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Opinion

Plant condensates: no longer membrane-less?

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Cellular condensation is a reinvigorated area of study in biology, with scientific discussions focusing mainly on the forces that drive condensate formation, properties, and functions. Usually, condensates are called 'membrane-less' to highlight the absence of a surrounding membrane and the lack of associated contacts. In this opinion article we take a different direction, focusing on condensates that may be interfacing with membranes and their possible functions. We also highlight changes in condensate material properties brought about by condensate–membrane interactions, proposing how condensates–membrane interfaces could potentially affect interorganellar communication, development, and growth, but also adaptation in an evolutionary context. We would thus like to stimulate research in this area, which is much less understood in plants compared with the animal field.

Highlights

The formation of condensates through phase separation can help in the compartmentalization of the cell by locally restricting, concentrating, and enhancing or inhibiting the activities and functions of biomolecules.

Membranes can be enriched in so-called 'membrane-less condensates', but we know little about them in plant biology.

Condensates can show different material properties akin to liquids or solids and these properties are highly tunable, thereby, defining their functions and interactions with membranes or even other condensates.

Membrane-bound condensates may modulate the formation of links between organelles, such as those formed between the endoplasmic reticulum and the plasma membrane.

An evolutionary view of condensates, especially the membrane-bound ones, suggests that they may be responsible for many (heritable) traits and thus the manipulation of condensates could provide avenues for biotechnological applications.

The basics of condensates for plant biologists

At certain physicochemical conditions, molecules like proteins or RNAs separate from a diluted watery phase into a more condensed phase *in vitro*. This behavior can be also seen *in vivo*: the discovery that mesoscale dense 'blobs' (i.e., in the range of hundreds of nanometers), like the *Caenorhabditis elegans* **P-granules** (see Glossary), are ribonucleoprotein ensembles resembling liquid-like materials [1], paved the way for scientific discussion on the extent to which these properties are of biological significance. P-granules form through a type of phase separation known as **liquid–liquid phase separation (LLPS)**. LLPS can be a powerful mechanism underlying cellular compartmentalization and can take place when the attraction between biomolecules in the blob is stronger than with their surrounding environment. Following LLPS, molecules in the blob will form a more condensed phase (i.e., with higher local concentration). Thus, these blobs are collectively referred to as '**condensates**'; yet condensates may form through other mechanisms than LLPS, making condensates an umbrella term that does not imply formation through LLPS [2].

The realization that condensates are blobs of denser matter led to efforts focusing on understanding the forces driving their organization and compactness. The **Flory–Huggins theory** is often used to explain condensates' formation [3]: the separation of two or more phases that are polymer-rich (condensate) and polymer-poor (the watery phase around the condensate) depends on the net attraction between polymers. Polymers can be proteins and nucleic acids, and the condensation depends on total polymer concentrations, **valency**, and affinities (Box 1). Many of the proteins that drive phase separation show significant conformational heterogeneity and are referred to collectively as **intrinsically disordered proteins (IDPs)** and the corresponding sequences that drive phase separation as **intrinsically disordered regions (IDRs)** (Box 1). IDRs attain short-lived conformations which are energetically variable and very prone to even moderate alterations in conditions (e.g., pH, temperature, ions). Therefore, certain conditions can induce drastic changes in the condensation propensity of

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biomolecules [4], which makes condensates excellent sensors of the environment, as has been recently shown in plants (e.g., for salt [5]). Some IDPs with **prion-like domains (PrLDs)** can aggregate into amyloid fibrils, which can accommodate incoming protein monomers, thus propagating their aggregated form [6].

Even if born as liquids, when condensates are given enough time, they can attain a broad spectrum of so-called material properties. Material properties switches are likely because given enough time, IDPs are more likely to be entrapped in low-energy conformations (Box 1). Condensates can thus undergo maturation into semifluid gels, glasses, and solid aggregates. In the literature, the term 'condensate aging' has been used to imply a change in the material properties of condensate from a liquid to a more rigid and likely compact solid-like state (**liquid-to-solid phase separation**) [7]. We should note though that the term 'aging' is not necessarily associated with chronological age. Interestingly though, such transitions have attracted significant attention as they are key for many aging-related human diseases (neurodegenerative disorders, cancer, and others, as articulated in [8]). The key feature of liquid-to-solid transition or other types of transitions is that solid condensates show reduced fusion with one another and suppressed molecular rearrangements, due to their increased density and viscosity [8]. Liquid-to-solid transitions are widespread as they have even been observed even in bacteria cells. For example, the IDP PopZ forms condensates in the bacterium *Caulobacter crescentus* that undergo liquid-to-solid transitions to orchestrate the cell cycle [9]. Surprisingly, we know little about the material properties (and aging) of plant condensates.

Transitions in the material properties of condensates can be estimated *in vivo* and *in vitro* using various approaches (Box 2). The liquid-to-solid transitions can happen through the formation of clusters in condensates with fibrils or crystal-like aggregates such as amyloids, with IDP that have 'prion-like' IDRs having a significant role in the process [10]. However, increased liquidity of condensates may allow greater promiscuity for binding to different partners to increase the size and compositional diversity of the condensate. Furthermore, we assume that a liquid phase could in theory allow broader interactions at the boundaries of the condensate with other condensates or other biomolecules. Too little liquidity (too much solidity) would compromise this ability and potential functions [10], as discussed later. Furthermore, liquidity allows biochemical reactions as they tend to occur everywhere within a liquid through the random collision of reactants, while solid states may block them by restricting molecular diffusion. For example, the condensate Oskar in *Drosophila* embryo [11], shows a liquid phase permeable to RNAs, but as

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Box 1. Conscious atoms and curious matter: the grammar of condensation

Interactions of the following types, charge–charge, pi–pi, and cation–π, hydrophobic contacts, and hydrogen bonds, all contribute to LLPS. Residues most prone to such interactions include the aromatic residues Tyr, Phe, Trp, and His, carboxyl and carboxamide groups of Asn, Asp, Gln, and Glu, the guanidine group in Arg, and the exposed backbone peptide bond of Gly and other amino acids with small side chains. While hydrogen bonds are largely considered responsible for polar amino acid solvation, they also contribute to the self-association driving LLPS. Hydrogen bonding is also a major factor in the recognition of nucleotide bases and it is likely important for the incorporation of RNA and DNA into condensates. In addition to composition, the specific arrangement and sequence of amino acids also have an important role in LLPS.

Multicomponent (or 'multitypic') systems (with multiple IDPs) can display cophase separation scenarios whereby proteins that do not normally phase separate do so when they are mixed with other proteins. Alternatively, a protein that would normally be excluded from condensate is sequestered to it through interaction with a protein that can show LLPS. In this case, the former can be a protein that promotes LLPS and the latter a client protein. Post-translational modifications of phase-separating 'scaffold' proteins (i.e., driving LLPS), as well as partitioning 'client' molecules (i.e., those that partition to a condensate by interacting with scaffold proteins or RNAs), are important for condensate formation. Different components at different values of a control variable, which could be temperature, salt concentration, pH, and others, can affect condensation. In Figure I we summarize principles of condensation.

