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Liquid-liquid phase separation as a common organizing principle of intracellular space and biomembranes providing dynamic adaptive responses

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ABSTRACT

This work is devoted to the phenomenon of liquid-liquid phase separation (LLPS), which has come to be recognized as fundamental organizing principle of living cells. We distinguish separation processes with different dimensions. Well-known 3D-condensation occurs in aqueous solution and leads to membraneless organelle (MLOs) formation. 2D-films may be formed near membrane surfaces and lateral phase separation (membrane rafts) occurs within the membranes themselves. LLPS may also occur on 1D structures like DNA and the cyto- and nucleoskeleton. Phase separation provides efficient transport and sorting of proteins and metabolites, accelerates the assembly of metabolic and signaling complexes, and mediates stress responses. In this work, we propose a model in which the processes of polymerization (1D structures), phase separation in membranes (2D structures), and LLPS in the volume (3D structures) influence each other. Disordered proteins and whole condensates may provide membrane raft separation or polymerization of specific proteins. On the other hand, 1D and 2D structures with special composition or embedded IDRs can nucleate condensates. We hypothesized that environmental change may trigger a LLPS which can propagate within the cell interior moving along the cytoskeleton or as an autowave. New phase propagation quickly and using a low amount of energy adjusts cell signaling and metabolic systems to new demands. Cumulatively, the interconnected phase separation phenomena in different dimensions represent a previously unexplored system of intracellular communication and regulation which cannot be ignored when considering both physiological and pathological cell processes.

1. Introduction

To maintain homeostasis and quickly adapt to environmental changes, the vast majority of biochemical reactions inside cells are strictly organized in time and space. Some groups of processes proceed within the “classic” organelles, such as the nucleus, mitochondria, lysosomes, Golgi apparatus and Golgi vesicles, peroxisomes, endosomes, autophagosomes, and endoplasmic reticulum (as well as vacuoles and chloroplasts in plant cells), which are separated from the rest of the cytoplasm by the lipid bilayer membranes. Recent data show that in addition to the membrane-bound organelles, there are also cellular compartments which are separated from the rest of the cytoplasm/nucleoplasm/matrix without membranes [1]. Some of them are solid proteinaceous bodies (such as ribosomes and proteasomes) or insoluble aggregates containing cross-linked proteins and other biological macromolecules (e.g. LS-associated SOD1 aggregates [2]).

However, there are also numerous cellular bodies, so-called membrane-less organelles (MLOs), which represent liquid droplets formed as a result of liquid-liquid phase separation (LLPS). Recently, this phenomenon of biological LLPS and the associated MLO biogenesis have emerged as a new paradigm in the understanding of intracellular organization of both eukaryotes and bacteria [1,3–5]. It should come as no wonder that LLPS disturbances were shown to often serve as the cause of various pathologies [6]. This review briefly describes the basic principles of LLPS in living cells and emphasizes that, in addition to the volume phase separation (volume LLPS), two-dimensional LLPS within

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membranes (membranous LLPS), pseudo one-dimensional LLPS on DNA (within chromatin complexes), and cyto- and nucleoskeleton (LLPS-on-1D) are realized in cells as well.

While liquid separation is always a 3D process, 1D and 2D structural backbones can facilitate or limit its course, influencing biological functions. The schematic representation of different types of structures based on LLPS is represented in Fig. 1. Examples of the specific structures and their description will be provided in the following sections. We consider the structural and functional links between membranous, volume LLPS, and LLPS occurring on 1D scaffolds. This rod/fibril-membrane-volume coupling through LLPS may be an important and previously hidden part of intracellular signaling.

2. Liquid-liquid phase transitions as a mechanism for membraneless organelles formation

Phase separation in biological systems is commonly driven by special proteins that have intrinsically disordered regions (IDRs) and often (but not always) capable of RNA and/or DNA binding. LLPS is based on the multivalent weak interactions between biopolymers, but the exact mechanisms are still not completely understood. It is assumed that IDRs interact with each other, RNA/DNA, and water molecules, changing the local structure of the liquid and its physical properties [1,7]. In vitro experiments suggested that physical properties of formed liquid phases strongly depend on the polymer length and amino acid composition [8]. As a result, IDRs with different primary structures can form immiscible liquid phases allowing cells to form a large number of distinct membrane-less organelles MLOs. In recent years, an increasing number of MLOs have been discovered, the most prominent of which, found in eukaryotes, are presented in Table 1. Molecular condensates formed by LLPS are also present in bacteria [5,9,10].

Due to their liquid nature and lack of the membrane embodiment, MLOs formed by LLPS can quickly exchange molecules with the environment and have a highly dynamic nature – they can be formed and disintegrated in response to various external or internal signals. This property provides a means for quick and reversible adjustment of the metabolism and other cellular processes and functions to changes in the environment, without significant changes in gene expression, protein, and RNA levels. Liquid-liquid phase transitions (LLPTs) are reversible and occur when chemico-physical conditions make them energetically beneficial. Therefore, LLPTs represent a direct reaction of the cell interior to the environmental change and typically do not require additional energy to occur (though some energy may be needed for new phase seed formation). As a result, the use of LLPTs by biological systems is energetically and, therefore, evolutionarily beneficial. A striking example is given by the stress granule formation under stress-induced translation repression. Under these conditions, temporally unnecessary mRNA [65,66] and signaling proteins associated with anabolism [12,67] undergo LLPS to form stress granules. This mechanism provides both protection of temporarily unnecessary mRNAs from degradation and entanglement, and facilitation of the expression of stress defense-related proteins by eliminating competition.

