論文内容の要旨

Studies on the functional properties of outer-arm dynein heavy chains by use of a novel *Chlamydomonas* mutant

（クラミドモナス新規突然変異株を用いたダイニン外腕重鎖の機能に関する研究）

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Flagellar rhythmic beating is driven by sliding movements between microtubules and axonemal dyneins. Axonemal dynein is a molecular motor and is classified into inner-arm dynein and outer-arm dynein according to their positions in the axoneme. In the green alga *Chlamydomonas*, the outer-arm dynein comprises a single molecular assembly containing three dynein heavy chains (α, β, and γ DHCs), while the inner-arm dynein comprises seven major and three minor subspecies each containing one or two distinct DHCs. The N-terminal portion of each DHC is called the stem (or tail), and is bound with the intermediate chains (ICs) and most of the light chains (LCs). The proximal region of the stem is the site where the DHCs attach to the A-tubule of the outer doublet microtubule. The C-terminal portion of each DHC consists of six AAA^+ domains and a microtubule-binding stalk, and the general organization of this region is conserved in all dynein species. This portion forms a globular head and presents the motor activity.

Previous studies using *Chlamydomonas* have shown that the outer dynein arm and the inner dynein arm differ in functions and structures. Studies using mutants deficient in the outer-arm and inner-arm dyneins have shown that the outer-arm dynein affects the beat frequency, while the inner-arm dynein has an effect on the amplified waveform. The outer-arm dynein presents simpler species and could be isolated and purified more easily than the inner-arm dynein. Previous studies have been shown that different outer-arm DHCs display strikingly different in vitro motility. It is an interesting question that how these distinct dyneins assemble and coordinate to produce the axonemal beating.

The functional property of each dynein could be a key to understand the mechanism of flagellar beating movement. To achieve a better understanding of the function of each species of dynein, I aimed at isolating novel mutants lacking specific dyneins and analyzing their properties. In part I, I described screening of slow-swimming mutants, and seven novel motility-deficient mutants were isolated successfully. Two mutants, *#24* and *#34*, are deficient in the tubulin polyglutamylation, and

would facilitate studies to understanding how tubulin polyglutamylation affects flagellar motility in *Chlamydomonas*. Another outer-arm mutant, *#5* that lacks the motor domain of γ DHC but retains the α and β DHCs, is thought to be valuable for studies on the property and function of the γ DHC. All of seven motility-deficient mutants are expected to yield more important information about the mechanism of axonemal beating.

In part II, I characterized the outer-arm mutant *#5* and analyzed functional properties of outer-arm DHCs. The mutant *#5* was named *oda2-t*, because it is an allele of *oda2* having a structure mutation in *γDHC* and lacking the entire outer dynein arm, and presents a truncated γ DHC. This type of mutant was isolated for the first time and has been awaited since *oda11* lacking the α DHC and *oda4-s7* lacking β motor domain were isolated. The mutant *oda11* swims slower than wild type, but faster than mutants missing the entire outer arm, such as *oda2* and *oda4*. Thus outer-arm dynein containing only the β and γ DHCs can function in the axoneme, and the α DHC apparently increases the activity of the β or γ DHCs. The mutant *oda4-s7*, expressing only the N-terminal 160-kDa region of the β DHC and lacking its motor domain, assembles outer arms with the α and γ DHCs, whereas the mutant *oda4* deficient in the *βDHC* gene lacks the entire outer arm. In contrast to *oda11*, *oda4-s7* swims at almost the same speed as *oda4*. Thus, the β DHC motor domain appears to be essential for the function of the outer-arm dynein, and outer-arm dyneins containing only the α and γ DHCs are almost completely non-functional. The question arises to how significantly the γ DHC contributes to the overall outer-arm function.

