RIKEN BDR

Women and Future in Science Seminar

Structural and biochemical analysis of gene regulation by 3D genome folding

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Dissecting neuron-glia interaction using live STED microscopy and lattice light-sheet microscopy

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13:00-14:00 JST

← via Zoom Register here

https://zoom.us/meeting/register/tJ0ldeChrzojGt00NOWXaURX7mSC9HhqrFrD

Structural and biochemical analysis of gene regulation by 3D genome folding

Eukaryotic genomic DNA forms a hierarchical chromatin structure based on the disk-shaped nucleosome. Gene transcription is tightly controlled by the dynamic structural changes of genomic DNA. My research focuses on the "DNA-loop" that regulates transcriptional activation with the help of a multi-subunit co-activator called Mediator. Mediator directly connects activator which is bound to enhancer DNA with RNA polymerase II (Pol II) on the promoter DNA via a long DNA chain of several Kbps to Mbps. So far, we established a protein co-expression system for the core Mediator of 15 subunits, which is the smallest functional unit of the Mediator, and performed X-ray crystallographic analysis. The result clarified that it is involved in the transcription initiation of Pol II on the promoter (Nozawa., TR, Schneider., P, Cramer., 2017). On the other hand, recent reports indicate that the range of the DNA-loop is defined by a chromatin structure called Topologically Associating Domains, and its formation involves cohesin. Furthermore, the recently reported interactome analysis revealed the interaction of Mediators with nucleosomes, histone chaperones, and chromatin remodeling factors, complicating the overall picture of the DNA-loop. In this seminar, I will discuss the molecular mechanisms of the DNA-loop based on our newly discovered findings.

Dissecting neuron-glia interaction using live STED microscopy and lattice light-sheet microscopy

While accumulating evidence shows the involvement of astrocytes in neuronal circuits and cognitive functions, their activity and structure at individual synapses have been challenging to address with conventional light microscopy.

Using 3D-STED microscopy in living brain slices, we observed that the fine processes of astrocytes are composed of bulbous nodes and thin shafts. FRAP experiments and Ca2+ imaging revealed nodes to be biochemical compartments capable of supporting Ca2+ microdomains. Node Ca2+ signals were associated with individual synapses, identifying nodes as the likely synaptic partner. We also applied the SUper-resolution SHadow Imaging (SUSHI) technique to characterize the extracellular space which can shape the interaction between nodes and synapses.

To further characterize astrocytic nodes, we recently established Ca2+ imaging using lattice light-sheet microscopy, which reconciles high spatio-temporal resolution with low phototoxicity. We discovered that node Ca2+ transients can be < 100 ms, and may be elicited by glutamate uncaging. These findings indicate that nodes are equipped with the signaling machinery to respond to synaptic activity.

This work presents the framework for dissecting bidirectional crosstalk between neurons and glia with unprecedented spatial and temporal resolution, bringing us closer to evaluating the impact of astrocyte-neuron interaction on neural circuit function.

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