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Review article

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Biomolecular condensates in kidney physiology and disease

Guoming Gao[1,2], Emily S. Sumrall[1,2], Sethuramasundaram Pitchiaya[3], Markus Bitzer[4], Simon Alberti[5] & Nils G. Walter[2]

Abstract

The regulation and preservation of distinct intracellular and extracellular solute microenvironments is crucial for the maintenance of cellular homeostasis. In mammals, the kidneys control bodily salt and water homeostasis. Specifically, the urine-concentrating mechanism within the renal medulla causes fluctuations in extracellular osmolarity, which enables cells of the kidney to either conserve or eliminate water and electrolytes, depending on the balance between intake and loss. However, relatively little is known about the subcellular and molecular changes caused by such osmotic stresses. Advances have shown that many cells, including those of the kidney, rapidly (within seconds) and reversibly (within minutes) assemble membraneless, nano-to-microscale subcellular assemblies termed biomolecular condensates via the biophysical process of hyperosmotic phase separation (HOPS). Mechanistically, osmotic cell compression mediates changes in intracellular hydration, concentration and molecular crowding, rendering HOPS one of many related phase-separation phenomena. Osmotic stress causes numerous homo-multimeric proteins to condense, thereby affecting gene expression and cell survival. HOPS rapidly regulates specific cellular biochemical processes before appropriate protective or corrective action by broader stress response mechanisms can be initiated. Here, we broadly survey emerging evidence for, and the impact of, biomolecular condensates in nephrology, where initial concentration buffering by HOPS and its subsequent cellular escalation mechanisms are expected to have important implications for kidney physiology and disease.

Sections

[1]Biophysics Graduate Program, University of Michigan, Ann Arbor, MI, USA. [2]Department of Chemistry and Center for RNA Biomedicine, University of Michigan, Ann Arbor, MI, USA. [3]Department of Urology and Center for RNA Biomedicine, University of Michigan, Ann Arbor, MI, USA. [4]Department of Medicine, University of Michigan, Ann Arbor, MI, USA. [5]Technische Universität Dresden, Biotechnology Center (BIOTEC) and Center for Molecular and Cellular Engineering (CMCB), Dresden, Germany. e-mail: nwalter@umich.edu

Key points

- Biomolecular condensates have a broad impact on many cell types and organs, including kidneys.
- The physicochemistry that underlies the assembly of biomolecular condensates renders them highly reversible and switch-like, endowing them with powerful roles in cell biology.
- Biomolecular condensates have essential roles in kidney physiology — for example, in the formation of the glomerular filtration barrier and in the hyperosmotic stress response — and in kidney pathology.
- Hyperosmotic phase separation is a widespread cellular mechanism in kidneys, where it rapidly induces biomolecular condensates upon physiological osmotic shock.

Introduction

Osmoregulation is central to human physiology and health, with fluid or electrolyte imbalances leading to potentially life-threatening conditions[1–4]. In mammals, osmotic pressure is regulated in the kidney through the actions of highly sensitive sensors, transport mechanisms and sophisticated hormonal feedback loops to maintain osmotic homeostasis of the blood and consequentially the extracellular fluids of the body. Most osmoregulation is performed by distal tubular cells in the medulla, where a high osmotic gradient is maintained to concentrate and excrete solutes in the urine[5]. These mechanisms generate urine of widely varying osmolality depending on the body's hydration status. Although osmoregulation has been studied extensively for decades, providing detailed insights into human physiology, the subcellular and molecular changes caused by rapidly evolving osmotic fluxes and stresses have remained obscure.

Advances in high-resolution imaging have yielded insights into the composition and function of biomolecular condensates, defined as membraneless compartments, that typically contain proteins and RNAs that convey distinct biochemical functions[6–10]. Biomolecular condensates often form by the process of phase separation, where protein and RNA molecules self-assemble into 'droplets' through weak and transient, but multivalent, interactions. The transience of phase separation explains their highly reversible assembly, which maintains a boundary in the absence of a biological membrane[6,8,10–15]. Condensate assembly can be described by a phase diagram, where many physicochemical conditions, such as temperature, salt and pH[12], jointly determine whether or not condensates form (Fig. 1). Common cellular stressors and perturbations, such as heat, cold and hypertonicity, can directly change the condensate behaviour by shifting the 'tie line' in the phase diagram — a line that connects the equilibrium concentrations of condensed and dilute phases under given conditions (Fig. 1). Other stressors, such as oxidative stress or DNA damage indirectly reshape the phase diagram by influencing signalling cascades and/or post-transcriptional or post-translational modifications (PTMs) of composite RNAs or proteins[9,16]. The intrinsic, active sensing of many stresses by condensates makes them vital early regulators of the cellular stress response[14].

Hyperosmotic phase separation (HOPS) is the rapid (within ~10 s) process by which condensates are formed upon osmotic compression of cells[17–19]. HOPS has been observed for a large proportion of homo-multimeric proteins in many cell lines, including proximal tubule (HK-2) and clear-cell renal cell carcinoma (Caki-1) cells[18]. The high osmolarity in the kidney can trigger HOPS, as exemplified by the propensity of yes-associated protein (YAP) to condense in accordance with the osmolarity gradient from the cortex to the medulla of mouse kidneys[20]. Evolutionarily, life-saving responses to osmolarity changes would have been among the very first adaptations required by cells to rapidly adapt to their changing environmental conditions. Thus, HOPS may represent an ancient facet of cellular life[21], later assimilated into other biological processes.

Most stress responses, such as the integrated stress response signalling pathways and the resulting stress granule assembly, take minutes to hours to reach their full level of activation[22,23]. By contrast, HOPS initiates within seconds, and affects a set of proteins distinct from those found in stress granules[18]. Therefore, HOPS may represent a mechanism to rapidly detect and respond to osmotic changes, which then primes diverse downstream processes[18,19]. For example, HOPS-induced sequestration of the nuclear, pre-messenger RNA (pre-mRNA) processing protein, cleavage and polyadenylation specific factor 6 (CPSF6) induces changes in transcription termination in a significant subset of genes and leads to their transcription readthrough, with possible wider effects on RNA metabolism[18,24,25]. HOPS affects many cellular proteins[18–20,26–31], and is therefore expected to contribute to diverse processes involved in the osmotic stress response.

This Review surveys our current understanding of the role of biomolecular condensates in responding to extracellular osmolarity and other stressors in general, and specifically in the kidneys. We propose that condensate formation may be an immediate response to osmotic fluctuations in kidney cells and thus essential to kidney function. Connecting concepts and lines of evidence from both the biomolecular condensate and nephrology fields, we aim to draw attention to an underexplored condensation-driven stress response in kidneys and stimulate further research into the mechanisms by which HOPS may help to maintain kidney function in the context of inevitable fluctuations in osmolarity, and contribute to disease states.

Biomolecular condensates

Compartmentalization creates functionally specialized spaces in cells that separate biochemical reactions from one another[32–34]. For instance, it is widely known that eukaryotic cells contain a variety of subcellular compartments, including the nucleus, endoplasmic reticulum, mitochondria and Golgi apparatus[35–38]. These classical organelles are confined by semi-permeable biological (lipid) membranes, which define the organelle boundary and regulate organelle composition[39]. However, since the microscopic observations of neuronal substructures by Ramon Cajal in 1903, cell biologists have also known of droplet-like compartments within cells, termed bodies, foci, puncta, speckles or granules[40–42]. The introduction of the electron microscope led to the discovery that these droplet-like compartments do not have an enclosing membrane[43,44]. Since then, a fundamental question has been how these membraneless bodies maintain their specific molecular composition without a physical boundary such as a membrane. In 2009, Brangwynne et al. revealed that P granules — a class of membraneless organelles that are important for germline specification — are liquid droplets that are formed by phase separation, and that their polarized formation is caused by a lowered saturation concentration in only one part of a cell[45]. Since then, biophysicists have advocated for condensate formation by phase separation as a powerful framework with which to explain the formation and dynamics of such membraneless compartments in cells[12,45–48].

