New mutations in flagellar motors identified by whole genome sequencing in Chlamydomonas

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New mutations in flagellar motors identified by whole genome sequencing in Chlamydomonas

Huwen Lin, Nicholas P Nauman, Alison J Albee, Silas Hsu and Susan K Dutcher*

Abstract

Background: The building of a cilium or flagellum requires molecular motors and associated proteins that allow the relocation of proteins from the cell body to the distal end and the return of proteins to the cell body in a process termed intraflagellar transport (IFT). IFT trains are carried out by kinesin and back to the cell body by dynein.

Methods: We used whole genome sequencing to identify the causative mutations for two temperature-sensitive flagellar assembly mutants in Chlamydomonas and validated the changes using reversion analysis. We examined the effect of these mutations on the localization of IFT81, an IFT complex B protein, the cytoplasmic dynein heavy chain (DHC1b), and the dynein light intermediate chain (D1bLIC).

Results: The strains, fla18 and fla24, have mutations in kinesin-2 and cytoplasmic dynein, respectively. The fla18 mutation alters the same glutamic acid (E24G) mutated in the fla10-14 allele (E24K). The fla18 strain loses flagella at 32°C more rapidly than the E24K allele but less rapidly than the fla10-1 allele. The fla18 mutant loses its flagella by detachment rather than by shortening. The fla24 mutation falls in cytoplasmic dynein and changes a completely conserved amino acid (L3243P) in an alpha helix in the AAA5 domain. The fla24 mutant loses its flagella by shortening within 6 hours at 32°C. DHC1b protein is reduced by 18-fold and D1bLIC is reduced by 16-fold at 21°C compared to wild-type cells. We identified two pseudorevertants (L3243S and L3243R), which remain flagellated at 32°C. Although fla24 cells assemble full-length flagella at 21°C, IFT81 protein localization is dramatically altered. Instead of localizing at the basal body and along the flagella, IFT81 is concentrated at the proximal end of the flagella. The pseudorevertants show wild-type IFT81 localization at 21°C, but proximal end localization of IFT81 at 32°C.

Conclusions: The change in the AAA5 domain of the cytoplasmic dynein in fla24 may block the recycling of IFT trains after retrograde transport. It is clear that different alleles in the flagellar motors reveal different functions and roles. Multiple alleles will be important for understanding structure-function relationships.

Keywords: Kinesin-2, Cytoplasmic dynein, IFT81, Ciliary assembly, IFT recycling, Whole genome sequencing
simple picture is made more complex by examining the behavior of the BBSome in Caenorhabditis elegans [13]. The BBSome is a complex of seven proteins [14] that is postulated to be involved in the import of G protein coupled receptors in mammalian cells [15,16] and the export of cycling proteins in Chlamydomonas [17]. The BBSome may regulate anterograde IFT assembly and then rearrangement at the tip [13]. Cargo-specific adapter proteins may be important for IFT transport of cargo [18]. IFT is essential for mammalian development as mutants in various IFT proteins are lethal in mice (reviewed in Eggenschwiler and Anderson [19]). Mutations in several IFT proteins and cytoplasmic dynein cause asphyxiating thoracic dystrophy in humans [20-23].

A collection of temperature-sensitive mutants in Chlamydomonas that assemble flagella at the permissive temperature of 21°C, but lack flagella at the restrictive temperature of 32°C (Table 1) provides an important resource for the analysis of flagellar assembly [11,24,25]. Since many conditional mutants have reduced but sufficient function at the permissive temperature, this collection offers the opportunity to examine IFT in assembled flagella at the permissive temperature to ask about the effects of reduced function. For example, the temperature-sensitive allele in IFT172 suggests a role in remodeling IFT at the tip [26]. IFT is required to transport many of the flagellar proteins from the cytoplasm to the flagella. These include the inner dynein arm protein p28 that fails to be imported in the fla10-1 mutant [6]. Recent isobaric tags for relative and absolute quantitation (iTRAQ) experiments suggest that numerous proteins accumulate or are depleted in the presence of a mutant cytoplasmic dynein even when the length of the flagella has not changed [27], which shows the importance of retrograde movement for moving proteins back to the cell body.

The role of IFT differs between different axonemal proteins/cargos. Piperno et al [6] used temporary dikaryons, which are formed by the mating of two parental cells, to examine the kinetics and localization of proteins using antibodies to proteins in axonemal structures. The parental strains both carried the temperature-sensitive fla10-1 mutation in kinesin-2 [3] that stops IFT within 30 minutes after the shift to the restrictive temperature. One parent is otherwise wild-type, while the other parent has either an oda6 mutation that blocks assembly of the outer dynein arms [45] or an ida4 mutation that blocks assembly of a subset of inner dynein arms [46]. In ida4 x wild-type dikaryons at 21°C, IDA4 appear at the distal end of the mutant flagella by antibody staining and staining moved towards the proximal end with time after mating. In oda6 x wild-type dikaryons, ODA6 behave very differently. Staining appears along the entire length of the flagella 6 minutes after mating. The intensity increased with time. To test the role of IFT in the incorporation of dynein arm proteins, the parental cells were shifted to 32°C for 30 minutes to inactivate kinesin-2. The incorporation of IDA4 was blocked at the restrictive temperature, while ODA6 continued to be incorporated. Thus, the outer dynein arms appear to enter by diffusion or by a different motor complex [6], while the entry of the inner arm component requires kinesin-2. Transport of outer dynein arms also requires an adapter between the dynein arms and IFT. ODA16 functions as a cargo-specific adaptor between IFT particles and outer row dynein needed for efficient dynein transport into the flagellar compartment, as shown by its localization and interactions by immunoprecipitation and yeast two-hybrid experiments [18]. Recent results suggest that transport of tubulin into cilia is mediated by a weak affinity between tubulin and IFT81 and IFT74 [47].

Analysis of IFT using differential interference contrast (DIC) optics and kymographs showed that six of these conditional mutants have defects in the number of anterograde IFT particles or their velocity at 21°C, six have defects in either retrograde IFT particle number or velocity at 21°C, and four have no change in IFT particle number or velocity at 21°C (Table 1). As genes have been identified, it is clear that alleles in the same gene have slightly different properties; these differences must reflect the properties of the mutant alleles and not the function of the gene. For example, the fla1 and fla8 mutants both encode the other motor subunit of kinesin-2 [8] but show differences in the behavior of IFT particles [11]. These differences may reflect the degree of activity/concentration of the mutant proteins at the permissive temperature.

