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Dynein is a molecular motor that carries cargo in the direction of the centriole or basal body from the cell periphery along the microtubules in cells. The heavy chain is the dynein motor subunit, composed of three domains: stem, head, and stalk. The targeting of dynein to specific cargo may be related to the NH_2-terminal third constituting the ìstemî domain, where amino acid sequences are different between the two dynein families i.e., cytoplasmic and axonemal dynein. The COOH-terminal two-thirds, which constitute the ìheadî domain, exhibit sequence conservation even in distantly related species. The ìstalkî possesses an extended flexible structure which binds to microtubules in an ATP-dependent manner. Thus, dynein binds to microtubules at the "stalk" domain and to cargo at the "stem" domain.

The mechanism by which cytoplasmic and axonemal dyneins target the cargo has been gradually made clear in terms of the molecules concerned. Cytoplasmic dynein is linked with a dynactin complex containing at least 10 proteins. Via the dynactin complex it targets a receptor of cargo membranes. In flagellar and ciliary axonemes, outer and inner dynein arms are projected from the A-subfiber of peripheral doublet microtubules. These bind to the B-subfiber of neighboring doublet microtubules in an ATP-dependent manner. In this case, the A-subfiber corresponds to their cargo. Axonemal dynein barely detaches from the cargo, A-subfiber, while the cargo of cytoplasmic dynein is thought to be detached when it arrives at the cell center to recruit another motor. Thus, the targeting mechanism is different between the two dynein families.

When the Triton-model sperm are exposed to a high salt solution containing 0.5 M KCl or NaCl, the outer dynein arm detaches from the A-subfiber. Model sperm without an outer dynein arm swim with half the beat frequency of control sperm. Since the extracted sperm are able to recover normal beat frequency by both mixing with the extract and lowering the ionic concentration of the mixture, a high salt extract might contain certain proteins necessary for correct positioning of the outer dynein arm as well as certain scaffold proteins needed to mediate the binding of the outer dynein arm onto the A-subfiber. During the course of characterizing such proteins in high salt extract of the axonemes in sea urchin sperm, we found a novel protein with a molecular mass of 58 kDa which was designated ap58. Immuno-electron microscopy using antibodies raised against recombinant ap58 revealed gold-particles at ~25 nm repeats along the length of the axoneme coinciding with the repeat of the outer dynein arm (longitudinal sections of Figure 1). Thus, ap58 in situ binds to the outer dynein arm.

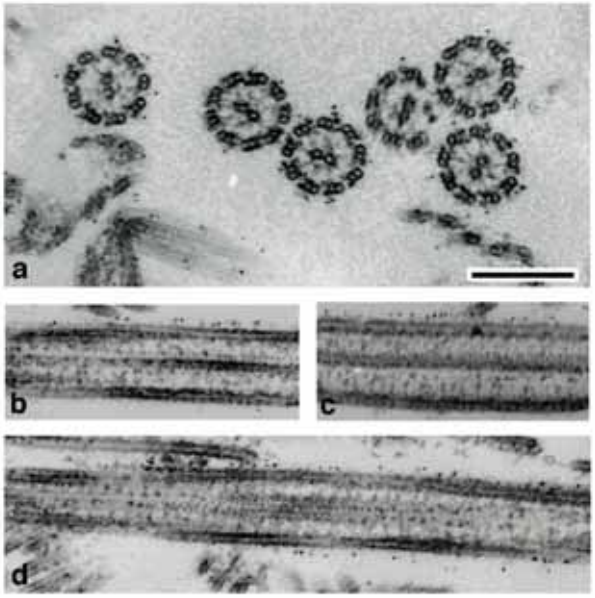

Figure 1. Immuno-gold localization of ap58 in the axonemes of sea urchin sperm. Cross section (a) and longitudinal images (b-d) are shown. The scale bar in (a) represents 200 μm for all images (Ogawa and Inaba).

Publication List:

Original papers

Ushimaru, Y., Konno, A., Kaizu, M., Ogawa, K., Sato, N., and Inaba, K. (2006). Association of a 66 kDa homolog of *Chlamydomonas* DC2, a subunit of the outer arm docking complex, with outer arm dynein of sperm flagella in the Ascidian *Ciona intestinalis*. Zool. Sci. *23*, 679-687.

Hozumi, A., Satouh, Y., Makino, Y., Toda, T., Ide, H., Ogawa, K., King, S.M., and Inaba, K. (2006). Molecular characterization of *Ciona* sperm outer arm dynein reveals multiple components related to outer arm docking complex protein 2. Cell Motil. Cytoskel. *63*, 591-603.

Ogawa, K., and Inaba, K. (2006). Ap58: A novel in situ outer dynein arm-binding protein. Biochem. Biophys. Res. Commun. *343*, 385-390.