Abstract. The kinesin superfamily of mechanochemical proteins has been implicated in a wide variety of cellular processes. We have begun studies of kinesins in the unicellular biflagellate alga, Chlamydomonas reinhardtii. A full-length cDNA, KLP1, has been cloned and sequenced, and found to encode a new member of the kinesin superfamily. An antibody was raised against the nonconserved tail region of the Klp1 protein, and it was used to probe for Klp1 in extracts of isolated flagella and in situ. Immunofluorescence of whole cells indicated that Klp1 was present in both the flagella and cell bodies. In wild-type flagella, Klp1 was bound tightly to the axoneme; immunogold labeling of wild-type axonemal whole mounts showed that Klp1 was restricted to one of the two central pair microtubules at the core of the axoneme. Klp1 was absent from the flagella of mutants lacking the central pair microtubules, but was present in mutant flagella from pft6 cells, which contain an unstable C1 microtubule, indicating that Klp1 was bound to the C2 central pair microtubule. Localization of Klp1 to the C2 microtubule was confirmed by immunogold labeling of negatively stained and thin-sectioned axonemes. These findings suggest that Klp1 may play a role in rotation or twisting of the central pair microtubules.

The kinesin superfamily of proteins has been implicated in a wide variety of microtubule-dependent motility processes, each of which requires a motor protein to provide the enzymatic link that converts the chemical energy of nucleotide triphosphates into mechanical force (for reviews, see Bloom, 1992; Skoufias and Scholey, 1993; Walker and Sheetz, 1993). Kinesin, the prototypical founding member of this family, is a microtubule plus-end-directed motor that was first isolated from squid axons (Brady, 1985; Vale et al., 1985), and has since been implicated in the movement of vesicles (Bloom et al., 1988; Vale et al., 1985), pigment granules (Rodionov et al., 1991), and cytoplasmic organelles (Dabora and Sheetz, 1988; Leopold et al., 1992; Schroer et al., 1988; Vale and Hotani, 1988). The NH2-terminal motor domain of the kinesin heavy chain (KHC) binds microtubules and generates the force required for motility (Hirokawa et al., 1989; Yang et al., 1989), while the KHC tail domain binds membranes tightly (Skoufias et al., 1994), tethering vesicles and organelles to microtubules. The tail domain of the KHC is also the region that allows kinesin to form an oligomeric protein (Hirokawa et al., 1989; Yang et al., 1989): KHC tails form homodimers, and each KHC tail binds a kinesin light chain molecule, yielding a tetrameric kinesin molecule composed of two identical heavy chains and two identical light chains (Bloom et al., 1988; Kuznetsov et al., 1988).

Since the initial molecular characterization of the Droso phila KHC (Yang et al., 1989), molecular techniques have greatly expanded the kinesin superfamily to include >30 members. Proteins of the kinesin superfamily are defined by a conserved motor domain that typically is 40% identical to the motor domain of the KHC (Endow and Hatsumi, 1991; Stewart et al., 1991). Though the motor domains of most kinesin-like proteins (kips) are found in the NH2-terminal region, examples of COOH-terminal motor domains and internal motor domains have also been described (reviewed in Bloom, 1992; Goldstein, 1993a). Most kips are thought to drive microtubule plus-end-directed movement, but kips that are microtubule minus-end-directed motors have also been described (McDonald et al., 1990; Meluh and Rose, 1990; Walter et al., 1990). It should be noted, however, that the directionality of transport for most kips has been inferred only from the position and sequence of the motor domain; for most kips, motor activities have yet to be documented. Functional specificity is conferred to each class of kips by a tail region that is unique to members of that class (Goldstein, 1991; Goldstein, 1993a; Yang et al., 1989). Kips are ubiquitously distributed among organisms and cell types, and a given organism may contain genes for >30 different kips (Endow and Hatsumi, 1991; Stewart et al., 1991). Kips
have been shown to be required for meiosis and mitosis (Nislow et al., 1990, 1992; Sawin et al., 1992; Walker et al., 1990; Wright et al., 1993; Yen et al., 1991, 1992; Zhang et al., 1990), as well as spindle pole body and nuclear migration in fungi (Enos and Morris, 1990; Hagan and Yanagida, 1990; Hoyt et al., 1992; Meluh and Rose, 1990; Roof et al., 1992; Saunders and Hoyt, 1992). In addition to microtubule-dependent motility, demonstrated in vitro activities for kips include microtubule sliding (Nislow et al., 1992), rotating (Walker et al., 1990), and bundling (Chandra et al., 1993; McDonald et al., 1990; Nislow et al., 1992). However, because most kips have been isolated and characterized solely by their homology to the head domain of kinasin, the function of most kips is unknown.

In a complementary approach to biochemical and genetic studies, antipeptide antibodies directed against highly conserved regions of the kinasin motor domain have been used to identify HKC and kinesin-related proteins (KRP s) at the protein level (Cole et al., 1992; Sawin et al., 1992) and to purify a trimeric complex containing two different KRP s (Cole et al., 1993). Recently, these antibodies have been used to detect the presence of KRP s in Chlamydomonas flagella (Fox et al., 1994; Johnson et al., 1994; Kozminski, K., and J. Rosenbaum, unpublished observation). We have begun studies to isolate and characterize these motors to learn about their function(s) in eukaryotic flagella. Using PCR, we have isolated the gene for a klp that is present in the flagella of Chlamydomonas, and we have characterized its gene product, the first non-dynein force generating protein isolated from the eukaryotic flagellum. Biochemical fractionation of isolated flagella and immunolocalization using an antibody specific for this klp indicate that it is part of the central pair microtubule complex. We discuss possible roles of this protein in relation to central pair rotation and flagellar beating.

**Materials and Methods**

**Strains and Growth Conditions**

Wild-type Chlamydomonas reinhardtii strain 21gr (mt−), cell wall-less strain CW15 (mt−, CC-400), radial spokeless mutant PF4 (mt−, CCI032), central pairless mutant PF5 (mt−, CCI033), and the CI-defective mutant PF6 (mt−, CCI034) were obtained from the Chlamydomonas Genetics Center (Duke University, Durham, NC). Cultures were grown in R/2 medium (Kindle et al., 1989) on a 14:10 h light/dark cycle.

**Molecular Cloning Procedures and Sequence Analysis**

DNA clones were isolated and manipulated using standard procedures (Sambrook et al., 1989). All cloning was done into plBluescript plasmids (Stratagene, San Diego, CA). Random sequencing of the partial KLP1 cDNA (Bankier and Barrell, 1983) was performed by the dideoxy method using Sequenase T7 DNA polymerase (U.S. Biochemical Corp., Cleveland, OH). All sequences were obtained from both DNA strands. Sequence assembly and analyses were done with the GCG sequence analysis software package available from the University of Wisconsin (Madison, WI). Database searches were performed using the BLAST program (Altschul et al., 1990). PCR reactions were performed in a DNA thermal cycler (Perkin-Elmer Cetus Instruments, Norwalk, CT).