Western-blot analysis indicated that *oda2-t* lacks the γ motor domain, but retains the stem portion of the γ DHC. The structure of the γ DHC in *oda2-t* was determined by RT-PCR analysis. The result showed that the γ DHC in *oda2-t* has only 1623 amino acids, containing a 1270 amino-acid sequence from the N-terminal region of the γ DHC and a 315 amino-acid sequence from the C-terminal region of the NIT1 protein, connected through a 38 amino-acid adapter. The molecular mass of the truncated γ DHC in *oda2-t* calculated from the determined sequence is 185379 Da, including 146931 Da of the N-terminal γ DHC sequence. The difference between *oda2* and *oda2-t* suggests that the stem portion of the γ DHC is essential for the stable assembly of outer dynein arm. A similar difference has been observed between *oda4-s7* and *oda4*, which has shown that the N-terminal portion of the β DHC is important for stable assembly of the outer arm.

The *Chlamydomonas* outer-arm dynein is composed of three (α, β and γ) DHCs, two intermediate chains (IC1, IC2) and eleven light chains (LCs). I carried out fractionation of dynein by ion-exchange chromatograpy (HPLC) to examine the outer-arm dynein composition of *oda2-t*. Analyses of SDS-PAGE and Western showed that *oda2-t* lacked the γ motor domain and LC1, a light chain that was known to be associated with the γ motor domain, but retained the other compositions of outer-arm dynein. The results also indicated that the truncated γ DHC remains stably associated with the αβ dimer, while the LC4, associated with the stem of the γ DHC, is stably associated with

the truncated γ DHC in the *oda2-t* axoneme.

The location of outer-arm DHCs in cross section has been determined using *oda11* and *oda4-s7*. Averaged outer-arm images in cross-section micrographs of the mutant axonemes located the α DHC at the outer-arm tip, and the β motor domain at an intermediate position between the base and tip. From the images of the double mutant *oda11oda4-s7*, the γ motor domain was predicted to localize to the inner lobe of the outer-arm image. In this study, outer-arm images of *oda2-t* in cross-section electron micrographs were classified and averaged using an image clustering protocol, and analyzed by Student *t*-test. The result showed that a major density difference between *oda2-t* and wild type was in the inner lobe of the outer arm. It is consistent with previous prediction, and suggests that the motor domain of the γ DHC is located in this region. In addition to the change in the inner lobe, a wedge-shaped area of density was observed on the outer side of the entire outer arm, as well as the tip area observed in *oda4-s7*. This difference could be an alteration in orientation of the total arm, which might be caused by the loss of the proximal structure. The mutant *oda2-t* appears to have a slightly impaired ability to assemble outer dynein arms on the outer doublets (average outer arms per nine outer doublets: *oda2-t*, 6.7; wild type, 7.9), and a similar reduction in the outer-arm number has also been observed in *oda4-s7*.

To study the function of the γ motor domain, I compared the swimming velocity and beat frequency in wild type, *oda2* (lacking the α, β and γ DHCs), *oda11* (lacking the α DHC), *oda4-s7* (lacking the β motor domain) and *oda2-t* (lacking the γ motor domain). The average velocity (standard deviation) for each strain (μm/s) was wild type, 161.6 (9.2); *oda11*, 120.4 (13.0); *oda4-s7*, 59.7 (7.4); *oda2-t*, 87.8 (9.4); *oda2*, 50.6 (6.6), and the beat frequency was: wild type, 69Hz; *oda11*, 56Hz; *oda4-s7*, 34Hz; *oda2-t*, 50Hz; *oda2*, 30Hz. These results suggested that the outer-arm dynein lacking the γ motor domain can function to some extent. Previous studies on the outer-arm structure, as well as this study, demonstrated that the γ motor domain is located very close to the microtubule, and may be critically important for the overall structure of the outer arm. Surprisingly, motility analyses indicated the outer arm retains partial function even without the γ motor doamin. From a phenomenological point of view, the β DHC is the most important power generator among the three DHCs of the outer arm.

