Supporting Information

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SI Text

Identification of the 70 kDa MPM2 Antigen as CrKinesin-13 and Generation of a Rabbit Antibody That Reacts with CrKinesin-13. We carried out immunoblot analysis of *Chlamydomonas* cells using the MPM-2 monoclonal antibody, which recognizes a phosphoepitope (pS/T-P) present on a set of proteins, including microtubule-associated proteins MAP-1, MAP-2, and NIMA kinase, that regulate microtubule dynamics during the cell cycle (1). Studies in the early 1990s had shown that the MPM-2 antibody stained the nuclear envelope in *Chlamydomonas* cells in mitosis (2) and that unidentified proteins of ≈90 and 34 kDa were detected in gametes undergoing flagellar regeneration (3). We used pH shock to activate vegetative cells to detach their flagella and then used immunoblot analysis with mAb MPM-2 to examine phosphoproteins in the cell samples. As shown in Fig. S1A (*left panel*), the MPM-2 blots of the pH-shocked cells contained a 70-kDa phosphoprotein that was not detected in control, non-deflagellated cells. Coomassie blue staining of the blot membrane documented equal loading (Fig. S1A, *right panel*). The relationship, if any, between this 70-kDa protein we detected in vegetative cells and *Chlamydomonas* proteins previously recognized (3) by the antibody in deflagellated gametes is unclear.

We used affinity purification with the MPM-2 antibody followed by mass spectrometry analysis to identify the 70 kDa phosphoprotein. To purify the antigen recognized by the MPM-2 antibody, control and deflagellated cells were extracted with cell lysis buffer A (50 mM Tris, pH 7.5, 10 mM MgCl2, 1 mM EDTA, 1mM DTT) containing 0.1% Nonidet P-40 with protease inhibitor (Roche, Switzerland) added. An agarose conjugate of the MPM2 antibody from Upstate Biotechnology Inc. was used for immunoprecipitation (IP) of the 70kDa phosphoprotein. A portion (1/10) of the IP pellet was used for confirmatory immunoblotting experiments (Fig. S1B, immunoblot). The remainder was subjected to SDS/PAGE, and proteins were detected by silver staining (Fig. 1*A*). The band migrating at a molecular mass of ≈70 kDa was subjected to mass spectrometry analysis (Protein Core Facility, UT Southwestern Medical Center).

Most of the peptides detected (328 of 453 peptides) were derived from a single protein, which is encoded by the gene C_730040 (protein ID 169327) in the *Chlamydomonas* genome database (JGI portal, chlre2) (GenBank accession no. XM_001695123). The protein product of the gene was annotated as a 647 aa-containing kinesin-like protein with a highly conserved 343 aa kinesin motor domain in the middle (Fig. S1*C*). cDNA clone MXL043b12 (Kazusa DNA Research Institute, Kisarazu, Japan), which contained nucleotide sequences from the 5′ UTR of gene C_730040, was sequenced in full, and contained an ORF identical to the annotated cDNA from the *Chlamydomonas* genome database v. 2.

Analysis of the crystal structure of mouse kinesin-13 (KIF2C) combined with biochemical analysis indicated that four specific and highly conserved domains are important for diffusional translocation of kinesin-13s along microtubules and for microtubule depolymerization (4–6). The KVD finger domain (L2) and domains L8 and α4 stabilize the curved conformation of protofilaments at the microtubule end and mediate ATP hydrolysis. The positively charged neck domain facilitates the diffusional translocation of the protein along the surface of the microtubule that accounts for its accumulation at the ends of microtubules (Fig. S2) (4, 6–8). The neck domain may also be important in deformation of the tubulin protofilaments during microtubule disassembly (9).

To characterize CrKinesin-13 further, we used a polyclonal anti-peptide, CrKinesin-13 antibody. A polyclonal antibody against CrKinesin-13 peptides (AA509–527: RERDRERASDRYPSPPRQQ; AA119–128: PPSEGEDPPK; AA373–383: ADTFDNNRQTR) was commercially generated (UT Southwestern Medical Center, Dallas, TX). Both serum and affinity-purified antibody (1:2000) exhibited similar staining in immunoblots (not shown). Immune, but not preimmune, serum recognized a predominant band of ≈70 kDa in cell samples (Fig. S3*A*), and immunoprecipitation/immunoblotting studies confirmed that the 70 kDa MPM-2 reactive protein was CrKinesin-13 (Fig. S3*B*).

