A *Chlamydomonas* Outer Arm Dynein Mutant Missing the α Heavy Chain

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Abstract. A novel *Chlamydomonas* flagellar mutant (*oda*-11) missing the α heavy chain of outer arm dynein but retaining the β and γ heavy chains was isolated. Restriction fragment length polymorphism analysis with an α heavy chain locus genomic probe indicated that the *oda*-11 mutation was genetically linked with the structural gene of the α heavy chain. In cross-section electron micrographs, the *oda*-11 axoneme lacked the outermost appendage of the outer arm, indicating that the α heavy chain should be located in this region in the wild-type outer arm. This mutant swam at 119 μm/s at 25°C, i.e., at an intermediate speed between those of wild type (194 μm/s) and of *oda*-1 (62 μm/s), a mutant missing the entire outer dynein arm. The flagellar beat frequency ($\sim$50 Hz) was also between those of wild type ($\sim$60 Hz) and *oda*-1 ($\sim$26 Hz). These results indicate that the outer dynein arm of *Chlamydomonas* can be assembled without the α heavy chain, and that the outer arm missing the α heavy chain retains partial function.

THE outer arm dynein of cilia and flagella has been the most extensively studied among all kinds of dynein. *Chlamydomonas* outer arm dynein contains three heavy chains (α, β, and γ), which have molecular masses in excess of 400,000 and ATPase activity, as well as several small and intermediate-sized subunits (Piperno and Luck, 1979; Huang et al., 1979; Pfister et al., 1982; King and Witman, 1988*a*,*b*). *Tetrahymena* outer arm dynein also has three heavy chains (Johnson and Wall, 1983; Toyoshima, 1987). On the other hand, outer arm dyneins from sperm flagella of sea urchin (Bell and Gibbons, 1982; Bell, 1983; Sale et al., 1985), trout (Gatti et al., 1989), and bull (Marchese-Ragona et al., 1987), and those from cilia of sea urchin embryo (Ogawa et al., 1990) contain only two heavy chains. Electron microscope studies have shown that the isolated outer arms have bouquet-like structures in which two or three heads are connected through a thin stem to a common base (Johnson and Wall, 1983; Witman et al., 1983; Goodenough and Heuser, 1984; Sale et al., 1985). Each of these heads appears to be composed of a heavy chain, because the number of the heads equals that of the heavy chains. It is not known, however, how these bouquet-like outer arms are folded when attached to the A-tubule of the outer doublets. It is not known either how the two-headed and three-headed dyneins differ in function, nor what specific function each heavy chain performs. Recent experiments using purified dynein subspecies have demonstrated that a partial assembly of sea urchin outer arms containing only one heavy chain (Sale and Fox, 1988; Vale et al., 1989), or that of *Tetrahymena* outer arms containing only two heavy chains (Vale and Toyoshima, 1989) can cause translocation of cytoplasmic microtubules in vitro. Thus partial outer arms retain function as mechanochemical transducers. It has remained to be studied whether these partial outer arms can function in beating axonemes.

In this paper we describe the isolation of an unusual *Chlamydomonas* mutant that lacks the α heavy chain of the outer arm. Because this mutant can swim at an intermediate speed between the speeds of the wild type and a mutant (*oda*) missing the entire outer arm, the mutant outer arm appears to retain some functional activity.

Materials and Methods

Strains

Chlamydomonas reinhardtii 137c (wild type; mating type + and −), wild isolate SID2, mating type −, (Gross et al., 1988), an outer dynein arm-missing mutant *oda*-1 (Kamiya and Okamoto, 1985; Kamiya, 1988), and the newly isolated mutant *oda*-11 were used.

The α chain-missing mutant *oda*-11 was obtained by a method modified from Kamiya (1988). A cell suspension of wild-type *Chlamydomonas reinhardtii* 137c mt^+ was irradiated for 5 min with an ultraviolet lamp (Toshiba GL-15) 40 cm above the sample. After the irradiation, cells were incubated in the dark for 24 h and then transferred to eight testtubes each containing 5 ml of Tris-acetic acid-phosphate (TAP)[1] medium (Gorman and Levine, 1965). The testtubes were kept under constant illumination for 3 d. Cells grown at the bottom of the test tube were streaked on 1.5% agar plates for clonal separation, and 24 colonies were saved from each testtube culture. Each colony was cultured in 0.5 ml of medium in a testtube without agita-

1. *Abbreviations used in this paper*: TAP, Tris-acetic acid-phosphate; TEDK, 30 mM Tris-HCl, 0.5 mM EDTA, 1 mM DTT, 25 mM KCl, pH 7.5, plus 0.1 mM PMSF.

tion. After 2 d of culture, the cells were examined for their motility under a dark-field microscope. Clones that displayed slower than normal swimming speeds were saved for further selection, since previous studies have shown that mutants lacking dynein components have reduced swimming speeds. Next, these slowly swimming cells were examined for their flagellar beat frequency. For this purpose, we used an instrument that can quickly (<10 s) measure the average frequency in a population of cells (Kamiya and Hasegawa, 1987). Cells that were judged to have lower swimming speeds and flagellar beat frequencies were examined for the composition of dynein heavy chains by SDS-PAGE; those strains that had abnormal SDS-PAGE patterns in the dynein heavy chain region were saved.

Genetic Analysis

Tetrad analysis between *oda*-11 and other mutants was carried out using standard techniques (Levine and Ebersold, 1960). In scoring the phenotypes, we measured the flagellar beat frequency when any one of the daughter cells displayed wild type–like motility. This was necessary because *oda*-11 swam so actively that it was difficult to distinguish it from the wild type by simple visual inspection.