In Chlamydomonas, seven genes needed for intraflagellar transport have been identified by conditional alleles (Table 1). Nonconditional mutations in nine additional genes as well as in three of the genes with conditional alleles have been identified. RNA depletion of two IFT genes has been analyzed in Chlamydomonas and result in short flagella (Table 1), which may occur because there is only partial knockdown of the genes. Eighteen of the IFT and motor protein genes have mutants or depletion results. In this report, we employed whole genome sequencing to identify the FLA18 and FLA24 genes. These genes encode a new allele in the FLA10 kinesin-2 motor subunit and a new allele in the cytoplasmic dynein DHC1b, respectively.

**Methods**

**Strains and culture conditions**

Strains were obtained from the Chlamydomonas Resource Center (University of Minnesota, St Paul, MN, USA): fla18, CC-3864; fla24, CC-3866; 137M, CC-124; 137P, CC-125; S1C5, CC-1952; and S1D2, CC-2290. Each fla strain was backcrossed three times to either 137P or 137M strains to remove any unlinked modifiers.
Whole genome sequencing

*Chlamydomonas* genomic DNA preparation for whole genome sequencing was prepared as described previously [30]. Three micrograms of DNA were submitted to Genome Technology Access Core (St Louis, MO, USA) for library construction, Illumina sequencing (San Diego, CA, USA), and initial data analysis. For multiplex Illumina sequencing, 7-nucleotide indexes were added to
individual DNAs during the library construction before the samples were subjected to sequencing. The \textit{fla18} and \textit{fla24} samples were tagged with TGAGGTT and GCTTAGA, respectively, and shared the same sequencing lane with two other samples. All resulting sequencing data were de-multiplexed before being subjected to sequence alignment and SNP calling.

dCAPS markers and segregation analysis

Restriction enzymes that provide differences between mutant and wild-type alleles are listed in Additional file 1: Table S1. For \textit{fla24}, NEBCutter (New England BioLabs, Ipswich, MA, USA) was used to find the appropriate restriction enzyme. However, no restriction enzyme distinguishes between CC-125 and \textit{fla18}. A dCAPS marker was designed using dCAPS Finder 2.0 (Washington University, St Louis, MO, USA) [48]. A forward primer (fla18-dcapF) introduces a mismatch immediately upstream of the point mutation that creates an \textit{MboI} recognition site in the wild-type PCR product (GAAGA(N)_8) but not in the \textit{fla18} PCR product (GAGGA(N)_8). The 132 bp PCR product, when digested with \textit{MboI}, generates 102 bp and 30 bp fragments from wild-type but is uncut in \textit{fla18}.

Flagellar isolation

Flagella were isolated as described previously [49,50] with the addition of Protease Arrest (G-Biosciences, St Louis, MO, USA).

Flagellar counts

Cells were grown overnight in a 21°C lighted incubator to a density of approximately $2 \times 10^6$ cells/ml. Cells were then transferred to a 32°C lighted incubator and samples were taken every hour. Samples were prepared by spotting 19 μL of cells onto a microscope slide and adding 1 μL of 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) directly to the spotted cells. A total of 200 cells from each strain at each time point were scored using phase optics (40x) for the presence or absence of flagella. Flagellar length was monitored by immunofluorescence with monoclonal antibody to acetylated α-tubulin (Sigma-Aldrich, St Louis, MO, USA) at a dilution of 1:1,000.

Immunoblots and immunofluorescence

The antibodies were: ift81.3 (a gift from Dr Doug Cole) for immunofluorescence at 1:200; α-tubulin (DM1α from Sigma-Aldrich) used for immunoblots at 1:5,000, DHC1b and D1bLIC (a gift from Dr George Witman, University of Massachusetts, Worcester, MA, USA) were used for immunoblots at 1:2,000 and for immunofluorescence at 1:100; and ε-tubulin was used at 1:500 for immunofluorescence [51]. For immunoblots, flagellar proteins were isolated and resuspended in HEPES/St/DTT/sucrose buffer [52]. All the protein samples were stored at −80°C before use. Protein concentrations were ascertained by using Bio-Rad protein assay (Bio-Rad, Hercules, CA, USA) following the manufacturer’s instruction. The proteins were boiled for 5 minutes and centrifuged for 1 minute before loading onto the gel. The proteins were size-fractionated on SDS-PAGE minigels (1.0 mm thick, 6% acrylamide gel (for DHC1b) or 10% acrylamide gel (for D1bLIC) prepared from 30% acrylamide and bis-acrylamide solution, 29:1 (BioRad, Hercules, CA, USA)) and transferred to Immobilon-P PVDF membranes (EMD Millipore, Billerica, MA, USA) in 25 mM Tris, 192 mM glycine buffer containing 20% methanol at 62v for one hour. The incubation with primary antibody was incubated overnight at 4°C. The blots were washed three times for 10 minutes each in PBST. The secondary antibody was incubated at room temperature for 1 hour. Goat anti-mouse HRP (BioRad) and goat anti-rabbit HRP (Sigma-Aldrich) were used at 1:5,000 dilution. SuperSignal West Femto Chemiluminescent Substrate (Thermo Scientific, Waltham, MA, USA) was used according to the manufacturer’s instructions. The blots were imaged on a FluorChem H2 imager (Alpha Innotech, Santa Clara, CA, USA). Signal quantification analysis was performed by ImageJ (National Institutes of Health (NIH), Bethesda, MD, USA).

For immunofluorescence, Alexa 488 goat anti-rabbit (Invitrogen, Grand Island, NY, USA) and Alexa 594 goat anti-mouse were used at a 1:500 dilution with published protocols [51], except newborn goat serum and fish gelatin were not used. All individual immunofluorescence channels were maintained with the same exposure time throughout the time courses.

Results

To identify and understand the function of genes that can be mutated to a temperature-sensitive flagellar assembly phenotype, we are using whole genome sequencing. Mutants with an anterograde IFT defect (\textit{fla18}) and a retrograde IFT defect (\textit{fla24}) were chosen for analysis [11]. Each mutant was crossed to wild-type (CC-124 or CC-125) to verify that the phenotype segregated as a single mutation in meiotic progeny. In 56 and 130 tetrads, respectively, the aflagellate phenotype at 32°C segregated two wild-type and two mutant progeny, which suggests a single mutation or several tightly linked mutations. Each mutant was subjected to whole genome sequencing. The coverage ranged from 51-fold for \textit{fla24} to 94-fold for \textit{fla18} (Table 2).

