Review of: "Transmembrane coupling of liquid-like protein condensates"

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In the article by Lee et al.,[1], a new technique was applied to study the phase separation processes on the membrane surface that convincingly proved the coupling of the formation of condensates on different sides of the membrane. Using model freestanding planar lipid membranes, they observed that liquid-like protein condensates on one side of the membrane colocalize with condensates on the other side. Among various methods, a system that imitates real processes in the cell signaling system was used, with a specific protein domain binding to phosphatidylinositol biphosphate. It was reasonably concluded that "signals originating on one side of a biological membrane, triggered by protein phase separation, can be transferred to the opposite side."

This work should be viewed in the context of previously published studies on condensate-membrane interactions and signaling, including our previously published theoretical model substantiating and predicting the existence of signal transduction across the membrane involving condensates.[2] This theory was based on a multitude of established facts, with many of its claims being further strengthened over the past couple of years. These included confirmation of the phenomenon that phase separation of proteins is facilitated on the membrane,[3] that the condensate can stimulate raft formation in the membrane,[4] and vice versa,[5], and that receptors can induce liquid-liquid phase separation (LLPS) in the perimembrane zone.[6] Even more, the condensate formation around membrane receptors has been demonstrated[7]. Although Lee et al. did not mention this body of literature, they verified one of the already proposed steps in the signal transduction mechanism[2]. Furthermore, this work partially compensated for the existing deficit of high-quality model systems for analysis of the condensate-membrane interaction. However, the discussion of this work should be expanded in light of the current state of research on the topic.

Cluster formation in a lipid membrane is a special case of liquid-liquid phase separation[2]. The cell membrane is a liquid crystal, so LLPS in the membrane occurs in two-dimensional space. The analog of condensates in membranes is rafts[2,8]. The experiments presented by Lee et al.[1] described only the simplest case of the condensation process in the membrane, since the model system did not contain integral membrane proteins. Disordered domains of membrane proteins can significantly affect the LLPS processes at the interface, while their intramembrane hydrophobic domains can stimulate the formation of rafts. The stimulating effect of the protein on the LLPS process used to form liquid condensates in such a simplified system on the membrane[1] can be partially explained by the effect of protein crowding near the membrane, which is known to affect membrane structure.[9,10] An important achievement of Lee et al.[1] is that they showed coupled

condensate formation on both sides of the membrane within a single experiment in a simple model system. A similar effect has been observed in a much more complex system of bacterial cells when a condensate-forming protein was overexpressed,[11] but the versatility of that system made it difficult to study the process in detail and elucidate its mechanism, which may be similar to that assumed for tight junctions.[12]

In real biological systems, when cluster sizes are small, it is often difficult to accurately distinguish whether proteins form a true liquid condensate or clusters of a different arrangement, such as supercomplexes or aggregates, which are solid entities. A prominent example is signaling in insulin and other cascades related to phosphatidylinositol phosphorylation. A whole plethora of signaling proteins possess special domains that bind to various phosphorylated forms of phosphatidylinositol and thereby interact with the membrane and form signaling hubs on it [13]. Importantly, one of the models used by Lee et al.[1] is constructed based on such domains. Although this type of signaling has been known for several decades, the importance of condensate formation for signal transduction in the insulin signaling system was recognized quite recently.[6] Phosphorylated forms of inositol neutralize the positive charges of proteins, reducing their electrostatic repulsion and thus promoting the formation of condensates.[14] Membrane-bound forms of phosphoinositols, as well as clusters of other charged lipids, can perform a similar function. In addition, interaction with the membrane promotes protein orientation, increasing the probability of interaction between their disordered domains. Other non-protein structural elements of the condensate may either be present on the membrane or come later if they have an affinity for the proteins accumulated on it. In addition to the well-known function of RNA as an LLPS-promoting element, proteoglycans could fulfill that role for condensates on the membrane[7].

We fully support the methodological approach used by Lee et al.[1] to investigate the interaction of condensates with the membrane. Their study addresses a number of gaps in this field of knowledge. However, the mechanisms assessed have been previously described [2]. It is expected that in the coming years the participation of LLPS will be shown in more signaling cascades, especially those that are coupled to the membranes.

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