ROADMAP

A framework for understanding the functions of biomolecular condensates across scales

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Abstract | Biomolecular condensates are found throughout eukaryotic cells, including in the nucleus, in the cytoplasm and on membranes. They are also implicated in a wide range of cellular functions, organizing molecules that act in processes ranging from RNA metabolism to signalling to gene regulation. Early work in the field focused on identifying condensates and understanding how their physical properties and regulation arise from molecular constituents. Recent years have brought a focus on understanding condensate functions. Studies have revealed functions that span different length scales: from molecular (modulating the rates of chemical reactions) to mesoscale (organizing large structures within cells) to cellular (facilitating localization of cellular materials and homeostatic responses). In this Roadmap, we discuss representative examples of biochemical and cellular functions of biomolecular condensates from the recent literature and organize these functions into a series of non-exclusive classes across the different length scales. We conclude with a discussion of areas of current interest and challenges in the field, and thoughts about how progress may be made to further our understanding of the widespread roles of condensates in cell biology.

Material properties
In the context of biomolecular condensates, physical properties of the assembly of constituent macromolecules including viscosity, surface tension and porosity.

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https://doi.org/10.1038/s41580-020-00303-z

Over the last decade, it has become broadly appreciated that many cellular structures consist of membraneless assemblies of proteins and/or nucleic acids. These structures, termed biomolecular condensates owing to their ability to selectively concentrate molecules in defined foci, are pervasive through biology and provide a fundamental mechanism of cellular organization.

A large number of studies using both natural and engineered proteins designed to form condensates, as well as theoretical and experimental results from polymer chemistry and physics, have provided a solid foundation explaining how condensates form in a regulated fashion. This work has shown that many condensates form via liquid–liquid phase separation (LLPS)[1,2], a thermodynamic process by which — above a threshold macromolecule concentration — separate, coexisting liquid phases form to minimize free energy. It is important to note, however, that alternate mechanisms of condensate formation can also exist (BOX 1). Material properties of condensates are distinct from their surroundings, including higher viscosity and internal structure that can exclude molecules in a size-dependent manner[1]. LLPS results in interfacial tension between the dense and dilute phases, resulting in spherical dense-phase droplets that relax upon fusion. In addition to forming three-dimensional droplets, condensates may also form on membranes[3].

Studies have revealed the central role of multivalent interactions in driving condensate formation[1,4], involving folded protein domains, intrinsically disordered regions[4,5], nucleic acids[4] and chromatin[6]. Now, we have substantial mechanistic insights into how these interactions contribute to condensate formation[7–12] and how they are regulated by post-translational modification[13,14], binding interactions[15–18] and environmental conditions[19–21]. A great deal of work has also demonstrated the relationship of aberrant condensates to diseases including neurodegeneration and cancer[22,23]. As mechanisms of condensate formation have become better understood and broadly appreciated, attention has begun to shift towards condensate functions. This has brought new challenges to the field and has required development of new technologies and experimental systems.

In this Roadmap, we highlight representative examples of condensate functions from the recent literature and organize these findings into a framework that allows recognition of mechanistic commonalities between condensates that act in different biological processes. The framework consists of functional classes organized by the length scales on which they operate (FIG. 1). Our goal is not to comprehensively discuss all known condensate functions, but rather to use examples to illustrate classes of functional mechanisms within this framework.

Box 1 | **Phase separation versus macromolecular assembly in condensates in vivo**

As interest in liquid–liquid phase separation (LLPS) as a mechanism for organizing macromolecules in cells has increased, so have criticisms of the quality of evidence for LLPS in vivo[141,195]. Other mechanisms for generating biomolecular condensates — broadly defined as concentrated foci lacking a surrounding membrane — have been proposed and the full range of mechanisms should be considered when interpreting experimental results.

Condensates can form via either active processes, which consume energy to organize molecules, or passive thermodynamic mechanisms, which do not require energy input. Both mechanisms may contribute to one condensate, which appears to occur for the nucleolus[196]. Active processes take many forms[24], for example deposition of molecules at a defined location by motors or constraint of molecules by applied forces, and we will not consider them further here.

For passive thermodynamic mechanisms, important considerations include assembly cooperativity, size scaling as a function of macromolecule concentration, stoichiometry of components within the condensate and condensate material properties. LLPS lies at one end of the spectrum for these parameters, as the transition between one-phase and two-phase regimes is infinitely cooperative, the dense phase volume grows without bounds as the macromolecule concentration increases before ultimately re-entering the one-phase dense regime and (for multicomponent systems) dense phases can form with a wide range of subcomponent stoichiometries[126]. Distinct material properties arise from LLPS, most prominently interfacial tension between the two phases.

At the other end of the spectrum, condensates may form through molecules binding to static cellular structures, for example RNA aggregates or genomic DNA. Formation of such condensates would be conceptually equivalent to ligands binding to a receptor with multiple binding sites, and may or may not exhibit cooperativity. In this case, the condensate size would be limited by the number of binding sites on the underlying structure. Likewise, the stoichiometry of components within the condensate would be dictated by binding site availability, interaction affinities between the condensate 'client' and the underlying structure, and total concentrations for each component. Importantly, assembly behaviour and composition of such condensates may resemble LLPS in certain affinity, cooperativity and concentration regimes.

Further challenges in defining mechanisms of condensate formation in vivo arise from the thermodynamics of small-number systems (containing only tens to hundreds of individual molecular species), where stochastic assembly state fluctuations become significant, as described elsewhere in this Roadmap (see below in subsection The question of size)[154,155]. Experimental findings such as rapid recovery of fluorescent signal after photobleaching are not sufficient to conclude a condensate forms via LLPS, and single-molecule imaging can provide more detailed information[141]. Similarly, the absence of a single, fixed threshold concentration for condensate formation is insufficient to exclude LLPS if the system consists of more than one component[128]. Researchers working to define condensate assembly mechanisms should therefore focus on the key factors of assembly cooperativity, size scaling, stoichiometry of subcomponents and material properties of dilute-dense phase interfaces, particularly the dynamics of molecules at such boundaries.

Finally, as this Roadmap aims, in part, to describe the cellular functions of biomolecular condensates, it is important to consider how proposed functions depend on specific condensate assembly mechanisms. For example, models invoking condensation to buffer gene expression noise[127] require formation via LLPS, but biochemical reaction rates could be enhanced in clusters of molecules bound to a static scaffold. Thus, researchers should consider whether functions ascribed to condensates require formation via a specific mechanism and whether a given formation mechanism excludes or indicates certain functional properties.

Interfacial tension
(Also known as surface tension). For separate liquid phases in contact with each other, the work required to increase the surface area of contact between the two phases. In the absence of external forces, interfacial/surface tension causes phase-separated liquids to form spherical droplets as spheres have minimal surface area for a given volume.

Multivalent interactions
Interactions occurring between macromolecules with multiple sites of interaction, such that each molecule can interact with multiple binding partners.

Intrinsically disordered regions
Protein regions that do not adopt any stable ordered three-dimensional structure.

Law of mass action
In the context of enzymology, the principle that the chemical reaction rate is proportional to the concentration of enzymes and substrates.

Ribulose bisphosphate carboxylase/oxygenase
(Rubisco). An enzyme acting in carbon fixation in photosynthetic organisms, catalysing the reaction between ribulose bisphosphate and atmospheric carbon dioxide.

However, we do provide a more comprehensive list of processes, organized by functional class (including some cases where functions are unclear or under debate), in TABLE 1. The mechanistic classes we discuss are not intended to be mutually exclusive, and multiple principles likely operate simultaneously for a given condensate depending on the relevant length scale(s). We feel that blurring of boundaries between these classes will serve as useful focal points for future experimental efforts. Finally, we suggest interesting avenues of future research, including the need for new technologies to address challenges in developing and confirming functional models. Throughout, we focus on condensate functions in normal physiology, and direct readers to excellent reviews on the physicochemical underpinnings of condensate formation[2,24,25] and the role of condensates in human disease[2,22,23].

Molecular-scale functions

Within biomolecular condensates, locally increased concentrations of specific proteins, nucleic acids and small molecules lead to several functions that depend on the proximity and interactions of individual molecules. We term these molecular-scale functions, which include enhancement or suppression of biochemical reaction rates and regulating the folding state of condensate-localized proteins or nucleic acids.

Regulating biochemical reaction rates

At the shortest length scale, condensate formation may enhance reaction rates (binding, catalysis and so on) due to the high local concentration of reactants in the condensate compared with the surroundings, according to the law of mass action (FIG. 2a). Recent data have borne out this prediction in various biological systems, and have shown different modes of regulation depending on which molecules are concentrated. Condensates may also enhance or suppress reactions through mechanisms beyond mass action, such as by controlling the structural organization or dynamics of molecules (FIG. 2b,c).

Enhancing activity through concentrating enzymes and substrates. Various studies in diverse biological systems have shown enhanced catalytic activity within condensates owing to co-concentration of enzymes and substrates (FIG. 2a). One such example has been proposed to accelerate the rate-limiting step in photosynthetic carbon fixation, carboxylation of ribulose bisphosphate (RuBP) by the enzyme ribulose bisphosphate carboxylase/oxygenase (Rubisco)[26]. Rubisco is a very slow

Cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) synthase
(cGAS). An innate immune signalling enzyme that senses cytosolic DNA, a pathogen-associated molecular pattern, and produces cGAMP, which activates the stimulator of interferon genes (STING) protein to induce pro-inflammatory transcriptional responses.

enzyme and has a non-productive side reaction, involving oxygenation of RuBP instead of carboxylation. To counter this, prokaryotic and eukaryotic photosynthetic organisms have independently evolved distinct multivalent ligands that interact with the octameric Rubisco to drive LLPS[27–31]. These condensates concentrate Rubisco and carbon dioxide, greatly enhancing carboxylation activity[32,33]. Mutations disrupting Rubisco condensation or carbon dioxide enrichment produce dramatic growth defects[28,34–36]. Condensation thus enhances both the reaction rate, via enzyme and substrate co-concentration, and the specificity owing to preferential concentration of carbon dioxide over the competing oxygen substrate (FIG. 2a). General mechanisms by which condensates concentrate small-molecule substrates are poorly understood. For example, certain intrinsically disordered region sequence features may promote interactions with hydrophobic or charged small molecules. Metabolomics experiments, coupled with computational studies, may help discern whether certain classes of small molecules are preferentially enriched in condensates and identify molecular mechanisms of enrichment.

A second example of co-concentration of enzyme and substrate can be found in the innate immune system, where the DNA sensor cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) synthase (cGAS) phase separates upon binding long, nucleosome-free, double-stranded DNA[37]. Structural studies indicate that DNA also induces activating conformational changes in the cGAS catalytic site[38]. Together, concentration and allostery dramatically increase the enzymatic activity of cGAS in vitro and lead to increased cGAMP levels in cells[39]. Mutations in cGAS that abrogate condensation have large effects on its activity[37]. Deconvolving the contributions of condensation and allosteric activation remains challenging, although both are likely necessary for maximal activation of enzymatic function.