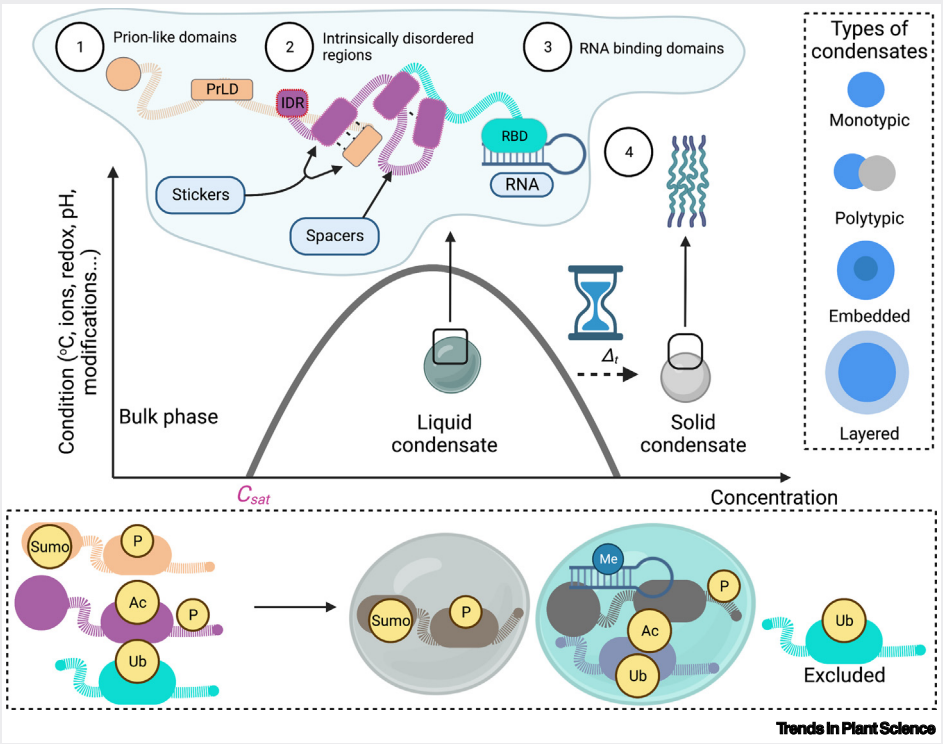

Figure I. Properties affecting the assembly of condensates. Protein and RNA molecules have attractive groups known as 'stickers' that form noncovalent and mainly weak interactions. At certain concentrations, which are determined by various factors (e.g., temperature, redox state, pH), interactions are enabled among intra- or intermolecular stickers (e.g., protein 1–protein 2 interaction on the cartoon). When reaching a system-specific threshold concentration ($C_{threshold}$ or C_{sat}), the whole system undergoes phase separation into two or more phases: a 'watery' phase (denoted as 'bulk') and a 'dense' phase (below the curve). Stickers are connected by 'spacers' that regulate the density transitions by orienting stickers. The 'stickiness' (or multivalency) depends on the attraction between residues, usually provided by the so-called 'intrinsically disordered regions' (IDRs) usually in the stickers [e.g., prion-like domains (PrLDs)]. Folded domains or nucleic acids also mediate phase separation [e.g., protein 3 in the cartoon with an RNA binding domain (RBD)]. Some molecules function as 'scaffolds' for the condensation process (much like 'nucleators') and likely are recruited first in 'precondensation' assemblies. Yet, other proteins are recruited to the condensate by scaffolds, including RNA binding proteins interacting with RNA molecules. These molecules are called 'clients' and may affect condensates by regulating their properties and activities, or even their interactions at their boundaries. Given enough time (Δt, hourglass symbol) or at high concentrations, condensates may form filaments/aggregates with solid-like material properties. Molecular components increasingly interact and lose their freedom to move as the condensates shrink and their density likely increases. Intrinsically disordered proteins (IDPs) can take many different molecular configurations, leading to complex energy landscapes with distributions of energy minima. Increasing relaxation times are likely related to the system exploring deeper energy minima that are increasingly inaccessible, but longer lived, as the system ages. Lower box: many post-translational modifications may affect interactions of scaffolds or clients, thereby affecting condensation and material properties (e.g., sumoylation, phosphorylation, ubiquitination, and acetylation); modifications on RNA can also affect the process (e.g., methylation). These modifications may promote the condensation of one protein, or many proteins together with RNAs, or lead to the exclusion of a protein from a condensate. Right box: condensates can be 'monotypic' (i.e., comprising a single protein) or 'multitypic' (i.e., comprising many proteins and RNAs). Furthermore, the condensate may have a multilayered organization, with the simpler being the 'core' (dark blue) and 'shell' (light blue) organization. Other types of organization are the 'embedding', which likely depends on differences in surface tension among concentrically placed condensates. For example, the nucleolus shows an embedded type of organization. Figure created with BioRender.com.

the condensate ages, its solidification blocks the incorporation of more RNAs while allowing proteins to get in.

Glossary

Affinity: the strength of the bonds formed, or the strength of the binding interaction between a single biomolecule (e.g., protein or DNA) to its ligand (e.g., drug or inhibitor).

Bet-hedging strategy: a risk-spreading strategy where (nearly) isogenic populations stochastically diversify their phenotypes, often resulting in maladapted individuals with lower fecundity. This fitness cost in a specific environment may turn out as an adaptive trait.

Coacervate: an aqueous phase rich in macromolecules such as synthetic polymers, proteins, or nucleic acids. It forms through liquid–liquid phase separation (LLPS), leading to a dense phase (nm to mm in size) in thermodynamic equilibrium with a dilute phase.

Colloid: a mixture in which one substance consisting of microscopically dispersed insoluble particles is suspended throughout another substance. The particles in a colloid are larger than the individual molecules in a solution but smaller than the particles in a suspension. The particles in a colloid do not settle out over time and the mixture appears to be homogeneous. Colloids show interesting behavior under certain conditions, such as the ability to form gels, change viscosity, or undergo phase transitions.

Colloidal phase separation: an organizing concept for the compartmentalization of living cells that describes the cytoplasm as a **colloid**.

Condensate: cellular 'blobs' that behave like liquids or solids, or they can show a gooey, gel-like consistency. Condensation is generally considered distinct from the formation of stoichiometric protein complexes with (well-)defined numbers of subunits.

Elastocapillarity: the ability of a capillary force of a liquid to deform an elastic (deformable) material. To give an example from our macrocosm, the coalescence of the wet hairs of a brush depends on this process.

Flory–Huggins theory: a mathematical lattice model (i.e., not defined in a continuum of space and time) of the thermodynamics of polymer solutions that takes into account the great dissimilarity in molecular sizes in adapting the usual expression for the entropy of mixing.

Box 2. Approaches for studying membrane-bound condensates

In vitro reconstitution approaches on artificial membranes, such as supported lipid bilayers (SLBs) and giant unilamellar vesicles, can be used to investigate phase separation in a simplified system [63,64]. We recently described an approach for *in vitro* condensation at SLBs with excess membrane reservoir (SUPER) templates, which can help examine the steric pressure on membrane lipids exerted by condensates or their dynamics on membranes with low tension (e.g., vacuolar, endoplasmic reticulum sheets) but also the $C_{threshold}$ reduction brought about by membranes [22]. LLPS in the bulk phase (e.g., cytoplasm) may also be modulated by various small molecules, such as chemical chaperones, and large molecular crowders, including polyethylene glycol (PEG). Often, adding those compounds is considered to better mimic the crowded *in vivo* conditions, but the exact mechanism of those additives is not understood in all cases [65]. When the binding of the condensate to the membrane requires additional proteins not present in the reconstitution system, attaching his-tagged purified proteins to SLBs (composed of Ni^{2+}-NTA modified lipids) has been successfully employed, including in the SUPER approach. Other approaches, such as quartz crystal microbalance with dissipation monitoring, which offset artifacts due to the weight of condensates that can affect precipitation approaches (e.g., co-sedimentation assays), can be used to examine the **affinity** of condensates for certain membrane lipids [32].