Restriction Fragment Length Polymorphism (RFLP) Analysis

Tetrads were dissected from a cross between *oda*-11 and wild-type strain S1D2. Products were scored for *oda*-11 phenotype by immunofluorescence using mAb C2.14, a mouse IgM specific for the outer arm α heavy chain (Mitchell and Rosenbaum, 1986) as primary antibody. To detect the primary antibody, biotinylated goat-anti-mouse-IgM and FITC-avidin (Vector Laboratories, Inc., Burlingame, CA) were used according to the manufacturer's recommendations. Unfixed cells were allowed to adhere to slides coated with 0.1% poly-L-lysine and were then permeabilized with 0.2% NP-40 and blocked with 3% BSA before incubation in primary antibody at 4°C for 18 h. Slides were then washed, fixed in 3% formalin, washed, incubated in secondary antibody, washed, incubated in FITC-avidin, washed, incubated in 100% methanol at −20°C, washed, and viewed on a Nikon fluorescent microscope. All wash, fixation and antibody incubation solutions were prepared in Tris-buffered saline (20 mM Tris, 150 mM NaCl, pH 7.5).

DNA prepared from tetrad products by the method of Weeks et al. (1986) was digested with KpnI, run on a 0.8% agarose gel, transferred, and probed with genomic clone EA2.2, a 2.7-kb SacI–SalI fragment of the α dynein gene (probe *6* in Fig. 5 *a* of Mitchell, 1989). Molecular techniques were as described in Sambrook et al. (1989).

Isolation of Axonemes and Extraction of Dynein

Culture of cells, isolation of flagella and preparation of crude dynein extracts have been described previously (Sakakibara and Kamiya, 1989). Briefly, cells were cultured on 10 800-cm^2 agar plates containing TAP medium for ~1 wk under constant illumination. This procedure yielded ~1 × 10^{11} cells. Upon completion of culture, cells were scraped off from the agar plate into 500 ml liquid TAP medium. Cells grew flagella after the suspension was kept stirred for 2 h. Flagella were isolated and demembranated by the method of Witman (1986). For dynein extraction, the axonemes were precipitated by centrifugation, suspended in 1 ml of HMDEK (30 mM Hepes, 5 mM $MgSO_4$, 1 mM DTT, 1 mM EGTA, 50 mM potassium acetate, pH 7.4) containing 0.6 M KCl. After being left at 0°C for 15 min, the extracted axonemes were separated from dynein by centrifugation. About 1 mg protein was obtained in the supernatant.

Sucrose Density Gradient Centrifugation

Sucrose density gradient centrifugation was carried out essentially by the method of King et al. (1986). The 0.6 M KCl extract was dialyzed for 8 h at 4°C against 500 vol of TEDK (30 mM Tris-HCl, 0.5 mM EDTA, 1 mM DTT, 25 mM KCl, pH 7.5, plus 0.1 mM PMSF). After being concentrated two- to threefold, the sample was centrifuged for 20 min at 13,000 *g* to remove aggregates of proteins. The supernatant, about 0.2 ml in total volume and with the protein concentration of ~2.0 mg/ml, was layered onto the top of a 4.9-ml linear 5–20% sucrose gradient prepared in TEDK containing 0.05 TIU/ml of aprotinin. The sucrose gradients were spun at 180,000 *g* (44,000 rpm) for 4.5 h at 4°C in an RPS55T-2 rotor (Hitachi Koki Co., Ltd., Tokyo, Japan) and collected in 0.25-ml fractions from the bottom of the tube. Bovine thyroglobulin (19.3S) bovine liver catalase (11.3S), *Escherichia coli* β-galactosidase (15.9S), and yeast alcohol dehydrogenase (7.6S) were also run on separate gradients as sedimentation coefficient standards. The apparent sedimentation coefficients were determined by the method of Martin and Ames (1961).

Electron Microscopy

Axonemes were fixed overnight at 4°C with 2% glutaraldehyde in the presence of 1% tannic acid, in 50 mM sodium cacodylate, pH 7.0. After postfixation with 1% OsO_4 for 1 h, the sample was block-stained with 0.5% uranyl acetate for 1 h at 4°C, dehydrated through a series of ethanol solutions at room temperature, and embedded in Epon 812. Golden thin-sections were cut and double-stained with aqueous solutions of 7.5% uranyl acetate and 0.4% lead citrate. A JEM100C microscope was used.

To examine the shape of outer dynein arms in mutants, images of outer doublet microtubules in cross-section micrographs were averaged using an IBAS image analyzer (Zeiss, Oberkochen, Germany), as used in a study of inner-arm mutants (Kamiya et al., 1991). Individual images of eight outer doublets (all but the one missing the outer dynein arm [Hoops and Witman, 1983]) in an enlarged cross-section micrograph of axoneme were taken with a TV camera and put into 8 frame memories of IBAS, which was equipped with 12 memories. In doing so, the orientation of the photograph was adjusted so that each "8"-shaped doublet microtubule image matched as closely as possible an overlay reference image that had been generated by binary contrast enhancement of an arbitrarily chosen doublet image. All the images in the memory were averaged after they were judged by visual inspection to be oriented well with respect to each other. Although this method involved such a subjective process that the image orientation was judged by eye, the averaged image did not change noticeably when the whole process was performed by different operators.

ATPase Assay

ATPase activities of fractionated dynein were measured by a malachite green method as described by Kodama et al. (1986). ATP-hydrolyzing reaction was allowed to proceed at 25°C in the presence of 10 mM Tris-HCl (pH 7.5), 5 mM $MgSO_4$, 0.5 mM EDTA, and 1 mM ATP.

Motility Assessment

The swimming velocities of the mutant and wild-type cells were measured by using a dark-field microscope at a total magnification of 100, a video recording system, and a personal computer. A red filter (cut-off wavelength 630 nm) was placed under the condenser lens to facilitate the measurement by suppressing the cells' response to light; when illuminated by white light, *Chlamydomonas* cells frequently display irregular swimming paths, which make the measurement difficult. About 50 samples were measured to obtain the average velocity of a given sample.