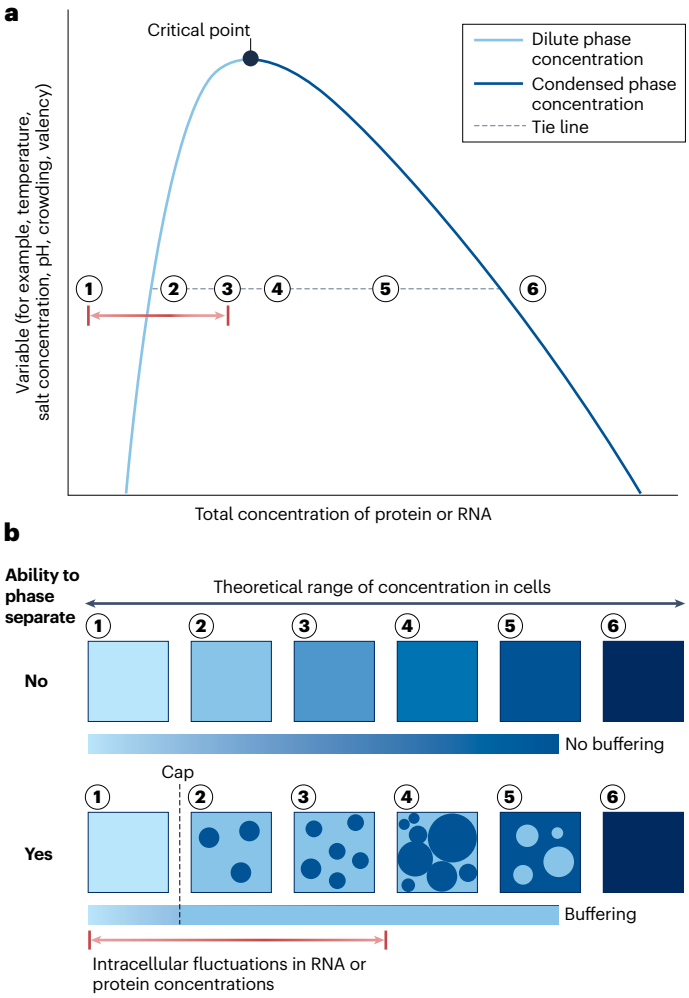

Fig. 1 | Biomolecular condensates and phase separation. a, A phase diagram is a standard tool for analysing a phase-separating system[6,10,12,96]. The phase diagram here describes an exemplary phase-separating system driven by a single protein or RNA. The *x*-axis represents a parameter that distinguishes between two phases, normally the concentration of the biomolecule that drives condensation. The *y*-axis represents a variable that tunes the condensation potential of the system so that, beyond a critical point, no condensation can happen, no matter how high the concentration of the biomolecule. The binodal curve describes a pair of concentrations at equilibrium, usually a lower one for the dilute phase (that is, saturation concentration (C_{sat})) and a higher one for the condensed phase ($C_{condense}$), defining the left and right arms of the curve, respectively. C_{sat} and $C_{condense}$ under a given set of conditions (for example, temperature) define a tie line. If the concentration is lower than C_{sat} or higher than $C_{condense}$, as in locations 1 and 6, the system is at equilibrium in a single phase (that is, not phase separating). If a perturbation such as hyperosmotic cell volume compression moves the biomolecule concentration to the non-equilibrium region below the binodal curve, as in locations 2–5, the system will form condensates (that is, it does phase separate). The relative volume ratio of the condensed and dilute phases, each of which has defined compositions as represented by the binodal line, will be determined by the total biomolecule concentration on the *x*-axis. Fluctuations of the total protein or RNA concentration within a cell are usually found at a range indicated by the red arrow. **b**, A comparison between a biomolecule capable of phase separation versus one that is incapable of phase separation along the same total concentration *x*-axis. For the biomolecule that does not phase separate, its concentration will fluctuate uniformly within the intracellular volume. However, if the biomolecule can phase separate, its concentration in most of the intracellular volume will remain at the dilute phase concentration determined by the left arm of the binodal curve. Any further increase in its total concentration will only change the volume ratio between dilute and condensed phase, but not the concentration. Therefore, the concentration is 'capped' at a fixed value despite any fluctuation in the total concentration, which is similar to a 'buffering' effect.

Phase-separation physicochemistry

A homogeneous one-phase system, such as a solution of protein and RNA molecules, can separate into a two-phase system consisting of a dilute and a dense protein or RNA-enriched phase when specific physicochemical conditions, such as appropriate temperatures and pHs, are met either in the test tube or in cells. This process is akin to water vapour condensing to form liquid droplets upon changes in pressure, temperature or humidity, demonstrating that the same set of molecules can form two distinct, but co-existing, phases[19]. Similarly, RNA–protein interactions can be altered by the physicochemical conditions in cells and condense into biomolecular condensates that are suspended in the complementary second liquid phase[9,47] (Fig. 1).

The term liquid–liquid phase separation was first introduced to describe the immiscibility of the condensate phase with its surrounding dilute or dense phase, and the liquid-like material properties of the condensates[49]. However, emerging evidence suggests that condensates are often not simple liquids, and that their formation can be driven by several physical processes other than liquid–liquid phase separation, as summarized elsewhere[10,50]. For example, one such physical process reported to have a role is a connectivity transition termed percolation[10,12,51] (Fig. 2), which involves the formation of a droplet-spanning network of interactions between molecules.

Biomolecular condensate formation

Biomolecular condensate formation is driven by multivalent interactions between proteins, RNAs, or both. The multivalency of a biomolecule is the number of intermolecular interactions it can establish. The condensing biomolecules must have the potential to interact with at least two other biomolecules simultaneously, contributing to an amorphous network of multivalent protein–protein, RNA–protein and RNA–RNA interactions[7,9,12,46,49,52]. Such a molecule can be described by a 'stickers'-and-'spacers' model, where the 'stickers' are the motifs that interact with each other and the 'spacers' are the inert linkers that exist between the 'stickers'[12]. According to this model, 'stickers' can be well-folded binding domains of a protein or RNA, interaction patches on molecular surfaces or single sticky amino acid residues[12].

Multivalent protein–protein interactions can originate from flexible intrinsically disordered regions (IDRs)[53–56] that often exhibit low sequence complexity[57–59], heterogeneous interaction domains[52,60,61] or self-interacting oligomerization domains[18]. Multivalent RNA–protein interactions can be achieved through the presence of repetitive RNA-binding domains, such as RNA recognition motifs (RRMs) or RGG (arginine–glycine-rich) regions in IDRs.[9] Multivalent RNA–RNA interactions can result from intermolecular base pairing or from the formation of G-quadruplexes, the latter of which are particularly associated with disease-causing RNAs that carry G-rich repeat expansions.[9,62,63]

Condensates are distinct from aggregates in their molecular interactions and the fact that condensates are usually biologically active, whereas aggregates are not (Fig. 2). However, condensates can also

harden into an aggregate-like state, suggesting that the molecular interactions inside a condensate can change (or mature) over time, with concomitant adjustments in structural and material properties, often associated with disease pathogenesis[51,64–67]. Biomolecular condensates can therefore broadly impact both physiological and pathological processes: physiological condensates can help to maintain the healthy homeostasis of a cell, whereas the disruption of physiological condensates or the formation of pathological aggregates may lead to ageing or disease.

Biomedical relevance of condensates

Biomolecular condensates have essential roles in fundamental cell biology[8,68,69], which are rarely organ specific. Examples include the centrosome in cell division[70,71], the nucleolus in ribosomal RNA biogenesis[72–74], nuclear speckles in pre-mRNA splicing[42,75,76], processing bodies (P-bodies) in RNA decay[77,78] and signalling clusters near the cell membrane in signal transduction[52,60,79]. Notably, many condensates are composed of both protein and RNA and fall under the category of ribonucleoprotein (RNP) granules, suggesting a close relationship between condensates and RNA metabolism in healthy cells[7,16,80]. Biomolecular condensates are critical organizers of cellular biochemistry and, therefore, are expected to have a role in the kidney, although this largely remains to be characterized.

Under disease conditions, physiological condensates are often dysregulated, and pathological condensates and aggregates can form and induce pathogenicity[14,81]. Genetic, disease-related mutations of proteins can alter the molecular interactions within condensates and thereby induce or disfavour condensate assembly or dissolution, leading to loss- or gain-of-function disease phenotypes[64,67,82–85].

For example, in cancer cells, oncogenic mutations can alter the landscape of chromatin condensates as well as lead to the dysregulation of condensates associated with downstream cellular processes from cell signalling to immune responses[81]. Oncogenic super-enhancers promote the formation of pathological transcriptional condensates that enrich transcription factors (TFs), transcription co-activators, and RNA polymerase II (RNAP II) to induce the overexpression of oncogenic genes[81].

Condensate formation is also strongly linked to ageing-related diseases such as neurodegeneration, where genetic defects lead to the dysregulation of condensates that regulate protein homeostasis and RNA metabolism[14]. Such dysregulation not only alters the composition of physiological condensates or their subcellular localization, but also changes their material properties, promoting a liquid-to-solid transition that is further accelerated by disease-specific perturbations of physicochemical conditions and PTMs[14,64,83]. Biomolecular condensates have been discovered in both healthy and diseased kidneys, as discussed later.

