別紙２

Results report

１．Title of Research and Development：Single molecule imaging of synaptic protein dynamics in neurodegeneration

２．Principal Investigator：Akihiro Kusumi

(Professor, Institute for Integrated Cell-Material Sciences, Kyoto University)

３．Counterpart Principal investigator：Giovanna Mallucci

(Department Chair and Professor, Department of Clinical Neurosciences, University of Cambridge (U.K.))

４．Results of Research and Development:

In many neurodegenerative diseases, impaired synaptic structural plasticity, which leads to synapse loss and ultimately neurodegeneration, have often been found. Among many studies detecting the relationship between impaired synaptic structural plasticity and neurodegenerative processes, the PI of the present project on the U.K. side, Prof. Giovanna Mallucci, discovered very clear evidence for the relationship. When the brain or neuron is cooled to 17˚C, many synapses are lost, but when they are rewarmed, synapses reform – a physiological form of structural plasticity. However, Prof. Mallucci found that such synaptic reformation upon rewarming does not occur in many neurodegenerative diseases. This shows that, by understanding the synapse reforming processes and mechanisms, we might be able to discover how synapses are impaired in neurodegenerative diseases. The objective of the present investigation is thus to understand the synaptic structural plasticity in the cooling-rewarming process. For this purpose, we image the entrance into and exiting from synapses of various synaptic molecules at the level of single molecules in the cooling-rewarming cycle.

During the previous fiscal year (FY14), Prof. Mallucci's group established that a heat-shock protein called RNA-Binding Motif protein 3 (RBM3) works as a switch for synaptic regeneration upon warming (Peretti et al. 2015 Nature). This opened a new possibility to regulate synaptic structural plasticity in both positive and negative ways. In FY15, we advanced the present project, incorporating this new finding.

In this fiscal year (FY15), the Mallucci lab established the conditions for synaptic regeneration in the cycle of cooling and rewarming using primary neuron culture obtained from the mouse hippocampus. While this experiment was ongoing, two young researchers from the Kusumi lab visited the Mallucci lab, and helped to establish the proper conditions, so that the same system works in the Kusumi lab. It has now become possible to perform single-molecule imaging of synaptic structural plasticity using the primary neuron culture in the Kusumi lab.

Meanwhile, the Kusumi group advanced the method for specifically labeling AMPA receptor with fluorescent probe molecules (GluA1 and GluA2; submitted). They also developed methods to simultaneously observe the clusters of Homer1b and single GluA1 and GluA2 molecules.

Previously, we only paid attention to proteins as synaptic molecules. However, in-out of synaptic molecules often depends on their diffusion within the plasma membrane, and therefore, we decided to observe the movements of lipids, entering and exiting from synapses. For this purpose, we developed fluorescent ganglioside analogs (submitted). In particular, the direct interaction of a ganglioside GM1 and AMPA receptor has been proposed. Therefore, we will examine the behaviours of fluorescent GM1 in the cycle of cooling and rewarming.