Chlamydomonas flagella display Ca^{2+}-dependent waveform conversion. The axonemes beat in an asymmetrical pattern at Ca^{2+} concentrations lower than 10^{-6}M, while they beat in a symmetrical pattern at Ca^{2+} concentrations higher than 10^{-5}M. Conversion to the symmetrical pattern is observed in live cells when displaying transient backward swimming upon illumination with intense light. Previous studies have shown mutants lacking the outer-arm dynein did not display the light-induced back swimming. To examine function of three DHCs at high Ca^{2+} concentration, I analyzed motilities of reactivated wiled-type and mutant axonemes under 10^{-4}M and 10^{-8}M Ca^{2+} conditions. At 10^{-8}M Ca^{2+}, the average beat frequency (standard deviation) for each strain (Hz) was wild type,

75.8 (11.7); *oda11*, 49.4 (5.4); *oda4-s7*, 38.4 (3.3); *oda2-t*, 47.1 (8.7); *oda2*, 31.0 (3.4). These frequencies are close to the flagellar native frequency. At high Ca^{2+} concentration, only <10% of *oda2* axonemes beat, and displayed moving straight with a symmetrical waveform of very small amplitude. The average beat frequency (standard deviation) for each strain (Hz) was wild type, 91.7 (14.1); *oda11*, 31.5 (6.9); *oda4-s7*, 42.6 (8.1); *oda2-t*, 19.6 (7.7); *oda2*, 15.9 (3.8), and the averaged velocity (standard deviation) for each strain (μm/s) was: wild type, 115.1 (24.2); *oda11*, 9.4 (2.3); *oda4-s7*, 20.8 (8.6); *oda2-t*, 10.2 (6.2); *oda2*, 3.5 (1.4). Axonemes of *oda2* beat at 1/6 the wild-type frequency, and the beating was so ineffective that it did not result in efficient propulsion of the axoneme, only moving at very small speed. The motility of *oda2-t* axonemes was a little higher than that of *oda2*, but much lower than that of *oda4-s7* axonemes. These results suggest that the outer-arm dynein lacking the γ motor domain appears to lose most of the motility at high Ca^{2+} concentration. Since LC4, a Ca^{2+}-binding light chain, has been suggested to play a role in the Ca^{2+}-dependent waveform conversion, I speculate that LC4 possibly controls waveforms through the γ DHC to which it binds, while the γ DHC produces the main power in the reactivated axonemes at high Ca^{2+} concentration.

The ATPase activities of the wild-type and mutant axonemes were measured in the presence of 1mM ATP in HMDEK at 25°C. Reactivated axonemes displayed beating for longer than 10 minutes, and checked that the phosphate liberation was linear with time for the initial 5 minutes. The average ATPase activity (standard deviation) for each strain (μmol phosphate /minute /mg axonemes) was wild type, 1.59 (0.08); *oda11*, 1.31 (0.10); *oda4-s7*, 0.60 (0.06); *oda2-t*, 2.03 (0.29); *oda2*, 0.35 (0.04). The activity was very low in *oda2* axonemes that lack the entire outer arm, suggesting that more than 75% of the ATPase activity in wild-type axonemes can be accounted for by the activity of outer-arm dynein. The ATPase activity of *oda2-t* axonemes was strikingly higher than that of wild type, indicating that the γ DHC suppresses the ATPase activity of the αβ dimer *in situ*. In addition, the ATPase activity of *oda4-s7* was markedly lower than those of *oda11* and wild type, suggesting the importance of the β DHC for the high ATPase activity in the axoneme. The observation that *oda11* and *oda2-t* axonemes have high ATPase activities yet display much poorer motility than wild type suggests that these axonemes have defects in mechanochemical coupling, which is likely to be regulated through interactions between the different DHCs or their associated components. The ATPase activities measured at high Ca^{2+} concentration showed the same results, and it showed that the ATPase activity of axonemes are not affected by Ca^{2+} concentration.

The mutant *oda2-t* lacking the γ motor domain, as well as *oda11* and *oda4-s7*, implies that outer-arm dyneins with any desired combinations of the three DHCs could be obtained by using these mutants and the double mutants between them. Thus this mutant should greatly advance studies on the structure and functional property of each DHC in outer-arm dynein.