The biological relevance of biomolecular condensates is further underscored by their critical roles in the cellular stress response. Many diseases are tightly associated with stress, whereby either the accumulation of chronic stress causes the disease, or the disease conditions provoke acute or chronic stress. All known stressors, such as heat, osmolarity, and oxidative stress, can induce the assembly of a major class of condensates known as stress granules[86–89], suggesting broad links between condensates and diseases.

The crucial roles of condensates in both physiological and pathological processes suggest that they may function as 'one-stop' hubs that regulate and coordinate a variety of cellular processes, including stress responses. These findings have raised strong commercial interest in therapeutic targeting of condensates for both the prevention and

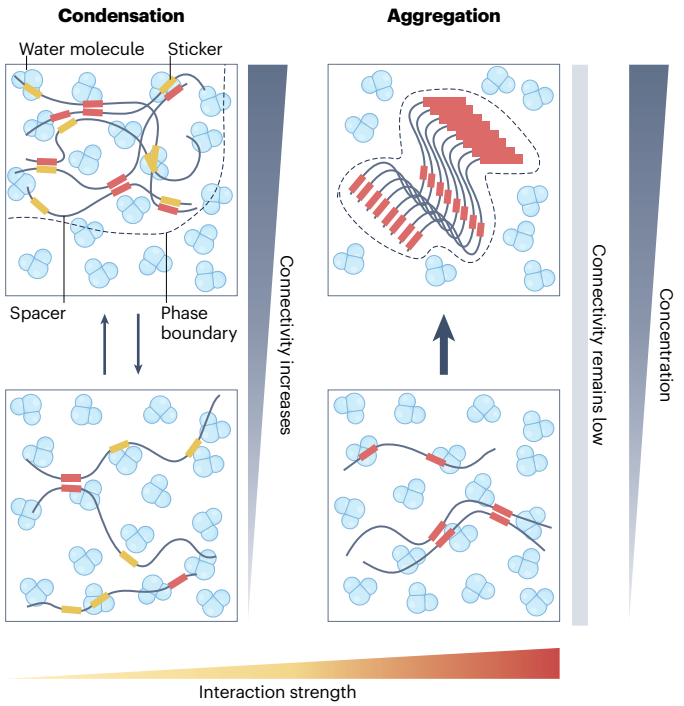

Fig. 2 | Condensates versus aggregates. Prior to our understanding of biological phase transitions, such as condensation, non-functional assemblies of proteins and/or RNAs that appeared as puncta in cell imaging were classically referred to as 'aggregates'. Today, a crucial distinction is made between condensates and classical aggregates. Condensation and aggregation both represent phase transitions that arise when the concentration of a protein or RNA rises above a specific saturation concentration (C_{sat}). Condensation signifies a reversible transition into a liquid- or gel-like phase, whereas aggregation indicates an irreversible transition into a solid-like phase. Both processes are driven by macromolecule–macromolecule interactions that are more favourable than the corresponding macromolecule–solvent interactions[46,187]; thus, both phenomena occur when the solubility of the macromolecule is low. However, there are at least three molecule-level distinctions between condensation and aggregation that explain their difference in reversibility. The molecular interactions can be depicted by the 'stickers'-and-'spacers' framework, where 'stickers' are parts of the biomolecule that interact with each other and 'spacers' are parts that do not interact. First, aggregates typically have much stronger, homotypic 'sticker'–'sticker' interactions than condensates[12], and thus do not allow any rapid movement of molecules within the phase or into the exterior environment. By contrast, condensates often exhibit weaker, heterotypic interactions between 'stickers' that enable them to exchange positions with each other and with molecules outside the condensate, which is the basis for their liquid-like behaviours[65]. Second, aggregates have much lower connectivity than condensates. When condensation occurs, all molecules within the condensed phase are interconnected and form a network of interactions with a high number of intermolecular connections per unit of volume (that is, there is high connectivity). By contrast, the connectivity will remain low during aggregation, and instead, homotypic stacking occurs between adjacent identical molecules rather than the formation of a complex, diverse network. Third, aggregates are not biologically active or functional because they are less mobile and exclude water molecules, disfavouring biochemical reactions[14]. By contrast, condensates have a water content as high as 60–70%[188–191], favouring enzymatic reactions that require water as a substrate or cofactor.

treatment of disease[81,90–92]. The discovery that drugs can specifically partition into condensates further supports this approach[93].

Molecular mechanisms of condensate function

The molecular mechanisms that underlie the biological consequences of biomolecular condensates are often similar across biological systems and diseases. To dissect the molecular impact of condensates, it is helpful to categorize the components in a phase-separating system as either 'scaffold' (host) molecules or 'client' (guest) molecules, where the scaffold molecules drive the formation of molecular condensates, and the client molecules are recruited to the condensates via interactions with the scaffolding molecules[8,52]. Condensation will cap the scaffold molecule concentration at the saturation point, buffering its fluctuations and thereby stabilizing the cellular processes it regulates[94–96] (Fig. 1). In addition, condensates can sequester or exclude different sets of client molecules to achieve distinct protein or nucleic acid functions. These modes of action are similar for both nuclear and cytosolic condensates (Fig. 3).

Modes of action in cytosol

In the cytosol, condensation can either increase the activity of enzymes by concentrating the enzymes and their substrates within condensates or, conversely, decrease enzymatic activity by separating them[34,52,60,97–99] (Fig. 3). For example, enrichment of the actin nucleation factor ARP2/3 complex in nephrin–NCK–neural Wiskott–Aldrich syndrome protein (N-WASP) condensates in kidney podocytes[52], or in T cell receptor (TCR) condensates in activated T cells[60], can drastically increase the actin nucleation activity. These two condensates are regulated by the phosphorylation level of nephrin and TCR, respectively. The TCR condensates also exclude phosphatases such as CD45, and thereby maintain the level of phosphorylation within the condensates[60]. More generally, enzyme regulation by biomolecular condensation can affect protein function by installing or removing PTMs. In addition to phosphorylation, PTM enzyme activities that are enriched or excluded by condensates include histone methylation or acetylation[81], sumoylation[8,34] and ubiquitination[8,82]. Metabolic enzymes[100] and complexes that are involved in protein quality control, such as the proteasome[14,26,30], can also be regulated by differential partitioning between the condensed and dilute phases.

Interestingly, an increase in enzymatic activity may not only be an effect of higher enzyme and substrate concentration within the condensate, but may also result from a direct increase in the Michaelis constant (K_M), invoking an emergent behaviour typical of complex systems[34]. Therefore, an increase in enzymatic activities in condensates can be the result of both classical enzyme reaction kinetics and complex system properties that are not yet fully understood.