Fluorescence recovery after photobleaching (FRAP) estimates the diffusion of nonbleached protein into the bleached region and can reveal information about material properties. Slower FRAP is anticipated when condensate is more solid because of the reduced molecular diffusion. The second measure of dynamics is the coalescence time of two condensates. Two condensates with liquid-like behavior merge into a final larger spherical condensate in which the components of the prefusion condensates intermix. The time it takes for this intermixing depends on the ratio of surface tension and viscosity. *In vitro*, more complicated, and accurate methods can be exploited, such as active or passive microrheology, where condensates can be deformed using two laser tweezers that act on two beads linked to a droplet through an attached protein [65].

Condensates can be examined *in vivo*, with, for example, 3D electron microscopy techniques, including correlative light and electron microscopy combined with electron tomography, and CryoET at the nanoscale [33]. At the dynamic mesoscale (few hundred nm), approaches such as live cell imaging super-resolution or total internal reflection microscopy (TIRFM) have been successfully used in plants [22,32]. Another successfully used *in vivo* approach in plants is fluorescence correlation spectroscopy, which can be used to examine the oligomeric states of scaffolds or clients in condensates [6].

In the solid state, condensates can attain soft glass-like states (in-between liquid and solid), which are easy to fluidize by changing their composition [7]. Soft glasses allow cells to slow down biochemistry while maintaining their softness, which can flexibly and rapidly respond to changing conditions. Glass-like aging of protein condensates may, therefore, offer cells a way to flexibly modulate the fluidity of condensates [7], while allowing for rapid response to changing environmental cues such as the compressive forces exerted upon wounding.

Condensates can fuse over time and, in extreme cases, a single large condensate remains. However, this behavior is rarely seen in cells, with few exceptions (e.g., P-granules and the plant carboxysomes) [12]. The specific factors that go against the thermodynamic equilibrium that render condensates in cells incapable of fusing into a singularity are a matter of speculation, but fission mechanisms, specific proteins, material properties as aforementioned, and/or energy squandering (e.g., ATP hydrolysis), or all of these at the same time, may play a role [13].

Condensates are everywhere in a cell and affect a vast number of processes, including immune signaling [14–16], regeneration, hormonal outputs, and development [6]. Many condensates can even interface with or engulf membranes in animal cells to modulate signals relevant to the cytoskeleton [17], immune cell activation, presynaptic active zones, and endocytosis condensates that engulf neuro-transmitting vesicles in synapses [18]; this is not an exhaustive list. This is likely counterintuitive, as condensates are usually referred to as ‘membrane-less organelles’.

Having introduced the basics of condensates, we discuss next how membrane–condensate interactions could be modulated and how these interactions could impinge on cellular and organismal properties.

Intrinsically disordered proteins (IDPs): proteins that lack a fixed ordered 3D structure are characterized by a biased amino acid composition, low sequence complexity, low proportions of bulky hydrophobic amino acids, and high proportions of charged and hydrophilic amino acids.
Intrinsically disordered regions (IDRs): hotspots of condensate assembly that represent the ‘wobbly’ polypeptide segments of proteins lacking fixed 3D structures, showing low complexity and biased amino acid composition that drives a conformational disorder and which interact with other molecules and solvents to guide phase separation.
Lipid rafts: plasma membrane dynamic assemblies with specific lipids and protein composition, also known as ‘membrane microdomains’ or ‘detergent-resistant membranes’.
Liquid–liquid phase separation (LLPS): a state that generates a subtype of colloid known as an emulsion that can coalesce from large droplets within a liquid.
Liquid-to-solid phase separation: a state that forms crystals/aggregates in gels, colloids, or suspensions within cells or extracellular secretions.
Micelles: amorphous substances such as starch and cellulose were proposed to consist of building block aggregates, packed in a loosely crystalline array: the ‘micelles’.
P-granules: the *Caenorhabditis elegans* ‘germ granules’, a class of perinuclear ribonucleoprotein condensates specific to the germline. They get their name from the ‘P lineage’, the embryonic lineage that gives rise to the germline.
Prion-like domains (PrLDs): protein domains that share certain characteristics with prion proteins, which are known to be associated with several neurodegenerative diseases such as Creutzfeldt-Jakob disease and mad cow disease. PrLDs are found in a wide range of proteins and are characterized by their ability to form self-perpetuating, aggregated structures. Like prion proteins, PrLDs can adopt alternative conformations, leading to the formation of insoluble protein aggregates. PrLD amino acid composition, like the IDP yeast prion domains, are usually enriched in Gln and Asn residues and depleted in hydrophobic and charged residues.

When membrane-less become membrane-rich: phase transitions in condensates could modulate their ability to interact with membranes

Among other processes, condensation depends on the formation of bonds with water. The exclusion of water molecules during conditions such as desiccation presents a challenge to proteins as they must switch to a completely desiccated state (anhydrobiosis) that cannot support cellular biochemistry, while preventing uncontrolled and irreversible protein aggregation. One would thus expect dramatic changes in the material properties of condensates. Recent evidence showed that in arabidopsis (*Arabidopsis thaliana),* during desiccation, the material properties of the PrLD FLOE1 control germination of seeds [19]. When the desiccated state ends through the imbibition (water uptake) of seeds, switches between liquid-to-solid states of FLOE1 drive a phenotypic variability in seed germination. This variability resembles a '**bet-hedging strategy**' that has been exemplified for prion-driven adaptation in yeast [20]. FLOE1 homolog IDR sequences differ even among ecotypes of the same species, allowing variability in germination. This variability in IDRs affects FLOE1 hydration-dependent phase separation in seeds, which might suggest a possible mechanism for the observed variation in germination. This strategy exemplified by FLOE1 implies that IDPs or condensates (i.e., assemblies comprising many IDPs and proteins) could promote adaptation by accepting mutations in their IDRs. This speculation is supported by the fact that IDRs are mutational hot spots [21]. Consequently, as IDPs mutate faster than structured proteins in non-plants, future studies in plants could reveal IDP potential in driving adaptation and to what extent their features can be tuned to introduce desirable traits or improve resilience.

The molecular function(s) of FLOE1 is rather speculative, as well as what type of properties it has in desiccated cells. Most importantly, it is unclear whether FLOE1 interacts with any membrane. We have recently identified FLOE1 homologs as putative constituents of the condensate **processing bodies** [22,23]. Processing bodies interface with membranes through the scaffolding protein DECAPPING 1 (DCP1). In support of the above speculation, structural predictions suggest that FLOE1 may interact with membranes (Figure 1, see legend). FLOE1 could potentially retain its interactions, even in the desiccated state, with membranes or polysaccharides. Furthermore, FLOE1 could sequester proteins that promote quiescence in seeds [19]. Investigations of FLOE1 paralogs (FLOE2 and FLOE3) and homologs and their links to other condensates (e.g., processing bodies) may deepen our understanding of plant condensate material switches.