Formation of separated liquid compartments allows not only for separation of the temporarily unnecessary elements from the rest of the cell, but also acceleration of the metabolic and other biological reactions by locally increasing the concentration of active components. The formation of metabolic reactors has already been described, for example, in the case of glycolysis [68]. Furthermore, an increase in the enzyme concentration in MLO may be necessary for further supramolecular assembly of some enzymes into the catalytic assemblies, like in the case of phosphofructokinase-1 filaments [69], in which catalytic domains are exposed to the outside promoting efficient supply and binding of substrates. It is likely that LLPS may be the driver of filamentation of other enzymes (for more examples of such enzymes see [70]). A similar self-assembly mechanism can not only enhance enzyme function but also inhibit it if catalytic domains become inaccessible [71]. The so-called “macromolecular crowding” phenomenon is also observed within the condensates, which, in fact, are overcrowded, since their local concentrations of macromolecules noticeably exceed those of the crowded cytoplasm and nucleoplasm [72]. Such crowding and overcrowding can additionally increase the rates of chemical reactions [73], promote aggregation [74], and alter folding [75]. The effects of macromolecular crowding have been described, for example, in nucleosomes [76,77].

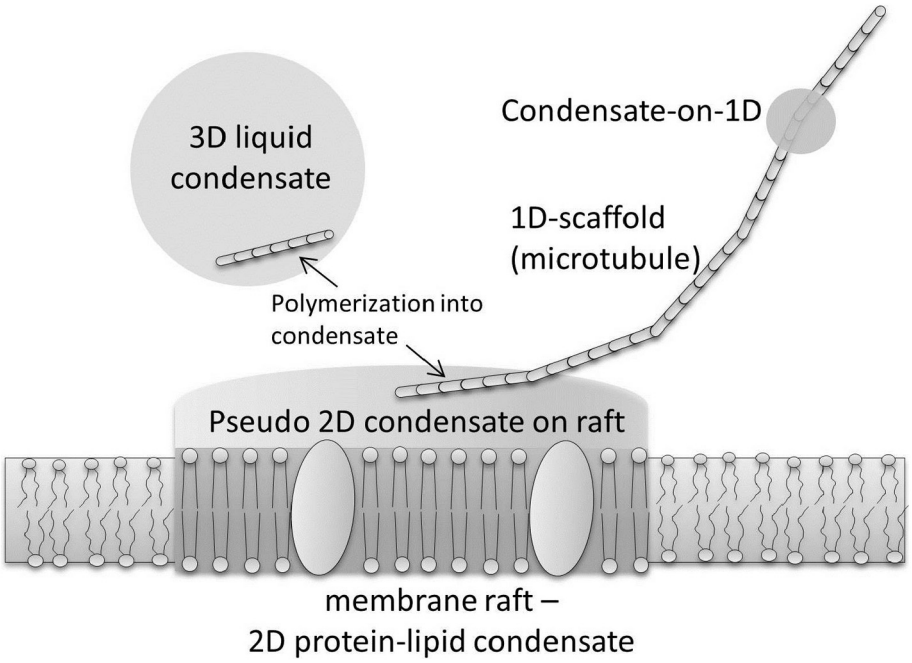

Fig. 1. Schematic representation of different types of cellular liquid condensates and their relationships. The difference between condensate-on-1D and true 3D-condensate is mostly in the larger size of the latter and independence from 1D-scaffold. The membrane rafts may be considered as true 2D condensates, while flattened (e.g. due to tethering to the membrane) protein condensates may be treated as pseudo-2D.

Table 1
The list of known MLOs and molecular condensates.