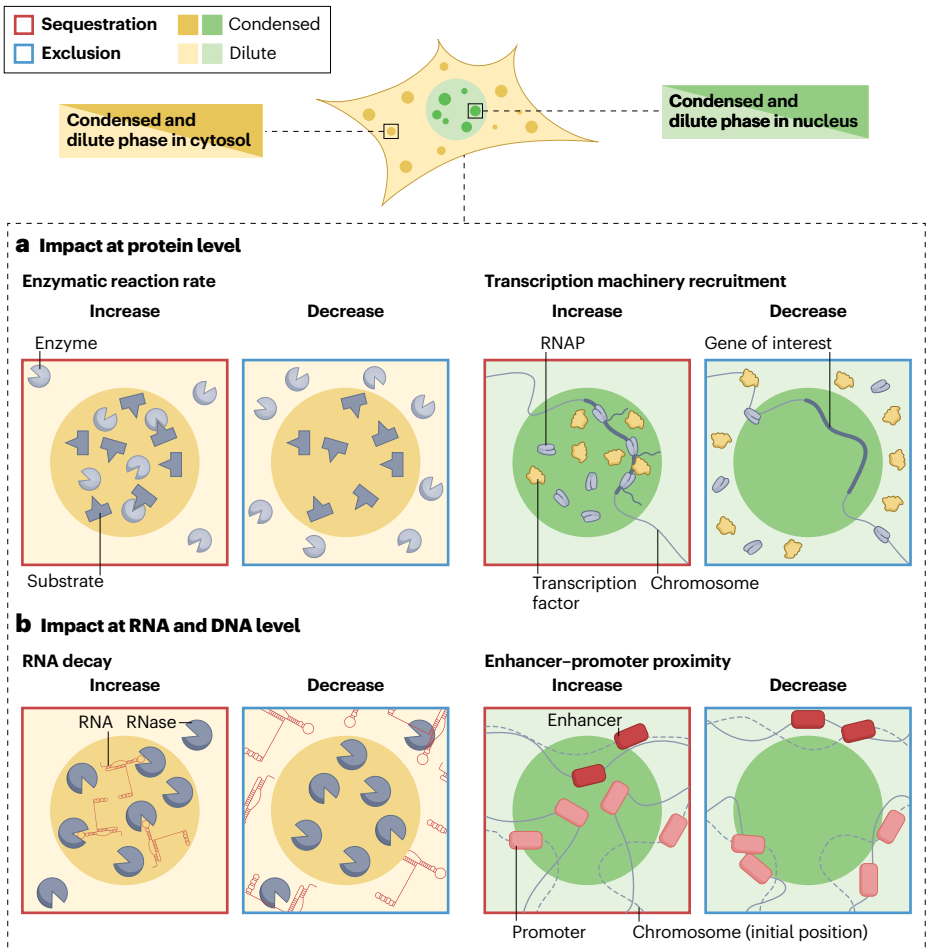

Fig. 3 | Condensates regulate cellular activities via selective sequestration and exclusion. Different condensates can selectively sequester or exclude specific biomolecules to differentially impact cellular activities. These two modes of regulation are broadly found across different biomolecules, regardless of whether their localization is cytosolic or nuclear. The functional outcome of condensation therefore varies depending on the composition of each condensate. **a**, Proteins can be the subject of regulation by condensates. For example, cytosolic condensates sequester both enzymes and substrates, increase the local concentration of both, provide a favourable physicochemical condition for reactions, and thus increase the enzymatic reaction rate. By contrast, they can also exclude enzymes or substrates to separate them and thereby reduce the reaction rate (left). Nuclear condensates can regulate nuclear protein function in the same way. The transcription reaction rate can be altered by the recruitment or exclusion of transcription machinery from nuclear condensates (right). **b**, Nucleic acids can be the subject of regulation by condensates. For example, cytosolic condensates that are enriched in RNA decay machinery, such as ribonucleoprotein (RNP) granules, can sequester or exclude specific RNAs and thus decrease or increase the half-life of specific RNAs (left). Besides RNA, nuclear condensates can sequester or exclude specific DNA elements, such as promoters and enhancers, and thus alter the proximity between them, regulating gene expression near that specific locus (right). Note that in this figure, sequestration is rendered to lead to an increase in function, whereas exclusion results in a decrease; the opposite can also be true.

The same principle of enzyme and substrate regulation applies not only to proteins, but also to RNAs within condensates (Fig. 3). The enzymatic regulation of mRNA degradation serves as a general mechanism for the control of gene expression by condensation[101,102]. Cytosolic condensates can either extend mRNA half-life by separating mRNAs from the RNA decay machinery or shorten it by recruiting mRNAs to their degrading enzymes[77,86,89,103]. Similarly, cytosolic condensates can affect the translation of specific mRNAs through selectively partitioning machinery components and mRNAs into an RNP granule[104].

Modes of action in the nucleus

In the nucleus, condensation can regulate gene expression through at least two distinct mechanisms. First, condensation can recruit transcription machinery, including TFs[105], transcription coactivators[106] and RNAP II[107], to specific genomic loci to activate the expression of downstream genes[105,108,109] (Fig. 3). These transcription condensates are often formed at special DNA elements, called super-enhancers, which determines the expression pattern associated with different cell identities and the healthy or diseased cellular states[105,106]. These condensates are also enriched for kinases such as cyclin-dependent kinase 9 (CDK9), which hyper-phosphorylates RNAP II[110], further boosting the efficiency of the transcription of nearby genes[107]. Similar to the exclusion effect in the cytosol, nuclear condensates can also regulate transcription by excluding transcription machinery. For example, Mediator is a conserved multisubunit complex that bridges TFs and RNAP II, and one of its subunits, MED1, co-condenses widely with many TFs and coactivators[105,106]. A variety of transcription condensates can assemble on different super-enhancers defined by distinct sets of TFs[105], and thus may compete against each other for MED1, resulting in exclusion of MED1 from some transcription condensates with low MED1 affinity.

Second, condensates can re-organize genome topology, changing the proximity between DNA motifs such as promoters and enhancers across the human genome (Fig. 3). For example, the transcription co-activator YAP undergoes HOPS to form nuclear condensates that exclude RNAP II within 5 min but then enrich for RNAP II after 2 h, suggesting that HOPS-induced YAP condensates dynamically regulate the transcription from specific gene loci over time[20]. Strikingly, HOPS-induced YAP condensates can also alter the organization of the accessible chromatin. Specifically, imaging studies with 3D transposase-accessible chromatin-photoactivated localization microscopy demonstrate that HOPS-induced YAP condensates colocalize with the transposase-accessible chromatin clusters, suggesting that the chromatin near the condensates adopts an 'open' conformation that favours gene expression[20].

Therapeutic targeting

The partitioning of drugs into condensates can also regulate condensate activity[81,90–93]. For example, small-molecule drugs can directly disrupt biomolecular condensates by disrupting the interactions between composite biomolecules[81], as demonstrated by the dissolution of stress granules by lipoamide[111] and bis-ANS[112]. The partitioning of chemotherapeutic drugs, such as cisplatin, into condensates can also promote their drug efficiency, at least in vitro[93]. Similarly, small molecules or engineered peptides can change the material properties of condensates to mitigate disease progression[92]. For example, the steroidal alkaloid cyclopamine and its chemical analogue A3E can harden respiratory syncytial virus inclusion bodies to prevent the replication of respiratory syncytial virus, which occurs predominantly in liquid-like inclusion body condensates[113]. Therefore, interference of pathological cellular activities through the targeting of signature condensates may represent a promising, yet understudied, therapeutic strategy for a variety of diseases, including kidney diseases.

Condensates in kidney physiology

Formation of the glomerular filtration barrier

The slit diaphragm is a specialized type of adhesion junction that connects podocyte foot processes and is a critical part of the glomerular filtration barrier[114]. The transmembrane protein, nephrin, is a core component of the slit diaphragm and has a prominent role in the development and function of the glomerular filtration barrier by interacting via its extracellular domain with its counterparts on the adjacent foot process[114]. The cytoplasmic domain of nephrin contains three tyrosine phosphorylation (pTyr) sites that can each bind the SRC homology 2 (SH2) domain of the adaptor protein Nck[52]. The three SRC homology 3 (SH3) domains on Nck can each bind to one of the six proline-rich motifs (PRM) on an actin regulatory protein called N-WASP[52]. These highly multivalent interactions among pTyr, SH2, SH3 and PRM drive the phase separation of nephrin together with a variety of scaffold and regulatory proteins that also contain the above-mentioned domains, forming nephrin–NCK–N-WASP condensates[52,115] as well as MAGI2–dendrin–CD2AP condensates that are enriched in nephrin[114] (Fig. 4a).

The function of nephrin-containing condensates is two-fold (Fig. 4a). First, they facilitate the formation of the slit diaphragm as they pull the cytoplasmic tails of multiple nephrin molecules into close proximity with one another, clustering them on the membrane for slit diaphragm assembly. Moreover, the condensates physically link the slit diaphragm to the actin cytoskeleton to stabilize the slit diaphragm[114,116]. Second, the condensates facilitate nephrin signalling and ensure maintenance of the slit diaphragm, as nephrin-containing condensates are enriched in downstream actin regulatory proteins, such as the actin nucleator ARP2/3 complex, and provide a favourable physicochemical environment for these proteins to enhance actin nucleation activity (Fig. 4a).

Other nephrin-associated proteins may also potentially contribute to the function of nephrin in podocytes through phase separation. Synaptopodin is another important protein that is associated with the nephrin complex and regulates the podocyte foot process stability via its actin-binding activity[117–119]. Synaptopodin co-localizes with MAGI2 clusters[120], which, as mentioned above, can co-condense with dendrin and CD2AP[114]. Although synaptopodin has not been shown to drive phase separation, it probably serves as a client molecule in condensates, where its function might be regulated by its partitioning into condensates. These observations suggest that biomolecular condensates could serve as regulation hubs to fine-tune the functions of key proteins in the kidney, which may be either scaffold or client molecules of the condensate.

