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RESEARCH HIGHLIGHT

Mesophasic Assembly of Inhibitory Postsynaptic Density

Guanhua Bai[1] · Mingjie Zhang[1,2]

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Neurotransmitter receptors are concentrated in a morphological specialization of synapses known as the postsynaptic density (PSD). A prominent PSD beneath the postsynaptic membrane in electron micrographs is a defining feature of excitatory synapses. Typically, the excitatory PSD (ePSD) has a disc-like shape 200–800 nm in diameter and 20–50 nm in thickness [1]. In contrast, the inhibitory synapse only has thickened postsynaptic membranes in classical EM studies [2, 3], thus whether inhibitory synapses contain PSD-like structures has been debated for decades. A few years ago, Bi and colleagues characterized the ultrastructure of excitatory and inhibitory synapses in cultured neurons by cryo-electron tomography (cryo-ET) and their high-resolution tomograms clearly showed electron-dense assemblies in both excitatory and inhibitory synapses [4].

The formation and maintenance of PSDs and the dynamic clustering of neurotransmitter receptors by PSD scaffold proteins are crucial for efficient and accurate synaptic transmission. However, elucidating the organizational principles of PSD scaffold proteins and receptors at individual molecule resolution *in situ* have not been possible, due to both the extreme complexities of PSDs (ePSD in particular) and the lack of suitable imaging methods. In a recently published paper in *Nature Neuroscience*, Bi and colleagues have been able to obtain the organization principles of $GABA_A$ receptors ($GABA_A$Rs) in the inhibitory PSD (iPSD) *in situ*, with the structure of $GABA_A$Rs resolved at 19-Å resolution, using the state-of-the-art cryo-ET method [5]. In this study, the authors developed a template-free classification method by oversampling high-resolution cryo-ET sub-tomograms to automatically identify $GABA_A$Rs on inhibitory postsynaptic membranes and obtain the structure of these receptors *in situ*. By analyzing the relative positions of $GABA_A$Rs, they found that neighboring $GABA_A$Rs show a characteristic distance of 11 nm with variable angles, such that the receptors self-organize into dense two-dimensional networks. Interestingly, the receptor networks contain sharp boundaries enclosing clustered receptors in iPSDs, indicating that the clustered receptors form a distinct "semi-ordered" state of organization similar to what is known as mesophase in soft-matter physics (Fig. 1A, B). These clustered $GABA_A$Rs are also aligned with presynaptic vesicle release sites, which may maximize the efficiency of synaptic transmission.

The formation and maintenance of ePSDs have been proposed to be *via* phase separation, by which the scaffolding proteins form membraneless self-organized condensates and cluster glutamate receptors both in solution and on lipid membrane bilayers [6, 7]. The mesophasic organization of $GABA_A$Rs at inhibitory synapses revealed by the cryo-ET study by Bi and colleagues suggests that iPSDs may also be formed *via* phase separation. The iPSD scaffold layer forms sheet-like condensates beneath the postsynaptic membrane (Fig. 1A) rather than the thick, disc-like condensates in ePSDs. Gephyrin is the most abundant scaffold protein and is thought to be the predominant organizer to cluster receptors in iPSDs. Gephyrin was initially identified as a glycine receptor (GlyR)-associated protein [8], containing a

✉ Mingjie Zhang
mzhang@ust.hk

[1] Division of Life Science, State Key Laboratory of Molecular Neuroscience, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China

[2] School of Life Sciences, Southern University of Science and Technology, Shenzhen 518055, China

