Yamamoto et al., http://www.jcb.org/cgi/content/full/jcb.201211048/DC1

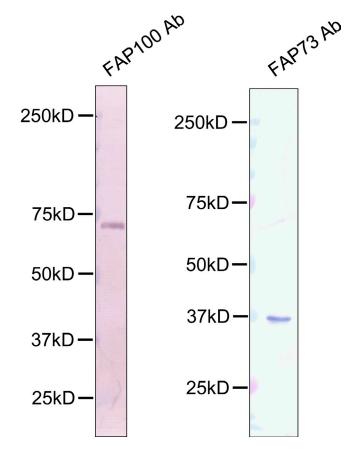
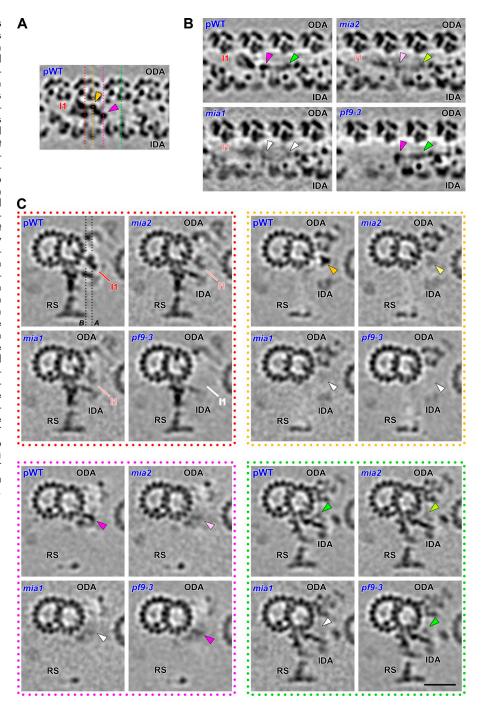


Figure S1. The FAP100 and FAP73 antibodies are specific for the Mia1 and Mia2 proteins. Immunoblots of wild-type axonemes were probed with the affinity-purified FAP100 and FAP73 antibodies. The FAP100 antibody detects a single band of \sim 70 kD, and the FAP73 antibody detects a single band of \sim 35 kD. The size of both bands is approximately consistent with the predicted mass for both the Mia1 and Mia2 proteins. Ab, antibody.

Figure S2. Structural defects in mia mutants revealed by cryo-ET. (A-C) Tomographic slices of the averaged 96-nm axonemal repeats from pWT, mia2, mia1, and pf9-3/ida1, viewed longitudinally from the front (A and B; proximal is on the left) and in cross section (C; from proximal to distal). (A) Same pWT image as shown in Fig. 6 B but with colored lines indicating the locations of the tomographic slices shown in C with the corresponding colored frames; arrowheads highlight densities that show reduction or are missing in the mia mutants. (B) Tomographic slices in the same orientation as A but with a cutting plane closer to the DMT; black lines in the pWT image of C (in red frame) indicate the cutting planes of A and B. Arrowheads point at regions where the densities are reduced in mia2 (light pink and light green arrowheads) or missing/dramatically reduced in mia1 (white arrowheads). The corresponding regions appear to be normal in both pWT and pf9-3/ida1 (pink and green arrowheads). Note that the density of 11 dynein is reduced in both mia mutants and missing in pf9-3/ida1. (C) Cross-sectional views from four different locations along the length of the axonemal repeat show the structural defects in mia mutant axonemes. The distal region of the 11 IC/LC complex (yellow arrowheads) and two additional regions (pink and green arrowheads) display defects in mia mutant axonemes. The defects observed are more severe in mia1 (white arrowheads) than in mia2 (arrowheads with light colors). In pf9-3/ida1, the regions highlighted in red and yellow in pWT (11 dynein) are also missing, whereas the two distal regions (pink and green arrowheads) are present at nearly wild-type level. The pWT and pf9-3/ida1 data were refined from data originally reported by Heuser et al. (2012). Bar, 25 nm; valid for A-C.