\textbf{FLA18 encodes a kinesin-2 subunit}

The \textit{fla18} mutant strain was crossed to the highly polymorphic strain SIC5 and one \textit{fla18} meiotic progeny was subjected to whole genome sequencing [8]. About 71% of the 101 bp reads align to the reference genome (Table 2). A total of 43,103 SNPs/indels unique to the \textit{fla18} mutant
strain were found after subtracting the SNPs/indels found in 15 other *Chlamydomonas* strains [53]. Among this set of SNPs/indels, 892 changes map to exons or intron/exon boundaries when synonymous changes are excluded (Table 2).

In our study of transcript levels following pH shock at various time points during flagellar assembly [54], we found that all of the IFT genes are upregulated at least 2.5-fold within 1 hour of flagellar amputation. Given the *fla18* mutant strain has a defect in anterograde IFT [11], we hypothesized that the *FLA10* gene is likely to be among the 1,850 upregulated genes. A comparison between the list of genes that have SNPs/indels in *fla18* and the list of upregulated genes identifies 85 SNPs/indels in 59 genes. One of the changes is a glutamic acid (GAG) to glycine (GGG) in the *FLA10* kinesin (*E24G*, Table 3). In the *fla10-14* mutant strain, the same glutamic acid is replaced by lysine (*E24K*) [8]. We then verified that all of the temperature-sensitive phenotype was linked to *FLA10* in 20 progeny from a cross of *fla18* x S1C5 (Table 3). The *FLA10* gene is located at 4.43 Mb on chromosome 17. The *fla18* maps 5 map units (mu) from a marker at 4.0 Mb, and markers at 2.23 Mb and 6.8 Mb show weaker linkage (20 and 16 mu, respectively, Table 3). These values conform to other crosses where approximately 100 kb corresponds to 1 mu and indicate that *fla18* is linked to the *FLA10* gene.

To confirm that this mutation is the causative change in *fla18*, we isolated revertants of *fla18* that regained the ability to swim at 32°C. Following UV mutagenesis, 46 independent swimming strains were isolated. A dCAPS marker that distinguishes the polymorphism in wild-type and *fla18* was used to analyze 8 of the 46 revertants/suppressors. The restriction enzyme *MboII* cuts the wild-type PCR product, but not the *fla18* PCR product. A representative gel of the PCR and digest is shown in Figure 1. Digestion by *MboII* indicates that these eight strains are likely to be true revertants. The restoration of the original codon was confirmed in these eight revertants by Sanger sequencing (Table 4). Thus, our revertant analysis indicates that mutation in the *FLA10* kinesin is the causative mutation in *fla18*. To examine the temperature-sensitive phenotype of the *fla18* mutant strain, it was shifted from the permissive temperature of 21°C to the restrictive temperature of 32°C, and the percentage of flagellated cells was determined. While wild-type cells remain approximately 80% flagellated, the *fla18* cells lose their flagella gradually and at 7 hours most cells are aflagellate (Figure 2A). The length of the flagella was measured and the length changed by only 2 μm (Figure 2B). The cells may be losing their flagella by detachment rather than by shortening.

**FLA24 encodes the cytoplasmic dynein heavy chain**

The *fla24* allele was mapped to chromosome 6 near the mating-type locus [31] and linkage to the mating-type locus was confirmed in an additional 243 tetrads (239:0:4; PD:NP:D:T). There is only one change in *fla24* in the mapped interval after subtracting changes found in other unrelated strains [53]. The candidate in the interval is DHC1b, the cytoplasmic dynein gene for retrograde IFT (Table 2). The T to C mutation predicts a L3242P change. We used a PCR-based assay to examine linkage of the flagellar phenotype with the alteration in the cytoplasmic dynein gene. The PCR product generated is 303 bp long in both the *fla24* and wild-type strains. The mutant product is cut by *NciI* but not cut by *AlwN1*, while the wild-type product is cut by *AlwN1* but not by *NciI*. This change cosegregates with the flagellar assembly defect in 59 meiotic progeny. To ask if this change is responsible for the phenotype, we again used reversion/suppressor analysis. Following mutagenesis, 64 independent swimming strains were recovered. Nine of the strains are no longer cut by *NciI*. Of these, *AlwN1* fails to cut three (Figure 3, Table 5).

**Table 2 Changes in *fla18* and *fla24* strains by whole genome sequencing**

| Mutant strain | Sequencing reads (101 bp, paired-ended) | Aligned reads % (coverage) | Number of changes across the genome | Chromosome | Position | Change |
|---------------|----------------------------------------|---------------------------|------------------------------------|------------|----------|--------|
| *fla18*       | 157/313244                             | 70.7% (94x)               | 892                                | 17         | 4323831  | gAg/gGg E to G |
| *fla24*       | 63540680                              | 95.3% (51x)               | 62                                 | 6          | 165063   | cTg/cCg L to P  |

**Table 3 Primers for mapping of *fla18* in crosses with CC-1952 (S1C5)**

| Primer name | Sequence F (5’ to 3’) | Sequence R (5’ to 3’) | Product size in *fla18* and restriction enzyme | *Fla18:S1C5* parental to recombinant progeny |
|-------------|-----------------------|-----------------------|-----------------------------------------------|---------------------------------------------|
| Ch17-0.5    | GCA CAG CGG CCC AAA AAGG AAG G  | CGT TTC TCG AAC TCA GCC ACT GT      | HindIII 180 bp                                | 119 Unlinked                                |
| LCS-2.32    | GGA CGG TGT GTA TGC ATT AG    | GCT GTC ACT ACG TGG TCT CG         | MspI 203 bp                                  | 155 (20 mu)                                 |
| Ch17-4.03   | ATA TTA CGG TTC TCC GAC AAG AAC | CAG CTT CTG TGT GCG CTT GTA CTT   | −271 bp                                      | 191 (5 mu)                                  |
| Ch17-6.28   | CAT CGA GCT GCT TGG AGG CGG CAT A | CGC TAT ACA CCA CAT ACG GTC GAG | −147 bp                                      | 164 (16 mu)                                 |

Markers without a restriction enzyme generate size polymorphisms due to CA repeats. All reactions were performed with an annealing temperature of 53°C.
Sanger sequencing verified that we recovered six true revertants that changed the proline at amino acid 3243 back to leucine, and three pseudorevertants that changed the proline to either serine (2) or to arginine (1).