**RT-PCR Cloning**

First-strand synthesis for reverse transcription PCR was performed using the cDNA Cycle Kit (Invitrogen, San Diego, CA). Poly(A) mRNA (1 μg) isolated from cells 40 min into flagellar regeneration was denatured with methylmercury as described by the supplier, and first-strand synthesis was primed with random primers. PCR primers against conserved regions of the kinasin motor domain have been described (Endow and Hatsumi, 1991), and were modified to contain restriction enzyme sites at the 5′ ends and to reflect the codon usage bias of Chlamydomonas (obtained via Gopher server from the Chlamydomonas Genetics Center, Duke University Botany Department). Primers consisted of the following oligonucleotides: primer 1 (sense primer for amino acids M/L/M/N/T/S/LVDSLAV), aatggcgcctcggtgaagtcctcgccctgg; primer 2 contained a 1:1 mixture of primers 2a and 2b (antisense for amino acids PYRD/E/K/N/SLKT), 2a: atatgctggtagcactgggaagtcctcgccctgg 2b: atatgctggtagcactgggaagtcctcgccctgg. Primer 3 (sense primer for amino acids F/L/FLAOGT): aatggcgcctcggtgaagtcctcgccctgg. PCR reactions were performed in a volume of 50 μl using Taq or AmpliTag polymerase. Reactions contained 1/50 of the random primed cDNA pool and degenerate primers at 20 μg/ml. Reactions containing reactants (minus Taq polymerase) were heated to 97°C for 10 min and quenched on ice. Addition of Taq was followed by four cycles of 95°C, 47°C, and 72°C, and 27 cycles of 95°C, 62°C, and 72°C (all incubations were 1 min). PCR products were run on low temperature gelling agarose gels and bands of the predicted length (200 bp for primer pair 1 and 2 and 600 bp for primer pair 1 and 3) were excised. A second round of amplification was performed using aliquots of isolated fragments as templates. Conditions for the second round of PCR were 30 cycles of 95°C, 62°C, and 72°C, 1 min each. Products from the second round of amplification were gel purified and subcloned directly into the Smal or EcoRV site of plBluescript plasmids, and individual clones were isolated and sequenced. PCR products that encoded five different kinesin-like motor domain sequences were obtained (including KLP1). Analysis of the non-KLP1 clones will be described at a later date. The cDNA library prepared from Chlamydomonas during flagellar regeneration was kindly provided by K. Wilkerson and G. Wimann (The Worcester Foundation for Experimental Biology, Worcester, MA).

**5′ RACE**

Rapid amplification of cDNA ends (RACE) was performed with the 5′ RACE System from Gibco BRL (Gaithersburg, MD). First-round synthesis contained poly(A) RNA (0.75 μg) isolated from cells 40 min into flagellar regeneration and 20 μg of the KLP1-specific primer gaatggcgcctcggtgaagtcctcgccctgttattgctg. All PCR reactions for 5′ RACE contained the anchor primer supplied by the manufacturer and a KLP1-specific primer with the sequence ggaatggcgcctcggtgaagtcctcgccctggtatctg at concentrations of 0.4 μM. First-round PCR conditions were four cycles of 95°C, 50°C, and 72°C (1-min incubations) and 27 cycles of 95°C, 62°C, and 72°C (1-min incubations), followed by 10 min at 72°C. The major PCR product of ~400 bp was gel purified and used in a second round of PCR (95°C, 62°C, and 72°C, 1 min each for 30 cycles; 10 min at 72°C). PCR products were digested with SalI and EcoRI, gel purified, and inserted into plBluescript II KS(+). Single PCR clones were isolated from bacteria and sequenced.

**RNA Analysis**

RNA was isolated from Chlamydomonas strain 21gr. For the RNA time course during flagellar regeneration, cells were concentrated to 1–2 × 107 cells/ml and deflagellated for 2 min with a mixer on setting 7 (Virtis Co., Inc., Gardiner, NY). At time points during regeneration, aliquots of cells were pelleted, frozen in liquid nitrogen, and stored at −70°C until RNA isolation. For large amounts of mRNA cells regenerating flagella, 10 μg/ml cycloheximide was added to cultures 15 min before deflagellation to stabilize induced mRNAs (Baker et al., 1986). Flagellar regeneration was allowed to proceed for 40 min, at which time cells were pelleted for RNA isolation. RNA was isolated by resuspending cell pellets in a 1:1 mixture of buffer (0.2 M Tris-HCl, pH 7.5, 0.5 M NaCl, 0.01 M EDTA, and 1% SDS) to organic (phenol/chloroform/isoamyl alcohol, 25:24:1), followed by disruption for 1 min with the tissue grider attachment of a homogenizer (OmniMixer; Sorvall-Du Font, Newtown, CT) and separation of phases by centrifugation at room temperature. The aqueous phase was extracted with an equal volume of chloroform. Reactions were performed in a volume of 50 μl using Taq or AmpliTag polymerase. Reactions contained reactants (minus Taq polymerase) were heated to 97°C for 10 min and quenched on ice. Addition of Taq was followed by four cycles of 95°C, 47°C, and 72°C, and 27 cycles of 95°C, 62°C, and 72°C (all incubations were 1 min). PCR products were run on low temperature gelling agarose gels and bands of the predicted length (200 bp for primer pair 1 and 2 and 600 bp for primer pair 1 and 3) were excised. A second round of amplification was performed using aliquots of isolated fragments as templates. Conditions for the second round of PCR were 30 cycles of 95°C, 62°C, and 72°C, 1 min each. Products from the second round of amplification were gel purified and subcloned directly into the Smal or EcoRV site of plBluescript plasmids, and individual clones were isolated and sequenced. PCR products that encoded five different kinesin-like motor domain sequences were obtained (including KLP1). Analysis of the non-KLP1 clones will be described at a later date. The cDNA library prepared from Chlamydomonas during flagellar regeneration was kindly provided by K. Wilkerson and G. Wimann (The Worcester Foundation for Experimental Biology, Worcester, MA).
Antibody Production

To produce antibody against the nonconserved portion of Klpl, the 1,500-bp PspI fragment of the Klpl cDNA, encoding amino acids 334-776, was obtained by RACE, which yielded three products, two corresponding to cloned genomic sequences and were found to be free of error (data not shown). The KLP1 cDNA (Fig. 1) was similar in length to the leader sequence of many other kinesin-like proteins, which is identical to the pre-mRNA leader sequence. Sequence analysis revealed (a) the presence of an in-frame ATG at nucleotide 174 and encoded a primary translation product of 776 amino acids and a relative molecular mass of 83,019. We believe translation of Klpl initiates as indicated for the following reasons: (a) the ATG at nucleotide 174 was the first initiation codon of the Klpl cDNA, and the next in frame ATG was 240 nucleotides downstream; (b) the leader sequence upstream of this ATG contained stop codons in every reading frame; (c) the leader sequence of 173 nucleotides was shorter in length. The sequences obtained by RACE were compared to cloned genomic sequences and were found to be free of error (data not shown).

The Klpl cDNA (Fig. 1) was 2,752 bp in length, excluding the poly(A) tail. The Klpl protein coding sequence began at nucleotide 174 and encoded a primary translation product of 776 amino acids and a relative molecular mass of 83,019. We believe translation of Klpl initiates as indicated for the following reasons: (a) the ATG at nucleotide 174 was the first initiation codon of the Klpl cDNA, and the next in frame ATG was 240 nucleotides downstream; (b) the leader sequence upstream of this ATG contained stop codons in every reading frame; (c) the leader sequence of 173 nucleotides was similar in length to the leader sequence of many other Chlamydomonas transcripts (Curry, A., and M. Bernstein, unpublished compilation); and (d) the initiation codon was part of the sequence AAAATGG, which is identical to the pre-

Immunoblotting and Indirect Immunofluorescence

Immunoblotting was performed with the cell wall-less strain BCIP/NBT (Harlow and Lane, 1988). Indirect immunofluorescence was performed with the cell wall-less strain (a) as described (Kozminski et al., 1993a). Affinity-purified anti-Klpl antibody and anti-α-tubulin serum were incubated in the Klpl antibody, diluted 1:1,000 in blocking buffer, for 2-5 h.

