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研究課題名（和文）Super-resolution imaging for synapse reveals structure-function correlation between sub-synaptic protein localization and synaptic plasticity

研究課題名（英文）Super-resolution imaging for synapse reveals structure-function correlation between sub-synaptic protein localization and synaptic plasticity

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交付決定額（研究期間全体）：（直接経費）　　2,200,000円

研究成果の概要（和文）：以前の研究で我々はシナプス活動に応じて後シナプスタンパク質のナノスケール局在が液－液相分離により制御されていることを明らかにした。特に、代表的なグルタミン酸受容体であるAMPA受容体とNMDA受容体の分離は記憶形成時のシナプス増強を説明することができる。
そこで本課題ではシナプスにおいてAMPA受容体とNMDA受容体のナノスケール局在を同時に観察するために、ニコン社の超高解像顕微鏡システムN-STORMの二チャネル化のセットアップを行った。イメージングバッファの最適化、568 nmレーザーの調整、レーザー光路の改善を行い、二チャネル化に成功した。

研究成果の学術的意義や社会的意義
Being able to study the interaction among synaptic proteins at nano-scale level by dual-channel STORM allows us to dig out the mechanism of synaptic plasticity which serves as molecular basis of memory that can lead medical invention of memory-related mental disorders and neurodegenerative diseases.

研究成果の概要（英文）：To investigate the molecular mechanism underlying the memory formation, we focused on elucidating the behavior of synaptic proteins. Previously, we have reported the synaptic activity-dependent organization of nano-scale localization of postsynaptic proteins via liquid-liquid phase separation (LLPS), which explains the potentiation of synapse during memory formation.
To observe the detailed structure of synaptic protein clusters requires super-resolution imaging. Thus, to expand our research, we first set up the super-resolution microscopy with Nikon STORM system. However, to observe the interaction between two synaptic proteins, dual-channel super-resolution microscopy is required. So, we extend our system from single-channel to dual-channel by optimizing imaging buffer, upgrading laser power and modifying the pathway of laser beam. Overall, we established the dual-channel STORM system that allow us to observe the interaction among synaptic proteins at nano-scale level.

研究分野：Neuroscience

キーワード：Memory formation　Synaptic plasiticity　Super-resolution imaging　STORM　liquid phase separation

１．研究開始当初の背景

Memory formation is a critical ability for animals to survive in this world. Transient information input to brain leads to persistent changes in neuronal network, thereby forming memory. The synaptic plasticity, the regulation of the efficacy of synaptic transmission, is considered to underlie this process. During synaptic transmission, neurotransmitters are released from pre-synaptic active zone and then reach the neurotransmitter receptors on post-synaptic membrane. Especially, AMPA type glutamate receptor (AMPAR) is a major mediator for excitatory synaptic transmission. The amount, stability and sub-synaptic localization of AMPAR is critical for synaptic transmission and plasticity. Various post-synaptic proteins, including AMPAR, form a huge macromolecular complex underneath the post-synaptic membrane that is widely known as post-synaptic density (PSD), by multiplexed interaction. However, the regulatory mechanism of PSD and its components remained unknown.

Recently, we reported that calcium/calmodulin-dependent protein kinase (CaMKII), a major component of PSD, undergoes LLPS with NMDA type glutamate receptor (NMDAR) in activity dependent manner. This confers PSD a mechanism to respond synaptic activity. Preferential binding of CaMKII with NMDAR resulted in the segregation of NMDAR and AMPAR as phase-in-phase structure (Fig. 1). In addition, Neuroligin, a synaptic adhesion protein between pre-synaptic releasing site and post-synaptic membrane was enriched with AMPAR. Given these, we hypothesized that sub-synaptic segregation of PSD proteins by CaMKII explains the increase of the efficacy of synaptic transmission (Fig. 2) after learning.

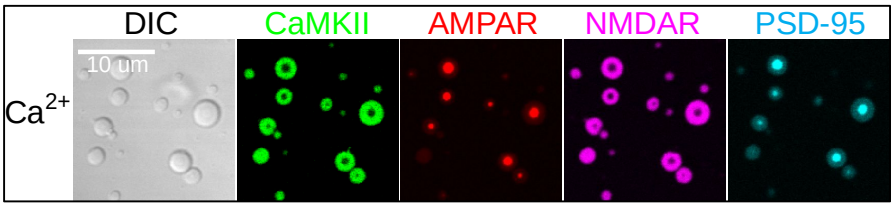

Fig. 1 Solution of purified proteins mixture are stimulated by Ca^{2+} and observed by fluorescent microscope. Activation of CaMKII results in the formation of the LLPS protein condensate with NMDAR, which segregates AMPAR and PSD-95 into a “phase-in-phase” structure.