Condensate	Description
Localized in cytosol	
Stress granules (SGs)	SGs form when translation initiation is impaired. Captures polyadenylic mRNAs, translation initiation factors and some other proteins, which are unnecessary under stress [11,12]. SGs prevent RNA entanglement [13] and promotes a translational switch from housekeeping to stress protein production [14].
Microtubule nucleation centers: centrosomes and TPX2 condensates	Centrosomes are condensed droplets of pericentriolar matrix around centrioles. They are involved in the process of cell division and microtubule nucleation [15,16]. TPX2 protein phase separates with tubulin into a co-condensate, which mediates microtubule nucleation and branching independently from centrosomes [17].
Tau condensates	Tau proteins and RNAs, under charge matching conditions, undergo LLPS in a process known as complex coacervation [18]. Tau condensates form selectively permissible barriers, spatially regulating the activity of microtubule-severing enzymes and the movement of molecular motors through their boundaries [19], providing microtubule nucleation [20].
Processing bodies (P-bodies)	Dynamic structures that function during cellular responses to stress and contain non-translating mRNAs, mRNA decay factors, and some signaling proteins [21,22].
RNA transport particles (neuronal granules, transport RNPs (ribonucleoprotein particles))	Membrane-less condensates of RNA binding proteins and RNAs that associate with motor proteins for their transport along microtubules [23,24].
Germ granules (nuage, germinal granules)	Characteristic cytoplasmic structures of animal germline cells. Associated with RNA metabolism, retrotransposon regulation, and interplay with mitochondria [25,26].
GW-bodies (mammalian processing bodies or P-bodies)	Contains proteins that govern microRNA-mediated silencing. GW-bodies most likely function as repositories for translationally silenced RNAs [27]. GW-bodies may also be present in nuclei.
U-bodies	U-bodies are cytoplasmic structures that contain uridine-rich small nuclear ribonucleoproteins and associate with P-bodies [28]. May be the storage of small nuclear ribonucleoproteins before their import into the nucleus. U-bodies increase in size in starved conditions [29].
Localized in nucleus	
Nucleoli	The nucleolus consists of at least three distinct phase-separated layers [30], participates in ribosome formation.
Nuclear pore complexes (NPCs)	NPCs regulate bidirectional traffic of macromolecules between the nucleus and cytoplasm. The permeability barrier is formed by phase separated nucleoporins in the interior of pore complex [31].
Heterochromatin	Heterochromatin has essential roles in nuclear architecture, DNA repair, silencing of transposon, and gene expression. Heterochromatin domains exhibit dynamics that are characteristic of liquid phase-separation [32,33].
Histone locus bodies (HLBs)	HLB concentrates factors necessary for histone gene transcription and pre-mRNA processing, enhancing the efficiency of histone mRNA biosynthesis [34].
Cajal bodies (CBs, coiled bodies)	CBs are sites for assembly and posttranscriptional modification of the splicing machinery of the nucleus [35]. Contains signature marker protein coilin.
Nuclear gems	Gems are closely related to CBs. Gems usually do not contain coilin, but do contain SMN (survival of motor neuron) protein whose function relates to small nuclear ribonucleoproteins biogenesis [36].
Nuclear stress bodies (nSBs)	nSBs are subnuclear organelles which form in response to heat shock. They are thought to participate in rapid, transient, and global reprogramming of gene expression through different types of mechanisms, including chromatin remodeling and trapping of transcription and splicing factors [37]. There are two distinct sets of nSBs [38].
DDX1 body	Contains DEAD box protein DDX1 which is a putative RNA unwinding protein that has been associated with RNA processing and RNA transport [39]. DDX1 rapidly redistributes to the sites of DNA double-strand breaks after cell expose to ionizing radiation [40].
Cleavage bodies	Contains factors for 3′-terminal processing of polyadenylated mRNAs [41]. DDX1 bodies, cleavage bodies, Cajal bodies, and gems associate with each other during S phase [39].
Nuclear speckles (NSs, splicing speckles, interchromatin granule clusters)	NSs are nuclear dynamic domains located in the interchromatin regions of the nucleoplasm of mammalian cells [42]. NSs contains numerous proteins crucial for epigenetic regulation, chromatin organization, DNA repair, and RNA modification [43].
Paraspeckles	Paraspeckles are discrete bodies in the interchromatin nucleoplasmic space that are often located adjacent to NSs [44]. Para-speckles are built on long noncoding RNA and regulate genes expression by nuclear retention of RNA [45].
OPT domains (Oct1/PTF/transcription domains)	OPT domains seem to act like nucleoli to bring particular genes on specific chromosomes together to a region where the appropriate transcription and processing factors are concentrated [46].
Polycomb bodies (PcG bodies)	Polycomb bodies are foci of polycomb group (PcG) gene-silencing proteins. They appear to be sites of gene repression [47] and possibly sumoylation centers [48].
Perinucleolar compartment (PNC)	The PNC is a dynamic body on the nuclear periphery, which is highly enriched in RNA-binding proteins and RNA polymerase III [49]. PNC forms exclusively in cancer cells, and the number of PNCs directly correlates with the malignancy of cancer cell population [50].
Sam68 nuclear bodies (SNBs)	SNBs are unique structures associated with the surface of nucleoli and involved in the RNA metabolism [51].
Promyelocytic leukaemia (PML) oncogenic domains (PODs, PML bodies, Kr bodies)	PODs play a role in transcriptional regulation and appear to be targets of viral infection [51], promote telomere lengthening and participate in the DNA damage response [52,53].
DNA damage foci	DNA damage response foci form due to LLPS of DNA damage response factors and provide DNA repair. Poly(ADP-ribose) can nucleate liquid demixing [54]. LLPS is also modulated by noncoding transcripts synthesized at DNA damage sites [55] and DNA damage-inducible intranuclear microtubule filaments [56].
Super-enhancers (SEs)	Clusters of enhancers that cooperatively assemble a high density of the transcriptional apparatus to drive robust expression of genes with prominent roles in cell identity [57,58].
Localized in mitochondria or chloroplasts	
Mitochondrial RNA granules (MRGs)	MRGs are sub-compartments formed of newly synthesized RNA, RNA processing proteins, and mitoribosome assembly factors [59]. MRGs associate with the inner mitochondrial membrane and their fusion coincides with mitochondrial remodeling.
Mitochondrial nucleoids	Nucleoid is the organizational unit of mitochondrial genome forming by LLPS and consisting of mitochondrial DNA and associated architectural proteins [60]. Oxidative damage induces fusion of nucleoids, contributing to mitochondrial dysfunction [61].
Mitochondrial RNA degradosomes	RNA degradosomes are formed in specific foci which co-localize with mitochondrial RNA and nucleoids [62]. It is likely that mitochondrial degradosomes are formed by LLPS as bacterial ones [63].
Chloroplast stress granules (cpSGs)	cpSGs contain poly(A)-binding protein and the small ribosomal subunit and mRNA. They form during oxidative stress and disassemble during recovery from stress [64].