Immunoelectron Microscopy

Whole mounts of axonemes were prepared essentially as described by Johnson and Rosenbaum (1992). All steps were performed at room temperature. Concentrated drops of cells were allowed to settle onto formvar-coated nickel grids, which were then reacted onto the grids with 3-5 min, incubated in 12-nm colloidal gold conjugated to goat anti-rabbit IgG (Zymed Laboratories, South San Francisco, CA) was used at a dilution of 1:250 for 2 h.

Results

Cloning of KLPI

The kinesin superfamily is defined by a motor domain that contains blocks of highly conserved amino acids dispersed among regions of variable sequence. Using reverse transcription PCR and oligonucleotides against conserved domains, we obtained five PCR products that encoded putative kinesin-like proteins. (A more detailed description of these clones will be presented at a later date.) These PCR products were used to screen a cDNA library prepared from Chlamydomonas cells undergoing flagellar regeneration, which yielded two identical partial cDNA clones encoding a kinesin-like protein, KLPI. The 5' end of the KLPI transcript was obtained by RACE, which yielded three products, two of which had identical 5' ends and a third that was 10 bp shorter in length. The sequences obtained by RACE were compared to cloned genomic sequences and were found to be free of error (data not shown).

The KLPI cDNA (Fig. 1) was 2,752 bp in length, excluding the poly(A) tail. The Klpl protein coding sequence began at nucleotide 174 and encoded a primary translation product of 776 amino acids and a relative molecular mass of 83,019. We believe translation of Klpl initiates as indicated for the following reasons: (a) the ATG at nucleotide 174 was the first initiation codon of the KLPI cDNA, and the next in frame ATG was 240 nucleotides downstream; (b) the leader sequence upstream of this ATG contained stop codons in every reading frame; (c) the leader sequence of 173 nucleotides was similar in length to the leader sequence of many other Chlamydomonas transcripts (Curry, A., and M. Bernstein, unpublished compilation); and (d) the initiation codon was part of the sequence AAAATGG, which is identical to the pre-

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Figure 1. KLPI sequence and coiled-coil probability. (A) KLPI cDNA sequence and deduced amino acid sequence. Underlined amino acids are tripeptide motifs shared by the Klpl protein and fungal members of the BimC subfamily of kinesin-like proteins. Underlined nucleotides indicate the FspI site used to subclone the nonconserved carboxy-terminal portion of Klpl for antibody production. The Klpl protein is predicted to be 776 amino acids with a relative molecular mass 83,019 and a pI = 7.0. (B) Probability of coiled-coil formation at each amino acid, calculated by the program of Lupas et al. (1991). These sequence data are available from EMBL/GenBank/DDBJ under accession number X78589.
Figure 2. Sequence comparison of kinesin motor domains. Kinesin motor domains were compared using the Pileup program (GCG computer software package; University of Wisconsin). Shaded regions are positions where Klpl is identical to at least two other proteins. Amino acid sequences were taken from the following sources:

- *S. cerevisiae* Kar3 (amino acids 389-728), Meluh and Rose (1990);
- *Arabidopsis thaliana* KatA (amino acids 439-789), Mitsui et al. (1993);
- *Aspergillus nidulans* BimC (84-223), Enos and Morris (1990);
- *S. cerevisiae* Kipl (55-417), Roof et al. (1992);
- *Drosophila melanogaster* KHC (15-340), Yang et al. (1989);
- *Chlamydomonas reinhardtii* Klpl (8-342), see Fig. 1A.

Sumptive translation start site of several other *Chlamydomonas* transcripts (Franzen et al., 1989; Goldschmidt-Clermont and Rahire, 1986; Stein et al., 1993; Yu and Selman, 1988; Zimmer et al., 1988).

The NH₂-terminal portion of Klpl, from amino acids 8-342, was homologous to the motor domains of other members of the kinesin superfamily (Fig. 2). These proteins share blocks of highly conserved regions separated by sequences that are highly variable. The motor domain of Klpl was 35-42% identical to each of the motor domains of 25 other kips whose sequences were available from the combined SwissProt, PIR, and GenPept databases via the BLAST search program (Altschul et al., 1990), which indicated that Klpl constituted the founding member of a distinct subfamily of klp. Consistent with this interpretation was the observation that the COOH-terminal portion of Klpl showed no significant homology to other proteins in these databases. However, as in other members of the kinesin superfamily, the COOH-terminal region of Klpl contained sequences that have a high probability of forming coiled-coil domains (Fig. 1B; Lupas et al., 1991).

Although the head region of Klpl constituted a distinct class of kinesin motor domain, we did recognize peptide motifs within the motor domain of Klpl that were common to fungal members of the BimC subfamily of klp (Fig. 3). These proteins include BimC from *A. nidulans*, cut7 from *Schizosaccharomyces pombe*, and Kipl and Cin8 from *Saccharomyces cerevisiae*, which are required for the separation of spindle pole bodies before mitosis (Enos and Morris, 1990; Hagan and Yanagida, 1990; Hoyt et al., 1992; Meluh and Rose, 1990; Roof et al., 1992; Saunders and Hoyt, 1992; Sawin and Endow, 1993). Two tripeptide motifs (FTI at amino acids 216-218 and NAL at amino acids 276-278 of the Klpl protein, Fig. 3) were found in Klpl and each of the Bernst.
**Figure 3.** Tripeptide motifs common to the motor domains of Klpl and the fungal BimC subfamily of klps. Boxed amino acids are tripeptide motifs that are present in the Klpl protein and fungal members of the BimC subfamily of klps. The amino acid residues for Motifs 1 and 2 are as follows: D. melanogaster KHC, 208-217 and 271-276 (Yang et al., 1989); A. thaliana KatA, 648-657 and 711-716 (Mitsui et al., 1993); C. reinhardtii Klpl, 209-218 and 274-279 (see Fig. 1A); A. nidulans BimC, 289-298 and 355-360 (Enos and Morris, 1990); S. pombe cut7, 270-279 and 348-353 (Hagan and Yanagida, 1990); S. cerevisiae Cin8, 392-401 and 454-459 (Hoyt et al., 1992); S. cerevisiae Kipl, 279-288 and 349-354 (Roof et al., 1992); S. cerevisiae Kar3, 596-605 and 659-664 (Meluh and Rose, 1990).

fungal members of the BimC subfamily. The NAL motif is highly specific for Klpl and the BimC subfamily members and was present in none of 34 other members of the kinesin superfamily, while the FTI motif was less restricted and was found in 6 of 31 other members of the kinesin superfamily. These highly conserved motifs, although small, may indicate regions of structural and/or functional homology among the motor domains of Klpl and the members of the BimC subfamily of klps.

