Internalized molecular localization of nAChR and MuSK by CLEM

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Neuromuscular junction (NMJ) is a synapse between a nerve terminal (presynapse) and a cytoplasmic membrane of muscle (postsynaptic membrane). In NMJ, acetylcholine is released from presynapse and received by nicotinic acetylcholine receptor (nAChR) on the postsynaptic membrane. On the postsynaptic membrane of NMJ, nAChR forms clusters with various cluster-related proteins such as agrin and muscle specific receptor tyrosine kinase (MuSK). Agrin is a secreted protein from presynapse, and MuSK is a postsynaptic membrane protein. Both agrin and MuSK are necessary for nAChR clustering, which is related to efficient signal transduction at the NMJ. It was reported that endocytic recycling of nAChR and MuSK is concerned with nAChR clustering by light-microscopic observation [1], [2]. It was also reported that agrin promotes endocytosis of MuSK by biochemical studies [2]. nAChR clusters can be matured on the cytoplasmic membrane of C2C12 myotubes by adding agrin into the culture medium. Time lapse imaging of C2C12 myotubes showed that small nAChR clusters began to assemble into a large cluster during maturation and the large clusters began to break into smaller clusters in a few hours [3]. However, the details of the molecular mechanisms of nAChR clustering remain largely mysterious.

We analyzed the internalized molecular localization of nAChR and MuSK of C2C12 myotubes during cluster maturing and breaking by correlative light and electron microscopy (CLEM). First, C2C12 myoblasts were differentiated into C2C12 myotubes. After nAChR and MuSK on the cell surface were labeled using a fluorescent ligand and gold particle-conjugated antibodies, agrin was added and the cultivation was continued. Then, we observed entire structure of nAChR clusters by fluorescent microscopy (FM), as shown in Figure 1(a). After that, we made sections of the cells and the nAChR cluster site imaged by FM was observed by electron microscopy (Figure 1(b)). As a result, we could detect internalization of nAChR and MuSK around nAChR clusters during maturing and breaking.

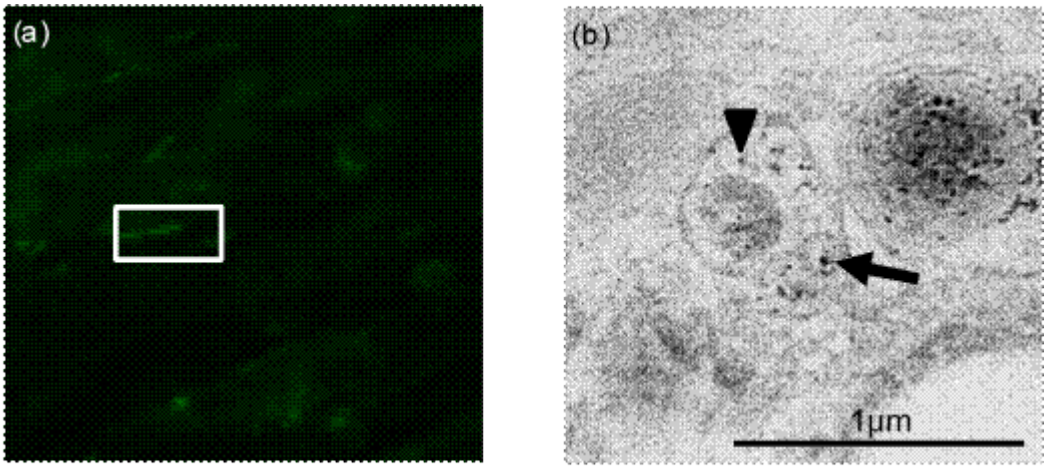

Figure 1 : CLEM images of nAChR cluster formed on C2C12 myotubes.
(a) A FM image of a nAChR cluster (shut in white square).
(b) A SEM image in the white square of (a). The arrow indicates MuSK labeled with 10nm-gold particles, and the arrow head indicates nAChR labeled with 5nm-gold particles.

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[2] D. Zhu et al., J Neurosci 28, (2008) 1688-1696
[3] T. Shyuan et al., J Cell Sci 125, (2012) 1531-1543