Flagellar beat frequencies of swimming cells were measured by analyzing the frequency of the bodily vibration of cells by means of a fast Fourier transform (FFT) analyzer, as described by Kamiya and Hasegawa (1987).

For measurements of beat frequency of the *cis*- and *trans*-flagella, uniflagellated cells were produced by subjecting 1 ml of 2–5 × 10^6 live cells to 5–10 strokes in a 2-ml Teflon homogenizer. This treatment produced cells lacking one or both of the two flagella, but left the cell bodies apparently intact. The mean beat frequency of uniflagellated cells in a sample did not change noticeably during an observation for ~40 min, after which time we replaced the sample with a freshly homogenized one because shortening of the existing flagellum and regeneration of the other flagellum became conspicuous. We took care not to use cells with flagella that appeared to be shorter than normal or bent in an abnormal way. Uniflagellated cells rotated in a small area at 2–5 rotation/s. The flagellum beating in such rotating cells was judged to be *cis* or *trans* by observing its position with respect to the eyespot, although the judgement was not always easy; when any doubt existed about the assignment of the flagellum, the data was not used. Use of an oil-immersion condenser was essential for clear visualization of the eyespot. Flagellar beat frequency in uniflagellated cells was measured also by the FFT method, as described by Kamiya and Hasegawa (1987). All the motility measurements were carried out at 25°C.

Other Methods

SDS-PAGE was carried out by the method of Laemmli (1970) as modified by Jarvik and Rosenbaum (1980). The gel was composed of a 3–5% acrylamide gradient and 3–8 M urea gradient. To see the intermediate- and low-molecular weight bands of dynein, the samples were also run on 20% acrylamide slab gels. All the gels were silver stained (Merril et al., 1981).

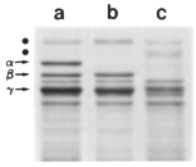

Figure 1. Part of a 3–5% SDS-urea PAGE pattern showing the high-molecular weight bands of (*a*) wild-type, (*b*) *oda*-11, and (*c*) *oda*-1 axonemes. Stained with silver. (*Arrows*) The three (α, β, γ) high-molecular weight chains of the outer arm. (*Asterisks*) Bands with contaminating membrane proteins.

Protein concentrations of the axoneme and crude extract were determined by the method of Bradford (1976).

Results

Isolation of an α Chain–Missing Mutant, oda-11

In our attempts to isolate dynein mutants by looking for slowly swimming phenotypes, we have encountered mutants missing the entire outer arm or subsets of the inner arm, but no mutants with partial defects in the outer arm (Kamiya and Okamoto, 1985; Mitchell and Rosenbaum, 1985; Kamiya, 1988). In this study, because we wanted to isolate mutants with more subtle defects in dynein arms, we looked for mutants whose swimming speed was only slightly lower than that of the wild type.

From a population of mutagenized cells, we first obtained 20 clones that appeared by visual inspection to have such slow swimming phenotypes. Of these, ten strains were found to have lower flagellar beat frequencies, e.g., 45–55 vs. 60 Hz of wild type at 25°C. SDS-PAGE analyses of the axonemes from these clones showed that one clone was missing the α heavy chain of outer arm dynein (Fig. 1). Because tetrad analysis revealed that this strain was not linked with previously isolated outer arm–missing mutants, *oda*-1–*oda*-10 (Kamiya, 1988) (Table I), we named it *oda*-11. We have, however, been unable to determine the locus for this mutation in the genetic map.

Dynein Heavy Chains

Fig. 1 shows a portion of the SDS-PAGE pattern of the *oda*-11 axonemes, along with those of the axonemes of wild type and an outer arm–missing mutant *oda*-1, to show the dynein high-molecular weight polypeptides. It clearly demonstrates that *oda*-11 is missing only the α chain, whereas *oda*-1 is missing the α, β, and γ heavy chains. Apparently normal amounts of β and γ chains are present in the *oda*-11 axoneme.

Table I. Tetrad Analysis between oda-11 and oda-1 to oda-10

oda-1	0:5:15*
oda-2	1:3:9
oda-3	5:2:11
oda-4	4:4:13
oda-5	3:0:38
oda-6	2:4:9
oda-7	4:4:15
oda-8	3:3:7
oda-9	0:1:9
oda-10	0:1:9

* Numbers of parental ditype/nonparental ditype/tetratype.