**KLPI Gene Expression**

In response to environmental stresses, such as low pH or mechanical shear, Chlamydomonas cells shed their flagella (Randall et al., 1967; Rosenbaum et al., 1969) through an active process that requires a second messenger (Cheshire and Keller, 1991; Quarmby et al., 1992) and the action of the Ca²⁺-dependent contractile protein, centrin (Sanders and Salisbury, 1989). Immediately after flagellar excision, cells upregulate genes encoding flagellar proteins and use newly synthesized proteins, as well as preexisting precursors, to regenerate full-length flagella in 80 min (Leffebvre and Rosenbaum, 1986). Fig. 4A illustrates that KLPI encoded a transcript of ~3,000 nucleotides (consistent with the length of the KLPI cDNA) that was upregulated during flagellar regeneration. As with many other genes encoding flagellar proteins, a low level of KLPI message was detectable in nonflagellated cells. With kinetics similar to tubulin (Fig. 4B) and other flagellar proteins (Curry et al., 1992; Youngblom et al., 1984), the KLPI message was upregulated within 10 min of flagellar removal, peaked at 45 min into regeneration, and returned to basal levels within 90–120 min after deflagellation. These results provided the first evidence that Klpl was a flagellar protein.

**Anti-Klpl Antibody**

As a probe for Klpl protein, we raised antisera directed against amino acids 334–776 of the nonconserved tail region of Klpl protein expressed in bacteria (see Materials and Methods). We would expect these antisera to recognize only Klpl or a Klpl homologue, but not other members of the kinesin superfamily. Though we report on only one antisera in this work, two independent sera gave identical results.
Indirect immunofluorescence. The cell wall-less strain cwl5 was probed using primary antibodies against Klpl or α-tubulin (Silflow and Rosenbaum, 1981) and a fluorescein-labeled secondary antibody. Klpl antibody (A and B) stains the flagella and cell bodies. B shows that the cell body associated Klpl is concentrated slightly around the nucleus and in the region between the nucleus and flagellar base. α-Tubulin antiserum (C) reveals heavy labeling of the flagella and cytoplasmic microtubules. (D) Minus primary antibody control. Exposure times for photographing indirect immunofluorescence were: Klpl, 60 s; α-tubulin, 30 s; and minus primary antibody control, 60 s. A and B are prints from a single negative with B printed to reveal the concentration of Klpl in the anterior region of the cell bodies.

Immunofluorescence

Affinity-purified Klpl antibody was used to localize Klpl in fixed and permeabilized Chlamydomonas cells by indirect immunofluorescence. As predicted from the finding that KLPI is upregulated during flagellar regeneration, we observed Klpl staining in the flagella of cells (Fig. 5 A). We also observed Klpl fluorescence within the cell body, with a higher level of staining in the regions surrounding the nucleus and between the nucleus and flagellar base (Fig. 5, A-B). However, Klpl was not concentrated on the flagellar roots (compare to Fig. 5 C), as might be expected if Klpl is bound to cytoplasmic microtubules. The Klpl observed in the cell body could represent precursors to flagellar-localized Klpl, since precursors of many flagellar proteins are found in the cytoplasm and transported to the flagella during flagellar regeneration. Alternatively, like the tubulins, Klpl could be localized stably within both the cell body and the flagella.

Klpl in the Flagellum and Cell Body

When used to probe immunoblots of Chlamydomonas flagella, the affinity-purified Klpl antibody recognized two proteins with an apparent molecular weight of 96 kD (Fig. 6 A). In every case examined, we observed this doublet, suggesting that at least a fraction of the primary Klpl translation product had undergone posttranslational modification, or that the KLPI gene, which is present in only one copy per
Figure 6. Klpl in flagellar fractions. Fractions from whole flagella (F), axonemal pellets (A), and membrane/matrix fractions (M) were immunoblotted and probed with Klpl antibody. Fractions were prepared from (A) wild-type flagella (B) pf5 (9+0, central pairless) mutant flagella (C) pf4 (radial spokeless) mutant flagella and (D) pf6 (Cl-deficient) mutant flagella. All lanes contain flagellar fractions from starting material of ~2 × 10⁶ cells, which corresponds to ~25 µg of protein for the flagellar and axonemal fractions, and <2 µg of protein for the membrane matrix fractions. Positions of protein standards (kD) are shown on the left.

Flagellar Fractionation and Localization of Klpl

To localize Klpl in the flagellum, we isolated flagella and extracted them with the nonionic detergent NP-40, which yielded a detergent-soluble membrane/matrix fraction and an insoluble axonemal fraction. After detergent extraction of wild-type cells, the majority of Klpl within flagella remained bound to the axoneme (Fig. 6 A). Furthermore, Klpl remained attached to axonemes after incubation in HMDEK + 5 mM ATP (data not shown). These results indicated that Klpl was neither tightly associated with the flagellar membrane nor in the soluble matrix fraction.

Figure 7. Klpl labeling of axonemal whole mounts. Electron micrographs of axonemes labeled with Klpl antibody and 12-nm gold particles. (A–C) light-positive stain; (D) negative stain. (A) A typical axoneme showing the frayed distal end and the arc of the central pair. Labeling is restricted to one side of the central pair microtubules; note the absence of labeling at the distal 200–250 nm. Bar, 1 µm. (B) More of the central pair is exposed revealing a twist that is matched by a cross-over of the Klpl labeling (arrow) from one side of the central pair to the other. Bar, 1 µm. (C) An axoneme showing labeling along the entire length of the central pair microtubules and three points of cross-over (arrows). Note labeling of the central pair protruding from the basal end of an adjacent axoneme (star). Bar, 1 µm. (D) Negative stain reveals the two microtubules of the central pair, and that Klpl staining is along only one microtubule. The side view of the central pair at the point of twist (arrow) shows projections from a single microtubule. Also visible is an outer doublet of microtubules with dynein arms projecting towards the top of the page. Bar, 250 nm.

We also assayed for the presence of Klpl in flagella defective for specific axonemal structures. In flagella from pf5 cells (Adams et al., 1981; Warr et al., 1966), which are missing the central pair microtubules and their attached projections (9+0 axonemes), the level of Klpl protein was severely reduced relative to wild-type flagella (Fig. 6 B). We also examined other flagella with the 9+0 phenotype (pf8, pf9, and pf20) (Adams et al., 1981; Warr et al., 1966) and found that the level of Klpl was also low in flagella from these strains (data not shown).

In Chlamydomonas flagella, as in other types of eukaryotic flagella, projections attached to the central pair microtubules are in close proximity to radial spokes (Goodenough and Heuser, 1985; Hopkins, 1970), which extend from the A tubules of the outer doublet microtubules. It is possible that the central pair projections interact with the radial spokes to form a bridge between the central pair and outer doublet microtubules (Goodenough and Heuser, 1985; Hopkins, 1970; Warner and Satir, 1974). To determine if radial spokes were required for proper localization of Klpl, we examined flagella from the radial spokeless mutant, pf4 (Piperno et al., 1981; Witman et al., 1978), and found that Klpl localization to the flagellum was unaffected (Fig. 6 C) and, as in flagella from wild-type cells, Klpl was not extracted from pf4 axonemes by ATP treatment (data not shown).

