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Spotlight

Tear down this wall: phosphorylation regulates the internal interfaces of postsynaptic condensates

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Can the fusion/fission of biomolecular condensates be regulated in cells? In a recent study, Wu *et al.* show that phosphorylation of a key scaffold protein that drives condensates in postsynaptic densities modulates the apparent miscibility of underlying components, thus enabling intracondensate demixing-to-mixing transitions.

Biomolecular condensates enable spatial and temporal organization of cellular matter. The biogenesis of these membraneless bodies involves a combination of spontaneous and driven processes that combine associative and segregative phase transitions of multivalent macromolecules [1,2]. The complex combination of reversible associations and different phase transitions may be referred to simply as condensation or macromolecular phase separation. Condensates represent distinct phases that coexist with other cellular bodies and the cellular milieu, and, depending on their composition, they form distinctive microenvironments that enrich or deplete specific types of proteins, nucleic acids, and metabolites [3–5].

The physicochemical determinants of territorial preferences, defined by extents of colocalization or delocalization of components within condensates, is a topic that is directly relevant to the spatial organization of biochemical reactions within condensates. Early work on this topic helped establish that differential solvation [6], which contributes to differential interfacial free energies, contributes the spontaneous driving forces that enable multiphasic or so-called 'phase-in-phase' patterns of condensates [7]. The interfaces between coexisting phases were recently shown to act as reactive centers that accumulate electrical charge to catalyze redox reactions [8] and impact their interactions with membranes [9]. Nowhere are these charged interfaces more crucial than at synapses, where multiple condensates often coexist to integrate the neuronal signaling cascades.

Synapses are specialized contact sites where the signaling molecules, neurotransmitters, are released in a calcium-dependent manner. These neurotransmitters diffuse across the intersynaptic space to bind and activate receptors at the plasma membrane of a recipient postsynaptic neuron. At excitatory postsynaptic sites, the glutamate receptors at the plasma membrane (e.g., ionotropic NMDA and AMPA receptors), together with the intracellular scaffolding protein and signaling enzymes, form a dense, proteinaceous structure referred to as postsynaptic density (PSD), which is a well-established example of a phase-separated region within a neuron [10,11]. Electron micrographs and biochemical analysis indicate that the PSD is subcompartmentalized [12]. Glutamate receptors, PSD-95, and SynGAP, a postsynaptic Ras-GTPase, form a PSD core, whereas the downstream signaling scaffold proteins Shank and Homer form the PSD pallium. Members of SAPAP scaffolds are distributed across both regions. During synaptic activity, the entire PSD is enlarged and the border between these subregions vanishes [11]. The growth and thickening of PSD are functionally coupled with an acute accumulation of calcium/calmodulin-dependent kinase II (CaMKII) in PSD.

In a recent study, Wu and colleagues partially reconstituted a PSD condensate comprising PSD-95, simplified SAPAP1 (also known as GKAP), truncated Shank-3, Homer-1, truncated SynGAP, and the cytoplasmic tail of stargazin (Stg, an auxiliary subunit of AMPA receptors) [7]. Mixing of these six PSD proteins resulted in the formation of two coexisting phases referred to by the authors as a 'phase-in-phase' pattern. The core comprised PSD-95/Stg/SynGAP (mimicking the PSD core) and the outer phase comprised Shank-3/Homer-1 (mimicking the PSD pallium). SAPAP family proteins were uniformly distributed in both phases (Figure 1). Such morphology strongly points to the differential solvation preferences of components of the PSD pallium versus the PSD core, which give rise to differences in interfacial free energies with respect to one another and with respect to the cellular milieu.

SAPAP1/GKAP contains several ~15-residue stretches that can bind the guanylate kinase (GK) domain of PSD-95, hence the name GK-binding repeats (GBRs). While the initial binding of GBRs to PSD-95 was weak (≥100 μM), phosphorylation of Ser residues within these repeats increased the apparent binding affinity by ~1000-fold [13]. In a systematic series of reconstitutions, CaMKII was included in the mixture that contained either wild-type SAPAP or SAPAP variants with Ser-to-Ala mutations (i.e., phosphonull variants) [7]. The data suggested that all three regions of SAPAP were targets of phosphorylation at the Ser-patch within GBR regions. This was found to be crucial for regulating GBR binding to PSD95 and, thus, for intracondensate mixing versus demixing. Even phosphorylation within a single SAPAP region was sufficient to cause two PSD subcompartments to fuse into one homogeneous compartment (Figure 1). Importantly, this process was reversible because dephosphorylation of SAPAP family proteins by a synaptic phosphatase PP2A caused fission of single-phase PSD