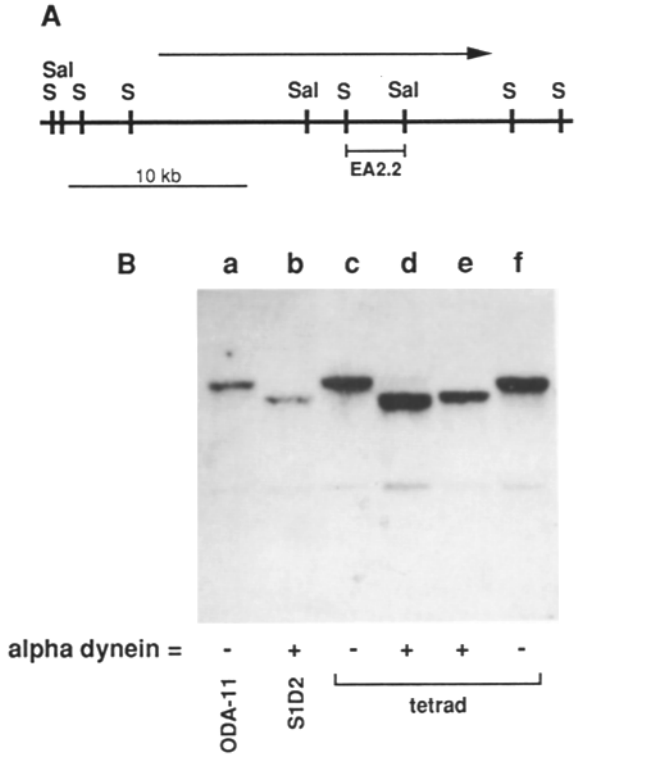

Figure 2. RFLP analysis of genetic linkage between *oda*-11 and the α dynein gene. (*A*) Restriction map of the α dynein locus showing the location of the α dynein transcription unit (*arrow*) and the 2.7-kb fragment, EA2.2, used as hybridization probe. *S*, SacI; *Sal*, SalI. (*B*) Southern blot of DNA from *oda*-11 (lane *a*), S1D2 (lane *b*), and from the four products of one tetrad of a cross between *oda*-11 and S1D2 (lanes *c–f*), digested with KpnI and probed with EA2.2. The bands are ~17 kb (137c/*oda*-11) and 9.5 kb (S1D2) in size. The presence (+) or absence (−) of an α heavy chain in the flagella of each strain is indicated below the lanes.

Linkage with the α Chain Clone

To determine whether the absence of an α heavy chain in *oda*-11 could result from a mutation in the α dynein structural gene, we performed RFLP analysis. DNA was isolated from *oda*-11 and from wild-type strain S1D2, which contains many RFLPs with respect to the laboratory strain (137c) used as a background for mutant selection. Samples were digested with several restriction enzymes and probed with clones from within the coding region of the α dynein gene, and an RFLP was observed for KpnI fragments detected with clone EA2.2. Fig. 2 *A* shows a restriction map of the α dynein gene with the location of the transcribed region (*arrow*) and fragment EA2.2 indicated. An example of the polymorphism at this locus is seen by comparing Fig. 2 *B*, lanes *a* (*oda*-11) and *b* (*S1D2*). Lanes *c–f* in Fig. 2 *B* show the hybridization pattern from the four products of a single tetrad. The presence or absence of an α heavy chain in flagella of each strain is indicated below the lanes, and shows that the absence of an α chain cosegregates with the *oda*-11 hybridization pattern in this tetrad. An additional nine tetrads were scored, and in every case absence of the α heavy chain cosegregated with the *oda*-11 RFLP pattern. The resulting ratio of 10:0:0 (parental ditype:nonparental ditype:tetratype

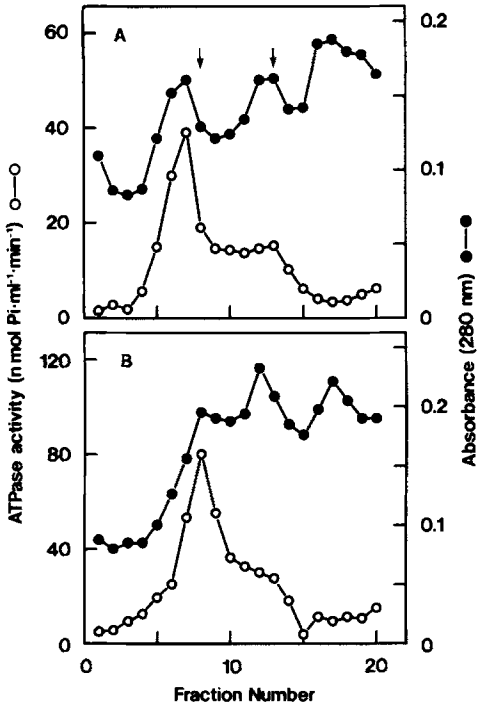

Figure 3. Sucrose density gradient centrifugation profiles of 0.6 M KCl extracts from (*A*) wild-type and (*B*) *oda*-11 axonemes. The bottom of the gradient is at left. (*Closed circle*) A_{280} protein absorbance of the fractions. (*Open circle*) Mg^{2+}-dependent ATPase activity expressed as nanomoles Pi per milliliter per minute. Arrows indicate the position of the sedimentation peaks of bovine thyroglobulin (19.3S) and catalase (11.3S). Solution composition for ATPase assay: 10 mM Tris-HCl (pH 7.5), 5 mM $MgSO_4$, 0.5 mM EDTA, and 1 mM ATP. Temperature: 25°C.

tetrads) indicates linkage between *oda*-11 and the α dynein gene at the 99% confidence level (Perkins, 1953) and further suggests that *oda*-11 and α dynein reside within the same 5 cM map interval.

Sucrose Density Gradient Centrifugation

For further analyses of dynein components in the mutant, 0.6 M KCl extract from axonemes was subjected to a 5–20% sucrose density gradient centrifugation. With the high salt extract from wild-type axonemes, major Mg-ATPase activities appeared in two peaks as reported by several investigators (Watanabe and Flavin, 1973; Piperno and Luck, 1979; Pfister et al., 1982; Mitchell and Rosenbaum, 1985) (see Fig. 3 *A*). The apparent sedimentation coefficient of the higher ATPase peak was 21–22S and the lower one was 12–15S in our experiment. It is likely that the 21–22S peak corresponds to the so-called 18S dynein and the 12–15S peak the 12S dynein described in previous reports, since it has been reported that the "18S" dynein has a sedimentation coefficient of 21.4S under the same conditions as used in this study (Pfister and Witman, 1984). SDS-PAGE has indicated that the peak fraction at 21–22S (fraction no. 7) contains the α and the β heavy chains and the 12–15S peak (fraction nos. 12-13) the γ chain, agreeing with the previous reports (Fig. 4). With the *oda*-11 axoneme extract, in contrast, an ATPase peak appeared at ~19S (fraction no. 8) instead of 21–22S (Fig. 3 *B*). SDS-PAGE showed that this peak contained the