3. Phase separation on one dimensional (1D) structures

Biomolecular condensates have liquid-like properties that commonly imply a spherical-like structure. However, biomolecular condensates may also be formed on linear quasi 1D-structures (such as DNA and the cyto- or nucleoskeleton), which are significantly longer than the diameter of biocondensates. In these cases, the condensate still remains as a three dimensional (3D) structure, but for some purposes may be reasonably considered as a point on an axis. Such LLPS-on-1D is a way to ensure protein-protein interactions on the cytoskeleton and DNA, because partner-search for a 1D-bound protein is limited by partner diffusion. In these conditions, partner recruitment is effective only for "special" proteins, e.g. for IDR-containing ones [78]. Disordered proteins have the advantage of being highly specific with low affinity [79], have enhanced binding rates [80] and enlarged capture radius, and have the potential to interact with multiple binding partners [81]. Of course, not all protein complexes formed in 1D are dynamic liquid drops. The formation of some protein complexes occurs due to classical highly specific interactions.

LLPS has a particularly important role on quasi-1D DNA chains, where it participates in transcription activation. The predicted propensity for LLPS formation appeared to be larger for transcriptional activators compared to repressors [82], which is consistent with the fact that transcription factors usually have IDRs [78]. IDPs and IDRs frequently include so-called molecular recognition features (MoRFs) that possess a unique ability to undergo disorder-to-order transition upon binding to their protein partners [83]. These MoRFs provide the means for regulation of transcriptional activity, allowing multistep activation and interactions with transcription co-regulators [78].

Classically, cooperative binding describes the phenomenon that the binding of one TF molecule to DNA impacts the binding of another TF molecule [84,85]. Interaction of a transcription factor's IDRs with coactivators induces formation of a nano-drop-like structures (called typical enhancers) on the double-stranded DNA axis, which serves to activate transcription (reviewed in [86]). More sophisticated structures can even combine two double-stranded DNA regions through 3D structures of biomolecular condensates. For the concerted enhancement of multiple genes expression, occurring in stem or tumor cells [57] or under viral infection [87], condensates from transcription factors and coactivators of different genes are combined, forming joint 3D-structures called super-enhancers [86]. Super-enhancers (SEs) are clusters of enhancers that are occupied by exceptionally high densities of transcriptional machinery and regulate genes with especially important roles in cell identity [88,89]. These condensates are large enough to be placed on par with other non-membrane organelles (Table 1). Other processes on DNA can also use biological condensates, e.g. DNA-damage-response [90,91] and co-translational RNA splicing [92].

Direct Interactions exist between biomolecular condensates and the cytoskeleton, which is involved in transport and remodeling of condensates (see [93] for a recent review). Some structural components of the cytoskeleton have IDRs [94,95], and cytoskeleton interacting proteins are characterized by high disorder [96] as in the case with DNA-interacting proteins. The cytoskeleton is a dynamic, changing structure of 1D filaments. Microtubules form the basis for intracellular transport and mitotic spindle. Microtubules are the base for self-organization of cellular units [97]. They are formed in special nucleation centers, such as TPX2 condensates [17], centrosomes [15], and tau condensates [20] (mentioned in Table 1). Actin microfilaments are concentrated at the outer membrane of the cell, maintaining the cell's shape by interacting with the plasma membrane. Hence they can regulate not only 3D and 1D phase separation, but also 2D separation in the membranes and near their surface, e.g. compartmentalization of caveolae-resident signaling components (such as GPCRs) [98], polarity of endothelial and epithelial cells, cell migration, mechanotransduction, lymphocyte activation, and neuronal growth and signaling (reviewed in [99]).

1D filamentous structures and liquid condensates are interconnected. For example, microtubule interacting tau protein forms 3D condensates, which spatially regulates the activity of microtubule-severing enzymes and the movement of molecular motors [19]. On the other hand, tau condensates form local tubulin nucleation centers providing tubulin polymerization [20]. So, in this case both 1D structures promote condensate formation and, in reverse, the condensate promotes 1D structure formation. A similar but slightly different situation occurs with actin, the polymerization of which occurs in 2D condensates formed by Nephrin receptors near the membrane surface [100]. As was mentioned, not only special proteins but also some enzymes are prone to polymerization [70]. This process also likely occurs in condensates, such as in the case of phosphofructokinase [69]. Interestingly, both polymerization of enzymes [101] and LLPS [102] in vivo accompany the changes in the metabolic state of the cell. So, polymerization in condensates formed due to environmental changes may be a mechanism of fast metabolic adaptation.