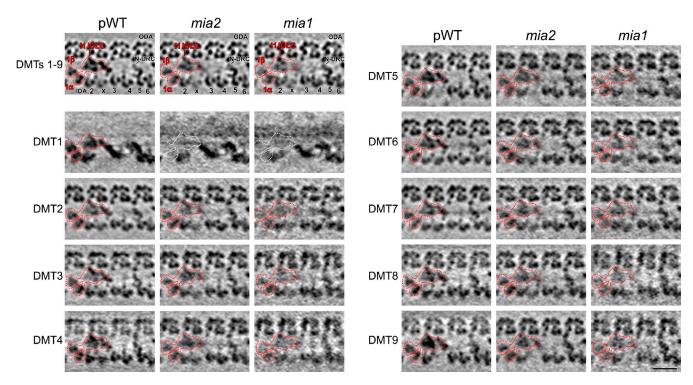


Figure S3. 11 dynein is assembled on outer DMTs 2-9 but missing on DMT1 in the *mia* mutants. Tomographic slices of averaged axonemal repeats from pWT, *mia2*, and *mia1*. Longitudinal front views of all doublets combined (DMTs 1-9) and of each individual doublet (DMT1 to DMT9) are shown. As shown in Fig. 7 A, the entire 11 dynein is missing or dramatically reduced on DMT1 from both *mia* mutants (white outlines). Consistent with the results of all doublets combined, the individual doublet-specific averages of DMT2 to DMT9 show a reduced density of the 11 dynein in *mia* mutants compared with pWT; this reduction appears more severe in *mia1*. The pWT data were refined from data originally reported by Heuser et al. (2009) and Lin et al. (2012). red outlines, 11 dynein complex. Bar, 25 nm.

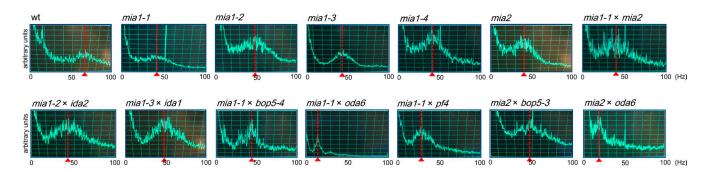


Figure S4. The *mia* mutants have reduced beat frequencies. Beat frequency measurements show that the *mia* mutants have reduced beat frequencies (~40–50 Hz) relative to wild type (60–70 Hz). Double mutants between the *mia* mutants and *oda6* or *pf4* have reduced beat frequencies (10–30 Hz). In contrast, doubles between the *mia* mutants and 11 dynein (*ida1*, *ida2*, *bop5-3*, and *bop5-4*) have beat frequencies similar to that of the *mia* alone (40–50 Hz). The fast Fourier transform power spectra show the approximate distribution of flagellar beat frequency and the peaks in the spectra represent the mean flagellar beat frequencies of 10–100 cells (red arrowheads and dotted lines; Kamiya, 2000). wt, wild type.

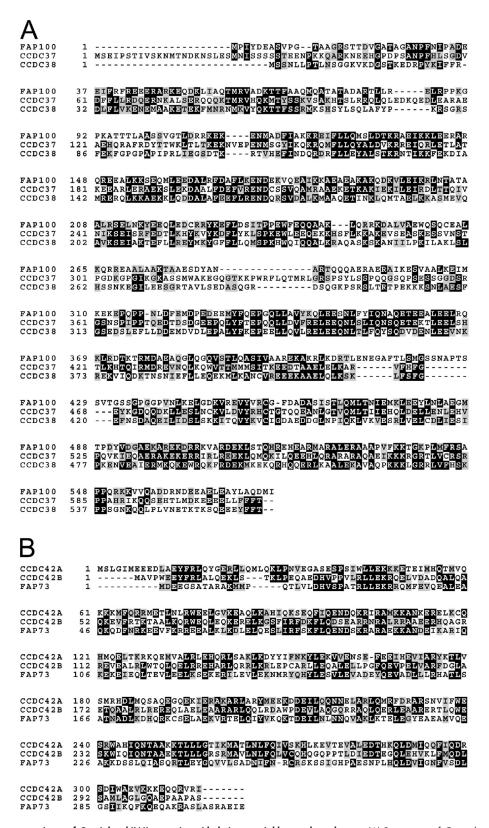
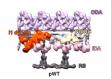
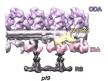


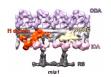
Figure S5. Sequence comparisons of *C. reinhardtii* Mia proteins with their potential human homologues. (A) Sequences of *C. reinhardtii* FAP100 and two potential human homologues, CCDC37 and CCDC38, were aligned using ClustalW, and the output was processed with BoxShade. Characters with black and gray backgrounds represent identical and similar amino acids, respectively. Accession numbers are as follows: *C. reinhardtii* FAP100, AB692780; Homo sapiens CCDC37, AAI01369; and Homo sapiens CCDC38, AAH95479. (B) Sequences of *Chlamydomonas reinhardtii* FAP73 and two potential human homologues, CCDC42A and CCDC42B, were aligned as described for A. Accession numbers are as follows: *C. reinhardtii* FAP73, AB692781; Homo sapiens CCDC42A, Q96M95; and Homo sapiens CCDC42B, NP_001138344. Accession numbers were obtained from the NCBI protein and NCBI nucleotide databases.