Currently, there are five other mutant alleles identified in the cytoplasmic dynein gene in *Chlamydomonas*. Three show nonconditional phenotypes; they assemble very short flagella [9,10], and two temperature-sensitive alleles have nonconditional phenotypes; they assemble very short flagella (Figure 5A, 32°C, 2 to 5 hours). The level of D1bLIC remain high in wild-type cells at 21°C and for at least 5 hours after cells are shifted to 32°C (Figure 5A). The signal shows further reduction as cells lose their flagella (Figures 2 and 4). The DHC1b signal remains detectable in the basal body area, but decreases with time at the restrictive temperature. By immunoblots, we showed that DHC1b is reduced by 18-fold in *fla24* flagella compared to wild-type flagella (Figure 4C). In contrast, the three revertants (D11, D12, and D42b) show similar signal intensities and locations to wild-type cells at both the permissive and restrictive temperatures (Figure 4B and Additional file 2: Figure S1).

The localization of D1bLIC in wild-type cells appears similar to that of DHC1b. The signal intensities of D1bLIC remain high in wild-type cells at 21°C and for at least 5 hours after cells are shifted to 32°C (Figure 5A). However, in *fla24* cells, even though D1bLIC shows similar localization, the signal intensity is greatly reduced when compared to wild-type cells at 21°C (Figure 5A). The signal shows further reduction as cells lose their flaggella (Figure 5A, 32°C, 2 to 5 hours). The level of D1bLIC

### Table 4 Reversion of *fla18* allele provides evidence of causality

| Strain name | Mboll allele | Sequence for digestion (underline)* | Amino acid | Sanger sequence |
|-------------|--------------|-------------------------------------|------------|----------------|
| Wild-type   | Cut          | GAAGAGGCAGAT                        | Glutamic acid | GAG             |
| *fla18*     | Not cut      | GAAGAGGCAGAT                        | Glycine    | GGG             |
| R1          | Cut          | GAAGAGGCAGAT                        | G to E     | GGS to GAG     |
| R2          | Cut          | GAAGAGGCAGAT                        | G to E     | GGS to GAG     |
| R3          | Cut          | GAAGAGGCAGAT                        | G to E     | GGS to GAG     |
| R5          | Cut          | GAAGAGGCAGAT                        | G to E     | GGS to GAG     |
| R6          | Cut          | GAAGAGGCAGAT                        | G to E     | GGS to GAG     |
| R7          | Cut          | GAAGAGGCAGAT                        | G to E     | GGS to GAG     |
| R8          | Cut          | GAAGAGGCAGAT                        | G to E     | GGS to GAG     |
| R9          | Cut          | GAAGAGGCAGAT                        | G to E     | GGS to GAG     |

*Underlined nucleotides in the third column indicate the recognition site. This site is created by the primers in the wild-type (FLA18) sequence but not in the mutant (fla18) sequence.*
is reduced, as shown by immunoblots of flagellar proteins at 21°C by 16-fold (Figure 4C). An immunoblot of D1bLIC using whole cell extract from wild-type and fla24 cells at 21°C indicates that similar to flagellar D1bLIC, the amount of D1bLIC in whole cell extract is reduced approximately 16-fold in the mutant (Figure 5C). We also observed approximately 3-fold reduction when wild-type cells were switched from 21°C to 32°C for 5 hours (Figure 5C). Consistent with the observation by immunofluorescence (Figure 5A), we were unable to detect the D1bLIC signal by immunoblot after fla24 cells were shifted to 32°C for 5 hours (Figure 5C). Similar to observation of DHC1b, the signal intensities of D1bLIC remain high in all three revertants (Figure 5B and Additional file 3: Figure S2).

Localization of IFT81 is perturbed at permissive temperature in fla24 but not in fla18
At the permissive temperature of 21°C, Lomini et al. found that fla18 IFT trains show a reduced anterograde velocity, and the fla24 IFT particles show a reduced retrograde velocity [11]. These mutant strains have
Table 5 Reversion of \textit{fla24} allele provides evidence of causality

| Strain name | AlwNl   | Nci    | Sequence for digestion (underline)* | Amino acid | Sequence |
|-------------|---------|--------|-------------------------------------|------------|----------|
| \textit{fla24} | Not cut | Cut    | CAGCTGCGGGG                           | Proline    | CCG      |
| Wild-type   | Cut     | Not cut | CAGCTGCTGGGG                          | Leucine    | CCG      |
| D7          | Cut     | Not Cut | CAGCTGCTGGGG                          | P to L     | CCG to CTG |
| D9b         | Cut     | Not Cut | CAGCTGCTGGGG                          | P to L     | CCG to CTG |
| D11         | Cut     | Not Cut | CAGCTGCTGGGG                          | P to L     | CCG to CTG |
| D12         | Not cut | Not cut | CAGCTGCGGGG                           | P to S     | CCG to CGG |
| D25b        | Cut     | Not Cut | CAGCTGCTGGGG                          | P to L     | CCG to CTG |
| D37         | Cut     | Not Cut | CAGCTGCTGGGG                          | P to L     | CCG to CTG |
| D42b        | Not cut | Not Cut | CAGCTGCGGGG                           | P to R     | CCG to CGG |
| D43         | Not cut | Not Cut | CAGCTGCTGGGG                          | P to S     | CCG to CTG |
| D57         | Cut     | Not Cut | CAGCTGCTGGGG                          | P to L     | CCG to CTG |

*The underlined nucleotides in the fourth column indicate the recognition site for Nci or AlwNl.

Figure 4 Localization of DHC1b in wild-type, \textit{fla24}, and \textit{fla24} revertants at 21°C and 32°C. DHC1b staining is shown in green (first column) and the flagella are labeled with acetylated α-tubulin (red, second column). Merged images of both staining are shown in the third column. Overexposed DHC1b signals are shown in the forth column to show the localization of DHC1b in the flagella. Cells were obtained from 21°C and various time points at 32°C, as indicated. (A) Wild-type and \textit{fla24} cells. (B) \textit{fla24} revertants. (C) Twenty micrograms of flagellar proteins were isolated from wild-type (FLA24) and \textit{fla24} cells at 21°C, and probed with DHC1b and D1bLIC antibodies. The membrane that used to probe D1bLIC was stripped and reprobed with α-tubulin to normalize the loading.
defects in the anterograde and retrograde motors, respectively. Therefore, we asked the whether the localization of IFT81, a complex B protein, is affected in these strains.

