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LS-4-O-3255 Observation of intracellular complexes of filopodia at molecular resolution with cryo-ET

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Introduction: Cryo-electron tomography (cryo-ET) give us nanometer resolution structure of biological samples. Samples which observed by cryo-ET are fixed at liquid nitrogen temperature so it can keep biological condition structures. This technique have developed dramatically during last decade. In our research we have tried to unveil the intracellular structure of filopodia with this technique. Filopodia are very important structure for cells. They have very thin and finger-like (needle-like) structure and have an important role like antennae for cells. This structure associate with a lot of cell processes such as cell migration, neurite outgrowth and so on. However mechanisms of filopodia are still unknown. Therefore we would like to reveal the formation mechanisms of filopodia with cryo-ET.

Methods: We use NG108-15 (neuroblastoma/glioma) as a model nerve cell. These cells are cultured on the QUANTIFOIL (Au 0.6/1) directly and manually plunge frozen by second cryogen such as liquid ethane. In this method the samples are rapidly frozen, so it is able to avoid the artifact from any chemical fixations. Hence we can reveal the detail 3D structure of biological condition samples with transmission electron microscope. We use Tecnai G² Polara, FEI Company operated at 200kV with the GIF 10eV energy filter. However hydrated biological samples are very week for electron beam, and so we have to minimize radiation of electron beam and EM images must be with low dose system. Thus it cause low signal-to-noise ratio (S/N ratio) of image, so we need to process the acquired images with computers.

Results: We succeeded to observe the ultrastructure of filopodia. In filopodia, actin filaments are bundled by fascin in 36 nm period and this is equal to the half pitch of actin filaments. Moreover we using helical averaging technique to intracellular acin filaments and get atomic resolution actin filament model, and the bundling region of actin filament by fascin are observed. However we could not observe any motor proteins such as myosin because of crowd environment in the cell.

Conclusion: At the first, we are going to reveal the actin bundling mechanisms by fasin. Next, we would like to unveil the mechanisms of motor protein like myosin. It is very important to understand the mechanisms of formation of filopodia. It will give us new insight to the mechanisms of intracellular transportation.
Fig. 1: (Upper Left) XY-plane, (Upper Right) YZ-plane, Segmented image of the reconstructed volume of filopodia.

Fig. 2: (Left) Interaction region between actin filaments and fascin of the reconstructed image. (Right) Helical averaged image of an actin filament.