Video 1. **3D visualization of pWT axonemes.** Shown is an animated 3D isosurface rendering of the averaged 96-nm axonemal repeat from pWT *C. reinhardtii* flagella. At the beginning of the video, the proximal end of the repeat is on the left side. Labels and coloring: 11 dynein (orange), 11 tether head (red), nexin–dynein regulatory complex (N-DRC; yellow), inner dynein arms (IDAs; rose), outer dynein arms (ODAs; purple), and radial spokes (RSs; dark gray).



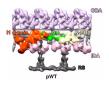
Video 2. **3D visualization of** *pf9-3/ida1* **axonemes.** Shown is an animated 3D isosurface rendering of the averaged 96-nm axonemal repeat from *pf9-3/ida1 C. reinhardtii* flagella. Note that the entire 11 dynein complex is missing. At the beginning of the video, the proximal end of the repeat is on the left side. Labels and coloring: nexin–dynein regulatory complex (N-DRC; yellow), inner dynein arms (IDAs; rose), outer dynein arms (ODAs; purple), and radial spokes (RSs; dark gray).



Video 3. **3D visualization of mia1 axonemes.** Shown is an animated 3D isosurface rendering of the averaged 96-nm axonemal repeat from mia1 C. reinhardtii flagella. Note that the distal density of the 11 dynein complex and the density between the 11 dynein and the N-DRC are significantly reduced. At the beginning of the video, the proximal end of the repeat is on the left side. Labels and coloring: 11 dynein (orange), 11 tether head (red), nexin-dynein regulatory complex (N-DRC; yellow), inner dynein arms (IDAs; rose), outer dynein arms (ODAs; purple), and radial spokes (RSs; dark gray).



Video 4. **3D visualization of mia2 axonemes.** Shown is an animated 3D isosurface rendering of the averaged 96-nm axonemal repeat from mia2 C. reinhardtii flagella. Note that the distal density of the 11 dynein complex is reduced. At the beginning of the video, the proximal end of the repeat is on the left side. Labels and coloring: 11 dynein (orange), 11 tether head (red), nexin-dynein regulatory complex (N-DRC; yellow), inner dynein arms (IDAs; rose), outer dynein arms (ODAs; purple), and radial spokes (RSs; dark gray).



Video 5. **3D localization of the MIA complex.** Based on a pWT-mutant comparison, the axonemal structures affected in the *mia* mutants are colored in green in the animated 3D isosurface rendering of the averaged 96-nm axonemal repeat from pWT *C. reinhardtiii* flagella. At the beginning of the video, the proximal end of the repeat is on the left side. Labels and coloring: I1 dynein (orange), I1 tether head (red), MIA complex and associated structures (green), nexin-dynein regulatory complex (N-DRC; yellow), inner dynein arms (IDAs; rose), outer dynein arms (ODAs; purple), and radial spokes (RSs; dark gray). Compare with Fig. 6 C.

Table S1. Potential MIA complex components identified by chemical cross-linking and immunoprecipitation

Protein number	Protein definition	Score		
FAP100-HA				
GI 159477649°	Flagellar inner dynein arm 11 IC, IC138	137		
GI 159471389	Flagellar-associated protein, FAP189	48		
GI 159486503	Flagellar-associated protein, FAP57	46		
GI 30580462°	Dynein-1-β HC, flagellar inner arm 11 complex	41		
GI 159463370	Flagellar-associated protein, FAP68	35		
GI 159485950	Flagellar/basal body protein, FBB10	35		
GI 30580468°	Dynein-1- α HC, flagellar inner arm 11 complex	33		
GI 159489898	SWI/SNF chromatin-remodeling complex component			
GI 159472759				
GI 159483703	159483703 Flagellar/basal body protein, PACRG-like protein			
GI 159467413	DNA-directed RNA polymerase II, largest subunit	25		
GI 1354832	RpoC2 protein, partial (chloroplast)	23		
GI 159471658	Predicted protein, partial	23		
GI 159474306	Flagellar-associated protein, FAP44	20		
FAP73-HA				
GI 30580462°	Dynein-1-β HC, flagellar inner arm 11 complex	107		
GI 159474916	RWP-RK transcription factor	31		
GI 159489926	5-enolpyruvylshikimate-3-phosphate synthase	26		
GI 159485936	Hypothetical protein CHLREDRAFT_167801	25		
GI 159477649°	Flagellar IDA 11 IC, IC138	24		
GI 159483705	Hypothetical protein CHLREDRAFT_141667, partial	24		
GI 159472228	Hypothetical protein CHLREDRAFT_196913	24		
GI 159483703	Flagellar/basal body protein, PACRG-like protein	23		
GI 159481544	Flagellar-associated protein, FAP43	23		
GI 159468520	Predicted protein	23		
GI 159481 <i>574</i>	Hypothetical protein CHLREDRAFT_151956, partial	22		
GI 159481750	Hypothetical protein CHLREDRAFT_152015, partial	21		
GI 159481859	T-type cyclin	21		
GI 159478731	Predicted protein, partial	21		
GI 159480608	Predicted protein	20		