In the \textit{fla18} mutant strain at the permissive temperature, no change of localization or reduction of intensity of IFT81 was observed when compared to wild-type cells (Figure 6). After cells were shifted to the restrictive temperature, the IFT81 signals remained in the basal body region and in the flagella until the cells became aflagellate at 6 hours. IFT81 appears unperturbed by the \textit{fla18} mutation.

In the \textit{fla24} mutant strain at the permissive temperature, the majority of the IFT81 protein is not localized at the basal body as observed in wild-type cells (Figure 6). After cells were shifted to the restrictive temperature, the IFT81 signals remained in the basal body region and in the flagella until the cells became aflagellate at 6 hours. IFT81 appears unperturbed by the \textit{fla24} mutation.

Discussion
Conditional mutants have been extremely useful in the study of essential genes in many cellular processes from ribosome assembly to cell division to secretion to synaptic transmission.
vesicles. A collection of temperature-sensitive flagellar assembly mutants in *Chlamydomonas* has allowed the analysis of intraflagellar transport; they have documented that anterograde movement requires kinesin and IFT complex B, and that retrograde movement requires cytoplasmic dynein and IFT complex A. In addition, remodeling of IFT trains at the tip requires IFT172, a protein in complex B, and a temperature-sensitive mutation in this protein leads to a retrograde defect (Table 1). Of the 21 extant conditional mutants, previous work together with this work has identified the causative lesion in 12 of them. All of these genes encode either IFT components or motor proteins (Table 1). Six have mutations in one of the three kinesin motor genes and four other mutations are in the cytoplasmic dynein motor. This bias suggests that conditionality may be more easily achieved in the motor subunits than in the IFT components.

Whole genome sequencing in *Chlamydomonas* has been fruitful when the gene is mapped to a region or chromosome [30]. We have developed a collection of changes in other wild-type and mutant strains that can be used to eliminate non-causative candidates [53]. In addition, the transcriptional profiles during regeneration of flagella are useful for identifying candidate flagellar assembly genes [54]. For *fla18*, we narrowed the list of candidates from 892 to 85 (Table 2) by combining the data from whole genome sequencing and transcriptional profiles. If we had used this strategy for *fla24*, only three of the 62 genome-wide candidates showed increased levels of transcript during regeneration. For *fla9* (unpublished data at *Cilia*), this strategy would have narrowed the 78 genome-wide candidates to eight. The use of both data sets may help to obviate the need for genome-wide fine mapping of flagellar assembly mutants.

The mutation in *fla18* affects the same amino acid that is mutated in the *fla10-14* strain. In *fla10-14*, the glutamic acid is changed to lysine but in *fla18* (now renamed *fla10-16*) it becomes a glycine. The two alleles have different kinetics of flagellar loss [8]; the E24K allele takes over 12 hours to see loss of 50% of the flagella compared to the E24G allele that takes only 6 hours to see complete loss (Figure 2A). This glutamic acid is conserved in all kinesin-2 molecules across the ciliated phylogenetic tree (n = 75, data not shown). As speculated previously [8], it seems likely that this amino acid may interact with the P-loop and be important for motor activity. Interestingly, the *fla2* mutant shows a ‘fragile’ phenotype [24]. Upon shifting cells to the restrictive temperature, the flagella detach rather than shorten. We observe similar detachment with the *fla18* allele. Since *fla18* greatly reduced anterograde IFT velocity, it is interesting to speculate that either a component is transported that maintains the integrity of the flagellar axoneme or a signal to maintain the integrity fails. This phenotype is allele-specific, which supports the idea that different alleles may provide different information about the functions of anterograde IFT.

The *fla1* mutation (now *fla8-2*) and the *fla8-1* mutation are in the second motor subunit of kinesin-2 [8]; they were thought to affect different phases of IFT [11]. The *fla10-1* and *fla8-1* alleles show similar phenotypes with normal anterograde velocity but a reduced ratio of anterograde to retrograde particles, while *fla8-2* and *fla18* show similar phenotypes with reduced anterograde...
velocity and a reduced ratio of anterograde to retrograde particles. Different mutations have different phenotypic effects on IFT.

*fla24* is a mutation in the cytoplasmic dynein. The mutant *fla24* allele has several helpful properties that will allow dissection of a successful IFT cycle. The IFT trains must be assembled at the basal bodies, turn around at the tip to change from anterograde to retrograde movement, and then be reloaded at the base for anterograde transport. In our study of *fla15* (IFT144) and *fla17* (IFT139) IFT complex A mutants, we observed that diploid cells heterozygous for *fla24* and either *fla15* or *fla17* were aflagellate at 32°C but flagellated at 21°C [31]. It is not unexpected that defects in complex A might show an enhancement of a cytoplasmic dynein mutant phenotype.

Since *fla24* is compromised by reduced mutant Complex B proteins, we considered that the IFT dynein function may also be sensitized to dynein inhibitors since the retrograde velocity is reduced to 0.9 μm/second from 3.1 μm/second for wild-type cells and the frequency of retrograde particles is reduced [11]. Ciliobrevin D is a small molecule that inhibits cytoplasmic dynein [55]. We asked if *fla24* cells were more sensitive to ciliobrevin D than wild-type cells. Surprisingly, with the addition of 100 μM ciliobrevin D, *fla24* cells showed no effect on flagellar length after 30 minutes (data not shown), although this concentration has been shown to reduce the retrograde particle frequency after 5 minutes [56]. Further experiments to examine IFT particle rates with the mutant and inhibitor will shed more light on synthetic interactions.