A third example occurs in RNAi, where mRNA deadenylation — the first and rate-limiting step in mRNA degradation — is thought to occur in cytoplasmic condensates (RNA granules) containing endonuclease argonaute 2 (AGO2). It was shown in biochemical reconstitutions that human AGO2 makes multivalent interactions with the microRNA-induced silencing complex component TNRC6B, driving LLPS[40]. The resulting droplets concentrate the deadenylation enzyme complex, CCR4–NOT, together with target mRNA substrates, accelerating the deadenylation reaction. Acceleration occurs either when both AGO2 and TNRC6B are above the LLPS threshold concentration or at sub-threshold concentrations when LLPS is induced by crowding agents, suggesting that deadenylation is increased specifically via condensation. The role of condensation in RNAi in vivo remains unclear, as previous work demonstrated little effect on RNA silencing upon condensate disruption[41,42]. A possible explanation is that RNA silencing may occur in condensates that are too small to observe using standard microscopy methods, a general challenge for evaluating condensates in vivo (discussed in more detail below). This possibility could be addressed through super-resolution imaging studies that, coupled with development of activity-dependent fluorescent probes, would allow simultaneous analysis of condensate formation, structure and enzymatic activity.

Nucleation-dependent processes are particularly sensitive to changes in concentration, and thus should be especially susceptible to regulation by condensates. This has been demonstrated in control of microtubule polymerization, a process that is rate-limited by nucleation and only occurs stably above a threshold concentration of the αβ-tubulin dimer[43]. Recent work biochemically reconstituted centrosomes — condensates that nucleate microtubules in vivo — through self-assembly of the key scaffold protein, SPD-5, along with other centrosome proteins, PLK1, SPD2, ZYG9 and TPXL1 (REF.[44]). When maintained in a liquid-like state, these reconstituted condensates recruited and concentrated αβ-tubulin, accelerating microtubule nucleation. Similarly, it was

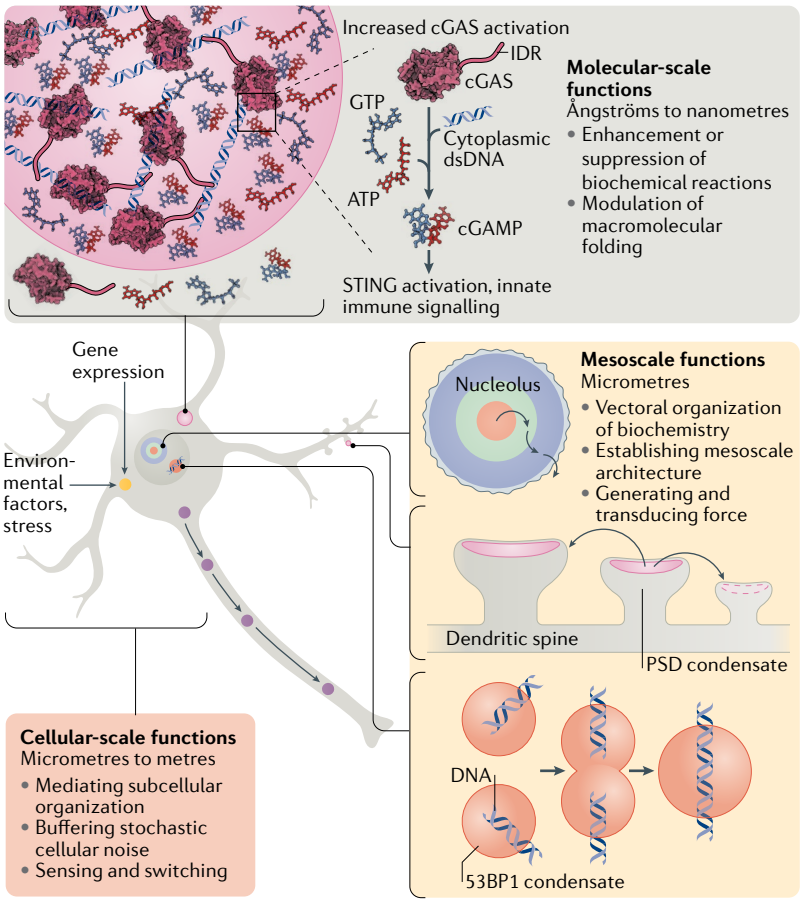

Fig. 1 | **Overview of biomolecular condensate functions across scales.** The functions of biomolecular condensates operate on multiple length scales, ranging from the atomic or molecular level to the cellular level, which, in principle, operates on metre length scales in cells such as mammalian neurons. This Roadmap has catalogued the different condensate functions reported in recent literature into functional categories and assigned them according to the length scale on which they operate. It is important to note that a condensate may have functions on more than one length scale, for instance participating in mesoscale vectoral organization of biochemistry while also enhancing reaction rates via mass action due to increased concentration of substrates and/or enzymes within condensates. By this classification — based on mechanistic commonalities between condensates with roles in different biological processes — we hope to provide a framework for understanding how condensates contribute to cell function. cGAS, cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) synthase; dsDNA, double-stranded DNA; IDR, intrinsically disordered region; 53BP1, p53 binding protein 1; PSD, postsynaptic density; STING, stimulator of interferon genes.

Table 1 | **Examples of the functional mechanisms of biomolecular condensates in biological processes**

Functional mechanism	Biological process	In vitro	In vivo
Enhancing activity through concentrating enzymes and substrates	Carbon fixation	28,30	31
	Innate immune signalling	37	37
	RNAi	40	–
	Microtubule nucleation/spindle assembly	44–47,171	44,171
	Cell–cell adhesion	49	48,49
	Autophagy	105	105
	RNA degradation	172	172
	RNA splicing	173	173
	Pre-mRNA processing	NA	174,175
Inhibition of activity through sequestration	Transcription regulation	NA	51,52
	Cell fate determination under stress	NA	176
Modulation of activity through exclusion of factors	Adaptive immune signalling	54	54
	Synaptic transmission	56	NA
Factors beyond concentration that modulate enzyme activity	RAS activation in signal transduction	61	NA
	Nucleation of actin filaments	60	60
	Regulation of mRNA translation and deadenylation	63	NA
	Bacterial cell polarization	NA	177
	Membrane protein trafficking	NA	65
Regulating macromolecule folding state	Nuclear protein homeostasis	NA	66
	RNA homeostasis	70,72,73	70–73
Vectoral organization of biochemistry	Ribosome biogenesis	76,79	77,78
	Transgenerational inheritance of RNAi	NA	80
Establishing mesoscale architecture	Synaptic transmission	56,84,85,178	85
	DNA damage response	91,95,96,98	91,95,96,98
	DNA replication	99	NA
	Autophagy	103–105,179	103–105,179
	Centriole biogenesis	180	180
Mediating subcellular organization	Localized neuronal translation	113	113
	Heterochromatin maintenance	123	123
	Germ cell fate maintenance	181	81
Buffering stochastic cellular noise	Enhancing robustness of cellular signalling processes	NA	127
Sensing and switching	Heat shock response	19,134	19,134
	Nutrient stress response	20,182,183	20,182,183
	Protein homeostasis	NA	184
Unknown or debated functions	Transcription	150,158,159,162,185,186	149–151,158–162,185,186
	Chromatin organization and heterochromatin compaction	6,118,120,187,188	120,187,188
	Viral replication/evasion of host innate immunity	NA	189–191
	Protein degradation	192	192
	Cell motility	NA	193
	Sorting mRNA to distinct granules	12	12
	Nucleocytoplasmic transport	194	NA

NA, not available.

Allostery
Regulation of enzyme activity via binding by a second molecule at a site other than the enzyme's active site, often by inducing a conformational change.

Scaffold
In simple molecular systems, a macromolecule that is required for condensate formation. The other, general group of condensate components are client molecules, which bind to and selectively partition into condensates without affecting condensate formation. In many natural condensates, this distinction is not absolute, and whereas some macromolecules act as pure scaffolds and some as pure clients, others can have varying impacts on the formation (threshold concentration) and composition of the compartment.

shown that TPX2, a protein that enhances nucleation of new microtubules that branch from the sides of existing microtubules, undergoes LLPS with αβ-tubulin[45]. These condensates were shown to interact with the microtubule lattice and accelerate branching nucleation in cell extracts. Nucleation enhancement in both cases correlated with αβ-tubulin enrichment, suggesting that the condensate functions by increasing the concentration to increase assembly rates. Similar mechanisms may be involved in the function of the mitotic