To immunoprecipiate CrKinesin-13 from flagella, flagella undergoing shortening induced by NaPPi treatment were isolated. The membrane/matrix fraction of the flagella was obtained by suspending the flagella in cell lysis buffer A containing 0.1% Nonidet P-40 followed by centrifugation (16,500 *g*) for 15 min at 4 °C. Preimmune and immune serum of CrKinesin-13 were used for immunoprecipitation following standard protocols (10).

DNA Constructs and Cell Transformation. The RNAi construct for knocking down CrKinesin-13 comprised a DNA fragment containing the 1st exon and intron of CrKinesin-13 followed by a DNA fragment of the reversed 1st exon was cloned between the HsRb promoter and the Rbsc terminator in plasmid pJP (11–13; data not shown). Briefly, an oligonucleotide containing the first exon and intron of CrKinesin-13 was amplified by PCR with primers CGCtctagaAGCCTGTTCTGAGCAGCTC and GTAggatccGGCCTGCATGTTTGAGAC containing flanking restriction sites of XbaI and BamHI, respectively and cloned into the XbaI and BamHI sites of plasmid pJP, generating plasmid pJPM1. The first exon of CrKinesin-13 was amplified by primers GATggatccCGTCGGCCAGTAGATCCC and GTAggatccATGGTTAGCTCATCAGTAG (with flanking BamHI sites) and cloned into pJPM1 at the BamHI site, generating the final RNAi construct pMCAKRNAi (4.45 kb). The sequence was verified by sequencing. The RNAi construct (1 μg) was transformed into wild-type strain 21gr by the glass bead method (1×10^8 cells used per transformation), together with pSI103 (0.5 μg), a 4.98-kb plasmid containing a paromomycin resistance gene (14). Two independent transformations were carried out and 62 transformants were obtained. Eight of the 62 transformants showed reproducible reduction of kinesin-13, and these eight transformants also possessed shorter flagella. Of ≈10 transformants with normal expression of CrKinesin-13 that were examined, all had flagella of wild-type length (not shown). Four of the transformants (92, 93, 99, and 115) were selected for further analysis. Measurements of their flagella ($n = 100$; lengths are expressed as mean ± SEM) yielded the following lengths: wild-type, 11.7 ± 0.2 μm; RNAi-92, 7.5 ± 0.1 μm; RNAi-93, 7.4 ± 0.1 μm; RNAi-99, 5.6 ± 0.1 μm; and RNAi-115, 5.9 ± 0.1 μm.

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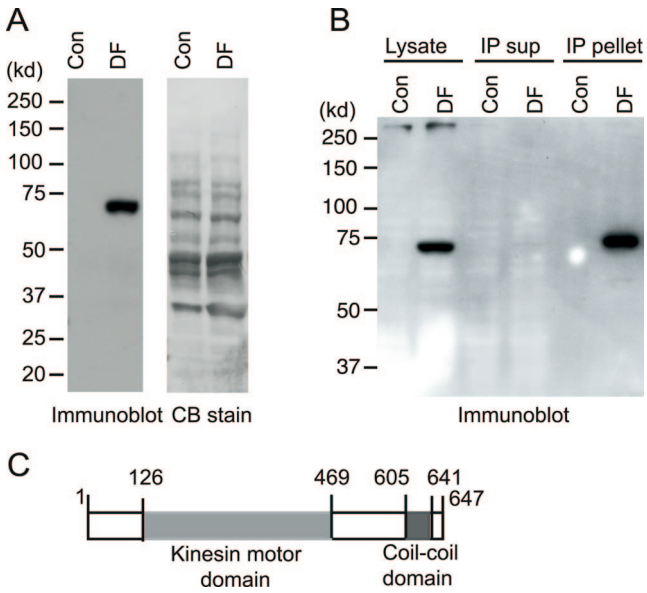

Fig. S1. Identification of the MPM2 70 kDa antigen. (*A*) Cell lysates from control (Con) and deflagellated (DF) were subjected to immunoblot analysis with MPM2 antibody. After immunoblotting, the immunoblot membrane was stained with Coomassie blue to document equal loading of the samples. (*B*) Cell extracts from control and deflagellated cells were used for immunoprecipitation with MPM2 antibody and analyzed by immunoblotting with the same antibody. The 70-kDa MPM2 antigen was depleted from the IP supernatant (Sup) of the deflagellated samples and enriched in the IP pellet. (*C*) Schematic diagram of CrKinesin-13 functional domains.

