Molecular distribution analysis of nicotinic acetylcholine receptor and MuSK on the cell surface by correlative fluorescent microscopy and cryo-SEM

Nishino, Y.[1], Kaise, M.[1] and Miyazawa, A.[2]

[1] University of Hyogo, Japan, [2] University of Hyoog, Japan

At the postsynaptic membrane of the neuromuscular junction (NMJ), nicotinic acetylcholine receptor (nAChR) forms clusters with muscle specific kinase (MuSK) and other associated proteins. The matured nAChR clusters are indispensable for the neuromuscular signal transmission. During the differentiation of the NMJ, nAChR clusters mature into large and dense clusters. Agrin secreted from a motor neuron is known to play a major role in the cluster maturation, however, the molecular mechanism of the cluster maturation has not been elucidated. In order to understand the matured cluster at the molecular level, molecular distribution of nAChR and MuSK in the cluster matured by agrin, has been analyzed by correlative light and electron microscopy (CLEM) using a myotube culture. However, CLEM of myotubes by fluorescent microscopy and conventional SEM has been difficult because myotube is easy to shrink non-uniformly during freeze-drying step, which is necessary to observe the surface of cultured cells by conventional SEM. Therefore, we examined CLEM by fluorescent microscopy and cryo-SEM to observe the molecular distribution on the myotube surface.

Myotubes derived from C2C12 cells were cultivated on a holy carbon coated gold grid, and nAChR clusters on the myotube surface were matured by adding agrin into the culture medium. nAChR and MuSK were labeled with a fluorescence-conjugated ligand and colloidal gold-conjugated antibodies. The entire feature of several micrometers of clusters were observed by fluorescent microscopy to check the maturity of each cluster by size and fluorescent intensity. After that, myotubes on the grid were rapidly frozen into liquid ethane. The myotubes were covered with a layer of ice consisted of phosphate-buffered saline. The surface ice was sublimated at low temperature in vacuum to reduce the thickness of ice layer above myotubes, and myotube surface structure and colloidal gold particles became detectable by secondary electrons and reflected electrons respectively. As a result of the observation, nAChR cluster consisted of small aggregations including nAChR and MuSK shown in figure 1. We clarified that mature clusters extended in the direction of the sarcomeric actin filaments, while shape of small aggregations was irregular and did not have direction.

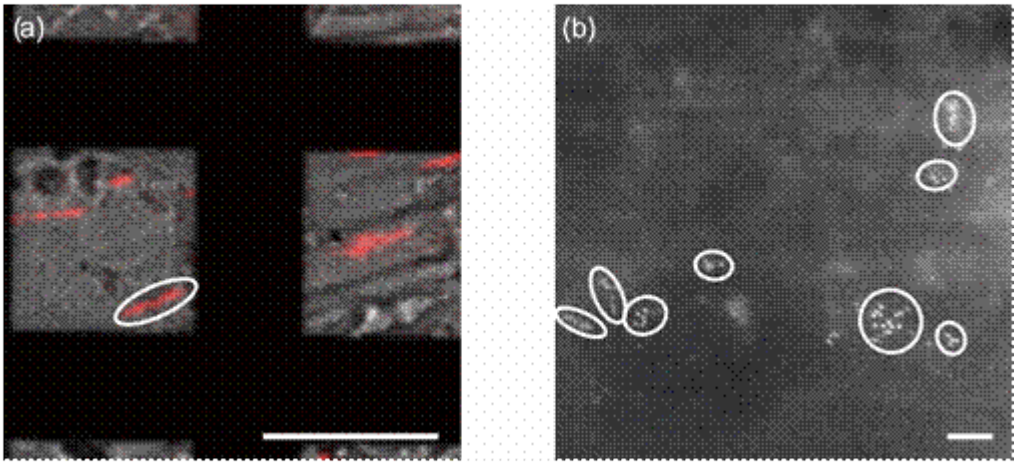

Figure 1: Correlative images of nAChR cluster labeled with a fluorescence-conjugated ligand and colloidal gold-conjugated antibodies

(a) A fluorescent image of nAChR cluster (red). The scale bar is 100 μm.

(b) A cryo-SEM image of nAChR and MuSK molecules (white dots) in the encircled cluster shown in (a). The white circles indicate small aggregation of nAChR and MuSK. The scale bar is 100 nm.