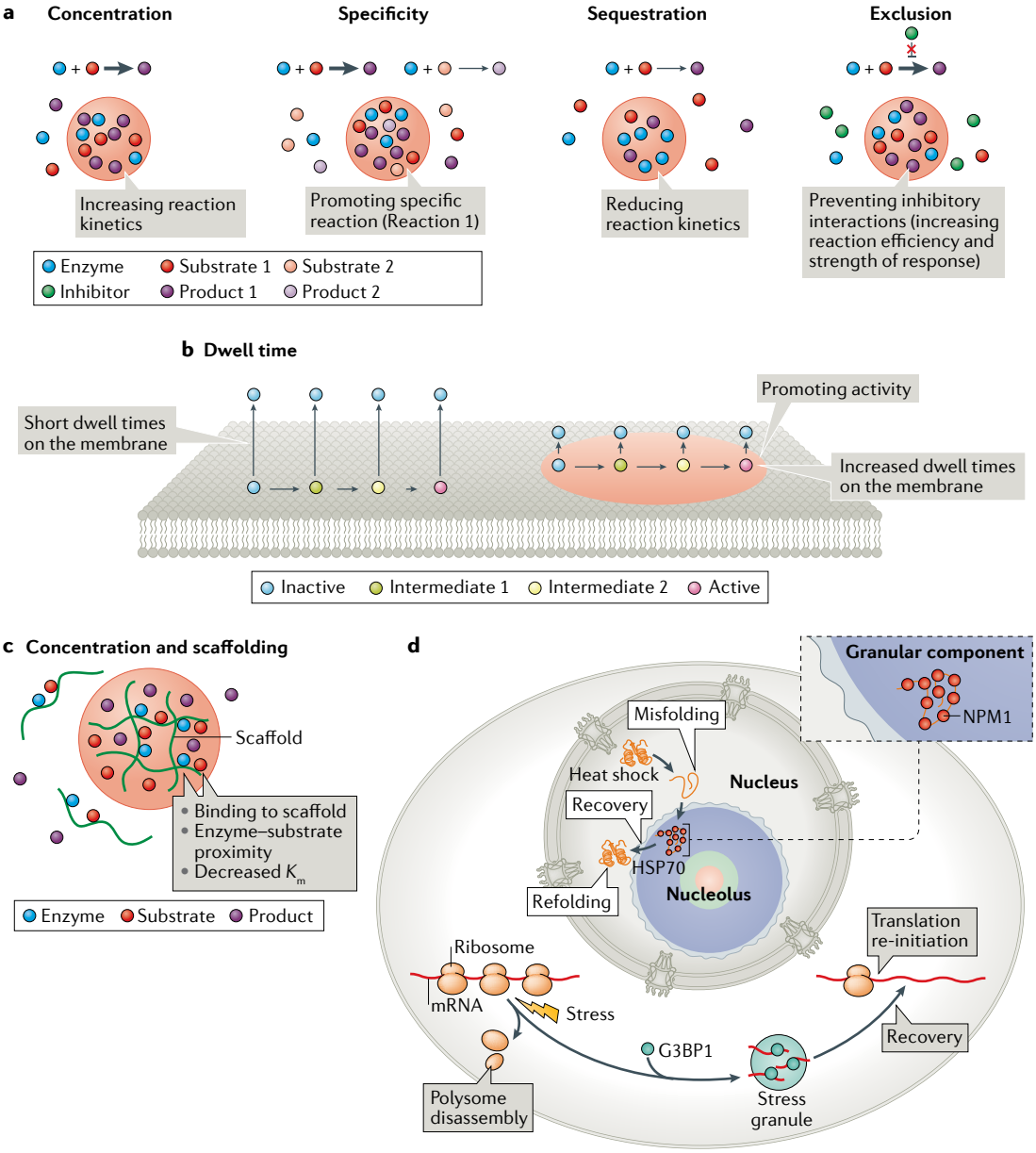

Fig. 2 | **Molecular-scale functions of biomolecular condensates. a** | Different effects of condensates on biochemical reaction rates related to mechanisms of mass action. Enrichment of both enzyme and substrate into condensates results in a substantial increase in product within the condensate, with unchanged or decreased activity in the surrounding bulk. Condensate enrichment of only one component — an enzyme, for example — will result in higher condensate activity of the enzyme but lower bulk activity due to enzyme sequestration away from its substrate. The overall biochemical activity decreases due to the relatively small condensate volume being unable to compensate for the loss of activity elsewhere. If there is an inhibitor that is excluded from the condensate, together with the concentration of enzyme and substrate within condensates, this will further promote product generation, as compared with concentration alone. **b** | Mechanisms independent of purely concentration/exclusion of components, such as increased dwell time on two-dimensional membrane-associated condensates, can also enhance reaction rates. For slow reactions, decreasing the rate at which a protein (such as an enzyme) diffuses away from the membrane increases the probability of a productive reaction (in this case, enzyme activation) by allowing more time for the intermediate steps to take place. **c** | Scaffolding of reaction components can further promote reaction rates, in a concentration-independent mechanism. In this case, close tethering of reactants (for example, enzyme and substrate) by a scaffold can result in an apparent decrease in the K_M value, accelerating the reaction. **d** | Roles of condensates in the response to macromolecule misfolding. Misfolded nuclear proteins (resulting from stress exposure, such as heat shock) are recruited into the granular component of the nucleolus, where they interact with resident proteins such as nucleophosmin 1 (NPM1). These interactions maintain misfolded proteins in a state where refolding can occur aided by molecular chaperones, including heat shock protein 70 (HSP70), after stress subsides[66]. In the cytoplasm, stress leads to polysome disassembly and mRNA release. Condensates termed stress granules form by interaction between mRNA and the essential stress granule assembly factor G3BP1, which prevents base pairing and aberrant RNA aggregation, allowing translation to resume when stress is relieved.

spindle protein BugZ, which undergoes LLPS in vitro and recruits αβ-tubulin and the spindle assembly factor Aurora A kinase[46,47]. Together, these studies illustrate the ways condensates may enhance formation of nucleated assemblies, which may occur in other biological contexts including cell–cell adhesion[48,49].

Beyond promoting biochemical activity per se, the ability to concentrate certain molecules over others may also afford reaction specificity (FIG. 2a). It has recently been reported that in a biochemical reconstitution of the SUMOylation cascade in engineered condensates, when two substrates were present, only the one recruited into the condensates was SUMOylated efficiently[50]. However, in this system, rate enhancement and specificity were constrained by the properties of enzymes and substrates, in manners that should be general. For example, the relative change in reaction rate due to association with a condensate depended on the K_M value for each individual substrate: substrates present at concentrations well above their K_M value exhibited little change in the reaction rate upon condensate formation because the enzyme was already saturated even in the initial homogeneous solution; by contrast, when substrates were present at concentrations well below their K_M value, formation of condensates produced strong reaction rate enhancement. Thus, reaction rates for specific substrates can be enhanced upon condensate formation, whereas for others these rates will change only marginally, dictated by the substrate concentrations, K_M values and degree of enrichment in the dense phase[50]. These considerations may be important in understanding specificity of pathways such as RNAi, where thousands of different substrates can compete for the same enzyme.

Inhibition of activity through sequestration. Apart from enhancing activity, condensates may act to decrease activity by sequestering molecules from their sites of action and/or substrates (FIG. 2a). As detailed below, this activity requires substantial depletion of molecules from the cytoplasm/nucleoplasm, which demands very large condensates and/or very high degrees of concentration in them.

Paraspeckles are condensates that appear to act by sequestration. The primary scaffold for paraspeckles is the long non-coding RNA, NEAT1, which recruits the transcription regulator protein SFPQ. Proteasome inhibition or viral infection upregulates NEAT1 transcription and enlarges paraspeckles, thereby depleting SFPQ by up to 50% from the nucleoplasm[51]. This depletion then leads to repression of SFPQ target transcripts, providing an example of how sequestration of proteins within condensates can exert regulatory functions.

A second example of sequestration comes from plants, in which the auxin response factor (ARF) proteins, ARF7 and ARF19, are localized primarily within cytoplasmic condensates. Cytosolic sequestration of these transcription factors inhibits their nuclear translocation, blocking their gene regulatory activities in response to plant hormone auxin[52]. Cell-to-cell differences in ARF7/19 nuclear localization give rise to varying auxin responsiveness, such that actively growing cells respond differently from quiescent cells even in the presence of the same hormone signal. Sequestration in cytoplasmic condensates thus represents a short length-scale biochemical inhibition that is coupled to cellular-scale effects on nucleocytoplasmic transport and response to signalling molecules, demonstrating how condensates can have interrelated functions at different length scales.

Together, these examples illustrate how condensates can inhibit activity through sequestration of transcription factors away from their sites of action, an effect that can be readily tuned and reversed upon environmental changes or signalling.

Modulation of activity through exclusion of factors. In addition to recruiting components, condensates can enhance activity by excluding a negative regulator (FIG. 2a). Two recent studies have demonstrated this effect in vitro. The first involves T cell receptor (TCR) signalling clusters, which are 2D, membrane-associated condensates that form in T cells upon antigen recognition[53–55]. In this system, TCR activation causes phosphorylation of the scaffold protein, LAT, which undergoes LLPS with a group of adaptor proteins. LAT phosphorylation is antagonized by the phosphatase CD45. In a biochemical reconstitution, LAT clusters partially exclude CD45 owing to charge-mediated repulsion, thereby stabilizing the clusters against dephosphorylation-triggered dissolution[54]. A possible cellular consequence of CD45 exclusion is to provide positive feedback in LAT phosphorylation, prolonging TCR signalling and increasing sensitivity to antigen stimulation.

The second study investigated condensation of components of the postsynaptic density (PSD), a protein dense structure that clusters postsynaptic ion channels for activation in response to neurotransmitter release[56,57]. Synapses can be excitatory or inhibitory, and their developmental pathways are mutually exclusive, suggesting competition between the two outcomes. Gephyrin is an inhibitory postsynaptic scaffold protein, which was found to be partially excluded from biochemically reconstituted PSD condensates by an unknown mechanism not based on molecular size. This exclusion could have ramifications for establishing either an excitatory or inhibitory synapse during development. These examples illustrate how exclusion of molecules from condensates can have both short length-scale effects via changing biochemistry and large length-scale effects on cellular function.

In general, the physical mechanisms of exclusion from condensates are unclear. These may involve specific constraints on charge as discussed above. Molecular size will also likely have a role, as the size of pores within the macromolecular networks composing condensates can impose diffusion barriers[58]. With improved physical models of condensates, it may be possible to determine constraints on charge density required for charge repulsion-mediated exclusion, and to discover specific molecular features that dictate the effective pore volume of condensates.

Factors beyond concentration that modulate enzyme activity. Recent studies have implicated mechanisms apart from mass action in regulating reactions within condensates. This can occur through changes in the

K_M
A parameter of the Michaelis–Menten model of enzyme kinetics, describing the concentration of a substrate molecule at which the rate of product formation reaches half of the maximum possible rate under a given set of conditions. If the rate of enzyme–substrate binding is rapid relative to catalysis, the K_M value approximates the dissociation constant for the enzyme–substrate complex.

Paraspeckles
Nuclear condensates implicated in RNA base editing as well as transcriptional regulation. Paraspeckles are formed from the long non-coding RNA NEAT1 and the DBHS family of proteins (NONO, SFPQ and PSPC1).

Kinetic proofreading
A biochemical error-correction mechanism favouring reaction pathways that lead to correct over incorrect products, wherein an irreversible step that leads to exit of reaction intermediates from the pathway is more likely to occur for incorrect intermediates.

membrane dwell time of constituent proteins (FIG. 2b) as well as through effects related to condensate architecture, including specific molecular organization and the presence of multiple coexisting phases (FIG. 2c).

In systems where phase separation occurs on lipid bilayers (such as nephrin condensates that control actin assembly in podocyte cells of the kidney), the membrane dwell time of constituent proteins is substantially higher within the condensate than on surrounding regions of the membrane. This property was suggested to lead to enhanced signalling when reactions are slow, multistep and driven out of equilibrium, through a process akin to kinetic proofreading[59]. Classically, kinetic proofreading occurs in multistep reaction pathways when desired and undesired substrates exhibit different rates of dissociation from an enzyme, resulting in enhanced specificity. In such systems, flux through the pathway is maximized when substrates exhibit low rates of dissociation at key proofreading steps. For membrane-associated condensates, the analogue of dissociation from an enzyme is the dwell time on the membrane. Thus, reaction flux increases within the condensate because the dwell time is longer there (FIG. 2b). These predictions were borne out in two recent studies. The first study examined the nucleation of actin filaments by membrane-associated nephrin signalling clusters, a process that meets the criteria for kinetic proofreading[60]. It was found that the specific activity (filament nucleation activity per molecule) of the two cluster components, N-WASP and Arp2/3 complex, were strongly correlated with their dwell time, which increased within the clusters. A parallel study examined the activation of the RAS GTPase by distinct, but analogous, signalling clusters[61]. This system also meets the criteria for kinetic proofreading, and RAS activation was dependent on the increased membrane dwell time of the guanine nucleotide exchange factor, SOS, at the signalling clusters. Both studies also reported that the dwell time was related to the connectivity between components forming the cluster, which is determined by relative stoichiometry of the components. Thus, interaction stoichiometry can control activities of molecules within the condensate. This mechanism represents a new means of regulation that is unique to the stoichiometrically variable nature of phase-separated structures. These observations demonstrate the importance of physical properties (which are determined by interaction kinetics and network structure), beyond simple concentration, in determining how condensates affect molecular activities.

In addition to the dwell time, the molecular organization imparted by condensate scaffolds can further enhance activity[50]. Specifically, scaffolds can tether the substrate and enzyme in close proximity, and in consequence decrease the K_M value of the reaction. This effect was shown to be scaffold-specific, with changes in valence of interactions of scaffold molecules abrogating the activity enhancement (FIG. 2c).

Some condensates consist of multiple immiscible phases, including, prominently, the nucleolus, in which multiphase organization is tightly coupled to biological function[62]. In addition to the nucleolus, multiphase organization has been recently documented for cytosolic condensates involved in translation[63]. Specifically, a system consisting of the RNA-binding proteins FMRP and CAPRIN1, together with RNA, can form either single-phase or two-phase condensates depending on which protein is phosphorylated. Single-phase condensates formed by phosphoFMRP and CAPRIN1 homogeneously recruited RNA and the deadenylase CNOT7. By contrast, two-phase condensates formed by FMRP and phosphoCAPRIN1 concentrated RNA and CNOT7 into distinct inner and outer phases, respectively. Despite spatial segregation, deadenylation activity was higher in the two-phase system than in the one-phase system, suggesting that co-enrichment does not always lead to higher activity. A similar lack of reaction enhancement was also observed in ubiquitylation reactions occurring within condensates of ubiquitin ligase substrate adaptor SPOP and its substrate DAXX[64]. In both systems, further quantitative studies could reveal whether the lack of enhancement is due to inhibitory activities within the condensate that counter the effects of concentration, or simply due to saturation of the enzyme systems at the concentrations employed (see discussion on the role of the substrate K_M value in above subsection Enhancing activity through concentrating enzymes and substrates).