Upon the shift of *fla24* cells to 32°C, the flagella are lost within 4 hours (Figure 2C); this is quite rapid compared to the other DHC1B alleles. The amount of DHC1b in flagella is greatly reduced as observed by immunofluorescence and immunoblot (Figure 4). The *fla24* cells have three interesting phenotypes at 21°C that may suggest roles for the AAA5 domain of cytoplasmic dynein. First, the retrograde velocity and number of particles are reduced [11]. Second, the level of light intermediate chain (D1bLIC) is reduced as indicated by immunofluorescence and immunoblots (Figures 4C and 5). Third, the IFT81 protein distribution is dramatically altered; instead
of localizing to the basal body and along the flagella, IFT81 has left the basal body region and is concentrated in the proximal approximate 1 μm of the flagella (Figure 7A). Unlike the dhc1b-3 allele that shows a reversal in the direction of phototaxis [27], we never observed a change in the phototaxis phenotype of the fla24 mutant over a 6-hour period (data not shown). Again, it is clear that different alleles have different phenotypic properties.

The cytoplasmic dynein molecule is composed of a central ATP-hydrolyzing ring that has six AAA modules arranged around the ring’s central pore. The fla24 mutation falls into an alpha-helix in the AAA5 domain that is extended into the alpha helical strut/buttress [57,58] (Figure 8). The strut/buttress is postulated to have a high degree of plasticity that may be important for its function in communicating between the microtubule-binding domain (MTBD) of the stalk and the AAA ring. The distal region of the strut interacts with the middle of the stalk and a deletion of the distal end of the strut removes this interaction. Allosteric communication between AAA1 and MTBD is postulated to be relayed through the C-sequence, the strut, and the stalk [57].

Two in-frame deletions of 6 and 7 amino acids in the cytoplasmic dynein of Neurospora crassa affect the strut [60]. The 3739 Δ6 amino acid deletion removes part of the first coil of the strut and is postulated to affect communication between the MTBD and the nucleotide status of the AAA1 domain. The dynein localizes distally in this mutant. The 3756 Δ7 amino acid deletion is in the first coil of the strut as well but causes aggregation of the dynein. It is postulated that this mutation may lock the structure of the dynein. The cytoplasmic dynein mutants in Neurospora also demonstrate that different alleles have very different phenotypic effects [52].

The reduced retrograde velocity of IFT trains [31], the reduction in D1bLIC at the basal bodies, and the accumulation of IFT81 in the fla24 mutant suggest that the mutant has a defect in moving along the microtubules and in remodeling the IFT trains at the proximal end of the flagella via cargo binding. In our screen for suppressor and revertants of fla24, we have identified at least three genes that suppress the restrictive temperature flagellar assembly defect. These strains may provide information about interactions with the dynein heavy chain that will speak to how the strut and other structures communicate flagellar assembly defect (data not shown).

**Conclusions**

Whole genome sequencing provides a fast and inexpensive means to identify chemically induced mutations in Chlamydomonas [30,53]. Identification of the remaining seven temperature-sensitive mutations will be greatly helped by this technique. The identification of multiple mutant alleles in kinesin and in cytoplasmic dynein that have different phenotypes will greatly help studies of the function of these proteins. The fla18 allele is unique among the motor mutants in that it appears to deflagellate after the temperature shift instead of shortening. The fla24 allele results in an abnormal localization of IFT81 near the basal bodies at the permissive temperature, which may suggest a defect in recycling IFT trains.

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![Figure 8](http://www.ciliajournal.com/content/2/1/14)

**Figure 8** The fla24 mutation falls in a conserved alpha-helix that leads to the strut/buttress structure. (A) Diagram of domain in the cytoplasmic dynein based on the structure of Dictyostelium cytoplasmic dynein [50]. The stalk is extended from AAA4 and contains the microtubule-binding domain (MTBD) (in darker yellow). The strut or buttress is extended from AAA5. (B) Alignment of Dictyostelium cytoplasmic dynein and Chlamydomonas cytoplasmic dynein 1b in the AAA5 domain into the strut. A leucine (L3243) is mutated in the fla24 allele. The alpha helices above the alignment show the extent of the helices in the Dictyostelium structure. (C) Logo of the region in panel B from 52 organisms [59] showing conservation based on height. L3243 at position 10 in the logo is conserved in all 52 organisms. (D) Diagram (redrawn from Pazour et al. [50]) showing the back side of the ring that may allow information to flow between the AAA1 domain and the MTBD through the C-terminus, strut, and stalk. L3243 (indicated by a black triangle) may be critical for this communication. MTBD, microtubule-binding domain.
Additional files

Additional file 1: Table S1. Primers for reversion analysis of fla18 and fla24.

Additional file 2: Figure S1. Localization of DHC1b in fla24 revertants at 32°C. Staining of DHC1b (green), acetylated α-tubulin (red), merged images, and overexpressed DHC1b signals are shown. Cells were obtained from various time points at 32°C, as indicated.

Additional file 3: Figure S2. Localization of D1B1C in fla24 revertants at 32°C. Staining of D1B1C (green), acetylated α-tubulin (red), merged images, and overexpressed D1B1C signals are shown. Cells were obtained from various time points at 32°C, as indicated.

Additional file 4: Figure S3. Localization of IFT81 in fla24 revertants at 32°C. Staining of α-tubulin (green), IFT81 (red), merged images, and overexpressed IFT81 signals are shown. Cells were obtained from various time points at 32°C, as indicated.

Abbreviations
dCAPS: Degenerate cleaved amplified polymorphic sequence; DIC: Differential interference contrast; DTT: Dithiothreitol; HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HRP: Horseradish peroxidase; IFT: Intraflagellar transport; ITRAQ: Isobaric tags for relative and absolute quantitation; KAP: Kinesin-associated protein; mu: Map unit; MTBD: Microtubule-binding domain; NIH: National institutes of health; PBST: Phosphate-buffered saline-tween; PCR: Polymerase chain reaction; PVDF: Polyvinylidene fluoride; RNAi: RNA interference; SNP: Single nucleotide polymorphism; UV: Ultraviolet.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
HL performed the analysis of the whole sequence, analysis of dynein subunit localization, and immunoblots. NPN measured flagellar length and number. AJA prepared and examined the samples for immunofluorescence, and examined the effect of ciliate brev. D. SH mapped meiotic progeny from crosses of fla24 x FLA24. SKD conceived of the study, performed the reversion analysis and PCR, and wrote the manuscript. All authors read and approved the final manuscript.