4. Two-dimensional LLPS in lipids – formation of membrane rafts

Lipid membranes existing in an aqueous environment can be considered as an example of phase separation of two liquid fractions having different chemical nature. Fatty acid chains of lipids aggregate with each other due to hydrophobic interactions, while hydrophilic "heads" preferentially interact with water. Lipids are not unique amphipathic substances. Some other polymers have similar two-faced properties [103]. It is well-known that water-lipid mixes do not form homogeneous phases, but lipids aggregate into micelles, liposomes, mono-, or multi-layers. The hydrophobic parts of lipid membranes retain liquid properties, which are well-manifested during lipid diffusion. However, despite the fact that the bilayer membrane is already a separate liquid phase, the multicomponent lipid membrane is capable of further phase separation, which occurs in two-dimensional space (laterally) [104].

Lipids in the membrane can separate into liquid ordered (Lo) and liquid disordered (Ld) phases [105]. These transitions may be accompanied by the formation of intermediate phases, such as the ripple phase, which in some systems is stable in a certain region of the phase diagram (temperature and pressure range). In model membranes, phase separation can be enhanced by physicochemical effects, such as changes in osmotic pressure [106], increases in concentration of the divalent cations [107], and addition of some peptides [108] and proteins, including cytochrome *c* [109,110], ATP synthase [111], and proteins containing IDRs [112,113]. The membrane rearrangement can also be caused by contact with a liquid condensate [59].

The membrane raft concept suggests that cell membranes are capable of spontaneous self-organization into structurally and functionally separated liquid domains. This separation is caused by structural differences between the lipid species and their tendency to preferentially interact with each other or certain proteins [114]. As recent experiments show, phase separation into large domains visible under a microscope is not observed in the membrane of living cells stretched over the cytoskeleton [115]. However, the formation of nanodomains of different phases still occurs, although they are smaller than fluorescence microscope resolution [116].

Regions rich in certain types of lipids can form around some proteins. Currently, a significant amount of literature data suggests that some lipids selectively bind to special protein sites. Detection and identification of such tightly bound lipids is possible even in extracted preparations using NMR [111,117], X-ray [118], and electron microscopy [119]. In addition, the formation of even very large domains (more than 100 nm) can be induced by stressful conditions, such as membrane damage [120] or curvature changes [121]. A striking example of a raft-like structure, formation of which is associated with the membrane curvature change, is given by caveola [122].

As aforementioned, the state of lipid membranes stretched over the cytoskeleton can significantly differ from that of liposomes. Therefore, transferring results from a model to living systems requires special care. The membrane-associated cytoskeleton illustrates interactions between 1D and 2D structures. On the one hand, it suppresses large-scale phase separation within the membrane below the phase transition temperature; on the other hand, it preserves phase separation above the transition temperature [123]. Protein scaffolds exist not only in cytosol, but also in some organelles. For example, in mitochondria, the topology of the inner membrane is determined by the formation of ATP synthase oligomers [124,125], as well as by special dynamin-like proteins such as Opa1 and some others [126]. The disruption of ATP synthase oligomerization or Opa1 deletion significantly alters membrane topology, leading to the smoothing of cristae folds, which leads to significant functional impairments [127,128]. Therefore, protein scaffolds significantly affect mitochondrial membranes. Membrane-shaping proteins also stabilize large lipid domains in the endoplasmic reticulum [129]. It is logical to assume that protein scaffolds also affect phase behavior of lipids in other organelles. Clarification of the backbone proteins role in the formation of membrane rafts in various organelles is a promising topic for future research.

It should be added that 2D condensates do not necessarily form only in the membrane itself. Biocondensates formed in the organelle interfaces [130] may be also considered as two-dimensional, when their surface is much larger than their thickness. The formation of pseudo-two-dimensional protein condensates is also recorded at the surface of non-joined membranes [100]. In this case, the two-dimensional surface of the membrane with embedded proteins serves as a seed for the condensation of partner proteins due to multivalent interactions. Such surface-bound condensates may be common in cells (see [131] for review).

5. Membrane rafts in the signaling system

Membrane nanodomains can serve as a seed for further clustering. Rafts are more densely packed, more viscous, rich in saturated lipids, sterol analogs, and lipidized proteins [132]. Currently, the most-known function of rafts is sorting and targeted transport of proteins [133]. Formation of rafts differencing in thickness and lipid composition provides the sorting of integral proteins and their clustering. The formation of lipid-protein domains in membranes modulates membrane-associated processes. Both excessive solidification (transition to the gel phase) and viscosity reduction (which can be triggered by specific environmental conditions, e.g., temperature change) makes the formation of rafts impossible, and leads to the inability to regulate membrane processes with their participation. It is known that bacteria and yeast adjust the lipid composition of the membrane in accordance with environmental conditions [134,135]. By maintaining the lipid composition, which allows nanodomain formation, unicellular organisms are likely optimizing the functioning of vital membrane processes, including the systems of Na^+, K^+, and pH homeostasis maintenance [136].