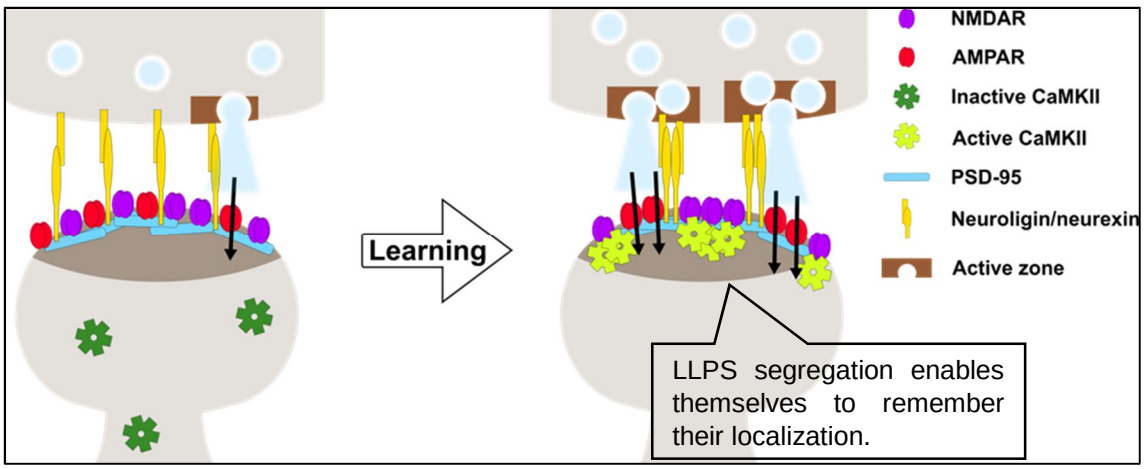

Fig 2. Synaptic proteins randomly distribute in synapse before learning (left). Calcium signaling during learning results in the formation of protein condensate which segregates glutamate receptors to optimize their localization and increase the efficacy of synaptic transmission. This segregation can be permanent beyond the metabolic turn-over of molecules.

２．研究の目的

It is critical to know the effect of excitatory stimulation for sub-synaptic localization of PSD proteins to understand synaptic plasticity, learning and memory. However, the spatial resolution of conventional fluorescent microscopy is not enough. Recently, stochastic optical reconstruction microscopy (STORM), one of super-resolution microscopy, starts to be used to image neuron and synapse. In this study, we established the STORM system to observe the sub-synaptic localization of major PSD proteins such as CaMKII, AMPAR, NMDAR and PSD-95 in cultured hippocampal neuron. Also, we extended the system from single-channel to dual-channel STORM, in order to analyze the overlapping ratio of each of protein pairs to evaluate their interaction.

３．研究の方法

We first set up and optimized the Nikon STORM system in our laboratory for the observation of synaptic proteins. At the same time, we established the low-density primary neuronal culture, and the neurons were stained with dye-labeled antibodies against target proteins to specifically image it. Concentrated region of PSD proteins on synapse is defined as Region of Interest. After the imaging being reconstructed into STORM imaging, we can find the segregated nanodomains of target proteins as sub-synaptic localization. The number of proteins, the area of nanodomain and the density of the protein were analyzed from STORM image. Also, the overlapping ratio of each of protein pairs were analyzed by dual-color STORM image.

４．研究成果

In the first annual year, we have set up the preparation of low-density primary hippocampal neuronal culture and successfully observed AMPA-type glutamate receptor with Nikon STORM system. Also, we have tested a novel fluorescent agonist against postsynaptic neurotransmitter receptor for STORM imaging, which has better resolution in principle.

In the second year, we improved the efficacy of fluorophore blinking which is related with the resolution of final image, of the second color channel by optimizing the imaging buffer. Furthermore, we upgraded the laser power and modified the pathway of the laser beam. After those efforts, we successfully completed the setup for dual-color STORM imaging.