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References
1. Kozminski KG, Johnson KA, Forscher P, Rosenbaum JL (1993) A motility in the eukaryotic flagellum unrelated to flagellar beating. Proc Natl Acad Sci U S A 90:5519–5523.
2. Cole DG, Chinn SW, Wedaman KP, Hall K, Vuong T, Scholey JM (1993) Novel heterotrimeric kinesin-related protein purified from sea urchin eggs. Nature 366:268–270.
3. Walther Z, Vashishtha M, Hall JL (1994) The Chlamydomonas FLA10 gene encodes a novel kinesin-homologous protein. J Cell Biol 126:175–188.
4. Kozminski KG, Beech PL, Rosenbaum JL (1995) The Chlamydomonas kinesin-like protein FLA10 is involved in motility associated with the flagellar membrane. J Cell Biol 131:1517–1527.
5. Cole DG, Diener DR, Himelblau AL, Beech PL, Fuster JC, Rosenbaum JL (1998) Chlamydomonas kinesin-II-dependent intraflagellar transport (IFT): IFT particles contain proteins required for ciliary assembly in Caenorhabditis elegans sensory neurons. J Cell Biol 141:993–1008.
6. Piperno G, Mead K (1997) Transport of a novel complex in the cytoplasmic matrix of Chlamydomonas flagella. Proc Natl Acad Sci U S A 94:4457–4462.
7. Mueller J, Perrone CA, Bower R, Cole DG, Porter ME (2005) The FLA3 KAP subunit is required for localization of kinesin-2 to the site of flagellar assembly and processive anterograde intraflagellar transport. Mol Biol Cell 16:1341–1354.
8. Miller MS, Espanjar JM, Lippa AM, Lux FG 3rd, Cole DG, Dutcher SK (2005) Mutant kinesin-2 motor subunits increase chromosome loss. Mol Biol Cell 16:3810–3820.
9. Porter ME, Bower R, Knott JA, Byrd P, Dentler W (1999) Cytoplasmic dynein heavy chain 1b is required for flagellar assembly in Chlamydomonas. Mol Biol Cell 10:693–712.
10. Pazour GJ, Dickert BL, Witman GB (1999) The DHC1b (DHC2) isoform of cytoplasmic dynein is required for flagellar assembly. J Cell Biol 144:473–481.
11. Iomini C, Babaev-Khaimov V, Sassaroli M, Piperno G (2001) Protein particles in Chlamydomonas flagella undergo a transport cycle consisting of four phases. J Cell Biol 153:13–24.
12. Pigino G, Geimer S, Lanzavecchia S, Paccagnini E, Cantele F, Diener DR, Rosenbaum JL, Lupetti P (2009) Electron-tomographic analysis of intraflagellar transport particle trains in situ. J Cell Biol 187:1335–1348.
13. Wei Q, Zhang Y, Li Y, Zhang Q, Ling K, Hu J (2012) The BBSome controls IFT assembly and turnaround in cilia. Nat Cell Biol 14:950–957.
14. Nachury MV, Lokev AV, Zhang Q, Westlake CJ, Peranen J, Merdes A, Slusarski DC, Scheller RH, Bazan JF, Sheffield VC, Jackson PK (2007) A core complex of BBS proteins cooperates with the GTPase Rab7 to promote cilium membrane biogenesis. Cell 129:1201–1213.
15. Berbari NF, Johnson AD, Lewis JS, Askwith CC, Mykytyn K (2008) Identification of ciliary localization sequences within the third intracellular loop of G protein-coupled receptors. Mol Biol Cell 19:1540–1546.
16. Berbari NF, Lewis JS, Bishop GA, Askwith CC, Mykytyn K (2008) Bardet-Biedl syndrome proteins are required for the localization of G protein-coupled receptors to primary cilia. Proc Natl Acad Sci U S A 105:4242–4246.
17. Lechtreck KF, Brown JM, Sampaio JL, Craft JM, Chevchenko A, Evans JE, Witman GB (2013) Cycling of the signaling protein phospholipase D through cilia requires the BBSome only for the export phase. J Cell Biol 201:249–261.
18. Ahmed NT, Gao C, Lucker BF, Cole DG, Mitchell DR (2008) ODA16 aids axonal outer row dynein assembly through an interaction with the intraflagellar transport machinery. J Cell Biol 183:313–322.
19. Eggenschwiler JT, Anderson KV (2007) Cilia and developmental signaling. Annu Rev Cell Dev Biol 23:345–373.
20. Schmidts M, Arts HH, Bongers EM, Yap Z, Oud MM, Antony D, Duijkers L, Roepman R, Arts HH (2011) Ciliopathies with skeletal anomalies and renal involvement. J Med Genet 50:309–323.
21. Eggenschwiler JT, Anderson KV (2007) Cilia and developmental signaling. Annu Rev Cell Dev Biol 23:345–373.
22. Beales PL, Bland E, Tobin JL, Bacchelli C, Tuysuz B, Hill J, Rix S, Pearson CG, Ka M, Hartley J, Johnson C, Irving M, Elcioglu N, Winney M, Tada M, Scambler PJ (2013) Exome sequencing identifies DYNC2H1 mutations as a common cause of axonotrophic spinal dystrophy (Jeune syndrome) without major polydactyly, renal or retinal involvement. J Med Genet 50:309–323.
23. Beales PL, Bland E, Tobin JL, Bacchelli C, Tuysuz B, Hill J, Rix S, Pearson CG, Ka M, Hartley J, Johnson C, Irving M, Elcioglu N, Winney M, Tada M, Scambler PJ (2013) Exome sequencing identifies DYNC2H1 mutations as a common cause of axonotrophic spinal dystrophy (Jeune syndrome) without major polydactyly, renal or retinal involvement. J Med Genet 50:309–323.
24. Beales PL, Bland E, Tobin JL, Bacchelli C, Tuysuz B, Hill J, Rix S, Pearson CG, Ka M, Hartley J, Johnson C, Irving M, Elcioglu N, Winney M, Tada M, Scambler PJ (2013) Exome sequencing identifies DYNC2H1 mutations as a common cause of axonotrophic spinal dystrophy (Jeune syndrome) without major polydactyly, renal or retinal involvement. J Med Genet 50:309–323.
35. Hou Y, Qin H, Follit JA, Pazour GJ, Rosenbaum JL, Witman GB (2007) A dynein light chain is essential for flagellar assembly and function in Chlamydomonas reinhardtii. J Cell Biol 7267–85

34. Pazour GJ, Wilkerson CG, Witman GB (1998) A dynein light chain is essential for mitosis and cell growth in Chlamydomonas. Cell Motil Cytoskeleton 43:215–22