Protein number shows the gene index (GI) number of the protein in NCBI Protein database. Protein definition is the definition of the protein in NCBI. Score shows MASCOT probability score of ESI/liquid chromatography /MS/MS analysis. SWI/SNF, switch/sucrose nonfermentable.

*Previously identified I1 dynein components.

Table S2. Mutant strains used in this study

Mutant	Affected protein	Axonemal defect	Reference		
137c (wild type)	N/A	None	Harris, 1989		
pWT (<i>pf2-4::PF2-GFP</i>)°	N/A	None	Rupp and Porter, 2003; Heuser et al., 2009		
bop5	IC138	Loss of 11 dynein regulatory subcomplex	Hendrickson et al., 2004; VanderWaal et al., 2011		
ida1/pf9	DHC1	Loss of 11 dynein	Kamiya et al., 1991; Myster et al., 1997		
ida2	DHC10	Loss of 11 dynein	Kamiya et al., 1991; Perrone et al., 2000		
ida3	Unknown	Loss of 11 dynein	Kamiya et al., 1991		
ida4	p28	Loss of dynein a, c, and d	Kamiya et al., 1991; LeDizet and Piperno, 1995		
ida5	Actin	Loss of dynein a, c, d, and e	Kato-Minoura et al., 1997		
ida6	Unknown	Loss of dynein e; defect in N-DRC	Kato et al., 1993; Porter, 2011		
ida9	DHC9	Loss of dynein c	Yagi et al., 2005		
mia 1-1	FAP100	Defect in the MIA complex	King and Dutcher, 1997; this study		
mia 1-2					
mia 1-3					
mia 1-4					
mia2	FAP73	Defect in the MIA complex	King and Dutcher, 1997; this study		
mbo1	Unknown	Loss of beaklike structure	Segal et al., 1984		
mbo2	Mbo2p	Loss of beaklike structure	Segal et al., 1984; Tam and Lefebvre, 2002		
oda 1	DC2	Loss of ODA	Kamiya, 1988; Takada et al., 2002		
oda2	DHC15	Loss of ODA	Kamiya, 1988; Wilkerson et al., 1994		
oda6	IC2	Loss of ODA	Kamiya, 1988; Mitchell and Kang, 1991		
oda7	Oda7p	Loss of ODA	Kamiya, 1988; Freshour et al., 2007		
pf3	DRC1	Loss of dynein e; defect in N-DRC	Piperno et al., 1992, 1994; Gardner et al., 1994		
pf4	PP2A	Loss of protein phosphatase 2A B subunit	Elam et al., 2011		
pf14	RSP3	Loss of RSs	Luck et al., 1977		
pf17	RSP9	Loss of RS heads	Harris, 1989; Yang et al., 2006		
pf18	Unknown	Loss of CP	Harris, 1989		

Table S3. Resolution of 3D structures obtained by cryo-ET

Mutant name	Strain	Averaged repeats	Resolution		
			DMT	ODA	II dynein
			nm	nm	nm
pW™	pf2-4::PF2-GFP	720	3.1	3.5	3.3
mia 1	mia 1-1	750	3.8	4.1	4.3
mia2	mia2	1,200	3.5	3.5	4.3
pf9/ida1 ^b	pf9-3	1,100	3.2	3.7	_

Resolution (0.5 Fourier shell correlation criterion) was measured at two different locations: the DMT usually has the highest resolution in the axonemal averages; the 11 dynein has usually a slightly lower resolution, which is typical for associated complexes. The minus sign indicates that 11 dynein is absent.

*Data were used as control and were refined from data originally published by Heuser et al. (2009) and Lin et al. (2012).

*Data were used as control and were refined from data originally published by Nicastro et al. (2006) and Heuser et al. (2012).

N/A, not applicable.

The pseudo-wild-type strain (pWT) is a rescued mutant that is biochemically, structurally, and phenotypically indistinguishable from wild type. It was generated by transforming the N-DRC mutant pf2 with a GFP-tagged PF2 gene from wild type.

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