Desiccation tolerance in several organisms depends on the disaccharide trehalose (or sucrose) [24]. Trehalose promotes the glass-like formation of noncrystalline solids (vitrification), encapsulating and protecting cellular materials during desiccation, or through water replacement, where stabilizing bonds initially made with water are replaced by bonds with sugars [25]. Some anhydrobiotic non-plant organisms do not accumulate or even possess the genes to make trehalose [26]. Instead, these organisms seem to exploit IDPs such as the late embryonic abundant (LEA) protein to induce vitrification. Interestingly, LEAs can undergo LLPS, likely forming condensates, but also vitrify upon desiccation [27]. Through the formation of condensates, LEAs can likely sequester proteins to promote their protection. Although both processes reduce molecular rearrangements, we should note that vitrification is fundamentally different from the solidification observed in aging condensates, as in vitrification almost all water molecules are lost from the dense phase. It is thus unclear whether LLPS of LEAs somehow promotes their vitrification or if LEAs in condensates coexist with populations of vitrified ones with other functions.

Importantly, LEAs interact with membranes, where they become more structured, likely through a scaffolding process [28]. LEAs become more structured also in a desiccated state [29], which might imply that water could lead to increased disorder and condensation (as seen for protein **micelles**). This water-induced conformational reduction could also be applicable to FLOE1

Processing bodies: also known as P-bodies; cytoplasmic ribonucleoprotein condensates primarily composed of translationally repressed mRNAs and proteins related to mRNA decay, suggesting roles in post-transcriptional regulation.
Shell: condensates can be viewed as a biphasic composition of two concentric layers, from inside out, the core (inner) and the shell (outer). Sometimes, the shell can be looser and more watery (e.g., in stress granules).
Stress granules: cytoplasmic ribonucleoprotein condensates that form in response to various stressors, including heat, oxidative stress, viral infections, and nutrient deprivation. Stress granules can act as sites for the sorting and processing of specific mRNAs, allowing for rapid changes in gene expression in response to stress.
Valency: bond number forming inter- or intramolecularly, between homotypic molecules (e.g., protein–protein) or heterotypic, RNA–protein.
Wetting: the ability of a liquid to maintain contact with a (solid) surface, resulting from intermolecular interactions when the two are brought together.

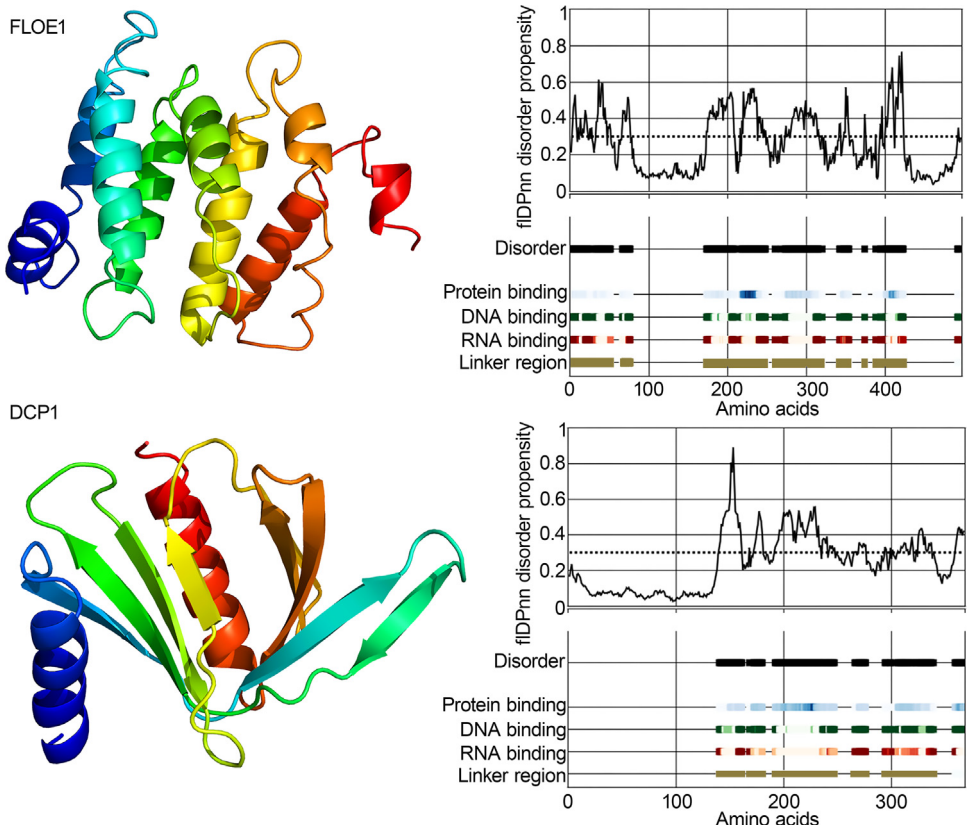

Figure 1. Scaffolding proteins for condensates can have lipid-binding motifs. Predictions for the structure of FLOE1 and DCP1. Upper: in FLOE1, 164 residues (33% of the sequence) have been modeled with 92.5% confidence by the single highest-scoring template; 77% of the FLOE1 sequence is predicted disordered. Lower: in DCP1, 123 residues (34% of the sequence) have been modeled with 100.0% confidence by the single highest-scoring template. In both models, as expected, intrinsically disordered regions (IDRs) could not be meaningfully predicted and the putative function- and linker-based disorder prediction using a deep neural network was used to infer and demonstrate IDRs (right). The templates used for FLOE1 were c5yz0C, c7nsuD, and c6o7xa, corresponding to ataxia Telangiectasia and Rad3-related protein (known as 'ATR'), plasma membrane ATPases, and bacterial pore-forming colicins, all of which interact with membranes. The template used for DCP1 was c2ifsA; interestingly, this template corresponds to the pleckstrin homology (PH) domain-like barrel structure of the N-WASP EVH1 domain. The EVH1 domain mediates the binding of N-WASP with phosphatidylinositol 4,5-bisphosphate lipids [66].

and could explain why imbibition could lead to its condensation. Furthermore, the anti-aggregation activity of some IDPs, like LEAs, is a consequence of physical interference. In particular, IDRs decrease the encounter frequency of (aggregating) proteins by intercalating between them through the stereochemical flexibility and the fluctuating molecular radii provided by IDRs [30]. We speculate that LEAs interface with FLOE1 and/or DCP1 at membranes but exert opposite functions: FLOE1 sequesters inhibitors of growth [19], while LEAs could be degraded to release growth-promoting factors.

Although it is unclear by which mechanism phase separation of IDPs (like DCP1 discussed earlier) takes place on membranes, this evidence suggests that condensation may implicate membranes in plants. Furthermore, although the internal forces of condensates are well-studied, we know little about their boundaries and how these could affect interactions with membranes (i.e., their external forces). These boundaries, which can correspond to the so-called '**shell**' in the jargon of the field, do

not necessarily reflect the properties of the innermost core (Box 1). For example, the plant condensate, formed by the auxin responsive factors (ARF) in the cytoplasm, has a more rigid shell due to strong intermolecular interactions between ARF monomers there [6]. Through condensates, the ARF condensate sequesters ARF molecules and restricts their entry into the nucleus. In this way, ARF condensates restrict auxin signaling. Unlike other condensates (e.g., the **stress granules** [31]), ARF has an inverted organization, with the hard part being outside, resembling a raw egg with a rigid shell outside and watery whites and yolk inside. Other condensates resemble peeled boiled eggs, with their harder core (the yolk) in the center and the softer whites representing the shell. We speculate that interactions between ARF monomers at the shell likely saturate accessible interacting sites, inhibiting bond formation with membranes or the dilute phase. Indeed, the ARF condensate is unlikely to interact with membranes, as we have observed *in vivo* (Liu *et al.*, *unpublished*), suggesting a mechanism by which some condensates could remain 'membrane-less'.