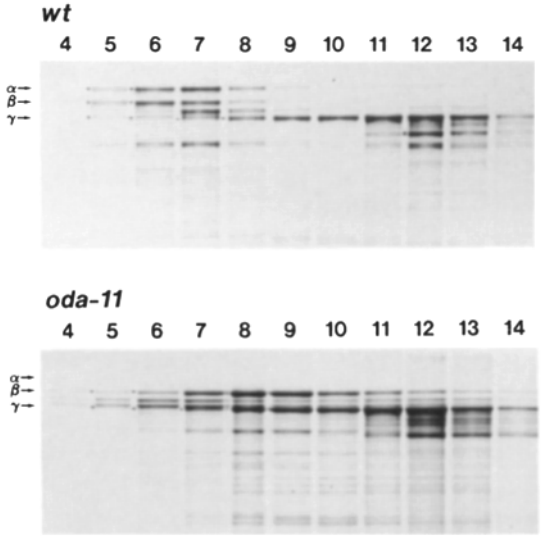

Figure 4. SDS-PAGE patterns of fractions from the sucrose density gradient centrifugation shown in Fig. 3. Only the high-molecular weight region of the 3–5% SDS-PAGE with 3–8 M urea gradient gel is shown. Equivalent volumes of the fractions were loaded. Arrows, the three (α, β, γ) high-molecular weight chains of the outer arm. (*Asterisk*) Two overlapped inner arm bands. Stained with silver.

β heavy chain, a small amount of γ chain, and several inner-arm chains (Fig. 4). Although only a shoulder appeared at the 12–15S region in ATPase profiles, SDS-PAGE analysis demonstrated that the γ chain, as well as several inner arm heavy chains, was sedimented in this region as in the wild-type extract. Probably the 12–15S peak appeared only as a shoulder because it was overlapped with the tail of the prominent 19S peak.

To see protein compositions in a lower molecular weight range, we also examined these fractions by SDS-PAGE on 20% acrylamide gels. A 16-kD light chain known to be associated with the α heavy chain (Pfister et al., 1982; Pfister

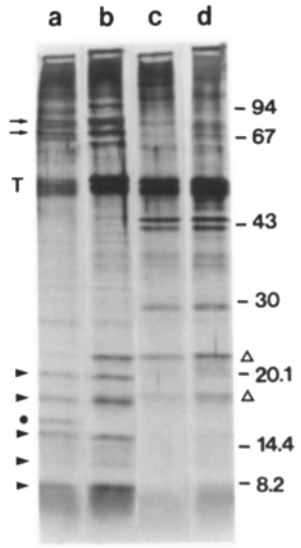

Figure 5. Intermediate-sized and low-molecular weight polypeptides separated on 20% SDS-PAGE. Fraction 7 (21S) of (*a*) wild-type, (*b*) fraction 8 (19S) of *oda*-11, (*c*) fraction 11 (13S) of wild-type, and (*d*) fraction 11 of *oda*-11 are compared. Arrows and arrowheads indicate the intermediate-sized (68 and 76 kD) and low-molecular weight (8.1, 11.5, 14.8, 17.5, and 20 kD) polypeptides normally associated with the β heavy chain (Pfister et al., 1982; Pfister and Witman, 1984; Mitchell and Rosenbaum, 1986). (*Asterisk*) Low-molecular weight polypeptides (16 kD) normally associated with the α heavy chain. (*Open triangle*) Low-molecular weight polypeptides (18.1 and 22 kD) normally associated with the γ heavy chain. *T*, tubulin. Bars and numbers at right, positions of molecular mass standards in kilodaltons. Stained with silver.

