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論文題目	CaMKII activation triggers persistent formation and segregation of postsynaptic liquid phase （CaMKII の活性化によるシナプス後部液相の持続的な形成と分離）		

（論文内容の要旨）

Transient information input to brain leads to persistent changes in neuronal network, thereby forming memory. Long-term potentiation of synaptic transmission (LTP) is considered to underlie this process. The major mechanism to induce and maintain LTP is the regulation of glutamate receptors, especially AMPA receptor (AMPAR). AMPARs have been found to form segregated clusters, called nanodomain, in activity dependent manner. This AMPAR nanodomain and presynaptic vesicle releasing site are aligned together, thereby forming trans-synaptic nanocolumns which can regulate the efficacy of synaptic transmission. However, the mechanism underlying AMPARs segregation and activity-dependent regulation remains unclear. Although the AMPAR nanodomains are formed on the postsynaptic membrane, there is no direct regulator on the membrane that defines their spatial distribution.

Recently, liquid-liquid phase separation (LLPS) of biological macromolecules was found to play a critical role in regulating the assembly and segregation of molecules within various intracellular structures, including postsynaptic density (PSD). For LLPS, a multimer-multimer interaction is required. In this regard, CaMKII, a highly abundant dodecameric protein kinase in the PSD, has ideal features to undergo LLPS with its binding partners. Ca^{2+}/calmodulin binding to CaMKII opens up a binding pocket called the T-site, which forms a stable complex with various synaptic proteins, such as the carboxyl tail of NMDA-type glutamate receptor (NMDAR) subunit GluN2B. Once bound, the complex persists even when cellular Ca^{2+} concentration decreases. Finally, the dodecameric structure of CaMKII allows multivalent interactions.

Given this, CaMKII was evaluated whether it has an ability to undergo LLPS with PSD proteins and, if it does, how it can affect the distribution and function of AMPAR nanodomains. The results showed that the Ca2+ activation of CaMKII allows the multivalent interaction between CaMKII and the carboxyl tail of GluN2B (GluN2Bc) which leads to the formation of LLPS with other PSD proteins, including PSD-95, and the carboxyl tail of stargazin (STGc, an auxiliary subunit of AMPAR critical for determining its synaptic distribution, as a proxy of the AMPA receptor). The CaMKII-mediated LLPS persisted in a manner requiring CaMKII T286 autophosphorylation. Furthermore, the two subtypes of glutamate receptors, AMPAR and NMDAR, were segregated through the formation of phase-in-phase structure.

The segregation of AMPAR and NMDAR nanodomains was recapitulated in neurons as revealed by direct stochastic optical reconstruction microscopy (dSTORM), a super-resolution imaging. Neuroligin-1 (NLGN1), a neuronal adhesion molecule, which clusters presynaptic neurexin and other active zone proteins, also segregates together with AMPAR. On the other hand, bidirectional regulation of synaptic strength has been reported as the synaptic plasticity. To test if the LLPS can be reversed, Camk2n1, the endogenous inhibitor of CaMKII which also interacts with CaMKII via the T-site, was applied to the CaMKII-mediated LLPS. The results showed that CaMKII-mediated LLPS collapsed by the Camk2n1. In addition, when the cell-permeable CN21, the minimum binding site to CaMKII of Camk2n1, was applied to the neuron, the overlapping area of AMPAR and NMDAR nanodomains increased, suggesting the segregation of AMPAR and NMDAR nanodomains requires the T-site binding to CaMKII.

Taken together, activated CaMKII can undergo persistent LLPS in PSD and establishes AMPAR nanodomain beneath active transmitter release site, thereby conducting a novel mechanism of activity-dependent and persistent synaptic plasticity.

（論文審査の結果の要旨）

AMPA 型グルタミン酸受容体(AMPAR)は神経伝達物質放出部位とアライメントされたナノカラムを形成する。しかし脳内での一過性の情報を持続的な記憶として変換する過程における AMPAR ナノカラム形成機構はよくわかっていない。

申請者は、シナプス活動依存的に Ca^{2+}/ カルモジュリン依存性キナーゼ（CaMKII）と NMDA 型グルタミン酸受容体(NMDAR) サブユニット GluN2B がシナプス後膜肥厚（PSD）内で持続的に液-液相分離（LLPS）することを見出した。この LLPS は、PSD-95 および AMPAR の局在を決定する Stargazin の相内相構造の形成を通じて AMPAR と NMDAR の分離に寄与することが分かった。また前シナプス終末と接続する Neuroligin-1 も AMPAR と一緒に分離した。

神経細胞におけるこれら分子の分離は超高解像顕微鏡法 dSTORM により確かめた。また、LLPS 形成を競合阻害する CN21 によりこの分離は阻害された。

これらのことは CaMKII の形成する LLPS 集合体が記憶形成の新たなメカニズムであることを示している。

以上の研究はシナプス活動依存的な蛋白質局在制御機構の解明に貢献し、記憶形成の分子機構の解明に寄与するところが多い。

したがって、本論文は博士（医科学）の学位論文として価値あるものと認める。

なお、本学位授与申請者は、令和３年２月９日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。

要旨公開可能日：　　年　　月　　日　以降