Condensates at specialized membrane sites: new functions?

Emerging evidence suggests that membrane-bound condensates regulate various processes in plants, such as the endo/exocytosis [22,32,33] and translational landscapes [22], as detailed later. Perhaps, the first evidence that a membrane condensate can regulate membranes in plants came from elaborate imaging studies of plant vacuoles [34]. Yet unknown condensates forming in the vacuole can regulate the vacuolar morphology. The vacuole is likely pinched off by stereochemical pressure brought about by these yet elusive condensates on the tonoplast, and these condensates could theoretically be controlled by alterations in the vacuolar pH.

Cohesive forces within condensates between their molecules (e.g., at their IDR stickers; Box 1) make them minimize their surface and, thus, look like droplets (i.e., small spheres), at least when they do not encounter other surfaces (e.g., membranes) or are subjected to mechanical application. This surface tension also drives the shape relaxation observed when condensates fuse: upon early steps of fusion, condensates resemble dumbbells, which are then reshaped ('relaxed') into a single spherical condensate. Surface tension may play a significant role in the interactions/fusions of condensates with one another or with surfaces like membranes (Figure 2A). When encountering a surface like a membrane, and if their components interact with this surface, condensates with low surface tension can transform into a lens-like shape in response to adhesion, known as '**wetting**', and if they stay there for long, they may undergo changes in their properties (Figure 2A). This shape can be determined by three energies associated with interacting interfaces: condensate–substrate (in this instance membrane), condensate–cytoplasm (also known as droplet surface tension), and cytoplasm–substrate [35]. These energies depend on nonspecific (e.g., electrostatic) and specific (e.g., protein–protein and protein–lipid) interactions and can alter the properties of the membrane through, for example, steric pressure (Figure 2B) [36]. If the condensate–membrane interaction is particularly favorable in comparison with the cytoplasm–substrate (i.e., membrane) interaction, the condensate will likely wet the membrane.

When encountering a membrane and subjected to wetting, optically, by forming lens-like films, condensates would resemble **lipid rafts** [37]. As such, condensates could perhaps be ignored or misinterpreted. More importantly, wetting can be partial and patchy, especially when condensates show a preference for specialized membrane domains (e.g., through specific protein–lipid interactions) or when there are not enough molecules offered by condensates to fully wet the membrane surface. Such partial wetting can be seen in a piecemeal type of autophagy ('fluidophagy'). This process results in condensate splitting, as the autophagosome wets part of the condensate, as revealed by microscopy [35,38]. Another mechanism of condensate splitting that also involves specialized membranes was recently described in arabidopsis. Through a mechanism that is not yet fully understood, the suppressor of the cAMP receptor (SCAR)–WASP

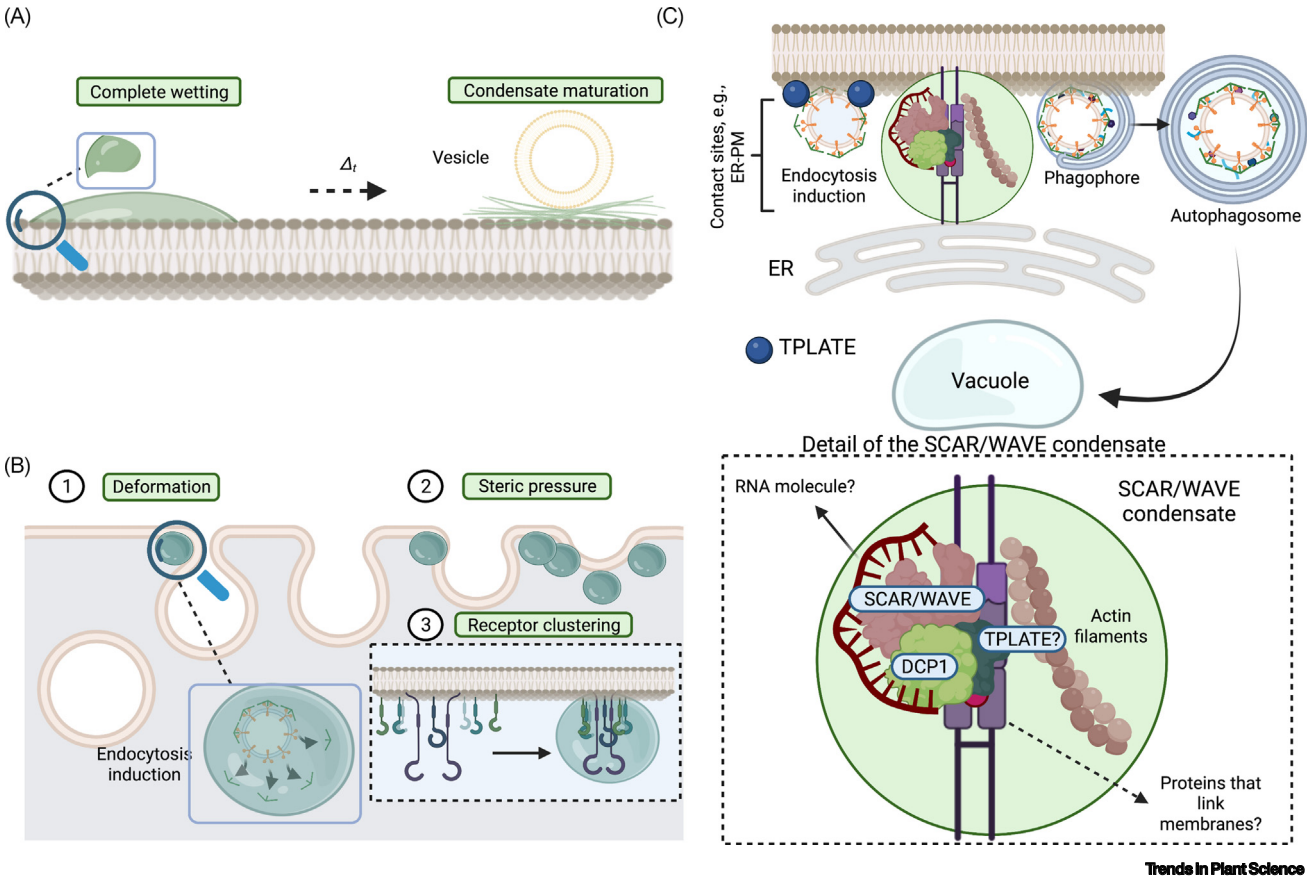

Figure 2. Membrane wetting by condensates affects both condensates and the membrane. (A) Condensates can wet membranes forming lens-like structures with rounded ends. After some time, they may change material properties and attain filamentous structure driving the initiation of cellular processes such as endocytosis [32]. (B) Condensates can wet membranes to deform them. Condensates attached to the membrane can induce mechanical stress to deform membranes under high tension, such as on the plasma membrane, and induce endocytosis (1); impose steric pressure, which can limit or modulate deformation (2); or induce receptor clustering through their material properties: their higher viscosity can reduce lateral motility of receptors that are encapsulated by these condensates (3). (C) Condensates can function as molecular hinges due to their increased density and viscosity, which can endow them with glue-like properties through elastocapillarity (much like the stickiness of honey). While the apposed membrane surfaces do not directly touch or fuse, protein assemblies at these interfaces facilitate tethering and signaling. Interestingly, the endoplasmic reticulum surface has a good tendency to attract condensates, as it, for example, is intertwined in human cells by a condensate known as TIS granule [52]. A blast search revealed AT1G60590 (putative pectin lyase-like) as a TIS granule homolog in arabidopsis (query coverage 18%, E value = 5e-23, identity 66.13%). Prediction of IDR by putative function- and linker-based disorder prediction shows that AT1G60590 is an intrinsically disordered protein (IDP) protein. Condensates, like, for example, TPLATE complex or DCP1, could in theory cluster molecules involved in membrane tethering, like VAPs (denoted as 'proteins that link membranes?'), and can promote condensation of actin nucleators (SCAR/WAVE) to enhance endocytosis and autophagy (lower part for details). RNA molecules may also participate in the process. The autophagosomes are then delivered to the vacuole. Perhaps, RNA molecules could also be involved in these processes. Recently, RNA–membrane interfacing with functional implications was shown [45]. Figure created with BioRender.com. Abbreviations: ER-PM, endoplasmic reticulum–plasma membrane contacts; SCAR, suppressor of the cAMP receptor; WAVE, WASP family verprolin homologous.