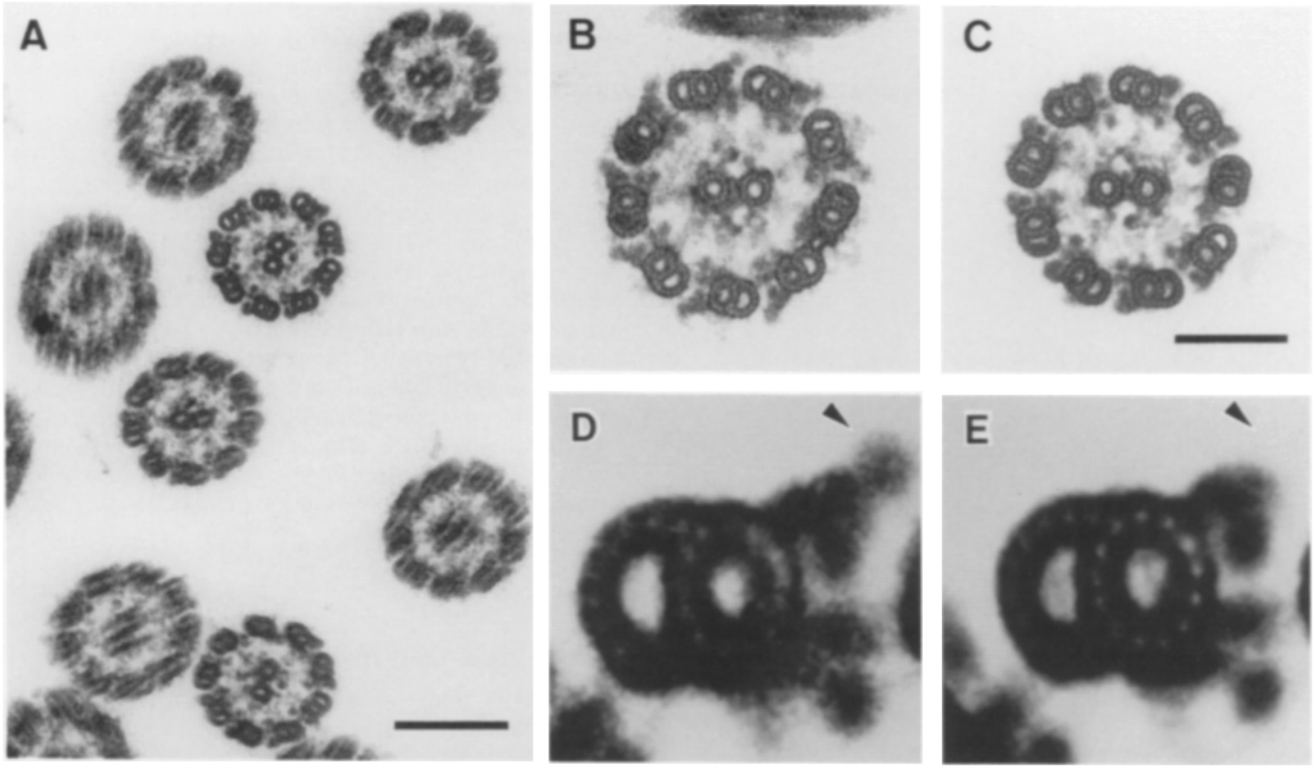

Figure 6. Cross-section electron micrographs of axonemes with averaged outer-doublet images. (*A*, *C*, and *E*) *oda*-11; and (*B* and *D*) wild-type axonemes. (*D* and *E*) Images averaged from eight outer doublets (all but one missing the outer dynein arm) in *B* and *C*. The outer dynein arm of *oda*-11 is missing the outermost appendage indicated by arrowheads. Bars: (*A*) 0.2 μm; (*C*) 0.1 μm.

and Witman, 1984; Mitchell and Rosenbaum, 1986) was absent in the 19S or other fractions of *oda*-11 extract (Fig. 5). On the other hand, intermediate-sized (molecular mass 68 and 76 kD) and low-molecular weight peptides (molecular mass 8.1, 11.5, 14.8, 17.5, and 20 kD) normally associated with the β heavy chain (Pfister and Witman, 1984; Mitchell and Rosenbaum, 1986) were present in the 19S fraction. Two other light chains of molecular mass 18.1 and 22 kD were normally found in the 12–15S fraction containing the γ chain. In no fractions of the *oda*-11 extract did we find a protein band that might represent a truncated form of the α chain, and no immunoreactive bands were detected on Western blots of *oda*-11 axonemes probed with anti-α dynein mAb C2.14.

Electron Microscope Observation on In Situ Outer Arms

Because the α heavy chain accounts for as much as about one-third of the total mass of the outer arm, we expected that *oda*-11 outer arm might look smaller than the wild-type outer arm in electron micrographs. We thus examined the outer arm image in cross-section micrographs of the axoneme. Like the wild-type axoneme, the *oda*-11 axoneme was found to have outer dynein arm on eight out of the nine outer doublet microtubules (Hoops and Witman, 1983). The outer arm in *oda*-11, however, looked significantly shorter than the wild-type outer arm. To see the structural defects in more detail, we used an image processor to average the eight outer arm images of both strains (Fig. 6). The averaged image of the outer dynein arms clearly indicated that the outer dynein arm in *oda*-11 is missing the outermost appendage. Thus, the α heavy chain should be located at this portion in the normal outer arm containing three heavy chains.

Swimming Speed and Flagellar Beat Frequency

To assess the functional activity of *oda*-11 outer arm dynein, we compared the swimming velocity and flagellar beat frequency between this mutant, wild type and *oda*-1 missing the entire outer arm. As the histograms in Fig. 7 show, the swimming speed of *oda*-11 was lower than that of the wild type but clearly higher than that of *oda*-1; the average velocities ± SD were 194.3 ± 23.3 μm/s in wild type, 61.7 ± 8.3 μm/s in *oda*-1, and 118.7 ± 17.0 μm/s in *oda*-11. Flagellar beat frequency of *oda*-11 (48–53 Hz) was also intermediate between the wild-type (58–62 Hz) and *oda*-1 frequencies (24–30 Hz). These results suggest that the outer dynein arm missing the α heavy chain retains some functional activity.

Difference in Beat Frequency between the Two Flagella

The two flagella on a wild-type cell (*cis*- and *trans*-flagella, distinguished by their position with respect to the eyespot) mostly beat at the same frequency when the cell is swimming, but tend to beat at different frequencies when the cell is captured by a micropipette (Ruffer and Nultsch, 1987) or rendered uniflagellated by mechanical treatment (Kamiya and Hasegawa, 1987). It is always the *trans*-flagellum (the flagellum farthest from the eyespot) that beats at higher frequencies. In contrast to wild type, the two flagella in *oda*-1 do not tend to beat at different frequencies. It thus has been suggested that outer arm dynein is involved in the mecha-

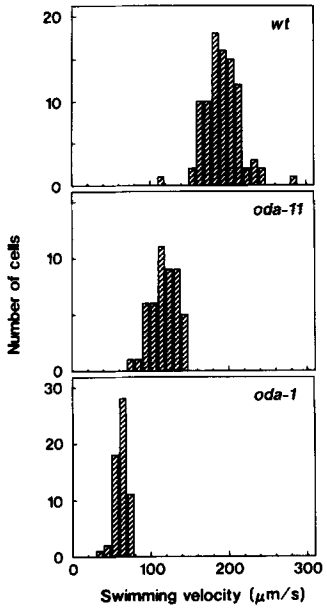

Figure 7. Swimming velocities of live cells of wild type (n = 92), *oda*-11 (n = 48), and *oda*-1 (n = 60). Temperature: 25°C.

nism that produces the frequency imbalance (Sakakibara and Kamiya, 1989).