Response to hyperosmotic shock

Currently known physiological condensates in kidneys also include a wide range of reversible condensates that are induced by hyperosmotic shock[18–20,26,28,31,121,122] (Fig. 4b–d). Osmotic fluctuations are prevalent in the kidney. Hence, cells of the renal tubule have evolved mechanisms to prevent adverse consequences associated with cellular hydration and dehydration through changes in renal cell volume. These mechanisms, termed regulatory volume increase (RVI) and regulatory volume decrease, initiate cell signalling cascades that promote the influx and efflux of solutes[2,123,124] and are, at least in part, regulated by the

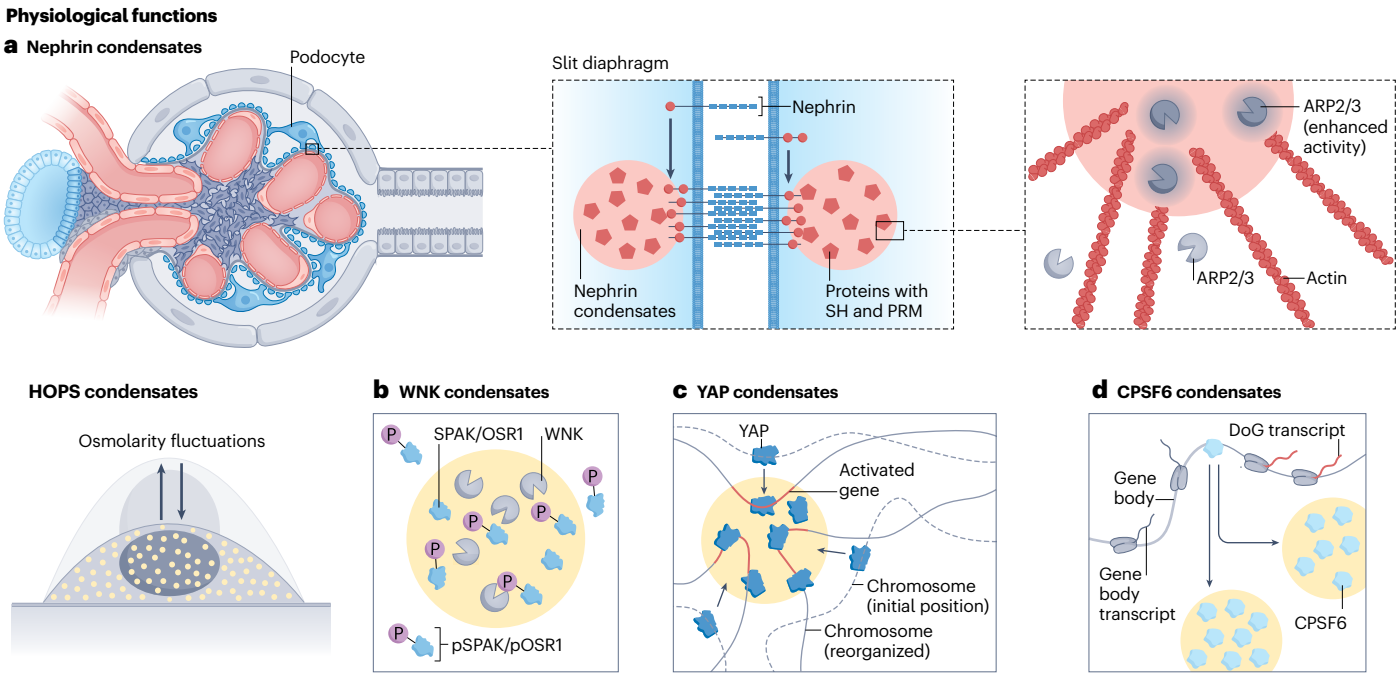
Physiological functions
a Nephrin condensates
Podocyte
Slit diaphragm
Nephrin
Nephrin condensates
Proteins with SH and PRM
ARP2/3 (enhanced activity)
ARP2/3
Actin
HOPS condensates
Osmolarity fluctuations
b WNK condensates
SPAK/OSR1
WNK
pSPAK/pOSR1
c YAP condensates
YAP
Activated gene
Chromosome (initial position)
Chromosome (reorganized)
d CPSF6 condensates
DoG transcript
Gene body
Gene body transcript
CPSF6
Pathological functions
e NRF2 condensates
Normal
Promoter
RNA↑
RNAP
DNA
Mediator
NRF2
Enhancer
KEAP1
ADPKD, NRF2↓
RNA↓
f CDYL condensates
Normal
CDYL
H3K18cr
CDYL (enhanced activity within condensate)
ADPKD, CDYL↓
Cyst-associated gene
g CRTC2 condensates
Phosphorylated CRTC2
Normal
RNA↓
P-TEFb complex
Inhibitor complex
cAMP
ADPKD, cAMP↑
RNA↑
RNAP, hyper-phosphorylated
Non-phosphorylated CRTC2
h ENL condensates
Normal, ENLwt
Histone acetylation
RNA↓
ENLwt
SEC complex
Wilms tumour, ENLmut
RNA↑
ENLmut

Fig. 4 | Functional and mechanistic implications of condensates in kidney cells. Biomolecular condensates probably contribute to both physiological (panels **a**–**d**) and pathological (panels **e**–**h**) functions in kidney cells. **a**, Nephrin co-condenses with scaffold or regulatory proteins that contain SRC homology domains (SH) and proline-rich motifs (PRMs)[52,114,115] to form the slit diaphragm, which connects adjacent podocyte foot processes[114], and to regulate their actin dynamics, for example, through co-condensation with the actin nucleator, ARP2/3 complex, to further stabilize the junction[52,115]. Hyperosmotic phase-separation (HOPS) condensates are induced by fluctuations in osmolarity (**b**–**d**). **b**, The formation of with-no-lysine (WNK) condensates phosphorylates and activates the WNK–SPAK–OSR1 pathway to restore cell volume[29,125]. **c**, YAP condensates re-organize chromatin structure and recruit the transcription machinery to activate gene expression[20,192]. **d**, HOPS-induced CPSF6 condensates sequester CPSF6 proteins away from transcription end sites and thus yield stress-induced extended downstream of gene (DoG) transcripts[18,24,25]. **e**, Healthy kidney cells with normal NRF2 expression levels form NRF2 condensates and recruit the Mediator complex to super-enhancers that maintain expression of cyst-suppressor genes. Decreased expression of NRF2 in autosomal-dominant polycystic kidney disease (ADPKD) cells disassembles NRF2 condensates and thus deactivates the cyst-suppressor genes[129]. **f**, The formation of CDYL condensates in healthy kidney cells increases CDYL activity and thus decreases histone crotonylation, suppressing cyst-associated genes. By contrast, the decreased CDYL expression in ADPKD cells results in the disassembly of CDYL condensates and promotes cyst formation[130]. **g**, Increased cAMP signalling, as occurs in ADPKD, induces the de-phosphorylation and import of CRTC2 to the nucleus, forming CRTC2 condensates that release the positive transcription elongation factor b (P-TEFb) from its inhibitor and promote P-TEFb-induced hyperphosphorylation of RNAP II and thus gene expression[132]. **h**, Mutations in eleven-nineteen-leukaemia protein (ENL) in Wilms tumour induce the formation of nuclear ENL condensates, which recruit the super elongation complex (SEC) for RNAP II hyperphosphorylation, which activates transcription from oncogenes[110].

formation of biomolecular condensates. For example, the with-no-lysine (WNK) kinase forms biomolecular condensates in the cytosol of distal convoluted tubule cells in response to changes in dietary potassium[125] (Fig. 4b). The formation of WNK condensates occurs on a very short timescale, within ~1 min, and is driven by an evolutionarily conserved IDR that endows the system with multivalency[29]. Both of these characteristics are typical of HOPS condensates[18,19].

WNK condensates enrich and promote the downstream phospho-activation of its effector kinases, SPAK (encoded by *STK39*) and OSR1 (encoded by *OXSR1*)[29]. This cascade leads to phosphorylation of the Na-K-2Cl cotransporter, NKCC1, which induces the influx of sodium, potassium and chloride ions and the phosphorylation of the K-Cl cotransporters (KCCs), which in turn blocks the efflux of potassium and chloride ions[29]. Therefore, the formation of WNK condensates helps to elevate intracellular osmolarity and normalize cell volume, thereby maintaining the volume homeostasis of distal convoluted tubule cells.

In addition to regulating kinase signalling, HOPS condensates can regulate the genome structure, as exemplified by the HOPS-induced YAP condensates, described above[20] (Fig. 4c). By inducing an 'open' chromatin conformation and recruiting TFs and RNAP II, HOPS-induced YAP condensates increase gene expression from newly accessible loci.

CPSF6 is a major component of the cleavage and polyadenylation (CPA) complex that binds and cleaves nascent pre-mRNAs at their transcription end sites (TES). Under conditions of hyperosmotic shock, CPSF6 favours homotypic self-interactions that drive phase separation away from its TES binding sites[18] (Fig. 4d). Therefore, HOPS-induced CPSF6 condensates promote readthrough transcription, which is a general hallmark of the mammalian cellular stress response. The resulting long non-coding RNAs, also termed downstream-of-gene (DoG)-containing transcripts, can be detected in association with a variety of stressors[24], including hyperosmotic shock. The formation of HOPS-induced CPSF6 condensates thus provides a molecular mechanism for the production of stress-induced DoGs, which can originate from as many as ~10% of all protein-coding genes in humans[18,25].