family verprolin homologous (WAVE) complex attached to the plasma membrane retracts the protein DCP1 from processing bodies, leading to their rapid splitting [23]. Interestingly, once removed from processing bodies, DCP1 forms condensates with SCAR/WAVE that presumably wet the plasma membrane at the cell edges and nucleate actin.

Another plant condensate interfacing with the plasma membrane in arabidopsis is the one formed by subunits of the TPLATE complex. This complex represents a specialized endocytosis machinery in plants [33]. The TPLATE component AtEH1/Pan1 colocalizes with the protein Vesicle-Associated Protein 27 (VAP27), which demarcates contact sites between the endoplasmic

reticulum and plasma membranes. In these sites, AtEH1/Pan1 recruits actin nucleating components of the SCAR/WAVE complex to initiate autophagy or endocytosis [33] (Figure 2C). Interestingly, this model for T-PLATE function is akin to yeast models, where a homolog of AtEH1, the EDE1, forms condensates to sequester abortive clathrin complexes from the plasma membrane through autophagy [39]. In a speculative scenario, as processing body components interact with both the TPLATE subunits and SCAR/WAVE [23,40], it is likely that TPLATE also coordinates the split of processing bodies by repurposing endocytosis or autophagy to execute a piecemeal split akin to fluidophagy (Figure 2C). When processing bodies split, RNA molecules residing in them could theoretically be freed and translated, with possible implications for plant physiology.

Condensates, in turn, could likely lead to the reinforcement of contact sites between membranes (e.g., endoplasmic reticulum–plasma membrane), functioning as molecular hinges. In these sites, an interplay between membrane elasticity and condensate wetting could lead to the physical phenomenon known as **elastocapillarity**. For example, condensates may form at endoplasmic reticulum–plasma membrane contacts, where complexes like TPLATE could stimulate them [13,41] (Figure 2C). A similar mechanism could be extrapolated for various types of contacts between organelles. The endoplasmic reticulum can make extensive and dynamic contacts with other membrane-bound organelles such as mitochondria, the *trans*-Golgi network, vacuole, and oil bodies. These condensate-driven contacts could likely drive the composition of these organelles, actin polymerization, or even the links between RNA–lipid interactions [42,43], thereby affecting multiple processes.

Mutual potentiation of membranes and condensates

Upon the formation of contacts between lipids of membranes and proteins in the condensates, the fluidity of membrane lipids can be affected [44]. Furthermore, the role of RNA molecules residing in condensates in the same context should not be excluded, as likely they can also form contact with lipids [45]. Collectively, such interactions could affect the formation of lipid patches enriched in certain lipids, as has been confirmed *in vitro* [46,47]. However, if these lipids embed receptors, this can lead to their clustering, thereby enhancing their ability for ligand binding or endocytosis, separate to the direct effects through steric pressure described earlier (Figure 2B). An example of condensate-driven ligand binding enhancement has been shown in animals for T cell receptors [48]. Whether similar processes exist in plants is unknown.

Interestingly, upon contact with surfaces, condensate viscosity increases in the vicinity of its boundary, allowing condensates to nucleate more easily on wetting substrates (e.g., membranes) compared with nonwetting ones [49]. Protein diffusion is restricted for condensates assembled on membranes, which can explain why condensates in cells do not grow very big. Yet, this diffusion restriction allows condensates to undergo energetically favorable nucleation, leading to a significant multifold reduction of the $C_{threshold}$ (or C_{sat}) considerably below that which can be observed in the cytoplasm (Box 1) [50]. Furthermore, condensate–membrane interfacing may involve specific anchoring by trans-membrane domains, lipid-anchors, or other interactions [46]. Accordingly, in animals, phase separation of argonauts (AGOs) on the endoplasmic reticulum is mediated by phosphatidyl inositol lipids [51], while the human condensate known as 'TIS granule' wets the endoplasmic reticulum through RNA anchors [52]. We speculate that the reduction and tuning of diffusion via membrane tethering through specific lipids may serve a general function in controlling condensate size and function throughout the cell.

Finally, membrane wetting by condensates could reduce or enhance their accessibility by secretory vesicles or biomolecules. This 'IDP wetting' could involve transmembrane or membrane-

bound proteins forming a presumable 'fence-like' structure, locally insulating the membrane [53]. Interestingly, ~45% of the IDPs are transmembrane proteins, with the IDRs being exposed mainly to the cytoplasmic face [54]. Experimental evidence in plants in this direction was recently provided showing that the lipid-transferase Sec Fourteen Homologue 8 (SFH8) is an IDP that can wet membranes and form condensates which can reduce fusion between artificial liposomes [32]. Similarly, other IDPs (e.g., the oleosins) cover oil bodies to reduce their fusion with one another, but it is unclear whether their IDRs play a role in this process [55].

Concluding remarks and future perspectives

Proteins tend to become more disordered during evolution, which suggests a positive selection pressure as cell biology tends to get more complicated. These increments in disorder suggest that more interacting-prone chunks of proteins could be available, enabling a wider network of interactions [56]. It is unclear, however, in the same context whether RNA molecules also encompass features that increase valency during evolution. Other advantages of accumulating disorder include the finer regulation of protein turnover because of their proteolytic degradation [57].

Looking deeper at membrane–condensate interfacing could help to further our understanding of evolution. The mechanism of piecemeal autophagy [35] might be a reminiscent process of the ancient formation of cells. The prime event in the origin of life could be some sort of macromolecular (**colloidal phase separation** of **coacervates**) phase separation [58]. In this type of archetype condensate, the density increase could allow reactions that would have been impossible outside because of low concentrations. The discovery of contemporary condensates suggests that this is likely [59]. Not surprisingly, condensates can be found in bacteria, for example, the BR-bodies that share features with the eukaryotic processing bodies and stress granules and that can interact with membrane proteins [60]. These examples of convergent evolution highlight the necessity of condensates.

Each condensate forms a unique microchemical compartment. Some small-molecule drugs concentrate in specific condensates due to chemical properties engendered by amino acid stretches or single residues in the proteins in those condensates [61]. The ability of rapid evolution within IDR regions may offer uniqueness when it comes to drug targeting, leveraging the control and targeting specificity of various processes in the cell. The increased IDRs stretches could likely associate with enhanced adaptation and signaling plasticity, but also with trade-offs such as aging and diseases. We are still unaware of such trade-offs in plants, making plants an excellent model for the artificial selection of IDPs through breeding. While speculative at this point, the divergent sequences of IDRs provide a mechanism for natural selection to tailor a condensate's material properties to a particular environmental niche.