To see how the absence of the α heavy chain affects the beat frequencies of the two flagella, we examined the beat frequencies of the *cis*- and *trans*-flagella of *oda*-11 separately, using cells that were rendered uni-flagellated by mechanical treatment. The histograms of beat frequencies in the *cis* and *trans*-flagella in such uniflagellated cells (Fig. 8) show that the two flagella in *oda*-11 cells, like those in *oda*-1 cells, beat at almost identical frequencies, whereas in wild type, the *trans*-flagellum beat at significantly higher frequencies than the *cis* as has been observed previously; the difference in mean frequency between the two flagella was only 3–4% in *oda*-1 and *oda*-11, whereas it was ~20% in wild type.

Behavior

The α chain is known to be phosphorylated in vivo (Piperno and Luck, 1981). Actually, it is the only outer arm heavy chain that undergoes phosphorylation. Since such phosphorylation on the dynein heavy chain might be involved in the regulation of flagellar activity, and since the two flagella of this mutant, unlike those of wild type, do not have the tendency to beat at different frequencies, we wondered whether *oda*-11 might have some defects in behavior such as phototaxis. However, in our qualitative observations on the mutant's behavior, *oda*-11 did not appear to have any significant behavioral defects. For example, when this mutant was suddenly illuminated with strong white light, it displayed a photophobic response, i.e., swam backwards for 0.2–1 s by changing the flagellar waveform from an asymmetric type to a symmetric one; this behavior is similar to what is observed with the wild-type cell (Hyams and Borisy, 1978). Also, when a population of *oda*-11 in a petri dish was illuminated from one side, the cells gathered around the brighter portion after 10–60 min; this is also as usually observed with the wild type. Thus, the effects of the absence of α heavy chain on the light-regulated behavior of *Chlamydomonas* is not clear.

Discussion

Mutant Missing the α Heavy Chain

In the present study we isolated a novel *Chlamydomonas* flagellar mutant, *oda*-11, missing the α heavy chain of outer arm dynein, and showed that its motility level is lower than that of the wild type but higher than that of a mutant missing the entire outer arm. No such mutants missing a specific subunit of outer arm dynein have been reported before.

Evidence for the absence of the α heavy chain in *oda*-11 axoneme came from SDS-PAGE of the axoneme and fractions from sucrose density gradient centrifugation of the axoneme extract. Moreover, the electron microscope observation that *oda*-11 lacks the outermost appendage of the outer arm indicates that a large part is lost in this mutant's outer arm. Therefore, if *oda*-11 axoneme should have a truncated form of α heavy chain, it must be much smaller than the native α chain. Since our SDS-PAGE analysis on 19S or 12S fractions showed no unusual protein bands over a 10–400-kD molecular mass range, it is likely that the mutant lacks practically the entire α chain. It is surprising that the outer

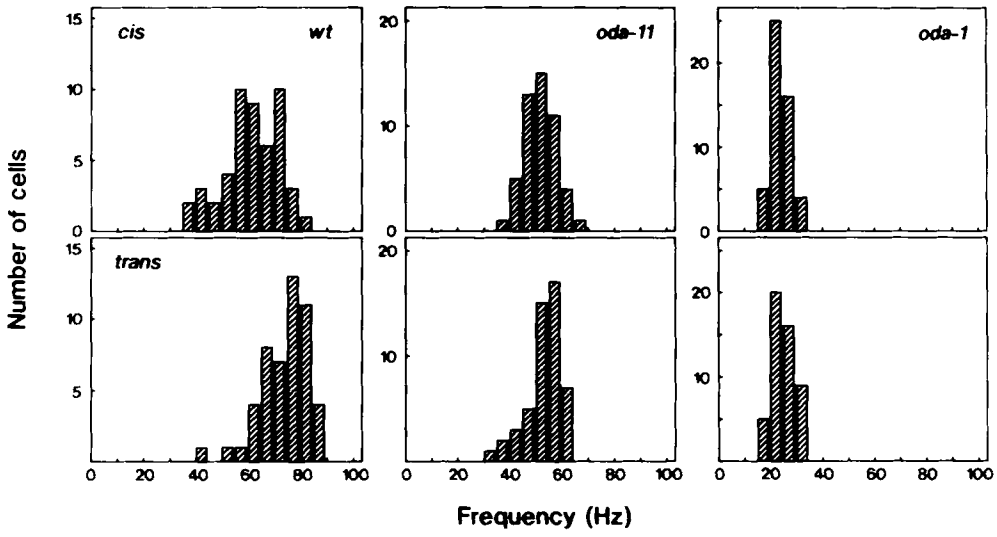

Figure 8. Beat frequencies of the *cis*- and *trans*-flagella in uni-flagellated cells of wild-type (*wt*) and outer-arm mutants. The mean frequencies ± SD in 50 measurements each are: 61.7 ± 10.7 Hz (wild-type *cis*) and 73.9 ± 9.2 Hz (wild-type *trans*); 51.9 ± 6.3 Hz (*oda*-11 *cis*) and 53.4 ± 6.4 Hz (*oda*-11 *trans*); and 23.8 ± 3.3 Hz (*oda*-1 *cis*) and 24.7 ± 4.3 Hz (*oda*-1 *trans*). Temperature: 25°C.

dynein arm can form without the α heavy chain, since a previous study has indicated that mutations in 12 independent loci, possibly including several loci for outer-arm subunits, resulted in total loss of the outer arm (Kamiya, 1988). The α heavy chain may be incorporated into the outer arm at a late stage of its assembly pathway.

The oda-11 Mutation

We have shown that *oda*-11 flagella lack both the α chain and a 16-kD light chain that is associated with the α chain in wild-type outer arm dynein. An assembly defect with this phenotype could potentially result from a defect in the α chain, the 16-kD light chain, another outer arm dynein protein that interacts with the α chain, or some nondynein protein, such as a protein kinase, that plays an essential role in outer arm assembly. Linkage analysis supports the first possibility.