Roles in kidney pathology

As alterations in the formation or dissolution of condensates results in loss-of-function or gain-of-function of specific biomolecules, they can also have pathological consequences. For instance, dissolution of condensates that maintain critical functions in normal kidney cells may diminish their canonical function, resulting in loss-of-function pathogenesis (Fig. 4e–f). Conversely, the formation of biomolecular condensates under pathological conditions may promote the expression of disease-related genes, resulting in gain-of-function pathogenesis (Fig. 4g,h). As an emerging field of study, pathological condensates in the kidney have so far been mainly examined in autosomal-dominant polycystic kidney disease (ADPKD) and certain kidney cancers, such as Wilms tumour. However, to uncover the identity of additional biomolecular condensates in the kidney and stimulate further research in this field, we have surveyed the UniProt database using a combination of gene ontology keywords to find known multivalent proteins that are related to kidney physiology or disease (Table 1). Of note, this list includes p53, p63 and p73, which are members of a protein family that has been linked to kidney cancer as well as ageing, with evidence of condensate formation.

Autosomal-dominant kidney disease

ADPKD is a common genetic kidney disease and affects one in every 400–1,000 people. It is characterized by the formation of numerous fluid-filled renal cysts that progressively replace normal kidney tissue. With no cures currently available, the disease progresses to kidney failure in the majority of patients. At the molecular level, ADPKD is characterized by mutations in PKD1 or PKD2, which encode members of the polycystin family of proteins that regulate calcium permeable cation channels and intracellular calcium homoeostasis. These aberrations result in impaired ion homeostasis and consequently elevated levels of reactive oxygen species in cells. Nuclear factor erythroid 2-related factor 2 (NRF2) is an essential transcription factor and master regulator of the cellular response to oxidative stress[126] (Fig. 4e). NRF2 is critical for normal kidney function and its impaired activity, largely via its reduced expression, contributes to ADPKD progression[127]. NRF2 can activate cytoprotective antioxidant gene expression programmes by binding to specific super-enhancers in a process that is driven by the Mediator complex, which bridges transcription factors to basal transcription machinery[128]. Thus, ADPKD progression and renal degeneration are probably caused – at least in part – by the reduced expression of NRF2-regulated genes. A 2020 study discovered that the Mediator complex and NRF2 co-condense, providing a viable model for the mechanism of NRF2-dependent gene activation in healthy kidney cells[106,127]. Specifically, NRF2 condensates were confirmed by the in vitro reconstitution and over-expression of NRF2 in the 9–12 ADPKD cells. In both cases, components of the Mediator complex partitioned into NRF2

Table 1 | Multivalent proteins of relevance to nephrology

Protein name (UniProt ID)	Relevance to nephrology	Disordered (D)/homo-X-mer (X)	PSP score[142]
p53 (P04637)	Kidney cancer biomarker and therapeutic target[143] Involved in kidney injury and repair[144]	D[145]	0.94
p73 (O15350)	Required for MDCK cell morphogenesis[146] p73 enhancer hypermethylated in Wilms tumour[147]	D[145,148]	0.94
p63 (Q9H3D4)	Biomarker expressed in a high percentage of urothelial carcinomas[149]	D, X = 4 (refs. 145,150)	0.94
BEND3 (Q5T5X7)	Associates with Sall4 and NuRD to repress transcription of PTEN and SALL1 in cystic kidneys[151,152]	D, AlphaFold[153]	0.93
JMJD6 (Q6NYC1)	Mediates histone lysyl 5-hydroxylation in embryonic kidney 293 cells[154] Mediates sunitinib sensitivity in renal cell carcinoma[155] Promotes stress granule formation by demethylating G3BP1 (ref. 156)	D, X = N[157]	0.88
α-Synuclein/SNCA (P37840)	Disruption of SNCA signalling in RPTECs contributes to the pathogenesis of renal tubulointerstitial fibrosis[158]	D, X = N[159,160]	0.61
PAX2 (Q02962)	PAX2 mutants are associated with abnormal kidney development, CAKUT, and kidney oncogenesis[161] Maintains expression levels of urea transporters and aquaporins in adult renal epithelia; deletion causes severe polyuria[162]	D, AlphaFold[153]	0.53
Glutaminase kidney isoform (O94925)	Heavily studied tumour promoter and drug target[163,164] Upregulated in many glutamine-dependent cancer cells[164] Important for growth of kidney cancer[165,166]	X = 4 (refs. 167,168)	0.16
SHMT (P34896)	Enhanced expression in kidneys; possible role in the fetal control of plasma serine levels[169]	X = 4, N[170,171]	0.12
RALDH-3/ALDH1A3 (P47895)	Higher expression level in kidneys than in other organs[172] *Aldh1a3* mRNA is found in the developing papilla, ureter and ureteric bud ends during nephrogenesis[173]	X = 4 (ref. 174)	0.04
KCTD1 (Q719H9)	Controls distal nephron differentiation and protects against renal fibrosis[175] KCTD1 I27N mutant causes kidney dysfunction[176]	X = N[177]	0.02
CPSF5/CFIm25 (O43809)	2-kb transcript of CFIm25 found in kidney, RNA-looping and alternative poly(A) site selection[178]	X = 4 (ref. 179)	0.01
RALDH-2/ALDH1A2 (O94788)	Controls retinoic acid concentration required for renal development[180] Expression is directly repressed by Wnt–β-catenin signalling in fetal kidney cells[181]	X = 4 (ref. 182)	0.01
TMABA-DH/ALDH9A1 (P49189)	High expression level in kidneys[183,184]	X = 4 (ref. 185)	0.00

The proteins listed in this table were derived from a UniProt search with the gene ontology terms 'protein trimerization [0070206]', 'protein homo-trimerization [0070207]', 'protein tetramerization [0051262]', 'protein homo-tetramerization [0051289]' and 'protein homo-oligomerization [0051260]', followed by manual verification of experimental evidence for multivalency and relevance in kidney function and disease. Multivalency was determined by both the presence of an intrinsically disordered region (IDR), denoted as 'D', and the ability to form homo-multimers, denoted as 'homo-X-mer' where 'X' is the number of monomers in the multimer and 'N' indicates a homo-oligomer. The label 'AlphaFold' implies that the majority of the protein structure is an IDR, as predicted by the machine-learning-based protein prediction algorithm AlphaFold[153]. A machine-learning based prediction algorithm was used to calculate a phase-separation protein (PSP) score for each protein to estimate its likelihood of forming intracellular condensates based on the presence of an IDR, an incomplete assessment that we further augmented with information on homo-multimerization, with the final score ranging from 0 to 1; the bigger the number, the higher the likelihood that protein is predicted to form biomolecular condensates[142]. Given that the database used to train the model was largely composed of IDRs[186], structured multivalent proteins generally have low PSP scores, despite the fact that they contribute to phase separation[18]. CAKUT, congenital abnormalities of the kidney and urinary tract; MDCK, Madin–Darby canine kidney; mRNA, messenger RNA; RPTECs, renal proximal tubular epithelial cells.

condensates, suggesting that the formation of NRF2 condensates in healthy kidney cells may recruit Mediator to activate the expression of NRF2-regulated genes. Accordingly, reduced NRF2 expression levels in ADPKD kidneys failed to maintain the assembly of NRF2 condensates, deactivating NRF2-regulated genes and inducing ADPKD progression[129] (Fig. 4e).

CDYL, a chromodomain Y-like transcription repressor and crotonyl-CoA hydratase, is another phase-separating protein that is suppressed in ADPKD. Overexpression of CDYL slows cyst growth in ADPKD mouse models[130] (Fig. 4f). Histone lysine crotonylation (Kcr) induces a more open chromatin state and thus activates gene expression[131]. Of note, CDYL can remove Kcr from histones, and the formation of CDYL condensates dramatically enhances its catalytic activity. A 2022 study found that CDYL phase separation is mediated by its lysine (K) and arginine (R) residues; a mutant form of CDYL in which all K and R were mutated to alanine (A) abolished its ability to assemble in condensates, both in cells and in test tubes. This non-condensing mutant also demonstrated a significantly reduced ability to remove Kcr from histones, supporting the important role of condensation in CDYL pathology[130]. In healthy kidney cells, CDYL condensates suppress cyst-associated genes by reducing their nearby Kcr marks. By contrast, the absence of CDYL condensates in ADPKD kidney cells promotes cyst growth and ADPKD disease progression[130] (Fig. 4f).