Many signaling cascades include secondary messengers that are located in the membrane or associate with it [137]. Lipid breakdown products, such as diacylglycerol, phosphatidic acid, and inositol 1,4,5-trisphosphate are themselves modulators of important biological processes serving as activators of protein kinases (PKC [138], mTORC1 [139]) and receptors (IP3R [140]). Charged lipids, such as phosphatidylinositol phosphates, activate 3-phosphoinositide-dependent kinases, which participate in insulin, growth factor [141], and NF-kB signaling cascades [142]. These lipids can be incorporated into membrane rafts [143,144], which should affect the membrane binding and activity of associated signaling proteins [145]. The compartmentalization into membrane rafts is important for insulin signaling [141,146–148].

Nanodomains also affect GPCR functioning [149,150] and phospholipase activity [150–153], which are mediators of many signaling cascades. The activity of enzymes is influenced not only by the lipid composition and thickness, but also by the membrane curvature [154], which increases upon clustering of conically shaped lipids and proteins [155]. The asymmetric distribution of lipids between inner and outer membrane leaflets also plays an important role in signaling processes. For example, plasma membrane phosphatidylserine is exposed on the outer leaflet of cellular membrane during apoptosis or necrosis, and works as an “eat me” signal for macrophages [156]. Cumulatively, the receptor signal can stimulate the formation of the membrane raft, which will nucleate and organize large protein complexes. The participation of IDR-containing proteins in these complexes leads to the formation of a liquid condensate (Fig. 2a).

It should be noted that raft formation can be activated without specific receptors, simply by changing the environmental conditions. As mentioned above, phase separation in the membrane can be caused by divalent cations, pressure, and protein or peptide binding. Phase separation in one membrane leaflet can cause separation in another, leading to the formation of a raft, interacting with proteins on both sides of the membrane and affecting their functioning. If a membrane raft has an affinity to any protein with IDRs, it can lead to the synchronous condensation of the liquid phase in the vicinity of the membrane (Fig. 2b). In addition, this process may lead to an increase in the membrane raft itself, thereby providing amplification through positive feedback, enlarging signaling domains up to micrometer scales [100].

Therefore, the induction of lipid phase separation by the receptor signal, physicochemical change, or special protein binding organizes the integral and membrane-interacting proteins, which in turn may induce condensate formation near the membrane raft. By this mechanism, a signal can be transmitted from one side of the membrane to the other with the participation of transmembrane receptors (Fig. 2a) or even without them (Fig. 2b). Such signal transmission may be essential for the processes tightly associated with membrane phase separation: phagocytosis [157], cytokine secretion [158], regeneration [159], endocytosis [160], formation of intercellular [161], and inter-organelle contacts [162].

6. On the possibility of signal transmission through the phase transition wave

In addition to condensing certain proteins in a separate liquid phase, the phase separation process can serve as a signal itself [163], because in some cases, even small changes in external or internal conditions can induce phase separation (Fig. 3). The new phase can move or spread from the place of its initial appearance. This can occur due to ATP-dependent movement of a granule by attached motor protein [164] or due to diffusion and incorporation of disordered proteins. Therefore, phase movement can occur like an autowave. Such a wave may obey the same physical and mathematical laws as the non-equilibrium chemical reaction of Belousov-Zhabotinsky (see review [165]) or may represent another type of autowave, such as switching or trigger wave [166] (like so called “domino effect”).

For autowave propagation, an activator and an inhibitor are required. In the case of phase separation, the process catalyzing phase separation can be a structural change of an activator protein that can be triggered, for example, by its post-translational modification, changes in interactions, or pH change. The process inhibiting or reversing phase separation, on the other hand, may be the opposite structural changes in the IDRs. This process may have oscillating behavior if there are appropriate negative feedbacks in the system.

One important factor in the control of LLPS is a local pH change. In many systems, liquid-liquid phase transitions (LLPTs) can be caused by a pH decrease [167]. Such a decrease (acidification) occurs in yeast and Drosophila cells under energy stress [102]. In mammalian cells, a pH decrease occurs under conditions of ischemia or hypoxia. A decrease in pH is a trigger for cellular adaptation, including the initiation of mitophagy and autophagy [168]. Recent data show that these processes are associated with LLPS [169]. Therefore, due to the control over the