In Chlamydomonas and other species, the two central pair microtubules of the flagellum are not structurally or biochemically equivalent (Dutcher et al., 1984; Linck et al., 1981). Attached to each central pair microtubule are characteristic projections that differ between the C1 and C2 microtubules (Adams et al., 1981; Hopkins, 1970), and a Chlamydomonas mutant (pf6) has been isolated, in which the C1 central pair microtubule is unstable relative to the C2 central pair microtubule (Dutcher et al., 1984). We examined flagellar fractions from pf6 cells by immunoblotting, and found that the localization of Klpl in these mutant flagella was identical to wild-type flagella (Fig. 6 D), suggesting that, at least in these mutant flagella, Klpl was capable of quantitative localization and stable binding to the C2 microtubule.

Immunolocalization of Klpl

The low level of Klpl in 9+0 flagella suggested that Klpl was bound specifically to the central pair apparatus of the axoneme. To test this hypothesis, we used anti-Klpl antibody for immunogold electron microscopy of axonemal whole mounts (Fig. 7). On every axoneme in these preparations, we found that where the axoneme had splayed open, Klpl labeled a single microtubule of the central pair microtubules, which were
identified by their characteristic arc in frayed axonemes, striated appearance, and distinctive plus-end cap (Dentler and Rosenbaum, 1977; Hopkins, 1970; Johnson and Rosenbaum, 1992). In regions where the central pair microtubules twisted and crossed over each other, the Klpl signal also crossed from one side to the other of the central pair microtubules (Fig. 7, B–C). Interestingly, gold labeling was always observed on the microtubule oriented towards the inside of the curved central pair microtubules. In regions where the axoneme had frayed extensively, or where the central pair microtubules had been released from the outer doublet microtubules, labeling was observed along the length of the central pair microtubule. However, labeling was rarely observed over the distal end of the central pair microtubules (~0.25 μm), a region that appears to lack central pair projections (Dentler and Rosenbaum, 1977). No labeling was observed in regions where the axoneme had remained intact, similar to what has been observed with other proteins that are found within the cylinder of the axoneme (Johnson and Rosenbaum, 1992). Labeling of negatively stained whole mounts confirmed that Klpl was associated with a single central pair microtubule (Fig. 7D) and suggested that Klpl labeling was in register with the central pair projections. However, the resolution of this technique is insufficient to determine if Klpl is attached to these projections or directly to the central pair microtubule. As controls, the Klpl antibody showed no labeling over 9+0 axonemes, and preimmune whole serum showed no labeling of axonemes above background (data not shown). Thus, in wild-type cells, Klpl was localized asymmetrically to the central pair apparatus, and was confined to only one of the two central pair microtubules or a structure associated with only one of the two central pair microtubules.

Postembedding labeling of thin sections was performed to determine which of the central microtubules binds Klpl. As predicted, Klpl antibody efficiently labeled the central pair apparatus in axonemal thin sections (30% of the transverse sections labeled, n = 200), and Klpl labeling was rarely observed over other regions of the axoneme (1% of the transverse sections, n = 200). Control samples lacking primary antibody failed to label any axonemal structures and antibodies directed against other axonemal components, e.g., radial spoke proteins, yielded only background label over the central pair microtubules (data not shown). The central pair microtubules can be distinguished from each other by the size of their projections, which in transverse section are ~18 nm in length on the C1 microtubule and ~8 nm on the C2 microtubule (Dutcher et al., 1984; Hopkins, 1970; Witman et al., 1978). In fixed and embedded axonemes, the projections on the C1 microtubule could be resolved, which allowed us to determine that Klpl labeling was always (n = 32) associated with the C2 microtubule (Fig. 8). Usually, only a single gold particle was present on each of the labeled transverse sections, and these particles were located on or near the C2 microtubule on the side away from the C1 microtubule. In ~10% of the labeled transverse sections, two or more particles were seen, also closely associated with the C2 microtubule (Fig. 8F). Since the C2 projections were not apparent in our preparations, we cannot say if Klpl was present on the C2 microtubule or on the C2-associated projections.

**Discussion**

Proteins of the kinesin superfamily are ubiquitous and have varied functions. We have cloned a gene from *Chlamydomonas, KLPI*, which encodes a protein with a typical kinesin motor domain. Using a Klpl-specific antibody, we have detected the Klpl protein in flagella, the first kinesin superfamily member of the eukaryotic flagellum to be cloned and characterized. Analysis of paralyzed flagellar mutants indicated that Klpl is part of the central pair apparatus, and immunogold localization showed that Klpl is bound specifically to central pair microtubule C2 or to projections that are attached to C2.
**Klpl Protein Structure**

The general design of Klpl is similar to many other members of the kinesin superfamily. The conserved kinesin motor domain at the NH₂-terminal region of Klpl suggests that Klpl, like most other characterized members of the kinesin superfamily, may encode a plus-end-directed microtubule dependent motor. The COOH-terminal tail domain of Klpl is novel, but as found in other members of the kinesin superfamily, it contains regions that have a high probability of forming coiled-coil structures. Thus, Klpl may form homodimers, as has been demonstrated for the *Drosophila* KHC polypeptide (de Cuevas et al., 1992; Kuznetsov et al., 1988), bind light chains (Gauger and Goldstein, 1993; Kuznetsov et al., 1988), or associate with other KRP s in a heteromeric complex (Cole et al., 1992, 1993). On immunoblots, Klpl appears as a 96-kD doublet, indicating that Klpl exists in multiple isoforms. Southern blot analysis showed that Klpl is a single copy gene in *Chlamydomonas* (unpublished data), indicating that isoforms arise either from alternative splicing of *KLP1* RNA or posttranslational modification of the Klpl protein. Many *Chlamydomonas* flagellar proteins are modified, including isoforms that are phosphorylated (Piperno et al., 1981; Williams et al., 1989) or acetylated (L’Hernault and Rosenbaum, 1985), suggesting that Klpl also undergoes posttranslational processing.

The head region of Klpl shares two tripeptide motifs with the fungal members of the BimC subfamily of kinesins (Fuller and Wilson, 1992; Goldstein, 1993b). Though small, the motifs found among Klpl and the BimC subfamily of proteins suggest that these proteins share conserved structural and/or functional features at these positions, as it has been shown that as few as three amino acids can constitute a structurally and functionally conserved motif. For example, the tripeptide sequence of N-X-S/T is necessary and sufficient to act as a site for addition of N-linked oligosaccharides to a protein (Kornfeld and Kornfeld, 1985), and peptide sequences of four amino acids have been shown to have a conserved structure that is sufficient to act as an internalization signal for cell surface receptors (Collawn et al., 1990, 1991).

Though the head domain of a kip generates force, it is the tail region of each protein that is thought to provide the functional specificity of a given motor protein by binding to a particular substrate (Goldstein, 1991; Goldstein, 1993a). The tail region of Klpl is novel and has no known homologue, suggesting that Klpl function is unique among the known members of the kinesin superfamily. To be functional, the tail domain of a kip must bind its substrate tightly, as is the case for the tail region of kinesin, which binds lipids with a high affinity and allows vesicles to be tethered to and moved along microtubule tracks (Henson et al., 1992; Leopold et al., 1992; Skoufias et al., 1994; Vale et al., 1985). The localization of Klpl on a central pair microtubule and a lack of vesicles within the core of the flagellum make it unlikely that the tail of Klpl binds to membranes. ATP hydrolysis dissociates the motor domain-microtubule interaction of kinesins (Chandra et al., 1993; Vale et al., 1985) leading to the extraction of some, though not all (Chandra et al., 1993; McDonald et al., 1990; Nislow et al., 1992; Sawin et al., 1992a; Sawin et al., 1992b; Yen et al., 1992), kips. Since ATP treatment does not release Klpl from the axoneme, it is probable that Klpl is bound tightly to the axoneme via its tail domain.