ADPKD kidney cells not only show a reduction in the formation of condensates with beneficial physiological activity but also induced the formation of pathological condensates. CREB-regulated transcription

coactivator 2 (CRTC2) is an example of a phase-separating protein that forms condensates in cystic epithelial cells from mouse and human ADPKD kidneys, but not in healthy kidney cells (Fig. 4g). The expression of CRTC2 is induced by cAMP signalling — which is elevated in ADPKD — and promotes the translocation of CRTC2 from the cytosol to the nucleus, where it forms condensates[132]. The positive transcription elongation factor b (P-TEFb) hyperphosphorylates the C-terminal domain (CTD) of human RNAP II to stimulate transcription elongation[133]. Cyclin T1, a component of P-TEFb, can also form condensates via its IDR, and the hyperphosphorylation of the CTD of RNAP II by P-TEFb is promoted in the condensate environment[107]. Similarly, CRTC2 condensates can promote RNAP II-mediated elongation by promoting P-TEFb-mediated hyperphosphorylation of RNAP II. These steps lead to upregulation of cyst-associated genes, promoting cystogenesis[132] (Fig. 4g).

Role in kidney cancer

The gain-of-function assembly of pathological condensates can also promote kidney cancer. For example, mutations in the eleven-nineteen-leukaemia protein (ENL) enhance its self-association and thus phase separation, forming condensates that increase ENL occupancy on chromatin and elevating downstream gene expression[110]. ENL is a reader of histone acetylation via its YEATS domain, a region in which hotspot mutations can cause Wilms tumour[110]. Although mutant ENL does not demonstrate increased acetyl-lysine binding affinity, its enhanced clustering recruits more super elongation complex (SEC) to the acetylated histone region[110]. The abnormally high concentration of the SEC component, CDK9, at the acetylation region hyper-phosphorylates RNAP II and increases the expression of oncogenes related to Wilms tumour[110].

Rapid condensation by HOPS

HOPS is the rapid biomolecular condensation of multivalent proteins directly triggered by an elevation in extracellular osmolarity and the resulting water efflux and hyperosmotic cell volume compression[18,19]. Upon hyperosmotic shock, a notable fraction of the human proteome (~10%) undergoes HOPS[18,25], with each phase-separating protein reversibly forming 300 or more condensates in the nucleus or cytosol within as little as 10 s[18] (Fig. 5a). HOPS has been observed in healthy mouse kidneys[20] and cultured kidney cell lines, including HK-2 cells from the proximal tubule[18], Caki-1 from clear-cell renal cell carcinoma[18] and HEK293 from embryonic kidneys[20,28,29]. Moreover, HOPS occurs independently of osmolyte identity, as it is induced by a range of solutes in cell culture medium[18,20,29,121], including sodium chloride, sucrose and sorbitol. The injection of macromolecular crowders into cells can also directly induce HOPS[29]. Thus, kidney cells might use HOPS to sense changes in molecular crowding within cells, enabling them to monitor fluctuations in extracellular osmolarity. Different segments of the nephron experience different levels of osmolarity fluctuations under diuretic and antidiuretic conditions (Fig. 5b), and therefore HOPS may have distinct effects on kidney cells depending on their location in the nephron.

The fundamental signature of HOPS is its rapid onset, making it a first responder to hyperosmotic stress. HOPS condensates start to form within 10 s of exposure to osmotic pressure fluctuation, with the number of condensates stabilizing within ~2 min[18]. By comparison, the formation of hyperosmotic-induced stress granules is 1–2 orders of magnitude slower, with condensates forming around 10 min after exposure and maturing at around 30 min[22]. This difference is consistent with the fact that HOPS is a purely physicochemical process, whereas stress granules form upon activation of the integrated stress response pathway and phosphorylation of eIF2α[22,134]. The significantly faster response time of HOPS enables cells to sense changes in extracellular osmolarity at a high frequency and may prime slower stress responses such as stress granule formation or downstream signalling pathways. This rapid adaptation is especially essential for kidney cells that are exposed to an environment with frequent osmolarity fluctuations, such as the cells that line nephron segments, where osmolarity fluctuations can be dramatic[135] (Fig. 5b).

Use of an immunofluorescence screen has shown that many proteins with structural multivalency domains (that is, at least homo-trimerization) or IDRs undergo HOPS, underlying the observation that condensation affects a considerable proportion of the human multimeric proteome[18]. To date, four HOPS proteins have been cross-validated by different laboratories: mRNA decapping enzyme 1 A (DCP1A)[18,20], YAP[18,20], fused-in-sarcoma (FUS)[18,31,122] and TAR DNA-binding protein 43 (TDP43)[18,31]. The existence of additional HOPS proteins is supported by strong evidence of roles of HOPS-induced condensates in a variety of cell activities, such as WNK[29,125], the proteasome complex[26], apoptosis signal-regulating kinase 3 (ASK3)[28,136] and Bcl2-associated athanogene 2 (BAG2)[30], as described below. Although HOPS has been extensively validated phenomenologically, the functional consequences of HOPS remain partly elusive.

Proteasomes are sophisticated macromolecular complexes that are involved in protein degradation. In the case of ubiquitin-dependent protein degradation, proteasomes are highly selective for lysine 48 (K48)-linked polyubiquitinylated substrates[26]. Proteasomes do not partition into most nuclear condensates, such as nuclear bodies of promyelocytic leukaemia protein (PML) or Cajal bodies[26]. However, a 2020 study in retinal pigment epithelial hTERT RPE-1 cells found that proteasomes undergo phase separation upon hyperosmotic shock[26]. These proteasome condensates assemble within 1 min under 0.2 M sucrose shock, and are driven by two multivalent ubiquitin-associated domains on proteasome substrate-shuttling factor RAD23B and four or more copies of K48-linked polyubiquitin on its substrates[26]. Both of these features — that is, its rapid onset and the multivalency of 'scaffold' molecules — align with the key characteristics of HOPS[18]. Hyperosmotic shock also perturbs ribosome biosynthesis so that ribosomal proteins that fail to incorporate into ribosomes are degraded. HOPS-induced proteasome condensates thus help to degrade unassembled orphan ribosomal proteins[26].

ASK3 is a kinase that is involved in the apoptosis signalling pathway. It can also suppress the aforementioned WNK–SPAK–OSR1 pathway through bidirectional hypo-osmotic and hyperosmotic-driven effects on ASK3 phosphorylation and thus activity[137,138]. Experiments performed in the embryonic kidney cell line, HEK293A, show that under hyperosmotic shock, ASK3 is dephosphorylated by protein phosphatase 6 (PP6)[138], which diminishes its ability to suppress WNK[137], and thus promotes RVI via the WNK–SPAK–OSR1 pathway[138]. The assembly of ASK3 condensates under hyperosmotic shock occurs within 5 min and relies on the multivalency provided by its coiled–coil domain, IDR and a PAR-binding motif (PBM) that binds to an important signalling polymer, the poly(ADP-ribose) (PAR)[28]. The characteristic short timescale of this process supports the involvement of HOPS in this process[18]. HOPS-induced ASK3 condensates are enriched in PP6, which promotes ASK3–PP6 interactions and facilitates ASK3 dephosphorylation and RVI[28]. Thus, HOPS-induced ASK3 condensates function in a similar manner to HOPS-induced WNK condensates, working to maintain cell volume homeostasis. Notably, the liquidity of ASK3 and DCP1A HOPS condensates, but not HOPS-induced WNK condensates, is enhanced