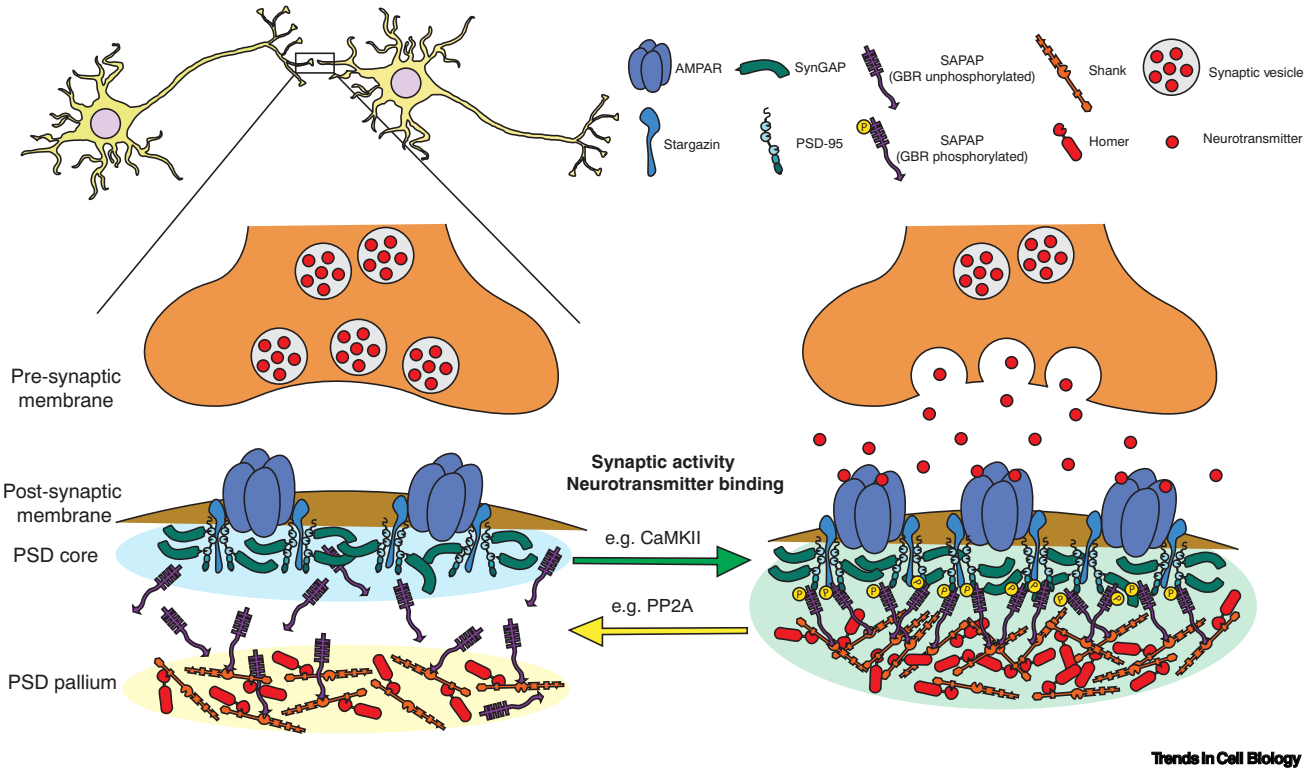

Figure 1. Activity-dependent phosphorylation regulates the postsynaptic density (PSD). (Left) At rest, a postsynapse contains two distinct condensates, a PSD core (blue) and a PSD pallium (yellow). (Right) Upon neurotransmitter binding, scaffold proteins from the SAPAP family are phosphorylated, triggering the fusion of the PSD core and pallium into a single condensate. This process is reversible upon dephosphorylation. Abbreviations: AMPAR, AMPA receptor; CaMKII, calcium/calmodulin-dependent kinase II; GBR, guanylate kinase binding repeats.

into two distinct subcompartments, mimicking the molecular events at repeated rounds of synaptic stimulation.

The observed synaptic effects of postsynaptic condensate (de)mixing as a function of SAPAP phosphorylation in mice make a strong case for the biological relevance of this mechanism in regulating synapse functioning. The neurons in culture from SAPAP3 knockout mice had impaired maturation compared with wild-type neurons. These defects could only be rescued by expressing by phosphorylation-fit SAPAP3 but not by SAPAP3 phosphonull mutants, validating the impact of PSD phosphorylation state on synaptic growth [7].

Post-translational modifications, such as phosphorylation in the case of SAPAP family proteins, provide a mechanistic link to the overall activity of the synapses. At low synaptic activity and low calcium-dependent kinase activation, PSDs are demixed and synapses tend to be small in volume; by contrast, the augmented activity and active kinases trigger PSD mixing and enlargement, subsequently enlarging the synapses. Similar mechanisms may act at the presynaptic terminal, where synaptic vesicles condensates [14] and their interaction with the presynaptic active zone [15] may, in fact, be a consequence of the unique phosphorylation signature of the participating protein components.

The findings of Wu *et al.* show the relevance of intracondensate mixing versus demixing of components as an important signaling switch in cell physiology. That similar regulatory control might underlie other switch-like functionalities of condensates is an intriguing possibility that will likely inspire many follow-up investigations.

Declaration of interests

The authors declare no competing interests.

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