A further understanding of condensate functions and their interfacing requires first getting a view of what is in but also around them. The interactions among their components are usually weak, and when we try to isolate them, they fall apart. Yet, the advent of new approaches could enable the identification of condensate composition. For example, RNA editing, proximity ligations (that define 'proxitomes' of condensates [23]), or the orthogonal organic phase separation approach for the nonadenylated RNAs [62], are some of the methodologies that could extend the compositional determination of condensates. This knowledge could spur new thinking in the direction of alternative strategies, which may provide the field with much-needed insights.

Here, we discussed how the condensate–membrane interface could reinvigorate studies in phase separation in plants and help in the understanding of structures and functions. We did that in a rather limited context, as the corresponding field in plants is in its infancy. On the membrane–condensate interfaces, more than one phase can coexist; thus, identifying the exact states

Outstanding questions

How general is the phenomenon of membrane wetting by condensates in plants? We know only a handful of such condensates (i.e., TPLATE, DCP1, and SFH8). As recent evidence suggests that condensates can be both cytoplasmic and reform on membranes, further research in this direction is merited.

Membrane rafts: to what extent do they have condensates embedded in them? As condensates optically would attain a lens-like structure when encountering and wetting membranes, further microscopic examination with advanced microscopic methods, *in vitro* reconstitutions on membranes, or microrheology experiments may reveal that they are embedded in known membrane rafts.

Is the membrane a storage depot for scaffolding proteins of condensates? Much like transcription factors that can be linked to membranes and retained there in an inert state away from their action site (the nucleus), scaffolding factors of condensates may be retained until a lipid signal, for example, releases them, allowing their phase separation. This event will be followed by the incorporation of client molecules and the condensate will grow.

What is the hierarchy of protein and RNA modifications and interactions during the formation of a condensate? The case of stress granules shows that condensation takes place around a hardened scaffold core comprising ribonucleoproteins that are either phosphorylated or methylated (i.e., RNA molecules). On this core, a consecutive addition of client proteins and RNA molecules follows. Yet, the hierarchy of these events for other condensates remains unclear. Are membrane-bound condensates built in a similar manner, utilizing a scaffold core, and what type of modifications could be required for their formation?

Why do some condensates not grow larger in size and why can some condensates form a singularity in the cell? Apart from the suggested decrease in diffusion due to membrane interfacing, which can bring about reduced size, the incorporation of some

in the cell, especially at membrane interfaces, will remain challenging (see Outstanding questions).

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Declaration of interests

No interests are declared.

References

1. Brangwynne, C.P. *et al.* (2009) Germline P granules are liquid droplets that localize by controlled dissolution/condensation. *Science* 324, 1729–1732
2. Musacchio, A. (2022) On the role of phase separation in the biogenesis of membraneless compartments. *EMBO J.* 41, e109952
3. Brangwynne, C.P. *et al.* (2015) Polymer physics of intracellular phase transitions. *Nat. Phys.* 11, 899–904
4. Banani, S.F. *et al.* (2016) Compositional control of phase-separated cellular bodies. *Cell* 166, 651–663
5. Cuevas-Velazquez, C.L. *et al.* (2021) Intrinsically disordered protein biosensor tracks the physical-chemical effects of osmotic stress on cells. *Nat. Commun.* 12, 5438
6. Powers, S.K. *et al.* (2019) Nucleo-cytoplasmic partitioning of ARF proteins controls auxin responses in *Arabidopsis thaliana*. *Mol. Cell* 76, 177–190
7. Jawerth, L. *et al.* (2020) Protein condensates as aging Maxwell fluids. *Science* 370, 1317–1323
8. Alberti, S. and Hyman, A.A. (2021) Biomolecular condensates at the nexus of cellular stress, protein aggregation disease and ageing. *Nat. Rev. Mol. Cell Biol.* 22, 196–213
9. Lasker, K. *et al.* (2022) The material properties of a bacterial-derived biomolecular condensate tune biological function in natural and synthetic systems. *Nat. Commun.* 13, 5643
10. Day, K.J. *et al.* (2021) Liquid-like protein interactions catalyse assembly of endocytic vesicles. *Nat. Cell Biol.* 23, 366–376
11. Bose, M. *et al.* (2022) Liquid-to-solid phase transition of oskar ribonucleoprotein granules is essential for their function in *Drosophila* embryonic development. *Cell* 185, 1308–1324
12. Wang, H. *et al.* (2019) Rubisco condensate formation by CcmM in beta-carboxysome biogenesis. *Nature* 566, 131–135
13. Lee, J.E. *et al.* (2020) Endoplasmic reticulum contact sites regulate the dynamics of membraneless organelles. *Science* 367, eaay7108
14. Cho, H.Y. *et al.* (2022) Ethylene modulates translation dynamics in *Arabidopsis* under submergence via GCN2 and EIN2. *Sci. Adv.* 8, eabm7863
15. Zhang, Y. *et al.* (2022) Phase separation of HRLP regulates flowering time in *Arabidopsis*. *Sci. Adv.* 8, eabn5488
16. Kim, J.H. *et al.* (2022) Increasing the resilience of plant immunity to a warming climate. *Nature* 607, 339–344
17. Su, X. *et al.* (2016) Phase separation of signaling molecules promotes T cell receptor signal transduction. *Science* 352, 595–599
18. Wu, X. *et al.* (2020) Liquid-liquid phase separation in neuronal development and synaptic signaling. *Dev. Cell* 55, 18–29
19. Dorone, Y. *et al.* (2021) A prion-like protein regulator of seed germination undergoes hydration-dependent phase separation. *Cell* 184, 4284–4298
20. Halfmann, R. *et al.* (2012) Prions are a common mechanism for phenotypic inheritance in wild yeasts. *Nature* 482, 363–368
21. Tsang, B. *et al.* (2020) Phase separation as a missing mechanism for interpretation of disease mutations. *Cell* 183, 1742–1756
22. Liu, C.M. *et al.* (2021) The dynamic composition of an archetypal plant condensate highlights a tug-of-war between condensates and cell vertex. *SSRN* Published online October 15, 2021. https://doi.org/10.2139/ssrn.3943616
23. Liu, C. *et al.* (2023) An actin remodeling role for *Arabidopsis* processing bodies revealed by their proximity interactome. *EMBO J.* Published online February 6, 2023. https://doi.org/10.15252/embj.2022111885
24. Tapia, H. *et al.* (2015) Increasing intracellular trehalose is sufficient to confer desiccation tolerance to *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U. S. A.* 112, 6122–6127
25. Pagnotta, S.E. *et al.* (2010) Water and trehalose: how much do they interact with each other? *J. Phys. Chem. B* 114, 4904–4908
26. Hesgrove, C. and Boothby, T.C. (2020) The biology of tardigrade disordered proteins in extreme stress tolerance. *Cell Commun. Signal.* 18, 178
27. Belott, C. *et al.* (2020) Liquid-liquid phase separation promotes animal desiccation tolerance. *Proc. Natl. Acad. Sci. U. S. A.* 117, 27676–27684
28. Bremer, A. *et al.* (2017) Folding of intrinsically disordered plant LEA proteins is driven by glycerol-induced crowding and the presence of membranes. *FEBS J.* 284, 919–936
29. Cuevas-Velazquez, C.L. *et al.* (2016) The unstructured N-terminal region of *Arabidopsis* group 4 late embryogenesis abundant (LEA) proteins is required for folding and for chaperone-like activity under water deficit. *J. Biol. Chem.* 291, 10893–10903
30. Zamora-Briseno, J.A. *et al.* (2018) Does water stress promote the proteome-wide adjustment of intrinsically disordered proteins in plants? *Cell Stress Chaperones* 23, 807–812
31. Kosmacz, M. *et al.* (2019) Protein and metabolite composition of *Arabidopsis* stress granules. *New Phytol.* 222, 1420–1433
32. Liu, C. *et al.* (2022) Phase separation of a nodulin Sec14-like protein maintains auxin efflux carrier polarity at *Arabidopsis* plasma membranes. *bioRxiv* Published online March 27, 2022. https://doi.org/10.1101/2022.03.26.485938
33. Dragwidge, J.M. *et al.* (2022) AtEH/Pan1 proteins drive phase separation of the TPLATE complex and clathrin polymerisation during plant endocytosis. *bioRxiv* Published online March 17, 2022. https://doi.org/10.1101/2022.03.17.484738
34. Kusumaatmaja, H. *et al.* (2021) Wetting of phase-separated droplets on plant vacuole membranes leads to a competition between tonoplast budding and nanotube formation. *Proc. Natl. Acad. Sci. U. S. A.* 118, e2024109118
35. Kusumaatmaja, H. *et al.* (2021) Intracellular wetting mediates contacts between liquid compartments and membrane-bound organelles. *J. Cell Biol.* 220, e202103175
36. Wang, H.-Y. *et al.* (2022) Coupling of protein condensates to ordered lipid domains determines functional membrane organization. *bioRxiv* Published online August 3, 2022. https://doi.org/10.1101/2022.08.02.502487