33. Matsuura K, Lefebvre PA, Kamiya R, Hirono M (2002) Kinesin-II is not essential for retrograde particle movement of intraflagellar transport (IFT). J Cell Biol 15262–266

32. Witman GB (2012) Dynein and Intraflagellar Transport. In: King SM (ed) Interactions in Chlamydomonas. Genetics 183:885–896

36. Lucker BF, Miller MS, Dziedzic SA, Blackmarr PT, Cole DG (2010) Direct interactions of intraflagellar transport complex B proteins IFT88, IFT52, and IFT81 subunits. J Biol Chem 285:21508–21518

38. Deane JA, Cole DG, Seeley ES, Diener DR, Rosenbaum JL (2001) Localization of intraflagellar transport protein IFT52 identifies basal body transitional fibers as the docking site for IFT particles. Curr Biol 11:1591–1594

37. Brazelton WI, Amundsen CD, Sifflow CD, Lefebvre PA (2001) The bdi1 mutation identifies the Chlamydomonas osm-6 homolog as a gene required for flagellar assembly. Curr Biol 11:1591–1594

39. Lucker BF, Miller MS, Dziedzic SA, Blackmarr PT, Cole DG (2010) Direct interactions of intraflagellar transport complex B proteins IFT88, IFT52, and IFT46. J Biol Chem 285:21508–21518

40. Pazour GJ, Baker SA, Deane JA, Cole DG, Dickert BL, Rosenbaum JL, Witman GB, Besharse JC (2002) The intraflagellar transport protein, IFT88, is essential for mitosis and cell growth in Chlamydomonas. Cell Motil Cytoskeleton 51:195–201

41. Behal RH, Miller MS, Qin H, Lucker BF, Jones A, Cole DG (2012) Subunit interactions and organization of the Chlamydomonas reinhardtii intraflagellar transport complex A proteins. J Biol Chem 287:11591–11594

42. Hou Y, Pazour GJ, Witman GB (2004) A dynein light intermediate chain, D1bLIC, is required for retrograde intraflagellar transport. Mol Biol Cell 15:4382–4394

43. Qin H, Wang Z, Diener D, Rosenbaum J (2007) Intraflagellar transport protein 27 is a small G protein involved in cell-cycle control. Curr Biol 17:193–202

44. Fan ZC, Behal RH, Geimer S, Wang Z, Williamson SM, Zhang H, Cole DG, Qin H (2010) Chlamydomonas IFT70/CyF1 is a core component of IFT particle complex B and is required for flagellar assembly. Mol Biol Cell 21:2696–2706

45. Mitchell DR, Kang Y (1991) Identification of odak1 as a Chlamydomonas dynein mutant with the wild-type gene. J Cell Biol 113:835–842

46. LeOzet M, Piperno G (1995) Ida, 4–1, ida–2, and ida–3 are intron splicing mutations affecting the locus encoding p28, a light chain of Chlamydomonas axonemal inner dynein arms. Mol Biol Cell 6:713–723

47. Bhogaraju S, Cajanek L, Fort C, Blisnick T, Weber K, Taschner M, Mizuno N, Lamia S, Bastin P, Nigg EA, Lorentzen E (2013) Molecular basis of tubulin transport within the cilium by IFT74 and IFT81. Science 341:1009–1012

48. Neff MM, Turk E, Kalishman M (2002) Web-based primer design for single nucleotide polymorphism analysis. Trends Genet 18:613–615

49. Piperno G, Huang B, Luck DJ (1997) Two-dimensional analysis of flagellar proteins from wild-type and paralyzed mutants of Chlamydomonas reinhardtii. Proc Natl Acad Sci U S A 74:1600–1604

50. Rupp G, O’Toole E, Gardner LC, Mitchell BF, Porter ME (1996) The sup-p2 mutations of Chlamydomonas alter the activity of the outer dynein arms by modification of the gamma-dynein heavy chain. J Cell Biol 135:1853–1865

51. Dutcher SK, Morrissette NS, Preble JM, Raclecy C, Stanga J (2002) Epiflament tubulin is an essential component of the centrode. Mol Biol Cell 13:3859–3869

52. Dutcher SK, Huang B, Luck DJ (1984) Genetic dissection of the central pair microtubules of the flagella of Chlamydomonas reinhardtii. J Cell Biol 98:229–236

53. Lin H, Miller M, Granas D, Dutcher S (2013) Whole genome sequencing reveals distinct functions of a protein phosphatase 2A in Chlamydomonas mating. PLoS Genet 9:e1003841

54. Albeau AJ, Kwan AL, Lin H, Granas D, Stormo GD, Dutcher SK (2013) Identification of cilia genes that affect cell-cycle progression using whole-genome transcriptome analysis in Chlamydomonas reinhardtii. G3 3:979–991

55. Firestone AJ, Weingar JS, Maldonado M, Barlan K, Langston LD, O’Donnell M, Gelfand VI, Kapoor TM, Chen JK (2012) Small-molecule inhibitors of the AAA+ ATPase motor cytoplasmic dynein. Nature 484:125–129

56. Shih SM, Engel BD, Kocaba F, Bilyard T, Geimer S, Wakabayashi K, Hirono M, Witman GB, Kamiya R, Marshall WF, Wiltz A (2013) Intraflagellar transport drives flagellar surface motility. Elife 2:e00744

57. Kon T, Otama Y, Shimo-Kon R, Imamura K, Shimma T, Sutoh K, Kurisu G (2012) The 2B.A crystal structure of the dynein motor domain. Nature 484:345–350

58. Schmidt H, Gleave ES, Carter AP (2012) Insights into dynein motor domain function from a 3.3-A crystal structure. Nat Struct Mol Biol 19:492–507

59. Crooks GE, Hon G, Chandona MJ, Brenner SE (2004) Weblogo: a sequence logo generator. Genome Res 14:1188–1190

60. Savagnerunathan S, Schmittler RR, Razafsky DS, Nandini S, Plamann MD, Thacker P, Wolpert LA, Malmström A (2005) Identification of cilia genes that affect cell-cycle progression using whole-genome transcriptome analysis in Chlamydomonas reinhardtii. Genetica 119:113–119

61. LeDizet M, Piperno G (1995) Ida, 4–1, ida–2, and ida–3 are intron splicing mutations affecting the locus encoding p28, a light chain of Chlamydomonas axonemal inner dynein arms. Mol Biol Cell 6:713–723

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