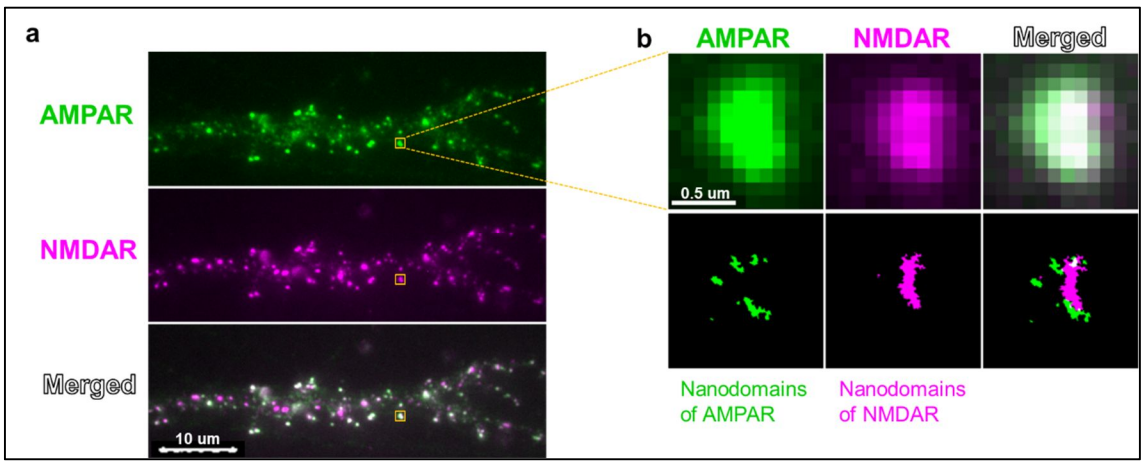

Fig 3. Dual-color STORM imaging of AMPAR/NMDAR nanodomains in synapse. **a**, Dendrite of single neuron and punctate signals of AMPAR and NMDAR in conventional fluorescent microscopy. Scale bar, 10 μm. **b**, 20x magnification of **a** for single synapse (upper panel). dSTORM imaging for the same single synapse (lower panel). Scale bar, 0.5 μm.

５．主な発表論文等

〔雑誌論文〕　計4件（うち査読付論文　4件／うち国際共著　1件／うちオープンアクセス　4件）

１．著者名 Liu Pin-Wu、Hosokawa Tomohisa、Hayashi Yasunori	４．巻 69
２．論文標題 Regulation of synaptic nanodomain by liquid?liquid phase separation: A novel mechanism of synaptic plasticity	５．発行年 2021年
３．雑誌名 Current Opinion in Neurobiology	６．最初と最後の頁 84～92
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.conb.2021.02.004	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

１．著者名 林 康紀、細川 智永、劉 品吾、實吉 岳郎	４．巻 93
２．論文標題 CaMKIIの新しいシナプス可塑性機構	５．発行年 2021年
３．雑誌名 生化学	６．最初と最後の頁 191～202
掲載論文のDOI（デジタルオブジェクト識別子） 10.14952/SEIKAGAKU.2021.930191	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

１．著者名 Hosokawa Tomohisa、Liu Pin-Wu、Cai Qixu、Ferreira Joana S.、Levet Florian、Butler Corey、Sibarita Jean-Baptiste、Choquet Daniel、Groc Laurent、Hosy Eric、Zhang Mingjie、Hayashi Yasunori	４．巻 24
２．論文標題 CaMKII activation persistently segregates postsynaptic proteins via liquid phase separation	５．発行年 2021年
３．雑誌名 Nature Neuroscience	６．最初と最後の頁 777～785
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41593-021-00843-3	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Hosokawa Tomohisa、Liu Pin-Wu	４．巻 12
２．論文標題 Regulation of the Stability and Localization of Post-synaptic Membrane Proteins by Liquid-Liquid Phase Separation	５．発行年 2021年
３．雑誌名 Frontiers in Physiology	６．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fphys.2021.795757	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

〔学会発表〕　計3件（うち招待講演　0件／うち国際学会　2件）

1．発表者名 Liu Pin-Wu、Hosokawa Tomohisa、Hayashi Yasunori
2．発表標題 Super-resolution imaging for subsynaptic architecture of glutamate receptor subtypes regulated by liquid-liquid phase separation
3．学会等名 2020年度 生理学研究所 シナプス研究会
4．発表年 2020年

1．発表者名 Liu Pin-Wu、Hosokawa Tomohisa、Cai Qixu、Ferreira Joana S.、Levet Florian、Butler Corey、Sibarita Jean-Baptiste、Choquet Daniel、Groc Laurent、Hosy Eric、Zhang Mingjie、Hayashi Yasunori
2．発表標題 Subsynaptic architecture of glutamate receptors and trans-synaptic nanocolumn regulated by CaMKII-mediated liquid-liquid phase separation
3．学会等名 SfN Global Connectome: A Virtual Event（国際学会）
4．発表年 2021年

1．発表者名 Liu Pin-Wu
2．発表標題 Regulation of synaptic nanodomain and nanocolumn by liquid-liquid phase separation
3．学会等名 International Symposium on Development and Plasticity of Neural Systems（国際学会）
4．発表年 2022年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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6．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

7．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関