TIS granules are another counter-intuitive example. These endoplasmic reticulum-associated condensates formed by the RNA-binding protein TIS11B are necessary for assembly of complexes of membrane proteins and the protein SET, despite SET being present at higher concentration outside the condensates[65]. This suggests that mechanisms beyond mass action are at play in enhancing the assembly reaction within TIS granules. Together, these data suggest that localization both to and within condensates can have significant, and sometimes non-intuitive, effects on function.

Overall, these examples demonstrate that condensates can modulate enzyme reactions through both mass action and other physical mechanisms. The frequent observation of such modulation in mechanistic studies suggests that control of function on short, biochemical length scales is likely to be pervasive in condensate biology.

Regulating macromolecule folding state

Organisms have evolved elaborate mechanisms for preventing aggregation of biomolecules during heat shock or other stresses, and the unique properties of condensates relative to bulk cytoplasm or nucleoplasm may facilitate these processes. A recent study on protein misfolding in the nucleus suggests that the nucleolus is involved in preventing pathological aggregation. Specifically, upon heat stress, thermally unstable proteins migrate into the nucleolus, where the nucleolar granular component — one of the coexisting phases within the multiphase nucleolus — appears to exhibit non-enzymatic chaperone activity, whereby transient associations of misfolded proteins with nucleolar proteins such as nucleophosmin (NPM1) prevent their irreversible aggregation. After stress subsides, unfolded proteins exit the nucleolus and refold dependent on activity of the ATPase chaperone HSP70 (REF.[66]) (FIG. 2d). Dissolving the nucleolus abolished the chaperone activity of the granular component, resulting in amyloid-like aggregation of nuclear proteins.

Processing bodies
Cytoplasmic condensates found in yeast and humans that contains mRNA, RNA decapping and RNA degradation machinery. P bodies are thought to either store or degrade mRNA during stress.

Argonaute
A protein component of the RNA-induced silencing complex that binds several classes of small non-coding RNAs, which direct the complex to mRNA targets via sequence complementarity to downregulate expression through endonucleolytic mRNA cleavage or translational inhibition.

Transgenerational epigenetic inheritance
Biological processes that allow transmission of epigenetic regulatory molecules or modifications, such as RNAi factors or DNA methylation, from parent to offspring without altering DNA sequences.

P granules
Biomolecular condensates formed by liquid–liquid phase separation in *Caenorhabditis elegans* composed of RNA and proteins involved in the maintenance of germ cell fate via post-transcriptional regulation and small RNA biogenesis.

Z granules
Biomolecular condensates in *Caenorhabditis elegans* containing the proteins ZNFX1 and WAGO4 required for transgenerational epigenetic inheritance of RNAi. Associates with both P granules and Mutator foci, forming a bridge between the two condensates.

Mutator foci
A type of biomolecular condensate in *Caenorhabditis elegans* consisting of proteins encoded by *mutator* class genes, originally discovered in genetic screens for activation of transposons in the germline. Functions in siRNA amplification and RNA silencing.

RNA can also form aberrant aggregated structures, which have been implicated in neurodegenerative diseases[11] and may form during stress-induced translation arrest when mRNA is released from polysomes[67]. Upon insults including heat, osmotic or oxidative stresses, cytoplasmic condensates termed stress granules form dependent on the RNA-binding protein G3BP1 (REFS[68–71]). G3BP1 forms condensates with long unstructured RNA, thereby inhibiting formation of entangled, base-paired RNA species[72]. This may facilitate recovery of mRNA and re-initiation of translation after stress subsides (FIG. 2d). DEAD-box RNA helicases may have similar roles in other RNA granules, including processing bodies, as they form condensates with RNA. These condensates dissolve upon stimulation of the ATPase activity of these helicases, which allows release of RNA[73].

The physical mechanisms underlying folding chaperone activity in condensates are not well understood. For G3BP1 in stress granules, co-condensation with unstructured RNA appears to sterically occlude potential base-pairing interactions. A conceptually similar mechanism may operate in the nucleolus, with aggregation-prone unfolded protein regions bound by NPM1. Alternatively, the material properties of the condensate itself may favour certain conformations of macromolecular chains or assemblies in a manner that suppresses aggregation or favours folding. Condensates formed by the intrinsically disordered region of DDX4 have been shown to destabilize duplex DNA and favour compact single-stranded oligonucleotide conformations. This is because the free energy of the system is minimized when oligonucleotides adopt conformations that minimally distort the mesh-like structure of the condensate interior[74]. The chemical environment within condensates may also play a role, as measured solvent polarities within condensates are lower than that of water, reducing both the hydrophobic effect and charge screening by solvent molecules[13,75]. Nuclear magnetic resonance studies may allow these mechanisms to be distinguished by interrogating conformations, interactions and dynamics of misfolded macromolecules within condensate dense phases. Additionally, fluorescence resonance energy transfer reporters for the conformation of misfolded proteins could be developed to enable in vivo interrogation of how folding is chaperoned in condensates.

Mesoscale functions

Apart from effects on biochemical reaction rates, the unique material properties of condensates result in functions that can organize cellular structures or processes on size scales larger than individual macromolecules. We term these mesoscale functions, which include vectoral organization of biochemistry in condensates with multiple coexisting dense phases, establishing the architecture of large intracellular structures and sensing or transducing forces.

Vectoral organization of biochemistry

Efficient production of biomolecules requires high catalytic efficiency without product inhibition, competing back-reactions and formation of dead-end products by non-specific activity. Two properties of condensates formed via LLPS provide potential solutions to these constraints. First, solutions containing multiple macromolecules can produce multiple dense phases, allowing different enzymes within a cascade to be concentrated in different compartments[76]. Second, condensates can concentrate or exclude molecules to varying degrees depending on the physical properties of the molecules[14,54,56], so condensates could recruit substrates and then exclude them following enzymatic modification. These properties combine to yield micrometre-scale organization, where a molecule can be vectorially transferred from one dense phase to another in a manner dependent on enzymatic modification in each phase (FIG. 3a). The best-studied example of vectoral organization of biochemistry via biomolecular condensation is the production of ribosomes in the nucleolus, where pre-ribosomal RNA is transcribed in the innermost phase and is processed and assembled with ribosomal proteins as it transits through the outer phases[76–79]. As this process is reviewed elsewhere[62], we will discuss another, recently discovered condensate with properties that may also facilitate vectoral biochemistry.

A genetic screen in *Caenorhabditis elegans* identified a requirement for the protein ZNFX1 and the Argonaute homologue WAGO4 in transgenerational epigenetic inheritance of RNAi[80]. In early germline progenitor cells, ZNFX1 and WAGO4 co-localized in P granules[81]. Later in development, ZNFX1 and WAGO4 transitioned to perinuclear puncta termed Z granules, which localized immediately adjacent to P granules and exhibited properties consistent with formation via LLPS. In adult germline cells, Z granules associate with yet another type of punctate body — Mutator foci that contain proteins involved in siRNA amplification and RNA silencing[82] — forming tri-condensate assemblages (PZM granules). This organization of condensates suggests an important role in regulating the processing steps of non-coding RNAs involved in transgenerational epigenetic inheritance and warrants further investigation of these mechanisms with the vectoral processing framework in mind. New technologies for tracking vectoral processes in real time would be beneficial, such as enzyme activity reporters that could be read out spatially via fluorescence microscopy[83] (BOX 2).

Establishing mesoscale architecture

The previous sections describe mechanisms that modulate or organize biochemical reactions occurring at sub-micrometre length scales. How might condensates contribute to cellular function at larger scales?

The elaborate cellular morphologies of neurons provide two such examples. Both presynaptic axon terminals and postsynaptic dendrites contain local zones of electron-dense material. The mesoscale architectures of both the presynaptic active zone and the PSD (see above) may be organized by condensates formed by LLPS[56,84,85], with conceptually similar roles[57,86]. Two components of the active zone, RIM1α and RIM-BP, undergo LLPS in vitro[84]. Importantly, these RIMα–RIM-BP condensates recruit and concentrate voltage-gated calcium channels. In this case, the voltage-gated calcium channel density was similar to estimates of native synapses obtained from electron