**Multiple Kinesin Like Protein within the *Chlamydomonas* Flagellum**

A single microtubule array, such as the mitotic spindle, may contain multiple classes of motor proteins (Sawin and Endow, 1993; Skoufias and Scholey, 1993). Similarly, in addition to the well characterized flagellar dyneins, the flagella of *Chlamydomonas* contain proteins that are putative members of the cytoplasmic dynein, myosin, and kinesin superfamilies (Kozminski, K., and J. Rosenbaum, unpublished). Among these non-dynein motor proteins are multiple members of the kinesin superfamily. We have obtained two PCR clones, distinct from *KLPl*, that encode members of the kinesin superfamily and which recognize mRNAs that are upregulated during flagellar regeneration (data not shown), implying that the kips encoded by these genes are flagellar proteins. Recently, other workers have used immunological and biochemical techniques to identify putative kips in the flagella of *Chlamydomonas*, though the identification of these proteins as bona fide members of the kinesin superfamily remains to be established. An antibody against the head domain of *Drosophila* KHC (Rodionov et al., 1991) has been used to identify a 110-kD flagellar protein (Johnson et al., 1994) and, using antipeptide antibodies against conserved regions of the kinesin motor domain (Cole et al., 1992; Sawin et al., 1992b), Fox and co-workers (1994) identified a different 110-kD protein, a 125-kD protein, and a 97-kD protein, which was shown to bind brain microtubules in vitro. It is unlikely that this 97-kD protein corresponds to Klpl because the 97-kD protein of Fox et al. (1994) reacted with an antipeptide antibody directed against the sequence HIPYRE-SKLT (Fox et al., 1994; Sawin et al., 1992b), whereas Klpl contains the sequence YVFQRTKLT at this position and does not react with the HIPYR antibody (data not shown). These results indicate that the flagella of *Chlamydomonas* contain at least five members of the kinesin superfamily.

**Targeting of Klpl**

Our results showed that in wild-type cells, Klpl was localized to the C2 central pair microtubule at the core of the flagellum. In cells lacking central pair microtubules, the amount of Klpl within the flagellum was severely reduced, indicating that in the absence of these structures, Klpl either failed to become concentrated within the flagellum or was degraded upon delivery to flagellum. In the absence of central pair microtubules, Klpl may lack a site of attachment, and so does not accumulate in the flagellum. Alternatively, in the central pairless mutants examined, Klpl may fail to be transported into the flagellum. Radial spoke proteins are thought to form partially assembled complexes within the cytoplasm of the cell body before transfer and complete assembly within the flagellum (Tao, W., D. Diener, and J. Rosenbaum, unpublished observation). A mutation in one radial spoke protein, rsp3, prevents transport and flagellar assembly of all radial spoke proteins (Piperno et al., 1977; Wittman et al., 1978), while mutations in other radial spoke proteins, rsp4 and rsp9, block transport and flagellar assembly of the subset of radial spoke proteins that form the spoke head (Huang et al., 1981; Piperno et al., 1977). Proteins associated with the central pair microtubules may also form preassembly complexes within the cell body, and only one of the proteins within such a complex needs to have a flagellar targeting signal. If complexes fail to assemble because of
mutations in any member of the assembly, then the localization of all members of the complex that lack their own localization signal would be blocked. Such a model is consistent with the failure of Klpl to localize in all mutants that lack central pair microtubules. A 110-kD flagellar klp (Johnson et al., 1994) is also targeted to the central pair apparatus. However, in contrast to Klpl, the central pairless mutants do not block flagellar accumulation of this 110-kD protein, suggesting that either Klpl and the 110-kD protein assemble into different structures of the central pair complex, or that the 110-kD protein contains an active flagellar localization signal.

Klps have been shown to be concentrated in specific regions of microtubule arrays, such as the midzone (Nislow et al., 1990, 1992; Yen et al., 1992), kinetochore (Yen et al., 1992), or spindle pole regions (Sawin et al., 1992a) of the mitotic spindle. Klpl is also associated with microtubules. However, Klpl shows a much more highly restricted localization pattern, being bound to only a single microtubule within the flagellum, which contains a total of 20 microtubules (nine outer doublet microtubules and two central pair microtubules). In *Chlamydomonas*, all microtubules are composed of identical α/β tubulin dimers (James et al., 1993; Younghblom et al., 1984). Yet, Klpl is bound specifically to the C2 microtubule, or to a C2-associated structure, indicating that Klpl (or a complex that contains Klpl) is capable of discriminating the C2 microtubule from the other 19 microtubules of the axoneme. Other axonemal structures are also bound to a subset of microtubules, e.g., the beads that are found within the B tubule of three of the nine outer doublet microtubules and the bridge structure that connects a single pair of outer doublet microtubules (Hoops and Witman, 1983). Other structures, though not restricted to a single microtubule or microtubule doublet, are assembled along the axoneme with highly characteristic spacings, e.g., radial spokes (Goodenough and Heuser, 1985; Hopkins, 1970; Warner and Satir, 1974; Witman et al., 1978), and dynein arm complexes (Goodenough and Heuser, 1985; Mastronarde et al., 1992; Warner, 1983).

**Function of the Klpl Protein**

We localized Klpl to one of the two central pair microtubules of the flagellum, where, presumably, it functions by exerting mechanical force. However, the dynamic result of Klpl activity remains undefined. Kinesin, the founding member of the kinesin superfamily, traverses the length of microtubules by moving along a single protofilament (Gelles et al., 1988; Kamimura and Mandelkow, 1992; Ray et al., 1993) and, via interactions through its tail domain (Skoufias et al., 1994), transports vesicles towards the plus-end of microtubules. Although the inside of the axonemal cylinder, where the central pair microtubules are localized, contains no vesicles, it is known that axonemal precursors are transported to and assembled at the distal end of the axoneme (Johnson and Rosenbaum, 1992; Rosenbaum et al., 1969; Witman, 1975). In central pairless mutants lacking Klpl, however, flagellar assembly is normal, indicating that Klpl is unlikely to be required for the transport of axonemal precursors.

To convert the sliding of outer doublet microtubules into flagellar beating, the dynein motors that drive sliding must be coordinately regulated (Warner and Satir, 1974). Two lines of evidence indicate that the central pair microtubules may be important in this process. Though the flagella of the central pairless mutants of *Chlamydomonas* are paralyzed, the dynein arms present on the outer doublet microtubules of these mutants are still capable of generating the forces required for microtubule sliding (Witman et al., 1978), which indicates that these flagella are paralyzed because they fail to coordinate dynein activity. Secondly, rotation and twisting of the central pair microtubules have been documented in a variety of organisms (Melkonian and Preisig, 1982; Omoto and Kung, 1979, 1980; Omoto and Witman, 1981; Tamm and Horridge, 1970), including *Chlamydomonas* (Kamiya, 1982), and in some cases has been found to show a strong correlation with the position of the ciliary beat (Omoto and Kung, 1980; Tamm and Horridge, 1970), suggesting that the orientation of the central pair apparatus may regulate dynein arm activity (Omoto and Kung, 1980; Warner and Satir, 1974). It is unclear, however, whether central pair rotation is a general property of all flagella (Tamm and Tamm, 1981). Given its position on the central pair apparatus, it is possible that Klpl could function in the rotation or twisting of central pair microtubules. In either of these cases, it would appear necessary that Klpl move or apply force radially between adjacent protofilaments of a central pair microtubule, rather than linearly along a single protofilament, as is the case for kinesin (Gelles et al., 1988; Kamimura and Mandelkow, 1992; Ray et al., 1993). This interpretation is consistent with the possibility that Klpl is part of projections attached to C2. Interestingly, in an in vitro motility assay, one klp, the minus-end-directed motor *ncd* has already been shown to generate a torque that twists microtubules (Chandra et al., 1993; Walker et al., 1990).