RFLP analysis with an α gene probe shows that *oda*-11 is linked to the α chain locus. Although such linkage data cannot prove the identity of these two loci, it suggests that *oda*-11 is an α dynein structural gene mutation, especially since previously-characterized outer arm dynein assembly loci are widely scattered on the genetic map, with no observable tendency to cluster (Kamiya, 1988). Further molecular and biochemical analyses will be required to determine the exact nature of the *oda*-11 mutation.

Sedimentation Properties of Extracted Dynein

Chlamydomonas outer arm dynein has been shown to dissociate upon extraction with high salt into two particles: an 18S particle containing the α and β chains and a 12S particle containing the γ chain. In our centrifugation analysis, dynein extracted from wild-type axonemes had peaks at 21–22S and 12–15S. Since the sedimentation coefficient of the 18S particle has been reported to be ~21S (Pfister and Witman, 1984), this result seems reasonable except that 21–22S peak contains a small amount of γ chain as well as the α and β chains (Fig. 4). We suppose that the presence of the γ chain in the 21S peak is due to the overlap of the two peaks, although we cannot rule out the possibility that it is due to incomplete dissociation of the γ chain from the other two heavy chains.

The extract from *oda*-11 axoneme, on the other hand, had a peak of ATPase activity at ~19S, which contained both β and γ chains as well as two intermediate chains and light chains known to be associated with the β chain (Pfister and Witman, 1984; Mitchell and Rosenbaum, 1986), and a shoulder at 12–15S; in this case also, γ chain may be present in the 19S peak simply because its wide distribution overlapped with the distribution of the particle containing the β chain. Pfister and Witman (1984) have reported that the α and β heavy chains in the 18S particle can be dissociated by dialysis against a low-ionic strength buffer, and that the dissociated particle containing the β chain has a sedimentation coefficient of 14.5S. Since their 14.5S particle seems to contain most of the peptides present in our 19S peak, except for the γ heavy chain, the reason for the disagreement in the sedimentation coefficient between the two samples is unclear. It may be that the particle obtained by low-ionic strength dialysis of 18S particle and the one obtained from the high salt extract from the mutant axoneme differ in conformation. Alternatively, the distribution of the particle containing β chain in our sedimentation experiment may have shifted to heavier fractions through interaction with the γ chain. Clearly, however, the β and γ chains tended to distribute in different fractions in the sucrose density gradient centrifugation. Hence, a future improvement on the extraction/fractionation conditions will enable us to purify the β chain from *oda*-11 axonemes relatively easily. The *oda*-11 axoneme should be an excellent source of the β chain for the study of its in vitro properties.

Motility in the Mutant

We have shown that the motility level in *oda*-11 cells is intermediate between those of wild type and a mutant missing the entire outer arm. Hence the outer arm dynein without the α chain appears to retain some functional activity. In other words, the α chain is not absolutely necessary for outer arm function, but seems to have the function to amplify the activity of the outer arm composed of β and γ chains. This functional amplification may be brought about through a simple addition of activities of the three heavy chains, through some cooperative interactions between them, or through regulatory action of the α chain on β and γ functions.

In terms of effects on motility, the relationship of the α heavy chain to the total outer arm is analogous to that of the outer arm to the total axonemal dynein system; that is, the outer arm is not absolutely necessary for the generation of axonemal beating, but functions to increase the motility of axonemes containing only inner arms. Just as the entire outer arm is redundant for the axonemal beating mechanism, the α heavy chain is redundant for the function of the outer arm.

Because the redundant α chain and outer arm have survived evolution, these components must have endowed cells with a significant advantage. One possibly significant merit in having the α chain is that it allows the *cis*- and *trans*-flagella to beat at different frequencies. In a previous study (Sakakibara and Kamiya, 1989), we showed that the outer arm dynein extracted from wild-type axonemes can be combined with *oda*-1 axonemes in vitro, and that the outer arm–combined *oda*-1 axonemes beat at higher frequency in a reactivation medium. Unexpectedly, however, the *cis*- and *trans*-axonemes of *oda*-1 still beat at the same frequency even when the higher beat frequency was restored by addition of the outer arm. From these observations we suggested that the *cis*- and *trans*-flagella of wild-type axonemes probably have different outer arms, which may differ in the degree of modification such as phosphorylation. The *cis*- and *trans*-axonemes of *oda*-1 beat at the same frequency after the addition of outer arms because the two different types of outer arms probably combine with both flagella unselectively in vitro. The present finding that the *cis*- and *trans*-flagella in *oda*-11 beat at similar frequencies supports the hypothesis that phosphorylation is involved in the *cis–trans* frequency imbalance, since the α heavy chain is the only outer-arm heavy chain that undergoes phosphorylation.

Hence, a function of the α chain of the outer arm may be to make the two flagella to beat at different frequencies. However, it is not understood how significant this property is for the life of a *Chlamydomonas* cell. One possibility is that environmental factors control the phosphorylation of α chain, which in turn controls the balance between the activities of the two flagella and thereby regulates cellular behavior. Although this idea is fascinating, our qualitative ob-

servations have not revealed a significant defect in the light-dependent behavior of *oda*-11. The defect in behavioral control, if any, may be more subtle, or related to the cell's response to environmental factors other than light conditions. Hence, an understanding of the functional importance of the α chain needs further quantitative studies on the behavior of this mutant as well as wild-type *Chlamydomonas*.

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Note Added in Proof. We have recently found that *oda*-11 is linked with *ac*-6 (PD:NPD:T = 13:0:18). This and data from the cross with *oda*-5 (Table I) locate *oda*-11 to the far left side of linkage group VII.

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