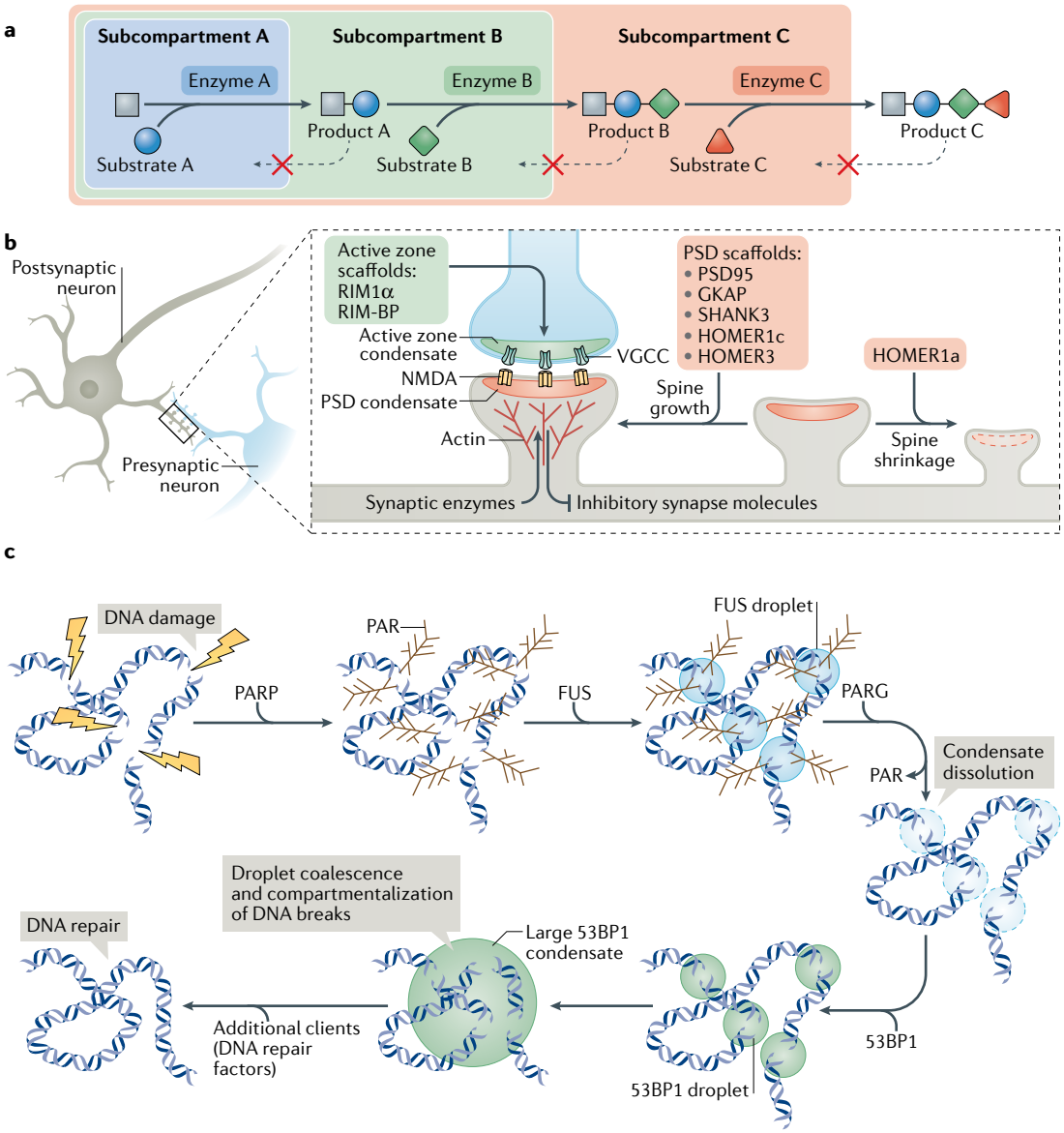

Fig. 3 | **Mesoscale functions of biomolecular condensates. a** | In condensates with more than one subcompartment, differential enrichment of enzymes in different subcompartments can lead to vectoral modification of substrates if substrate molecules are enriched in a given phase but reaction products are excluded from the phase[76,78]. This organization minimizes side reactions and improves efficiency of the pathway. **b** | Condensates have been implicated in mesoscale organization of synaptic connections. Both presynaptic active zones and the excitatory postsynaptic density (PSD) have been proposed to be organized by condensates formed by liquid–liquid phase separation, involving RIM1α and RIM-BP for the active zones, and postsynaptic density protein 95 (PSD95), guanylate kinase-associated protein (GKAP), SH3 and multiple ankyrin repeat domains 3 (SHANK3) and Homer scaffold protein 1c (HOMER1c) or HOMER3 for the PSD. The active zone clusters voltage-gated calcium channels (VGCCs), and keeps them in close proximity to synaptic vesicles. The PSD clusters excitatory *N*-methyl-D-aspartate (NMDA) glutamate receptors. These condensates were also shown to promote concentration of synaptic enzymes, while excluding gephyrin, a component of inhibitory synapses. In addition, SHANK3 directly interacts with cortactin, an activator of the actin-nucleating Arp2/3 complex, providing a connection between the PSD and the actin cytoskeleton. HOMER1a disperses PSD condensates, potentially controlling the size of postsynaptic dendritic spines. **c** | Condensates orchestrate repair of DNA double-strand breaks. Shortly after induction of DNA damage, the RNA-binding protein fused in sarcoma (FUS) and poly(ADP-ribose) (PAR) polymerase 1 (PARP1) localize to double-strand breaks, where FUS binds to PARP1-generated PAR and establishes phase-separated droplets. PAR removal by PAR glycosylase (PARG) causes FUS condensate dissolution. FUS foci can be replaced by foci formed by p53 binding protein 1 (53BP1), which can further coalesce into larger condensates, committing the break to repair by non-homologous end joining[95,96]. In this case, condensates could be acting to mechanically reshape the genome to allow efficient repair of multiple double-strand breaks.

Box 2 | **Key open questions and means to address them**

Scientific advances are often driven by technological advances. As the great molecular biologist Sidney Brenner once said: "Progress in science depends on new techniques, new discoveries and new ideas, probably in that order." Below, we list some of the questions currently of interest in the field (Q), why they are important (I) and technological advances that would foster their answers (A).

Q: What are the compositions of proteins, RNA and small molecules in condensates, and how do they arise from molecular properties and environmental conditions? Relatedly, how are molecules (both macromolecules and small molecules) excluded from certain condensates?
I: Composition is a primary determinant of condensate physical properties and functions.
A:

- Combined development of high-throughput cell engineering, quantitative imaging and mass spectrometry, as well as improved probes, particularly for small molecules
- Development of new theories to integrate experimental data and understand how multivalent, multicomponent physical interactions drive partitioning into condensates

Q: What functions arise specifically from higher-order assembly of individual molecules?
I: This issue is at the centre of condensate biology, and its answer will explain why condensates exist and how they arose through evolution.
A:

- Methods to precisely and quantitatively enhance or inhibit higher-order molecular assembly (for example, phase separation) without altering activities of individual molecules — current methods to achieve this are generally either non-specific (for example, hexane-1,6-diol, solution conditions) or perturb interactions known to be functionally important
- Improved understanding of how surface properties of folded protein domains, sequence patterns of disordered proteins and sequence/structural features of RNA lead to assembly and/or phase separation (to design perturbations)
- Spatially and temporally precise perturbations, for example using optogenetics

Q: How can biochemical activities be measured inside condensates in cells?
I: Understanding the biochemical consequences of condensate formation in vivo remains a major challenge in the field.
A:

- Novel fluorescence techniques for rapid, live cell imaging at super-resolution and/or with single-molecule sensitivity
- High-resolution imaging mass spectrometry to quantify small molecules in cellular condensates (see REF.[197] for an exciting recent example)
- Novel probes for real-time, spatially localized imaging of small-molecule enzyme substrates and products, and activity-specific protein and RNA probes, such as a recently developed fluorescent probe for mRNA decapping[83]

Q: What is the internal structure of condensates, and how does it relate to composition, dynamics and function?
I: Increasing data show that many condensates are not homogeneous but are composed of subcompartments and structured elements. Structure–function relations are central in structural biology as they explain how activity arises from atomic organization. The issue is similar in condensates, but at larger length scales.
A:

- Multicolour super-resolution imaging of fixed and live cells, single-molecule imaging
- Correlative cryo-super-resolution fluorescence imaging and cryo-electron tomography
- Means of perturbing internal structure in cells and creating it biochemically, to observe effects on molecular behaviours and functions
- Improved structure-specific probes (for example, for amyloid fibres) for cellular analyses

Q: What is the range of mechanisms by which condensates form in cells, and how can these be clearly distinguished?
I: Formation of condensates is closely tied to their regulation and sometimes to their functions.
A:

- Advanced single-molecule and super-resolution imaging, quantitative imaging in general
- High-throughput cell engineering to observe and manipulate multiple molecules simultaneously
- Improved theoretical descriptions of condensate formation and behaviours, particularly for small molecule numbers

Q: How do macroscopic material properties contribute to condensate function?
I: Some condensates sense and transduce forces, and it is unknown how the material properties of such condensates affect these activities. Material properties can also be regulated and are defective in neurodegenerative diseases.
A:

- Improved methods to exert quantitatively defined forces on condensates and monitor their functional responses in vivo, including optical trapping[198], generation of intracellular flows[199] and others
- Approaches to specifically modulate condensate material properties in cells — genetically, chemically or optically[78] — combined with measurements of function
- Methods to disrupt force transduction by condensates in vitro and in cells, for example laser ablation.

Q: Is the subcellular spatial organization of condensates functionally important?
I: Many condensates are not evenly distributed throughout the cytoplasm and nucleoplasm, but, for many, the reason for this is unknown.
A:

- Optical trapping[198] or generation of intracellular fluid flow[199] to allow condensates to be moved or held in place
- Optogenetics to assemble condensates in local fashion

Voltage-gated calcium channels
Membrane protein channels that allow ingress of calcium into the cell at presynaptic terminals of neurons when activated by membrane depolarization. Calcium activates exocytosis of neurotransmitter vesicles.

microscopy studies, consistent with a role for LLPS in organizing the active zone. On the postsynaptic side, major proteins of the PSD, PSD95, GKAP, SHANK3 and HOMER1c (a splice isoform of HOMER1) or HOMER3, also undergo LLPS and form condensates anchored to membranes via interaction with the carboxy-terminal tail of the *N*-methyl-D-aspartate (NMDA) receptor, thereby clustering and dramatically concentrating the receptor tail[56,85]. In addition, SHANK3 directly interacts with cortactin, an activator of the actin-nucleating Arp2/3 complex, providing a connection between the PSD and the actin cytoskeleton. In a regulatory mechanism, HOMER1a (another splice isoform of HOMER1) disperses PSD condensates, potentially providing a mechanism to control the size of dendritic spines and, thus, synapse strength (FIG. 3b). Beyond synapses, condensates may also have roles in the mesoscale organization of other membrane-bound structures, including clustering of mitochondria in dormant

N-Methyl-D-aspartate (NMDA) receptor
A postsynaptic membrane protein channel activated by the excitatory neurotransmitter glutamate, allowing ingress of cations to depolarize the postsynaptic neuron.

Dendritic spines
Small protrusions on postsynaptic dendrites that are sites of excitatory signalling by glutamate neurotransmitter receptors.

Balbiani body
A condensate specifically found during oocyte development that includes nuage, mitochondria and rough endoplasmic reticulum. Although the function is not fully understood, it is thought to preserve eggs in a dormant state prior to ovulation.

Optical trapping
The use of highly focused laser beams to apply force to ('trap') very small objects.

Chemogenetic approaches
A class of experimental techniques that introduce proteins or protein domain fusion constructs that have engineered small molecule-dependent activities into cells or in vitro biochemical reactions to achieve control over cellular or biochemical activities.

Optogenetics
A class of experimental techniques using light-responsive proteins or engineered protein domain fusions to acutely modulate cellular or protein activities by illuminating cells or in vitro biochemical reactions.

oocytes by the Balbiani body[87], and potentially in organization of the Golgi apparatus[88]. These examples demonstrate how condensates can provide a physical means of maintaining cellular structures — such as membrane channels — at specific cellular locations associated with their activity, and illustrate tight links between condensate assembly/disassembly and cellular functions.

A second recent example where condensates have an important architectural role, generating mesoscale organization, is in DNA damage response (DDR) (FIG. 3c). DNA double-strand breaks are toxic to cells, and can lead to chromosomal translocations if misrepaired. DDR poses a spatial challenge for the enzymes involved, as DNA ends can diffuse away from each other. It is also not clear how multiple sites of DNA damage can be efficiently repaired. It has long been known that various DNA repair factors form distinct foci at sites of DNA damage[89,90], and now we have substantial evidence that DDR involves LLPS. Shortly after induction of DNA damage, FUS, a protein known to phase separate and form amyloid fibres, and poly(ADP-ribose) (PAR) polymerase 1 (PARP1) localize to double-strand breaks, where FUS binds to PAR generated by PARP1 (REFS[91,92]). FUS compartments are dynamic and, after PAR removal by PAR glycosylase, rapidly dissolve, which likely serves to allow access of DDR factors to damaged DNA[91]. It has been shown that FUS foci can be replaced by foci formed by the DDR factor p53 binding protein 1 (53BP1)[93,94], which forms liquid-like condensates via interactions with long non-coding RNA transcribed near the double-strand breaks[95,96], committing the break to repair by non-homologous end joining. Mechanistically, it has been shown that phase separating proteins targeted to specific genomic loci can mechanically exclude chromatin while preferentially incorporating distant targeted loci via coalescence of multiple condensates, indicating that properties of nuclear condensates — potentially including DDR foci — can reshape the mesoscale architecture of the genome[97]. A further example occurs in budding yeast, where DNA damage foci not only form and fuse but are physically translocated by nuclear microtubules to the nuclear periphery where repair takes place[98]. Condensates formed by the metazoan DNA replication machinery may serve analogous architectural roles by bringing distant replication origins into close proximity in replication factories, the spatial organization of which is believed to coordinate firing between different origins[99–101].