A second 110-kD klp in *Chlamydomonas* (Johnson et al., 1994) has a localization pattern that is strikingly similar to Klpl, i.e., association with a single microtubule of the central pair apparatus. We have no direct evidence on whether Klpl and this 110-kD protein bind to the same or to a different central pair microtubule. We did note, however, that in axonemal whole mounts Klpl labeling was always observed on the microtubule oriented towards the inside of the curved central pair microtubules, while the labeling associated with the 110-kD protein was always oriented towards the outside of the central pair microtubules, suggesting that the proteins are on different microtubules. If both Klpl and the 110-kD protein are required for central pair rotation or twisting, they could encode redundant or overlapping functions, or distinct functions that could be either synergistic or antagonistic. It will be interesting to determine how these klp move microtubules in vitro.

In the future, molecular cloning will identify the other klp present in the *Chlamydomonas* flagellum. These proteins may be associated with the central pair apparatus or they may be associated with the outer doublet microtubules, where they could be required for other dynein-independent movements within the flagellum. In particular, a number of movements have been described that are associated with the flagellar membrane, including gliding motility (Bloodgood, 1981; Lewin, 1952), bead movement (Bloodgood, 1977), tipping of glycoproteins during mating (Goodenough and Jurivich, 1978), and submembranous intraflagellar transport (Kozminski et al., 1993b). Additional members of the kinesin superfamily remain likely candidates for the motors that drive these processes.
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References

Adams, G. M., B. Huang, G. Piperno, and D. J. Luck. 1981. Central-pair microtubular complex of Chlamydomonas flagella: polypeptide composition as revealed by means of antibodies. J. Cell Biol. 91:69-76.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403-410.

Baker, E. J., L. R. Keller, J. A. Schloss, and L. J. Rosenbaum. 1986. Protein synthesis is required for rapid degradation of tubulin mRNA and other deflagellation-induced RNAs in Chlamydomonas reinhardtii. Mol. Cell. Biol. 6:54-60.

Bankier, A. T., and B. G. Barrell. 1983. Shotgun DNA sequencing. In Techniques in the Life Sciences. Biochemistry-Vol. BS. R. A. Flavell, editor. Elsevier, Cambridge, MA. pp. 1-34.

Bloodgood, R. A. 1977. Motility occurring in association with the surface of the Chlamydomonas flagellum. J. Cell Biol. 75:983-989.

Bloodgood, R. A. 1981. Flagella-dependent gliding motility in Chlamydomonas. Protoplasma. 106:183-192.

Bone, G. S., V. D. S. N. Patel, and T. J. Brady. 1988. Native structure and properties of bovine brain kinesin and identification of the ATP-binding subunit polypeptide. Biochemistry. 27:3409-3416.

Brady, S. T. 1985. A novel ATPase with properties expected for the fast axonal transport motor. Nature (Lond.). 317:73-75.

Chandra, R., E. D. Salmon, H. P. Erickson, A. Lockhart, and S. A. Endow. 1993. Structural and functional domains of the Drosophila naked microtubule motor protein. J. Biol. Chem. 268:9005-9013.

Cheshire, J. L., and L. R. Keller. 1991. Uncoupling of Chlamydomonas flagellar gene expression and outgrowth from flagellar excision by manipulation of Ca²⁺. J. Cell Biol. 113:1561-1569.

Cole, D. G., W. Z. Cande, R. J. Baskin, D. A. Soufas, C. J. Hog, and J. M. Scholey. 1992. Isolation of a sea urchin egg kinesin-related protein using peptide antibodies. J. Cell Biol. 101:291-301.

Collawn, J. F., L. A. Kuhn, F. P. Weedsman, K. Hall, T. Vuong, and J. M. Scholey. 1993. Novel heterotrimeric kinesin-related protein purified from sea urchin eggs. Nature (Lond.). 366:268-270.

Collawn, J. F., L. A. Kuhn, F. P. Weedsman, K. Hall, T. Vuong, and J. M. Scholey. 1993. Novel heterotrimeric kinesin-related protein purified from sea urchin eggs. Nature (Lond.). 366:268-270.

Hoch, L. A., K. E. Sawin, and W. S. Sale. 1994. Kinesin-related proteins in Chlamydomonas. J. Cell Biol. 118:109-120.

Huang, B. G., G. Piperno, Z. Ramani, and D. J. Luck. 1981. Radial spokes of Chlamydomonas flagella: genetic analysis of assembly and function. J. Cell Biol. 88:80-88.

James, S. W., C. D. Silflow, P. Stroom, and P. Lefebvre. 1992. A mutation in the al-tubulin gene of Chlamydomonas reinhardtii confers resistance to anti-microtubule herbicides. J. Cell Sci. 106:209-218.

Johnson, K. A., M. A. Haas, and L. J. Rosenbaum. 1994. Localization of a kinesin-related protein to the central pair apparatus of the Chlamydomonas reinhardtii flagellum. J. Cell Sci. In press.

Johnson, K. A., and L. J. Rosenbaum. 1992. Polarity of flagellar assembly in Chlamydomonas. J. Cell Biol. 119:1605-1611.

Kaminska, S., and E. Mandelkow. 1992. Tubulin protofilaments and kinesin-dependent motility. J. Cell Biol. 118:865-877.

Kamiya, R. 1982. Excursion and rotation of the central-pair microtubules in detergent-treated Chlamydomonas flagella. Cell Motil. (Suppl.). 1:169-173.

Kindle, K. L., R. A. Schnell, E. Fernandez, and P. A. Lefebvre. 1989. Stable nuclear transformation of Chlamydomonas using a gene for nitrile reductase. J. Cell Biol. 109:2589-2601.

Kornfeld, R., and S. Kornfeld. 1985. Assembly of asparagine-linked oligosaccharides. Annu. Rev. Biochem. 54:631-664.

Kozminski, K. D., C. D. Silllow, P. Stroom, and P. Lefebvre. 1992. A mutation in the al-tubulin gene of Chlamydomonas reinhardtii confers resistance to anti-microtubule herbicides. J. Cell Sci. 106:209-218.

Kornfeld, R., and S. Kornfeld. 1985. Assembly of asparagine-linked oligosaccharides. Annu. Rev. Biochem. 54:631-664.

Kozminski, K. D., C. D. Silllow, P. Stroom, and P. Lefebvre. 1992. A mutation in the al-tubulin gene of Chlamydomonas reinhardtii confers resistance to anti-microtubule herbicides. J. Cell Sci. 109:2589-2601.

Kornfeld, R., and S. Kornfeld. 1985. Assembly of asparagine-linked oligosaccharides. Annu. Rev. Biochem. 54:631-664.