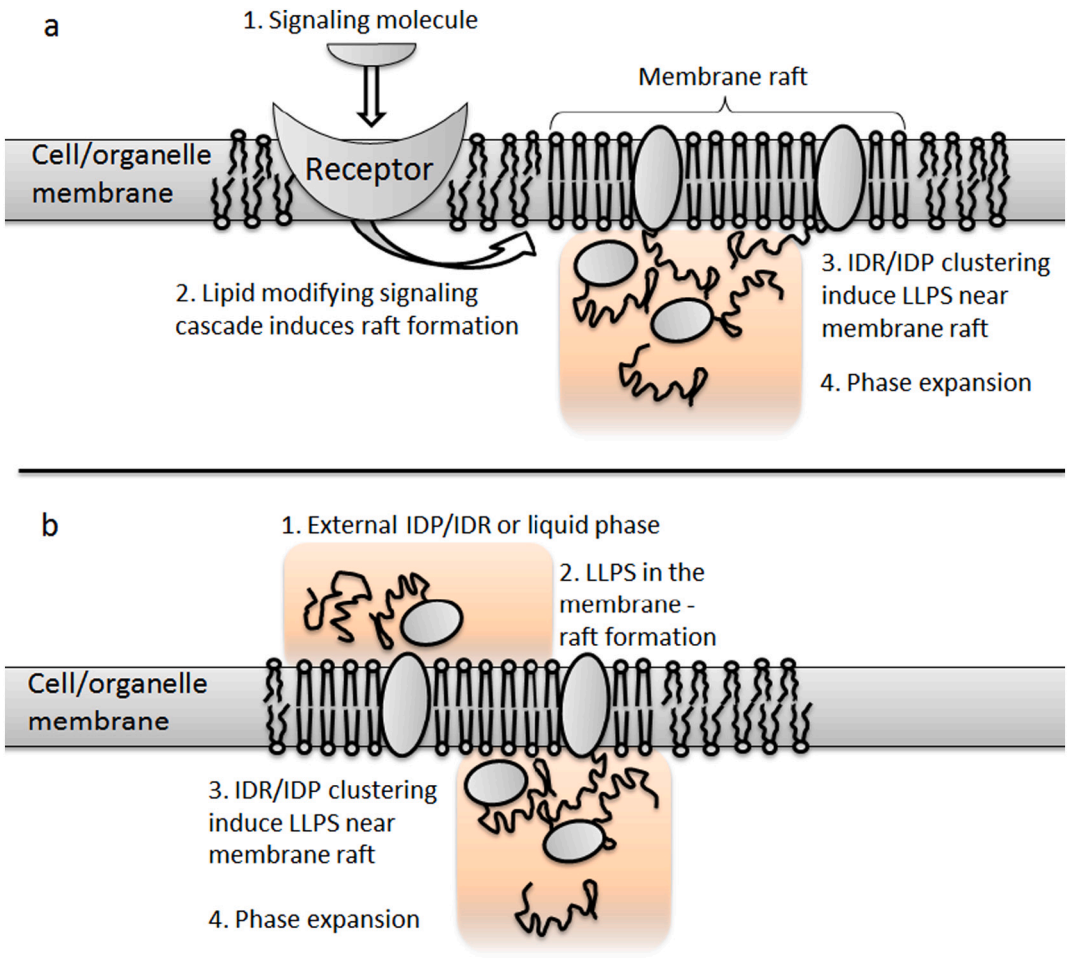

Fig. 2. Illustration of the coupling between 2D and 3D LLPS (i.e., LLPS in membrane and in volume). Two mechanisms of phase separation induction are proposed: a). Raft formation is initiated by a signal from membrane receptor; b). Raft formation is initiated by the contact of the membrane with IDR-containing proteins or a separated liquid phase.

activation of the key catabolic adaptation process (autophagy), LLPTs initiated by physicochemical changes may switch the metabolism. Importantly, the signal may be transduced through autowave mechanism for a long distance (in suitable environment) and can even serve as a synchronization mechanism within the cell. To understand the role of the time factor and its significance for cell signaling, it is necessary to conduct special experiments on the kinetics of phase separation processes in living cells in combination with the study of known signaling cascades and functional changes in the cell.

7. Discussion

The formation of separate phase droplets potentially provides an answer to one of the most important questions of cell signaling – how relatively small changes in protein physico-chemical properties (for example, phosphorylation) might cause significant rearrangements of its interactions, sometimes associated with the formation or disassembly of large signal clusters. If such interactions are based on random protein contacts, then this step would be limiting for fast processes and would reduce efficiency of entire system, especially taking the high viscosity of the cytosol into account.

The situation changes if the phosphorylated protein becomes a seed for a phase formation, which significantly increases its effective interaction radius. First, the surface of the condensate is much greater than the effective surface of an individual protein. The presence of interphase boundary allows for surface potential formation, which can further facilitate the attraction of some proteins into the condensate. Therefore, three-dimensional condensates can sort proteins similar to what the two-dimensional phase (membrane rafts) does. Secondly, the restructuring of the medium (water) and the emergence of interphases should change the kinetics of redox reactions proceeding according to the Marcus mechanism [170]. This can accelerate the required metabolic reactions and suppress unwanted parasitic reactions, such as oxidative damage of proteins [171].

It is interesting to note that the view on living cells as a separate mixture of biopolymers in a colloidal state was long present in literature. These ideas were systematically presented by Vladimir V. Lepeshkin as early as the beginning of 20th century [172]. The view on life matter as a separated protoplasm, in which water is absorbed on peptide chains, was also actively promoted by Gilbert N. Ling [173]. Not all elements of the stated theories are confirmed, but the idea of liquid-liquid phase separation was correctly noted by these researchers. After the discovery of membrane-less organelles, the realization of this phenomenon in living cells was no longer doubted, and the accumulated information on water properties associated with biopolymers is in good agreement with some of Lepeshkin's and Ling's conclusions.

The key role in the LLPS belongs to water molecules [1,7]. The difference in water ordering leads to the fact that two liquid phases, both of which are colloidal aqueous solutions of polymers (proteins, RNA, DNA), become so different in physicochemical parameters that they do not mix with each other. The static dielectric constant of water bound to lipid membranes at their surface can be as low as 2–10 [174], which is comparable to the dielectric constant of the hydrophobic parts within the lipid membrane or within the hydrophobic core of globular proteins.

