DIVISION OF Cell Structure

Associate Professor: *OGAWA, Kazuo*

Dynein is a molecular motor that carries cargoes to the direction of centriole from the cell periphery along the microtubules in a cell. It is composed of three domains: stem, motor, and stalk. Targeting of dynein to specific sites of cargoes may be related to the NH_2-terminal one-thirds of molecule constituting stem where amino acid sequence diversity was found between dynein superfamily (cytoplasmic and axonemal dynein), while the COOH-terminal two-thirds of molecule showing sequence conservation in distantly related species constitutes motor. Having interrupted long heptapeptide repeats in the sequence, the stalk takes an extended flexible structure and binds to microtubule in an ATP-dependent manner. Thus, dynein binds to microtubule at stalk domain of itself and cargoes at stem domain of itself.

The mechanism how cytoplasmic and axonemal dyneins target the cargoes has been gradually made clear in terms of molecules participated in. Cytoplasmic dynein is linked with dynactin complex containing at least 10 proteins. It is via dynactin complex that dynein targets to a receptor of cargo membranes.

In flagellar and ciliary movement, outer and inner dynein arms are projected from the A-subfiber of peripheral doublet microtubule of axoneme corresponding to the cargo. They bind to the B-subfiber of neighboring doublet microtubule in an ATP-dependent manner. Axonemal dynein is hardly detached from the A-subfiber being the cargo, while the cargoes of cytoplasmic dynein are thought to be detached from the motor after arrival to cell center to recruit the motor. Thus, there may be different targeting mechanism for dynein superfamily to specific sites of the cargoes.

When Triton-model sperm were exposed to hard condition such as 0.5 M KCl or NaCl (so called chemical dissection), outer dynein arm was detached from the A-subfiber. Triton-sperm losing outer dynein arm swam with a half beat frequency of control sperm. Re-binding of outer dynein arm onto the A-subfiber was possible by remixing the extracted Triton-sperm with the extract in a low salt concentration solution. Recovered Triton-model sperm swam with a normal beat frequency. Thus, a high salt extract might contain some proteins necessary for correct positioning of outer dynein arm and scaffold proteins to mediate binding of outer arm dynein onto the A-subfiber. During the course of characterizing proteins containing in the extract, we found a novel protein with molecular mass of 58 kDa designated as ap58. Immuno-electron microscopy using antibodies raised against recombinant ap58 shows that gold-particles are found at 25 nm repeat along the length of axoneme coinciding with the repeat of outer dynein arm (longitudinal sections of Figure 1). Thus, we conclude that ap58 is binding to in situ outer dynein arm.

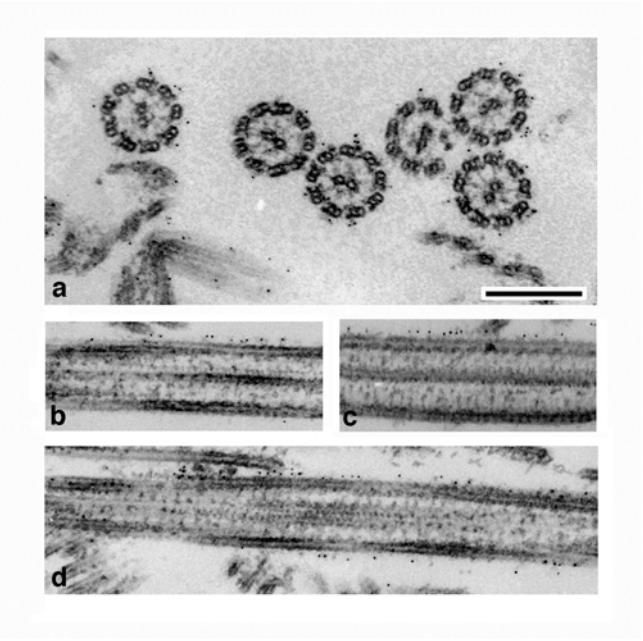

Figure 1. Immuno-electron micrographs to show the localization of ap58 within the sea urchin sperm axonemes. a: cross section; b-d: longitudinal sections (Ogawa and Inaba).

Publication List:

Original paper

Morii, H., Shimizu, T., Mizuno, N., Edamatsu, M., Ogawa, K., Shimizu, Y., and Toyoshima, Y.Y. (2005). Removal of tightly bound ADP induces distinct structural changes of the two tryptophan-containing regions of the ncd motor domain. J. Biochem. *138*, 95-104.