Kozminski, K. D., C. D. Silllow, P. Stroom, and P. Lefebvre. 1992. A mutation in the al-tubulin gene of Chlamydomonas reinhardtii confers resistance to anti-microtubule herbicides. J. Cell Sci. 109:2589-2601.

Kornfeld, R., and S. Kornfeld. 1985. Assembly of asparagine-linked oligosaccharides. Annu. Rev. Biochem. 54:631-664.

Kozminski, K. D., C. D. Silllow, P. Stroom, and P. Lefebvre. 1992. A mutation in the al-tubulin gene of Chlamydomonas reinhardtii confers resistance to anti-microtubule herbicides. J. Cell Sci. 109:2589-2601.

Leopold, P. L., A. W. McDowall, K. K. Pfister, G. S. Bloom, and S. T. Brady. 1989. Stable nuclear transformation of Chlamydomonas using a gene for nitrile reductase. J. Cell Biol. 109:2589-2601.

Kornfeld, R., and S. Kornfeld. 1985. Assembly of asparagine-linked oligosaccharides. Annu. Rev. Biochem. 54:631-664.

Kozminski, K. D., C. D. Silllow, P. Stroom, and P. Lefebvre. 1992. A mutation in the al-tubulin gene of Chlamydomonas reinhardtii confers resistance to anti-microtubule herbicides. J. Cell Sci. 109:2589-2601.

Kornfeld, R., and S. Kornfeld. 1985. Assembly of asparagine-linked oligosaccharides. Annu. Rev. Biochem. 54:631-664.

Kozminski, K. D., C. D. Silllow, P. Stroom, and P. Lefebvre. 1992. A mutation in the al-tubulin gene of Chlamydomonas reinhardtii confers resistance to anti-microtubule herbicides. J. Cell Sci. 109:2589-2601.

Kornfeld, R., and S. Kornfeld. 1985. Assembly of asparagine-linked oligosaccharides. Annu. Rev. Biochem. 54:631-664.

Kozminski, K. D., C. D. Silllow, P. Stroom, and P. Lefebvre. 1992. A mutation in the al-tubulin gene of Chlamydomonas reinhardtii confers resistance to anti-microtubule herbicides. J. Cell Sci. 109:2589-2601.

Kornfeld, R., and S. Kornfeld. 1985. Assembly of asparagine-linked oligosaccharides. Annu. Rev. Biochem. 54:631-664.

Kozminski, K. D., C. D. Silllow, P. Stroom, and P. Lefebvre. 1992. A mutation in the al-tubulin gene of Chlamydomonas reinhardtii confers resistance to anti-microtubule herbicides. J. Cell Sci. 109:2589-2601.

Kornfeld, R., and S. Kornfeld. 1985. Assembly of asparagine-linked oligosaccharides. Annu. Rev. Biochem. 54:631-664.
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Quarmby, L. M., Y. G. Yueh, J. L. Cheshire, L. R. Keller, W. J. Snell, and Ray, S. 1993. Kinesin follows Sawin, K. E., T. J. Mitchison, and L. G. Wordeman. 1992b. Evidence for Sannders, W. S., and M. A. Hoyt. 1992. Kinesin-related proteins required for Sawin, K. E., K. LeGuellec, M. Philippe, and T. J. Mitchison. 1992a. Mitotic Rodionov, V. I., F. K. Gyoeva, and V. I. Geifand. 1991. Kinesin is responsible Rosenbaum, J. L., J. E. Moulder, and D. L. Ringu. 1969. Flagellar elongation Sanders, M. A., and J. L. Salisbury. 1989. Centrin-mediated microtubule Structural and control processes in the basal bodies and flagella of Chlamydomonas reinhardii. Dev. Biol. 111:511-522. Omoto, C. K., and C. Kung. 1980. Rotation and twist of the central-pair stalk components. J. Cell Biol. 87:33-46. Omoto, C. K., and G. B. Witman. 1981. Functionally significant central-pair rotation in a primitive eukaryotic flagellum. Nature (Lond.). 290:708-710. Page, B., and M. Snyder. 1992. CIRK: a developmentally regulated spindle pole body-associated protein important for microtubule functions in Saccharomyces cerevisiae. Genes & Dev. 6:1414-1429. Piperno, G., B. Huang, and D. J. Luck. 1977. Two-dimensional analysis of flagellar proteins from wild-type and paralyzed mutants of Chlamydomonas reinhardii. Proc. Natl. Acad. Sci. USA. 74:1600-1604. Piperno, G., B. Huang, Z. Ramanis, and D. J. Luck. 1981. Radial spokes of Chlamydomonas flagella: polypeptide composition and phosphorylation of stalk components. J. Cell Biol. 88:73-79. Quarmby, L. M., Y. G. Yueh, J. L. Cheshire, L. R. Keller, W. J. Snell, and R. C. Crain. 1992. Insoluble phospholipid metabolism may trigger flagellar excision in Chlamydomonas reinhardii. J. Cell Biol. 116:737-744. Randall, J., T. Cavalier-Smith, A. McVittie, J. R. Warr, and J. M. Hopkins. 1967. Developmental and control processes in the basal bodies and flagella of Chlamydomonas reinhardii. Dev. Biol. 1(Suppl.):43-83. Ray, S., E. Meyhofer, R. A. Milligan, and J. Howard. 1993. Kinesin follows the microtubule protofilament axis. J. Cell Biol. 121:1083-1089. Rodionov, V. I., F. K. Gyoeva, and V. I. Geifand. 1991. Kinesin is responsible for centriugal movement of pigment granules in melanophores. Proc. Natl. Acad. Sci. USA. 88:4956-4960. Roof, D. M., P. B. Meluh, and M. D. Rose. 1992. Kinesin-related proteins required for assembly of the mitotic spindle. J. Cell Biol. 118:95-108. Rosenbaum, J. L., J. E. Moulder, and D. L. Ringo. 1969. Flagellar elongation and shortening in Chlamydomonas. The use of cycloheximide and colchicine to study the synthesis and assembly of flagellar proteins. J. Cell Biol. 62:387-399. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. Sanders, M. A., and J. L. Salisbury. 1989. Centrin-mediated microtubule severing during flagellar excision in Chlamydomonas reinhardii. J. Cell Biol. 108:1751-1768. Saunders, W. M., and A. H. Hoyt. 1992. Kinesin-related proteins required for structural integrity of the mitotic spindle. Cell. 70:451-458. Sawin, K. E., and S. A. Endow. 1993. Meiosis, mitosis and microtubule motors. Bioessays. 15:399-407. Sawin, K. E., K. LeGuellec, M. Philippe, and T. J. Mitchison. 1992a. Mitotic spindle organization by a plus-end-directed microtubule motor. Nature (Lond.). 359:540-543. Sawin, K. E., T. J. Mitchison, and L. G. Worderman. 1992b. Evidence for kinesin-related proteins in the mitotic apparatus using peptide antibodies. J. Cell Biol. 101:303-313. Schröer, T. A., B. J. Schnapp, T. S. Reese, and M. P. Sheetz. 1988. The role of kinesin and other soluble factors in organelle movement along microtubules. J. Cell Biol. 107:1785-1792. Silflow, C. D., and J. L. Rosenbaum. 1981. Multiple α- and β-tubulin genes in Chlamydomonas and regulation of tubulin mRNA levels after deflagellation. Cell. 24:81-88.