Formation of condensates has also been implicated in regulating autophagy, wherein a double-membrane structure termed the autophagosome is synthesized around cytoplasmic components, facilitating their degradation by fusion with a lysosome[102]. Specifically, it has been shown that during selective autophagy of misfolded proteins, the autophagic cargo receptor protein, p62, undergoes phase separation in a manner dependent on polyubiquitylation of substrate proteins and these condensates are subsequently engulfed by autophagosomes[103]. In yeast, phase separation was documented for vacuolar hydrolase Ape1 — a well-established target for selective autophagy — whereby Ape1 droplets recruit the autophagic receptor Atg19 to their surface to drive autophagosomal engulfment[104]. Thus, condensate formation is a mechanism to generate discrete mesoscale structures that can be specifically targeted for autophagic degradation. In addition to these examples, the pre-autophagosomal structure (PAS), which forms on the yeast vacuole under starvation conditions, is a condensate formed by the complex composed of autophagy proteins Atg1, Atg13, Atg17, Atg29 and Atg31 (REF.[105]). The PAS then matures by recruiting downstream Atg proteins and vesicles, subsequently serving as the site of autophagosome formation. Notably, the kinase activity of autophagy initiating complex Atg1 is enhanced within the PAS condensate and must be balanced by phosphatase activity to prevent condensate dissolution, highlighting the fact that short length-scale biochemical functions of the condensate operate at the same time as its mesoscale functions in organizing autophagosome biogenesis.

Given the relatively simple reconstitution experiments performed thus far in this line of research, it is unclear to what extent condensates contribute to biological processes via organizing mesoscale structures versus modulating biochemical reactions, as both functions may occur simultaneously in ways that vary from system to system. To address this issue further, experiments that break the large-scale organization without disrupting the smaller-scale biochemistry are necessary. In the yeast DDR example, this could be, at least partially, addressed through pharmacological inhibition of microtubule assembly, but tools for manipulating mammalian condensates associated with DDR, active zones or PSD are less readily available. Addressing the mesoscale functions of these condensates might require optical trapping and chemogenetic approaches or optogenetics to induce or prevent fusion of multiple clusters, or to change their material properties. Finally, for the neuronal systems, improved live-cell and intravital imaging studies are also needed to better characterize the structure and function of active zones and PSD condensates in vivo.

Generating and transducing force

Evidence is emerging that biomolecular condensates can exert force, locally reshaping cellular architecture. Growth of condensates within the nucleus can create regions of low chromatin density by effectively pushing chromatin away from the condensate, and coalescence of chromatin-targeted condensates can bring distant genomic sites into proximity[97]. As another example, formation of condensates at sites of clathrin-mediated endocytosis may contribute to the forces that drive membrane invagination. Experiments and theory demonstrate that membrane-associated condensates formed by endocytic coat and adaptor proteins can perform work to deform the membrane, as the balance of condensate–membrane and condensate–cytosol interaction energies results in a regime where membrane invagination is energetically favourable[106]. Finally, condensate formation by zona occludens proteins, components of cell-adhesive tight junctions, has been implicated in a positive feedback regulatory system whereby increasing tension between adhered cells leads to increased accumulation of tight junction protein components at

sites of adhesion[48,49]. Mechanistic details are yet to be revealed, but one possibility is that zona occludens condensates mediate force-dependent signal transduction, possibly via conformational changes induced by binding to contractile actomyosin networks. Other examples are likely to emerge as the properties of condensates involved in, for example, regulation of the cytoskeleton are further studied.

Cellular-scale functions

At the largest length scale, the fact that condensates form discrete structures allows localization of cellular materials to specific locations within the cellular volume. Additionally, assembly and disassembly of condensates can serve as a sensor or switch for changes in the environment that require a homeostatic response. We term these cellular-scale functions of biomolecular condensates.

Mediating subcellular localization

Cells are highly spatially organized, from the micrometre-scale architectures of endomembrane systems through the potentially metre-scale organization of mammalian neurons. Such large-scale organization means proteins, RNAs or vesicles may be produced far from the site of their function. Cells have thus evolved active mechanisms to transport these structures to specific locations and retain them there[107]. Recent data have shown that biomolecular condensates have important functions in controlling subcellular localization of specific molecules and processes.

Membrane-bound organelles, such as endosomes and mitochondria, can traffic along microtubules through direct binding to microtubule motor proteins (reviewed in[108]). Although occurring in most cells, the need for microtubule-based transport is particularly acute in neurons, where molecules and assemblies must be transported from the cell body to the ends of axons and dendritic arbors, which can be metres away[109]. Protein translation in neurons often occurs locally at these distant sites, necessitating transport of numerous mRNA molecules and ribosomes over large distances[110]. Transport efficiency is greatly enhanced by packaging mRNA into RNA transport granules, which are liquid-like protein–RNA condensates akin to stress granules and P bodies in non-neuronal cells[111]. These condensates form in the cell body, are trafficked by motors along axonal cytoskeleton and deposit their cargo at the axon terminal[111,112] (FIG. 4a). A recent study reported that neuronal RNA granules are coupled indirectly to motors through tethering — via annexin A11 (ANXA11) — to lysosomes, which are directly coupled to motor proteins (hitchhiking mechanism)[113]. ANXA11 can form condensates, co-localize with RNA granules and bind to lysosomes. Mutations in ANXA11 that are associated with the neurodegenerative disease amyotrophic lateral sclerosis alter the dynamics of RNA granules and impair their interactions with lysosomes, causing defects in granule trafficking and mRNA delivery to axonal terminals[113].

Chromatin in the eukaryotic nucleus is highly heterogeneous and organized across a range of length scales into compositionally and functionally distinct domains[114–117]. One high level of organization involves the division into transcriptionally active euchromatin and transcriptionally repressed heterochromatin. A major question in chromosome biology is how this organization is maintained despite profound short-range and long-range DNA reorganization during DNA replication and cell division. Heterochromatin is defined in part through histone H3 trimethylated at lysine 9 (H3K9me3), a modification recognized by heterochromatin protein 1α (HP1α). HP1α undergoes LLPS, which is important for compaction and biochemical identification of heterochromatin, and studies from fission yeast suggest that HP1 protein oligomerization induces reorganization of nucleosomes to promote multivalent interactions of histones to drive LLPS of chromatin[118–120]. There are also new insights coming from fly larval neural precursors into how heterochromatin could be maintained in cells undergoing mitosis, when many gene regulatory proteins, including HP1 proteins, leave chromatin and become diffuse[121,122]. This process involves yet another condensate, formed by the Prospero transcription factor (fly homologue of mammalian PROX1), whose LLPS is responsible for its retention at pericentromeric heterochromatin throughout mitosis. This localization of Prospero allows rapid recruitment of HP1 and the H3K9 methyltransferase following mitosis, leading to heterochromatin compaction and spreading[123]. Mutations to Prospero that inhibit phase separation impair heterochromatin maintenance and can lead to cell dedifferentiation, highlighting the importance of maintaining heterochromatin throughout rounds of cell division and in differentiation. Thus, like RNA granules, condensation of Prospero into a discrete body allows it to maintain localization at a specific nuclear location despite dramatic reorganization of the genome over the course of mitosis.

Buffering stochastic cellular noise

Owing to the stochastic nature of gene expression, cells exhibit fluctuations in protein concentration over time, a phenomenon known as gene expression noise[124]. Despite this noise, biological processes are generally robust and precise in space and time. This robustness has been attributed to noise-resistant signal transduction network architectures, analogous to active noise-filtering concepts in engineering[125]. LLPS of individual proteins has been proposed to afford passive noise filtering, as, in the two-phase regime, fluctuations in total concentration alter the volume of the condensed phase — by changing the condensate size and/or number — allowing concentrations in both the dilute and condensed phases to be held constant[126,127] (FIG. 4b). Indeed, a theoretical model for a protein undergoing LLPS while its concentration is subject to fluctuations — for example, related to changes in synthesis and degradation — predicted a sharp decrease in protein concentration fluctuations as the concentration approached the threshold required for LLPS. The ultimate reduction in noise was shown to depend on the protein lifetime and rate of diffusion between the condensate and dilute phases: when

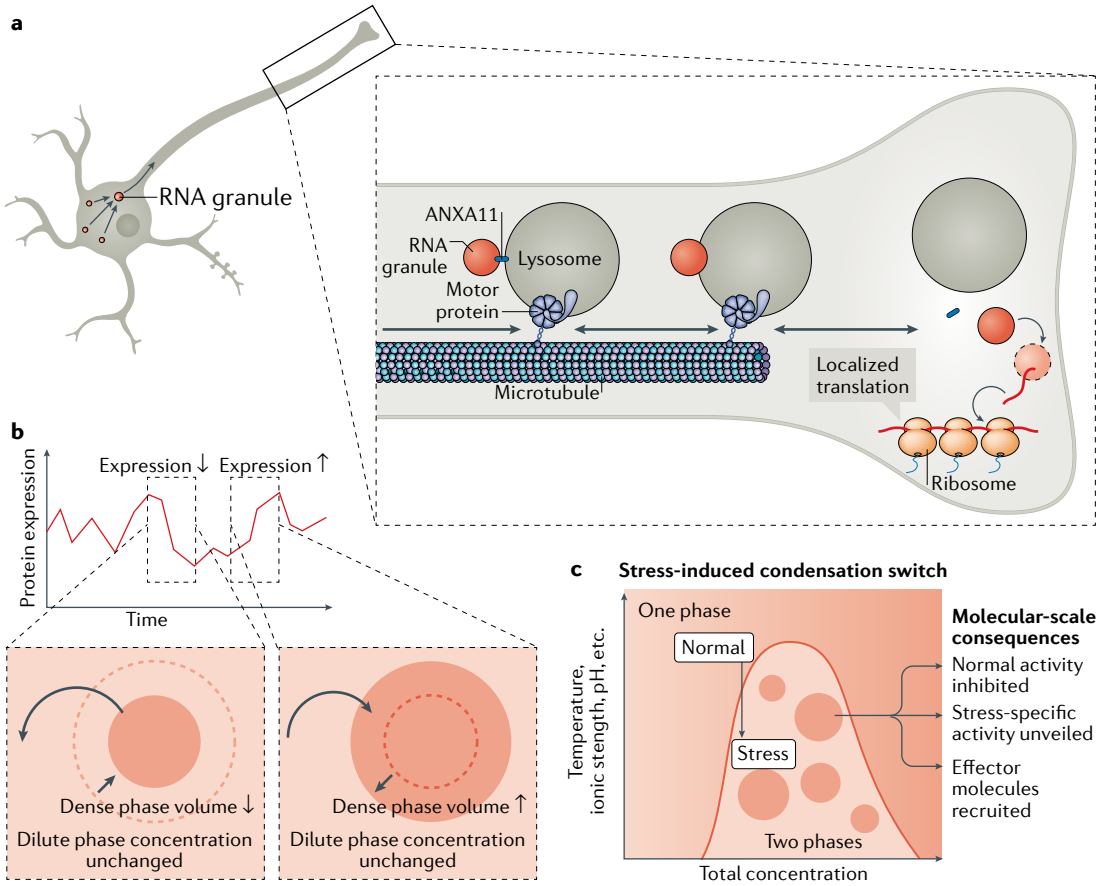

Fig. 4 | **Cellular-scale functions of biomolecular condensates. a** | Mechanisms based on condensates can drive subcellular organization. In the example shown here, condensates — RNA granules — which are generated in the cell body, hitchhike on lysosomes to the axon terminals, where RNA is released, thereby enabling localized translation. In this process, the protein annexin A11 (ANXA11) tethers RNA granules to lysosomes, which are transported along microtubules via motor proteins[113]. **b** | Condensates formed via liquid–liquid phase separation can integrate information about macromolecule concentration over the entire cellular volume to buffer stochastic fluctuations in gene expression. In the two-phase regime, transient fluctuations in protein concentration are buffered by transfer of molecules into or out of the dense phase (condensate), changing the dense phase volume (size and/or number of condensates) but leaving the dilute phase concentration unchanged[127]. **c** | Condensate formation integrates information at a cellular scale by coupling changes in the environment to the transition from the one-phase to two-phase regimes (sensing and switching). Such changes in condensate formation, controlled by external cues, can mediate activation of appropriate homeostatic responses via regulating molecular-scale (and mesoscale) functions of condensates[19,20].

transfer of protein molecules between phases was much faster than protein synthesis and degradation, the minimum noise approached the theoretical lower limit[127]. Phase separation of both a synthetic protein and the endogenously expressed nucleolar component NPM1 in cells resulted in reduced concentration fluctuations, consistent with the model[127].

