DIVISION OF CELLULAR COMMUNICATION

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The research in this laboratory is aimed at an understanding of the molecular mechanisms that regulate the assembly and function of cytoskeletal proteins. Specifically, we are currently studying the functional properties of axonemal dynein and actin in *Chlamydomonas*, an organism ideally suited for genetic and molecular biological studies.

I. Function of Multiple Axonemal Dyneins

It is well established that the beating of cilia and flagella is based on sliding movements of outer-doublet microtubules driven by motor proteins dyneins, but how the sliding is converted into axonemal oscillatory bending movement has not been made clear. Recently, various lines of evidence have suggested that dynein is crucially important also in the sliding-bending conversion mechanism. Thus our research effort is now focused on the properties of various dyneins.

Biochemical studies by us and other laboratories have established that a single flagellar axoneme contains at least eleven kinds of dynein heavy chains in inner and outer arms. The question is how different dynein heavy chains differ in function. To answer this question, we have been isolating and characterizing mutants that lack different kinds of axonemal dyneins. During the last ten years, we have isolated as many as 15 genetically different mutants lacking various subsets of dyneins. The isolation of these mutants greatly advanced our understanding of the function and organization of various dyneins within the axoneme, because only three mutants had been known to lack dynein heavy chains before we started mutant isolation.

The motility phenotypes of the isolated mutants have indicated that different dynein species differ in function in a fundamental manner. For example, the outer-arm heavy chains are important for flagellar beating at high frequency, whereas the inner-arm heavy chains are important for producing proper waveforms. Indirect evidence also suggests that the force generation properties differ greatly among different heavy chains. Interestingly, the axoneme can beat without some of these heavy chains, but cannot beat if certain combinations of heavy chains are lost. It appears that simultaneous presence of dyneins with different properties is necessary for the axonemal beating. Thus, it should be important to understand the mechano-chemical property of each dynein. To this end, we are currently trying to directly measure the force production in wild-type and mutant axonemes that lack various combinations of dyneins; we have constructed an experimental device to measure minute axonemal force with fine glass needles. Preliminary results indicate that the force produced in the mutant axoneme lacking the outer arm or part of the inner arm is reduced to about 1/3 of that in the wild-type axoneme.

As a by-product of these experiments, we have recently succeeded in detecting elasticity between the outer-doublet microtubules. Our results confirmed that there is an elastic component that connects adjacent outer-doublet microtubules, as has been postulated by theoretical studies of cilia and flagella. Such an elastic component has been considered crucial for axonemal beating, since it is regarded as responsible for restricting the amplitude of microtubule sliding and for generating oscillatory movements.

II. Function of Actin and an Actin-related Protein in *Chlamydomonas*

The inner dynein arms are known to contain actin as a subunit. Hence the two independent motility systems of eukaryotes - the actin-based and microtubule-based motility systems - should somehow cooperate in the inner arm dynein although the function of actin in dynein arms is totally unknown at present. Recently we found that the mutant *ida5*, lacking four out of the seven subspecies of inner-arm dyneins, has a mutation in the actin-encoding gene. Intriguingly, *Chlamydomonas* has been known to have only a single gene of conventional actin, and the mutant *ida5* was found to express no conventional actin at all. On close inspection, the cytoplasm and axonemes of this mutant were found to contain a novel actin-like protein (NAP) which displays exceptionally low homology (64%) to conventional actin. The mutant *ida5* is deficient in the formation of the fertilization tubule and thus has a low mating efficiency. However, it displays normal cell division and grows as rapidly as wild type, possibly because NAP can substitute for actin in important cellular functions. Thus conventional actin and NAP may overlap in some, but not all, cellular functions. It is interesting to note that NAP is expressed in significant amount only in the mutant lacking actin; i.e., the expression of NAP appears to depend on the presence of actin. We are currently investigating how such regulation takes place.

We have recently succeeded in transforming the mutant *ida5* with cloned actin gene and found that inner dynein arms become restored upon transformation. Transformation with NAP gene is underway. Studies with artificially mutated actin gene will enable us to determine what functions are carried out by actin and NAP and, in particular, whether actin or NAP is really essential for cytokinesis, assembly and function of inner dynein arms, or other fundamental processes in *Chlamydomonas*.

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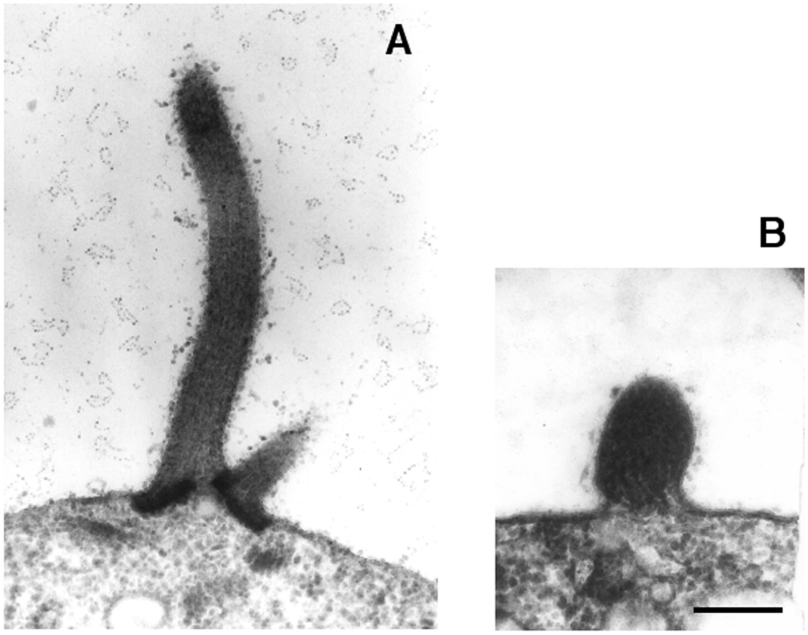

Fig. 1. Fertilization tubules in wild-type (A) and *ida5* (B) mt+ gametes produced in response to a 1 hour exposure to 10 mM dibutyryl-cAMP and 1 mM IBMX. Bar, 0.3 µm. Wild-type fertilization tubules have been shown to contain F-actin bundles.