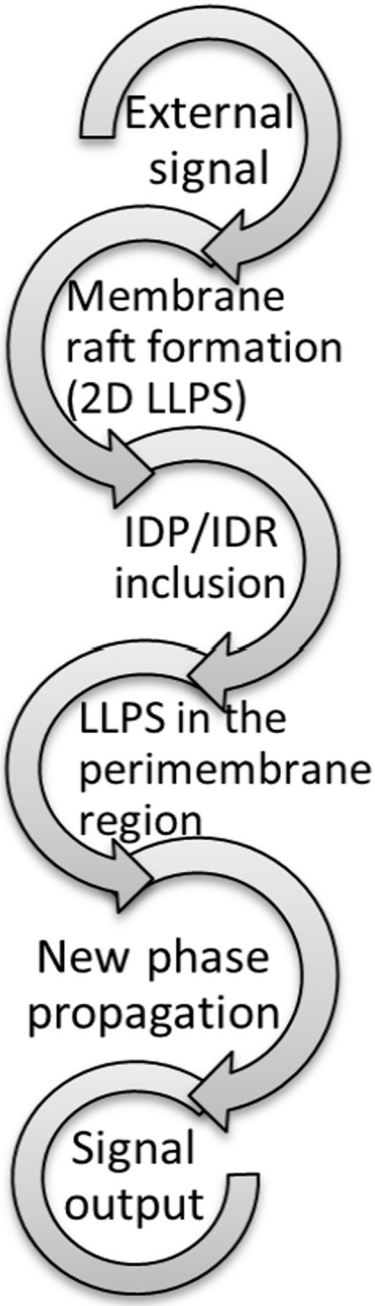

Fig. 3. Signal transmission sequence across the membrane using phase separation.

That suggests the absence of orientational polarization and indicates reduced mobility of water dipoles in the water-lipid interface.

A theoretical description of ordered water structures is possible using the method of modular design [175]. Even bulk water itself has a tendency to phase separation – ice-like clusters can be formed in the volume of distilled water [176]. Furthermore, a guest molecule placed in water (gas or polar molecules with large hydrophobic moieties) is encapsulated by a clathrate cage, in which water molecules bond to form complex networks forming cage-like structures of hydrogen-bonded, frozen water molecules. Spectroscopic data show that water molecules near hydrophilic residues of biopolymers form ice-like structures as well [177]. It is likely that hydrophilic biopolymers and membranes can catalyze the phase separation process inherent to water and stabilize the formed phases. These days multi subunit enzymatic systems are increasingly considered as molecular machines [178], stochastic machines [179], or even assembly lines [180]. Therefore, it can be said that there is a synergistic effect between ordered and hydrophilic disordered domains of machine-like enzymatic systems: disordered fragments stabilize water, ensuring the proper assembly of the whole complex and metabolite transfer between highly-ordered catalytic subunits.

It is difficult to overestimate the role of LLPS distortion in the pathogenesis of various diseases [6], and many phenomena have yet to be associated with the LLPS process. For example, new data showing that pH regulates autophagy by modulation of the LLPS make it possible to identify a mechanism providing the so-called pH paradox – the effect of faster cell recovery after ischemia in the case of low pH maintenance [181]. Also, phase separation can play an important role in the development and drug resistance of cancer, because, in contrast to normal cells, cancerous cells thrive in a more acidic environment with altered LLPS processes. This assumption is indirectly supported by the recently discovered ability of the key tumor suppressor p53 to form liquid-like droplets [182]. Indeed, altered LLPS in nucleus [183] and in cell membrane [184] often leads to tumorigenesis and tumor progression.

8. Conclusions

In this work, LLPS was considered as fundamental organizing principle of living cells. It was emphasized that LLPS occurs not only in the aqueous solution, but can also take place in lipid and protein-lipid membranes, which are two-dimensional liquids, or even on DNA and the cyto- and nucleoskeleton. Phase separation provides protein and metabolite sorting in the cell, accelerates the assembly of metabolic and signaling complexes, and provides efficient transport of substrates to them. In this work, we propose a model according to which the processes of polymerization (1D structures), phase separation in membranes (2D structures), and LLPS in the volume (3D structures) are closely interconnected. Membrane rafts and long polymers can nucleate a new bulk phase near themselves, and vice versa: the clustering of biomolecules in a condensate promotes polymerization, while the binding of specific IDR-containing proteins or separate liquid phase to the membrane enhances nanodomain formation. More than that, we hypothesized that propagation of LLPS can have an autowave character (most likely switching mode) and may represent the signal, which controls both signaling and metabolic systems.

CRediT authorship contribution statement

Semen V. Nesterov: Conceptualization, Study Design, Literature Search, Formal Analysis, Methodology, Visualization, Writing - Original Draft, Writing - Review & Editing. **Nikolay S. Ilyinsky**: Conceptualization, Study Design, Literature Search, Formal Analysis, Methodology, Writing - Original Draft, Writing - Review & Editing. **Vladimir N. Uversky**: Conceptualization, Study Design, Literature Search, Formal Analysis, Methodology, Writing - Original Draft, Writing - Review & Editing; Project administration; Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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