Although this example provides a convincing proof of concept, it remains to be seen whether native systems exploit LLPS to buffer noise in a functionally significant manner. As noise buffering via this model absolutely depends on condensate formation via LLPS, any native condensates proposed to serve a buffering role must be conclusively shown to form via LLPS. Further theoretical developments are also necessary to encompass any noise buffering effects of multicomponent phase-separating systems, which are more prevalent in vivo, where the saturation concentration will depend on the total concentration of each component[128] (see below).

Sensing and switching

Given the extraordinary sensitivity of phase transitions to solution conditions including temperature, pH and ionic strength[1,129,130], biological systems may have evolved to exploit them as a mechanism to sense potentially deleterious environmental conditions and induce appropriate homeostatic responses[131] (FIG. 4c). A compelling example of such a mechanism occurs in budding yeast, where the mRNA poly(A) binding protein Pab1 undergoes rapid condensation upon heat shock[19]. Pab1 condensation is exquisitely sensitive to temperature, with a 10 °C increase in temperature accelerating the rate of condensation by more than 300-fold, much greater than, for example, changes in ion currents for thermosensitive ion channels, which are also evolutionarily tuned for temperature sensing[132]. Mutations of Pab1 perturbing temperature sensitivity reduce cellular fitness. Pab1 releases bound mRNA upon heat shock and condensation, suggesting that translation of mRNAs with

A-rich 5′ untranslated regions (UTRs), which includes many heat shock protein mRNAs[133], will be selectively enhanced under conditions that favour Pab1 condensation. A parallel pathway operates by very similar means via heat-induced condensation of the translation initiation factor Ded1, an RNA helicase that facilitates ribosomal start-site scanning by resolving secondary structure in mRNA 5′ UTRs[134]. Under heat stress, transcripts with complex 5′ UTRs are preferentially downregulated, favouring expression of mRNAs with simple UTRs, such as heat-shock transcripts with A-rich UTRs described above. Similar mechanisms may be at play in stress sensing by the yeast translation termination factor Sup35, which forms condensates upon cytosolic acidification, resulting from nutrient deprivation and other stresses[20]. Finally, condensate formation may be regulated by cytoplasmic crowding, which can be controlled by regulating ribosome abundance via the mechanistic target of rapamycin (mTOR) pathway, a key sensor of nutrient status[135]. Condensates typically form more readily in crowded conditions, suggesting a link between condensate formation and nutrient status.

As the different environmental factors affect physiology at the level of cells or even whole organisms, such stress-triggered condensation serves to integrate information at long length scales. At the same time, the biochemical function of the condensing molecule is expected to change, as evidenced for compromised RNA binding, translation initiation and translation termination activities of Pab1, Ded1 and Sup35, respectively[19,20]. Delineating the distinctions between sensing large-scale environmental conditions and short length-scale biochemical interactions is likely to be a fruitful avenue for future experiments.

Future challenges and opportunities

Advances over the past several years have opened the door to interesting new opportunities in understanding the roles of biomolecular condensates, but have also revealed significant challenges. Below, we describe some of the areas that we are enthusiastic about for the future and ideas about how progress may be made. For key open questions in the field we also refer the reader to BOX 2.

Complexity of natural condensates

Most mechanistic studies of condensate function to date have been reductionist by necessity, both to reduce technical complexity and because basic principles were poorly understood. With technology advancing and basic principles increasingly clear, it will be important to understand how those principles are modulated by the complexity of native cellular condensates.

Natural condensates contain many molecular species with a wide range of physical properties. Proteomic and imaging data have shown that tens to hundreds of molecules can localize to individual condensates[136–140]. Concentrations of species can vary from <1 μM to >10 μM[136], with partition coefficient values ranging from ~1 to >100 (REFS[70–72,136]). Some molecules have been shown to be excluded from condensates (partition coefficient <1) in vitro[54,56] and in cells[141,142]. Different molecular species can also have widely variable dynamic properties; some exchange with the surroundings in seconds and others exchange only over minutes or longer[136].

In more collective aspects of complexity, molecules can contribute very differently to the formation and composition of condensates. An early model based largely on simplified synthetic systems classified molecules into scaffolds, whose assembly drives formation of the compartments, and clients, which are recruited into condensates through interactions with scaffolds[14]. It has become clear over time that in native condensates, the behaviour of molecules spans a continuum from absolute requirement for condensate formation to recruitment without altering condensate properties[70]. Condensates tend to be composed of few different scaffold molecules and many clients, but also species with intermediate behaviour, whose deletion shifts the saturation concentration of other components, affecting their propensity to phase separate. Mapping of genetic, proteomic and biochemical data onto known protein–protein and protein–RNA interaction maps has suggested that molecules with higher connectivity to other molecules in the condensate are likely to behave more scaffold-like, whereas those with lower connectivity are likely to behave more client-like[70,136]. It is important to note that with only two species phase separating together, the simple features of single-component phase separation often invoked in the literature[1,141,143–146] are not observed[14,128,147]. There is no longer a single phase separation threshold concentration, but rather the threshold of each species differs depending on the concentrations of the others. Moreover, the concentrations in the condensate and surroundings are no longer invariant, but shift as individual components change, generating cell-to-cell and, perhaps, even subcellular variability. Thus, effects of perturbations on the threshold concentration of a phase-separating condensate are also likely coupled to effects on composition, as highly connected molecules will recruit more species into the structure than those with only few interaction partners. Deleting more-connected scaffold-like molecules should more strongly impact both the formation of the condensate and the collection of molecules contained within it than deletion of less-connected molecules.

These features have important implications for the behaviours and functions of natural condensates. Different components will have different physical signatures depending on how they contribute to a condensate (measurable by various microscopies (BOX 2)). Scaffold-like components will show behaviours expected for a phase-separated molecule, with a threshold concentration for assembly, slow exchange dynamics and large contributions to material properties. By contrast, client-like components will behave more akin to molecules binding a porous, static scaffold[148], with Michaelis-like recruitment into the condensate, slow and fast dynamic components representing bound and free species, and less contribution to macroscopic condensate properties. The ways physical properties of condensate components are coupled to one another remain unclear, and it is thus unknown how condensate properties arise from various individual components. Likewise, it is

Partition coefficient
The ratio of molecular concentration within a biomolecular condensate relative to the concentration in the surrounding solution.

Michaelis-like recruitment
For the binding of a molecule to some structure, a non-linear, saturable relationship between the molecular concentration and the fraction bound described by the rectangular hyperbola of the Michaelis–Menten model of enzyme kinetics.

unknown, in general, how modification of components with, for example, scaffold-like or client-like behaviours affect other components upon either natural regulatory inputs or experimental perturbations. Importantly, it is also unclear how those physical properties, both the dynamics of individual molecules and material properties of the whole structure, will impact condensate functions on different length scales.

Thus, to fully understand the formation and activity of a condensate, it is necessary to build complete inventories of condensate components, with quantitative details on their concentrations, interactions, dynamics and position along the scaffold–client continuum. This will require combining proteomics analyses with biochemical and biophysical studies to develop a quantitative understanding of molecular composition and connectivity of natural condensates. These data must be further combined with physical theories that relate the properties of individual condensate species to the properties of the collective. The more complete this information, the better the physiology of a condensate can be captured in a biochemical reconstitution, and the more mechanistically one can understand the cellular behaviours and functions of the compartment.

The question of size

Micron-scale condensates such as the nucleolus and stress granules, and virtually all phase-separated condensates studied in vitro, contain tens of thousands of molecules. However, in vivo, many condensates are appreciably smaller, being <100–300 nm in diameter, and may contain only tens to hundreds of molecules[149]. These include foci involved in transcription regulation[149–151], some signalling puncta[152] and sub-diffraction assemblies of ribonucleoproteins[153]. It is unclear whether such small condensates have different properties from much larger condensates, and in general how condensate function scales with size.

This is an interesting conceptual question but also one of practical importance: if properties and functions differ dramatically as a function of size, it is essential that the size of condensates reconstituted in vitro closely matches their counterparts in vivo. Relatedly, it is unclear how large a condensate must grow before condensate-specific functions of its constituent molecules arise. This latter question is important in basic biophysics and biology, and also in development of therapeutic agents designed to disrupt condensates, a topic of considerable current interest (BOX 3). That is, if functionality is manifested in small molecular assemblies, a potential drug cannot merely eliminate the macroscopic, readily observable condensate but must reduce the system below the functional size threshold, which could be much smaller.

Although we are not aware of studies of macromolecules that address these issues, some information can be inferred from theory, computer simulations and experimental studies of colloidal and viral particles. These have shown that, unlike large-number systems, small systems do not show sharp phase transitions between discrete states[154]. Rather, transitions are broader, and intermediate states with intermediate compositions and densities exist. Stochastic fluctuations in assembly, existence and size are also significant for small assemblies[155–157]. Thus, very small condensates need not be long-lived and homogeneous, or form in switch-like fashion with changes in molecular concentration or environment. These considerations may be relevant to resolving current disagreements in the transcription field, where some groups have argued that transcriptional foci form through LLPS based on correlations between in vitro phase separation of the transcription machinery and cellular behaviours of these factors[149,150,158,159], whereas others have argued that the physical properties of transcriptional foci in cells (for example, stochastic fluctuations in their presence and size, lack of a single threshold concentration) are inconsistent with a LLPS mechanism of formation[141,151,160–162].

Surface tension is particularly important in small systems, as it can produce different structures and/or organizations of molecules in the centre of an assembly from those at the periphery, possibly altering molecular functions of assembly components. In simulations of assemblies of small numbers (≤100) of water molecules or non-adhesive particles, surface tension increases as the clusters grow, reaching values similar to those of macroscopic assemblies at ~50–100 particles[163,164]. Analogous behaviour has been observed experimentally in studies of weakly adhesive colloidal particles, with quantitative differences in surface tension depending on the magnitude of inter-particle forces[165]. Thus, for very small biological condensates, depending on molecular interaction strengths, surface tension effects on the structure, organization and activity of assembly components might be substantial. As an example of such a functional consideration, it has been proposed that the balance of interfacial tensions between phase-separated condensates and membranes and between condensates and cytosol can do mechanical work on the membrane[106]. Thus, as surface tension increases with the size of small droplets, the amount of work (and, thus, deformation of a membrane) could increase as droplets grow. Thus, theory, simulation and physical studies on the size dependence of the organization and activities of biomolecular condensates represent an important area for future investigation. In this regard, to obtain a more physiological picture of condensates, it will be necessary to ensure that the properties of macroscopic condensates reconstituted in vitro reflect all functional nuances of their much smaller counterparts in cells.