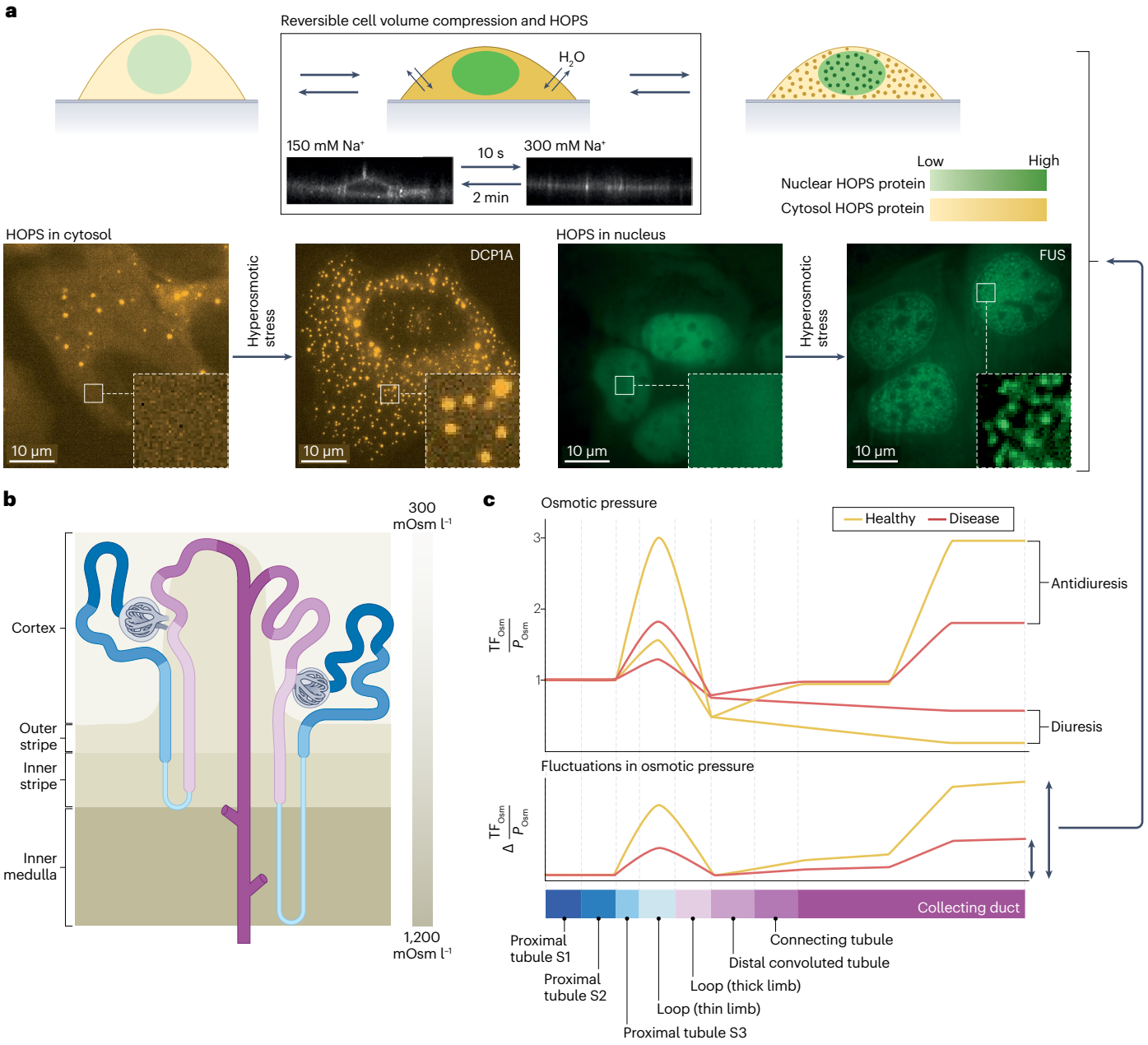

Fig. 5 | Induction of hyperosmotic phase separation by fluctuations in osmotic pressure in the nephron. a, Hyperosmotic shock causes cell volume compression and hyperosmotic phase separation (HOPS) within ~10 s. HOPS affects both nuclear and cytosolic proteins, each forming hundreds of condensates. Proteins enriched by other condensates can form HOPS condensates outside of their homo condensate. For example, the cytosolic RNA decapping enzyme mRNA decapping enzyme 1 A (DCP1A) is enriched in processing bodies under unstressed conditions and forms HOPS condensates outside of processing bodies during hyperosmotic stress. Similarly, fused-in-sarcoma (FUS) does not form nuclear condensates in unstressed U2-OS cells but, under hyperosmotic conditions, forms HOPS condensates in the nucleus, which are distinct from the stress granules FUS forms in the cytosol. **b**, Different segments of the nephron experience different levels of fluctuations in osmotic pressure[193]. **c**, Fluctuations in osmotic pressure along the nephron vary under diuretic and antidiuretic conditions and in response to disease. The fluctuation in osmotic pressure can be visualized by plotting the nephron segments against the osmotic pressure measured as a ratio between the osmolarity in the tubule fluid (TF_{osm}) and the osmolarity in the plasma (P_{osm}). The amplitude of such fluctuations can be estimated by taking the difference between two extreme osmotic pressure values, namely under antidiuresis and diuresis conditions, in both healthy and diseased kidneys. In acute kidney injury or chronic kidney diseases, kidney function is reduced and thus its ability to hold a strong osmolarity gradient decreases. Therefore, the osmolarity fluctuations in diseased kidneys are likely to be smaller than in healthy kidneys, as shown in the speculative disease curves. The *y*–*z* projection in part **a** is reprinted with permission from ref. 18, Cell Press. Part **c** is adapted with permission from ref. 194, Elsevier.

by RVI-induced sodium ion influx[136], suggesting that the RVI process may in turn regulate HOPS.

BAG2 is another stress response protein that acts as a co-chaperone and a nucleotide exchange factor (NEF) for the molecular chaperone heat shock protein 70 (HSP70)[139,140]. In the African green monkey kidney fibroblast-like cell line COS7, BAG2 forms condensates within 5 min of exposure to hyperosmotic stress[30]. These BAG2 condensates recruit the 20 S proteasome, its ubiquitin-independent activator PA28 and the cytotoxic tau protein, which degrades tau under stress conditions[30]. Thus, HOPS condensates may also serve as ubiquitin-independent degradation organelles to protect the viability of kidney cells under conditions of stress[30].

The induction of HOPS condensates by fluctuations in osmolarity suggests that HOPS may have a role in pathological conditions of the kidneys, where the amplitude and frequency of osmolarity fluctuations are altered (Fig. 5b). From one perspective, the increased or decreased ability of a protein to form HOPS condensates may provide insights into disease pathogenesis. Alternatively, alterations in the corticopapillary osmolarity gradient induced by an acute or chronic kidney disease may alter the amplitude of osmolarity fluctuations, with potential effects on the assembly of HOPS condensates. In the case of WNK, for example, we now know that the WNK kinase can form HOPS condensates[29,125] and that HOPS-induced WNK condensates amplify WNK signalling and thus RVI[29,125]. We also know that malfunction of the WNK signalling pathway causes salt-sensitive hypertension in patients with pseudohypoaldosteronism type II (PHAII)[141]. Consequently, the following hypotheses can be derived. First, the loss of HOPS condensates may serve as a driver for pathogenesis. For patients with PHAII who carry a mutant WNK, the mutations may hinder the ability of WNK to form HOPS condensates, thereby affecting the signalling strength of the WNK pathway. Second, the loss of HOPS condensates may be an intermediate step in disease pathogenesis. Patients with PHAII can carry mutants in non-WNK proteins on the WNK pathway, such as Kelch-like 3 (KLHL3) or cullin 3 (CUL3)[141], but may still have a reduced amplitude in osmolarity fluctuations, which could affect the condensation of WNK. Third, it is also possible that HOPS condensates have a role in other scenarios that are associated with alterations in osmotic gradients, including ureteral obstruction, venous congestion and ageing. However, no data are currently available to support these hypotheses. Whether dysregulation of HOPS is sufficient to drive disease and whether restoring normal function ameliorates diseases remain to be tested.

Conclusions

Despite the discovery of biomolecular condensates over 100 years ago[40–42], the phenomenon of phase separation has only gained widespread appreciation in cell biology in the past decade, with a growing number of studies demonstrating the importance of this process to the organization of cellular biochemistry[6–10]. The physicochemical principles that govern biomolecular condensates render the condensates very sensitive to physiological conditions and biochemical reaction products (Figs. 1,2), endowing them with the properties necessary to function as regulatory switches to maintain homeostasis, respond to stress or cause disease. Consequently, biomolecular condensates have a prominent role in a variety of cell types and organs, under both physiological and pathological conditions. A general framework of enrichment or exclusion by condensates could be used to dissect the molecular impact of condensates on different cellular processes (Fig. 3); such a framework transcends all currently known biomolecular condensates in heathy and diseased kidneys (Fig. 4).

As fundamental physicochemical processes, one may even speculate that phase transitions were among the evolutionarily first processes to which protocells had to adapt under inevitably changing environmental conditions, including fluctuations in temperature, pH and hydration state. HOPS in particular might therefore have long-standing evolutionary roots that cells eventually adopted to leverage to maintain homeostasis. The observation that condensates form in kidney cells upon osmotic stress implies that HOPS may be an early cellular response to osmotic fluctuations experienced by kidney cells. This evidence, combined with the biophysical understanding that HOPS buffers fluctuations in osmotic pressure, while sensing molecular crowding, supports an essential role for phase transitions in kidney function (Fig. 5). Deciphering the underlying subcellular and molecular mechanisms is worth a call to action. To spur further study of biomolecular condensates in the kidney, we present a bioinformatic analysis with manual verification, arriving at a proposed list of protein candidates for HOPS (Table 1). Our hope is to help the nephrology field to engage with an emerging, yet under-studied, physicochemical phenomenon that regulates important biological processes within kidney cells.

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Author contributions

All authors contributed substantially to discussion of the content and writing of the article. G.G., E.S.S. and N.G.W. reviewed and edited the manuscript before submission. G.G. and E.S.S. performed the bulk of the background research for the article.

Competing interests

The authors declare no competing interests.

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