clients may saturate interacting sites. These interacting sites would have otherwise been used for fusions with other condensates. Alternatively, interacting barriers (like in the case of the ARF condensate) may form, thereby insulating condensates and not allowing them to grow further.

What are the material properties of (membrane-bound) condensates (in plants)? Do they change with time or with external stimuli? These two questions relate to the ability of condensates to exchange materials with the surroundings, interface with membranes, or be subjected to deformations. Furthermore, if they can behave as viscoelastic material (a combination of elastic and viscous behavior), there might be some structural memory, which would be important for mechanotransduction.

37. Vequi-Suplicy, C.C. *et al.* (2010) Vesicles with charged domains. *Biochim. Biophys. Acta* 1798, 1338–1347
38. Agudo-Canalejo, J. *et al.* (2021) Wetting regulates autophagy of phase-separated compartments and the cytosol. *Nature* 591, 142–146
39. Wilfling, F. *et al.* (2020) A selective autophagy pathway for phase-separated endocytic protein deposits. *Mol. Cell* 80, 764–778
40. Case, L.B. *et al.* (2019) Stoichiometry controls activity of phase-separated clusters of actin signaling proteins. *Science* 363, 1093–1097
41. Huang, S. *et al.* (2022) The plant ESCRT component FREE1 regulates peroxisome-mediated turnover of lipid droplets in germinating *Arabidopsis* seedlings. *Plant Cell* 34, 4255–4273
42. Quon, E. *et al.* (2018) Endoplasmic reticulum-plasma membrane contact sites integrate sterol and phospholipid regulation. *PLoS Biol.* 16, e2003864
43. Encinar Del Dedo, J. *et al.* (2017) ORP-mediated ER contact with endocytic sites facilitates actin polymerization. *Dev. Cell* 43, 588–602
44. Lee, I.H. *et al.* (2019) Lipid raft phase modulation by membrane-anchored proteins with inherent phase separation properties. *ACS Omega* 4, 6551–6559
45. Czerniak, T. and Saenz, J.P. (2022) Lipid membranes modulate the activity of RNA through sequence-dependent interactions. *Proc. Natl. Acad. Sci. U. S. A.* 119, e2119235119
46. Mondal, S. and Cui, Q. (2022) Coacervation of poly-electrolytes in the presence of lipid bilayers: mutual alteration of structure and morphology. *Chem. Sci.* 13, 7933–7946
47. Andes-Koback, M. and Keating, C.D. (2011) Complete budding and asymmetric division of primitive model cells to produce daughter vesicles with different interior and membrane compositions. *J. Am. Chem. Soc.* 133, 9545–9555
48. Ditlev, J.A. *et al.* (2019) A composition-dependent molecular clutch between T cell signaling condensates and actin. *Elife* 8, e42695
49. Kurotani, Y. and Tanaka, H. (2020) A novel physical mechanism of liquid flow slippage on a solid surface. *Sci. Adv.* 6, eaaz0504
50. Zeng, M. *et al.* (2018) Reconstituted postsynaptic density as a molecular platform for understanding synapse formation and plasticity. *Cell* 174, 1172–1187
51. Gao, Y. *et al.* (2022) Lipid-mediated phase separation of AGO proteins on the ER controls nascent-peptide ubiquitination. *Mol. Cell* 82, 1313–1328
52. Ma, W. and Mayr, C. (2018) A membraneless organelle associated with the endoplasmic reticulum enables 3'UTR-mediated protein-protein interactions. *Cell* 175, 1492–1506
53. Mohammad, I.-L. *et al.* (2019) The disordered boundary of the cell: emerging properties of membrane-bound intrinsically disordered proteins. *Biomol. Concepts* 10, 25
54. Minezaki, Y. *et al.* (2007) Intrinsically disordered regions of human plasma membrane proteins preferentially occur in the cytoplasmic segment. *J. Mol. Biol.* 368, 902–913
55. Kretzschmar, F.K. *et al.* (2018) PUX10 is a lipid droplet-localized scaffold protein that interacts with CELL DIVISION CYCLE48 and is involved in the degradation of lipid droplet proteins. *Plant Cell* 30, 2137–2160
56. Martin, E.W. and Holehouse, A.S. (2020) Intrinsically disordered protein regions and phase separation: sequence determinants of assembly or lack thereof. *Emerg. Top Life Sci.* 4, 307–329
57. Liu, Z. and Huang, Y. (2014) Advantages of proteins being disordered. *Protein Sci.* 23, 539–550
58. Oparin, A.I. (1976) Evolution of the concepts of the origin of life, 1924-1974. *Orig. Life* 7, 3–8
59. Hansma, H.G. (2017) Better than membranes at the origin of life? *Life (Basel)* 7, 28
60. Al-Husini, N. *et al.* (2018) Alpha-proteobacterial RNA degradosomes assemble liquid-liquid phase-separated RNP bodies. *Mol. Cell* 71, 1027–1039
61. Kilgore, H.R. and Young, R.A. (2022) Learning the chemical grammar of biomolecular condensates. *Nat. Chem. Biol.* 18, 1298–1306
62. Queiroz, R.M.L. *et al.* (2019) Comprehensive identification of RNA-protein interactions in any organism using orthogonal organic phase separation (OOPS). *Nat. Biotechnol.* 37, 169–178
63. Ditlev, J.A. (2021) Membrane-associated phase separation: organization and function emerge from a two-dimensional milieu. *J. Mol. Cell Biol.* 13, 319–324
64. Stachowiak, J.C. and Kirchhausen, T. (2022) The beauty of simplicity in membrane biology. *Nat. Cell Biol.* 24, 1682–1685
65. Alberti, S. *et al.* (2019) Considerations and challenges in studying liquid-liquid phase separation and biomolecular condensates. *Cell* 176, 419–434
66. Volkman, B.F. *et al.* (2002) Structure of the N-WASP EVH1 domain-WIP complex. *Cell* 111, 565–576