In addition to physical considerations of small condensates, it is important to note the biochemical implications of molecules forming small condensates inside cells[136,141]. Where quantified, condensates only take up a small fraction of the cell volume. Yeast P bodies, for example, represent, on average, only ~1% of cytoplasm[136]; and promyelocytic leukaemia nuclear bodies represent only 0.2–2% of the nuclear volume in PML$^{-/-}$ HeLa cells re-expressing promyelocytic leukaemia protein (PML) (Allyson Rice, unpublished). Moreover, where quantified, most proteins are only concentrated 2-fold to 150-fold in cellular condensates, with most falling in the 2-fold to 20-fold range (it is possible that RNA molecules may partition more strongly owing to

Promyelocytic leukaemia nuclear bodies
Nuclear condensates formed by the promyelocytic leukaemia protein (PML). Fusion of PML to the retinoic acid receptor causes acute promyelocytic leukaemia. PML bodies are implicated in various processes, including transcription regulation, viral immunity, post-translational modification and apoptosis.

Box 3 | **Condensates as therapeutic targets**

Our increasing understanding of condensate function provides opportunities to target these structures in treatment of disease. Defects in condensates have been implicated in numerous diseases. Most notably, defects in stress granules due to mutations in RNA binding proteins hnRNPA1, hnRNPA2, FUS, TDP-43 and others, as well as RNA regulatory proteins, have been associated with the neurodegenerative diseases amyotrophic lateral sclerosis, frontotemporal dementia and multisystem proteinopathy[22,200]. Dipeptide repeat proteins expressed from the *c9Orf72* gene cause defects in multiple RNA-based condensates and the nuclear pore complex, and are also associated with amyotrophic lateral sclerosis and frontotemporal dementia[22]. Aberrant nuclear condensates produced by repetitive RNA sequences can produce different degenerative diseases[11]. Defective condensates have also been implicated in various cancers, which are driven by fusions of self-associating sequences from proteins such as EWS, FUS and EML4 to transcription factors or kinases[201–204]. As exemplified in the main text with specific condensate functions, condensates also have roles in many biological processes relevant to disease, including innate and adaptive immune signalling[37,54], DNA repair[92,98], gene regulation[150,158,160], cell adhesion[49] and synaptic transmission[56,84].

Thus, modulation of condensates has promise for broad impact in disease treatment. Many mechanisms can be envisioned for such modulation. Therapeutics could act directly on molecules within a condensate to disrupt their interactions and dissolve the structure. The small hydrophobic molecule lipoic acid has recently been reported to have such activity in vitro and in cells[205]. The formation and dissolution of many condensates are naturally regulated by covalent modification of components by enzymes[145,206] such as kinases[4,54,178,207–210], poly(ADP-ribose) polymerases[93], methyl transferases[13,16,188,211], ubiquitin transferases[103,212] and acetyl transferases[6,213], which could themselves be targeted therapeutically. Similarly, protein and RNA disaggregases control the turnover and dynamics of condensates[73,214], and could be targeted as well. These strategies could be used to destroy or enhance the formation of condensates, both of which could be valuable depending on the disease mechanism. In anticancer therapy with CAR T cells, increased phase separation through increased numbers of tyrosine phosphorylation sites could be used to increase the efficiency of signalling[54,215]. In neurodegenerative diseases, aberrant condensates appear to have slowed dynamics of their constituents, behaving more like solids than their natural liquids, and this change in material properties may contribute to pathogenesis[22]. In such cases, using sub-threshold amounts of disrupting agents, or distinct means of altering material properties, could reverse solidification and perhaps restore normal function. Capping amyloid fibre formation could be a mechanism to achieve this activity[216–219]. Because molecules act cooperatively to form condensates and define their material properties, it may be possible to target a disease-causing mutant protein indirectly, by altering the existence or activity of its neighbours in the structure. Finally, with a better understanding of the physical factors that control partitioning of small molecules into condensates, it may be possible to design inhibitors that selectively concentrate with their condensate-resident disease-causing targets[220]. Alternatively such compounds could be used to direct other inhibitory or activating molecules to such targets. Both approaches could enhance potency or specificity of target modulation.

Together, these strategies could provide novel means of treating disease based on the unique properties of biomolecular condensates. If these strategies could be generalized, they would also provide powerful tools to advance our basic understanding of condensate functions in biology. Such work thus presents exciting opportunities for future investigation, both practical and fundamental.

kinetic trapping)[70–72,136]. Taken together with small size, this means that most molecules of a given condensate component are not located within the compartment, but rather are in surrounding cytoplasm or nucleoplasm. In yeast P bodies, for example, even when correcting for potential undercounting due to sub-diffraction assemblies, only four components of 31 studied were found to have >50% of molecules partitioned into the condensed phase, whereas 25 components had <20% of their molecules within the condensates[136]. Models based on inhibitory sequestration by condensates (FIG. 2a) must account for such quantitative information. Similarly, models invoking enhanced activity within a condensate must also contend with the fact that much of the reaction can also take place through molecules outside the compartment. Because of these considerations, condensates may be most important biochemically in organizing cascades of reactions, where enhancements of reactivity and/or specificity in individual steps will be magnified when all components are concentrated together. In such cases, overall flux through a pathway could be vastly higher in the condensate than in the surroundings, and condensation could provide substantial increases in overall activity or specificity (FIG. 3a). This may explain why molecules involved in common processes are often co-concentrated into condensates.

CAR T cells
In cancer immunotherapy, T cells that express engineered T cell receptors (TCRs) where the native extracellular domains have been replaced by a heterologous binding domain targeted to a tumour-specific cell-surface protein in order to direct increased cytotoxic activity towards tumour cells.

Kinetic trapping
A phenomenon in which a thermodynamically less stable state is maintained due to the high energy barrier, and thus long time period, required to move to the more stable state.

Evolutionary considerations

This Roadmap has described various functions for biomolecular condensates, activities that have adaptive value and arose through mutation and natural selection. As knowledge of condensate functions advances, it will be important to understand how condensates and their functions evolve, as well as address intriguing hypotheses about how condensates might facilitate evolutionary adaptation.

Evolution of functional condensates requires selection for macromolecule-intrinsic biophysical properties that promote condensate formation in tandem with gene expression programmes that produce molecules at sufficient concentration for condensates to form. The ways in which these features evolve in parallel are unclear, and an understanding of these evolutionary processes may provide deeper insight into condensate functions. One possible route to addressing these questions is to examine homologous condensates found in related organisms that live at, for instance, high versus low temperatures. Given the sensitivity of LLPS to temperature and other physical parameters, organisms likely acquire adaptive mutations that favour condensate formation in the appropriate environmental conditions, as was recently demonstrated for heat-induced condensation of Ded1 (see above subsection Sensing and switching)[134].

Split enzyme system
The use of an enzyme that has been expressed as two separate polypeptide chains and is only active when the fragments are brought together to reconstitute the full enzyme.

These adaptations likely include changes to primary amino acid sequence that favour self-association, but regulatory adaptations in both mRNA and protein production and decay processes and post-translational modifications may also play important roles. There may be significant challenges to organismal fitness along the evolutionary trajectory towards condensate formation, as exemplified by pathological mutations in FUS where single amino acid changes are associated with aggregation and neurodegeneration[92]. Indeed, bioinformatic analyses indicate that aggregation-prone proteins are, on average, expressed at lower rates and turned over more rapidly, which is likely to keep total concentrations low and disfavour formation of deleterious aggregates[166,167]. This suggests that condensate-forming properties of proteins may evolve under regimes where the effects of deleterious, aggregation-promoting mutations are effectively masked by low expression and rapid degradation. Once mutations favouring functional condensate formation arise, regulatory mechanisms may then evolve to favour higher expression, and hence condensate formation.

How do functionally important protein–protein interactions arise? A single, random point mutation is unlikely to significantly change binding affinity between a protein and a non-specific interaction partner, meaning most mutations will not affect the abundance of protein–protein complexes and produce no selective advantage. However, if molecules are co-localized, the system is tuned differently, and the small changes in affinity resulting from a point mutation can manifest as considerable differences in the population of the protein–protein complex. If these new interactions have adaptive value, for example by allosterically modulating the activity of one or both molecules, over time these initial mutations may become fixed in the population, allowing for further adaptation to favour the novel function[168]. In this sense, the high concentration of molecules within condensates may have adaptive value simply in producing co-localization, and thus enabling generation of novel functions through mutation and selection. The experimental route for examining such possibilities is challenging, but a potential proof of concept could lie in engineered condensate systems designed to recruit components of a split enzyme system. With a suitable experimental evolution paradigm selecting for the split enzyme's activity[169], one would predict that mutations favouring formation of the functional enzyme would be fixed more rapidly in populations where the split enzyme was recruited into condensates.

As described above (see above subsection Sensing and switching), the yeast translation regulator Pab1 shows signs of selection for condensation upon heat shock, and, indeed, mutants that impair condensation are significantly less fit for growth under heat stress conditions[19]. Population-level analyses of fitness of similar systems under varying environmental conditions may reveal other ways that condensate formation has adaptive value for populations of cells. For example, as shown for a model protein, condensation into static, inactive aggregates can increase the phenotypic diversity of a population owing to differences between cells in the concentration of free, active protein. Depending on the function of the aggregating protein and the environmental conditions that the population experiences, higher phenotypic variation can increase the likelihood that at least a subpopulation survives and reproduces[170]. An intriguing possibility is that cells might regulate material properties of condensates — including via liquid–solid transitions — to tune population-level variability.

In summary, we envision that collaborations between biochemists, cell biologists, experimental evolution specialists and evolutionary biologists as well as theorists will be particularly fruitful for future development of the field.

Conclusions

We have organized the diverse functions of biomolecular condensates into a framework for understanding condensate functions based on the length scale on which they operate (FIG. 1; TABLE 1), with interrelated functions possibly occurring at different length scales. We hope this framework will provide a useful point of embarkation for studies of novel condensates and their associated functions, and will spur new thinking about molecular mechanisms, experimental approaches, technological advances, biological processes and therapeutic strategies associated with condensates. Finally, we hope that any gaps in our framework will be filled in with novel condensate functions as the field moves forward.

Published online: 09 November 2020

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Acknowledgements
Research on biomolecular condensates is supported in the Rosen laboratory by the Howard Hughes Medical Institute, a Paul G. Allen Frontiers Distinguished Investigator Award to M.K.R. and grants from the Welch Foundation (I-1544 to M.K.R.) and the National Institutes of Health (NIH) (F32 GM136058 to A.S.L.).

Author contributions
The authors contributed equally to all aspects of the article.

Competing interests
M.K.R. is a co-founder of the biotechnology company Faze Medicines. A.S.L and W.B.P. declare no competing interests.

Peer review information
Nature Reviews Molecular Cell Biology thanks M. Babu, S. Michnick and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.