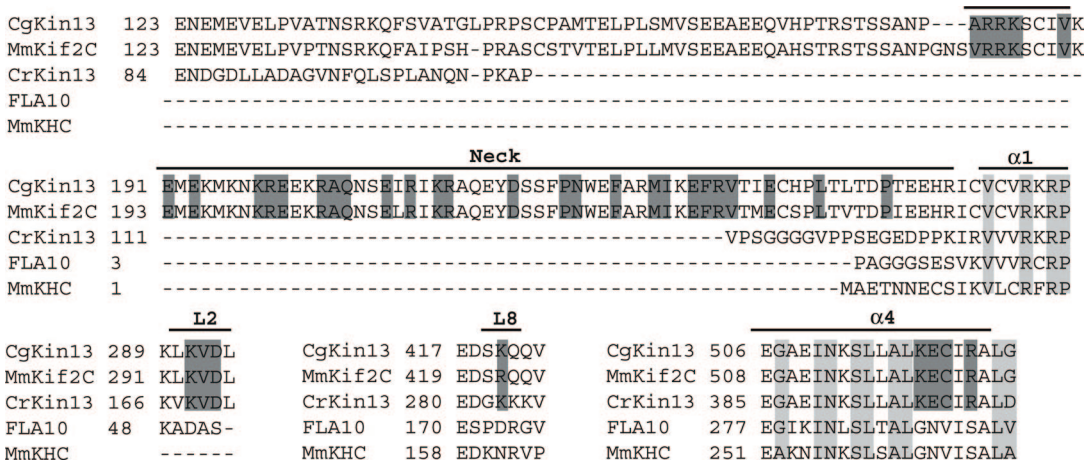

Fig. S2. Comparison of the amino acid sequence within specific domains of kinesin 13 family members and non-family members. Sequences used include the following founding members of the kinesin-13 family: Cgkinesin-13; a mouse orthologue of kinesin-13, MmKf2; IFT motor protein kinesin2 FLA10; and a mouse member of the kinesin superfamily MmKHC. Letters in gray show the conserved residues among the kinesin superfamily.

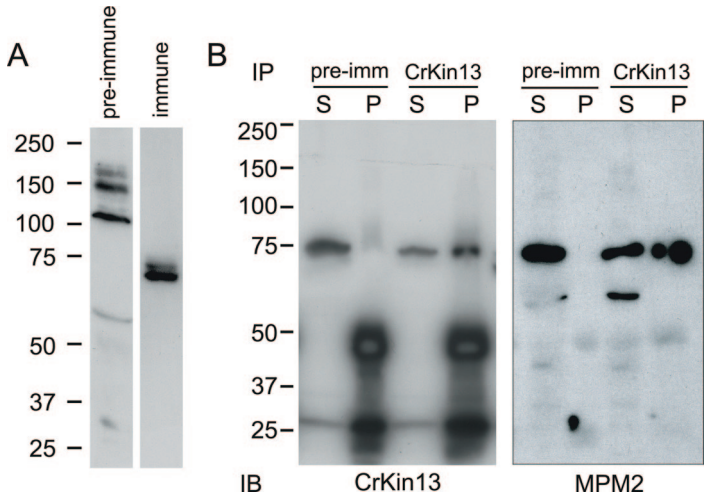

Fig. S3. Characterization of the anti-CrKinesin-13 antibody. (*A*) Specificity of the anti-CrKinesin-13 peptide antibody. Immune serum was tested with cell lysates from untreated wild-type 21gr. The protein band in the 70 kDa region appeared only in the immunoblot of the sample immunoprecipitated using anti-CrKinesin-13 serum. (*B*) Phosphorylated CrKinesin-13 is the MPM2 antigen. CrKinesin-13 was immunoprecipitated from deflagellated cells with anti-CrKinesin-13 and immunoblotted with anti-CrKinesin-13 and MPM2 antibodies.