular compounds that specifically target aberrant phase separation is one of the main research frontiers in the study of phase separation [62, 63]. A number of drugs have recently been developed to target abnormal biomolecular condensates in various cancers [62, 63]. Future studies are warranted to elucidate the mechanisms of phase separation involved in the pathogenesis of kidney diseases and to develop therapeutic agents targeting aberrant phase separation-mediated mechanisms for their treatment.

Conflict of Interest Statement

Dr. Jing Nie is an associate editor of *Kidney Diseases*. The other authors have no conflicts of interest to declare.

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Author Contributions

L.Z. wrote the manuscript; Z.L. and Y.L. prepared the figures; J.N. edited the manuscript; Y.C. conceived and wrote the manuscript.

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