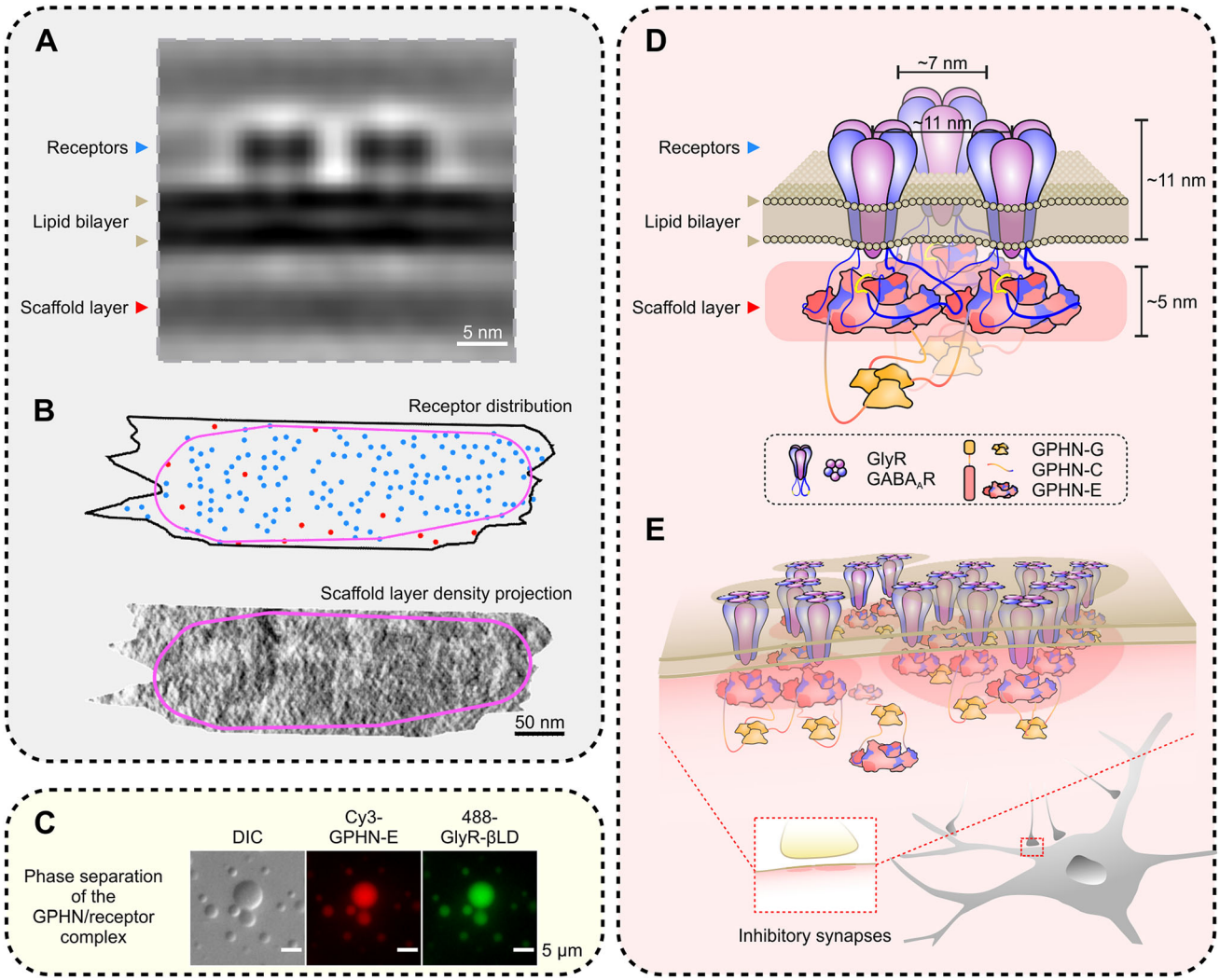

Fig. 1 Gephyrin-mediated receptor clustering in inhibitory synapses *via* phase separation. **A** An example of the sub-tomogram average of GABA$_A$R pairs in inhibitory synapses. **B** An example of GABA$_A$R distribution on the postsynaptic membrane (upper panel) and the corresponding density projection of the scaffold layer (lower panel) (blue dots, clustered receptors; red dots, solitary receptors; magenta lines, boundary of the mesophase formed by the iPSD receptor and scaffold layers). **C** Representative differential interference contrast (DIC) and fluorescence images showing the phase separation of the gephyrin (GPHN)/GlyR complexes. **D** Model of interactions between gephyrin and GABA$_A$R/GlyR pairs on the synaptic plasma membrane. The relative molecular scales are comparable to those in **A**. Note that the width of the gephyrin E-domain dimer is ~11 nm, and the distance between two GABA$_A$Rs in iPSDs is also ~11 nm. **E** Diagram showing the formation of the iPSD sheet *via* phase separation.

trimerized G-domain and a dimerized E-domain. Later, it was found that the E-domain is responsible for binding to both GABA$_A$Rs and GlyRs [9]. The densities in the scaffold layer found in the work by Liu *et al.* [5] match well with the two-fold symmetric structure of the gephyrin E-domain. In addition, their simulation studies showed that GABA$_A$Rs and gephyrin can form dense clusters *via* a self-organizing process. In a completely independent study from the authors of this Highlight (appeared on the same day as the paper by Liu *et al.* [5] in *Cell Research* [10]), it was demonstrated that the iPSD complexes formed between GABA$_A$Rs (or GlyRs) and gephyrin undergo phase separation both in solution and on membrane bilayers (Fig. 1C). Combining the cryo-ET studies of inhibitory synapses *in situ* [5] and the biochemical reconstitution studies of iPSD assembly *in vitro* [10], a compelling model depicting the gephyrin-mediated receptor clustering at inhibitory synapses *via* phase separation readily emerges (Fig. 1D, E). In this model, the gephyrin E-domain binds to the cytoplasmic TM3-4 loops of selected subunits of GABA$_A$Rs or GlyRs. The multivalent nature of GABA$_A$Rs or GlyRs can polymerize the gephyrin E-domain dimer, forming a sheet-like assembly *via* phase separation. The width of a gephyrin E-domain dimer is ~11 nm, matching the distance of the nearest-neighbor distribution of GABA$_A$Rs at inhibitory synapses measured

by cryo-ET. The thickness of the iPSD sheet also roughly matches the height of the E-domain dimer (~5 nm). The trimerization G-domain of gephyrin, which is likely located beneath the iPSD sheet distal to the plasma membranes, can promote the phase separation of the gephyrin/receptor complex by increasing the valency of the assembly.

The concept of phase separation is gaining immense popularity in biology. However, though dense molecular organizations in neuronal synapses have been observed by electron microscopy for more than five decades, the study by Liu *et al.* [5], by taking advantage of the recent developments of cryo-ET technology, is the first direct evidence supporting the hypothesis that the mesophasic-scale organization of the iPSD is formed *via* phase separation. In addition to making a major advance in synaptic biology as demonstrated in the work by Liu *et al.* [5], cryo-ET technology is poised to make many more revelations in the phase separation-mediated formation of biological condensates in diverse cellular systems.

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