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IC138 Defines a Subdomain at the Base of the I1 Dynein That Regulates Microtubule Sliding and Flagellar Motility

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To understand the mechanisms that regulate the assembly and activity of flagellar dyneins, we focused on the I1 inner arm dynein (dynein *f*) and a null allele, *bop5*-2, defective in the gene encoding the IC138 phosphoprotein subunit. I1 dynein assembles in *bop5*-2 axonemes but lacks at least four subunits: IC138, IC97, LC7b, and flagellar-associated protein (FAP) 120—defining a new I1 subcomplex. Electron microscopy and image averaging revealed a defect at the base of the I1 dynein, in between radial spoke 1 and the outer dynein arms. Microtubule sliding velocities also are reduced. Transformation with wild-type *IC138* restores assembly of the IC138 subcomplex and rescues microtubule sliding. These observations suggest that the IC138 subcomplex is required to coordinate I1 motor activity. To further test this hypothesis, we analyzed microtubule sliding in radial spoke and double mutant strains. The results reveal an essential role for the IC138 subcomplex in the regulation of I1 activity by the radial spoke/phosphorylation pathway.

INTRODUCTION

The dynein motors form the inner and outer rows of arm structures attached to the doublet microtubules of cilia and flagella (Porter and Sale, 2000; Smith and Yang, 2004). Several lines of evidence have indicated that the outer and inner arm dyneins are functionally distinct, differing in their subunit composition and organizational arrangement in the axoneme. In *Chlamydomonas reinhardtii*, a model genetic organism for cilia/flagellar studies, the inner dynein arms are composed of at least seven different dynein subspecies precisely organized in a 96-nm repeat pattern along with the outer dynein arms, radial spokes, and dynein regulatory complex (DRC) (Figure 1, Goodenough and Heuser, 1985a,b; Mastronarde *et al*., 1992; Porter *et al*., 1996; Porter and Sale, 2000; Nicastro *et al*., 2006; Wirschell *et al*., 2007; Bui *et al*., 2008; King and Kamiya, 2009). Genetic and phenotypic analyses have shown that the inner arm dyneins are responsible for control of the size and shape of the axonemal bend (Brokaw and Kamiya, 1987; Brokaw, 1994, 2008; Kamiya, 2002; King and Kamiya, 2009). However, we do not know how each dynein isoform is localized to its unique position in the 96-nm repeat structure or how the activity of each isoform is regulated.

To address general questions of assembly and regulation of dynein, we have taken advantage of motility mutants in *Chlamydomonas* and focused on the structural and functional properties of the I1 inner arm dynein, also known as dynein *f* (reviewed in Porter and Sale, 2000; Kamiya, 2002; Wirschell *et al*., 2007; King and Kamiya, 2009). I1 dynein is the only two-headed inner arm dynein and is located near the base of radial spoke 1, where it forms a trilobed structure at the proximal end of the axonemal 96-nm repeat (Figure 1). I1 is composed of two heavy chains (HCs), 1α and 1β; three intermediate chains, IC140, IC138, and IC97; and several light chains, including LC7a, LC7b, LC8, Tctex1, and Tctex2b (Goodenough and Heuser, 1985a,b; Piperno *et al*., 1990; Smith and Sale, 1991, 1992; Porter *et al*., 1992; Myster *et al*., 1997, 1999; Harrison *et al*., 1998; Perrone *et al*., 1998, 2000; Yang and Sale, 1998; DiBella *et al*., 2004a,b; Hendrickson *et al*., 2004; Wirschell *et al*., 2009).

Mutations that disrupt specific I1 subunits, or specific domains of those subunits, often result in assembly of incomplete or partial I1 dynein complexes, useful for revealing protein interactions, structural domains, and regulatory functions. For example, the *bop5-1* mutant expresses a truncated IC138 and assembles all of the I1 dynein subunits with the exception of LC7b and FAP120, a recently identified I1-dynein associated protein, revealing an interaction between the C terminus of IC138, LC7b, and FAP120 (Hendrickson *et al*., 2004; Ikeda *et al*., 2009; this study). Likewise, mutant strains expressing truncated dynein HCs that lack the motor domains of either the 1α or 1β HC still assemble the remaining I1 dynein subunits (Myster *et al*., 1999; Per-

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Abbreviations used: DRC, dynein regulatory complex; FAP, flagellar-associated protein; HC, heavy chain; IC, intermediate chain; LC, light chain; MBO, move backwards only; PKI, protein kinase inhibitor; TAP, Tris acetate phosphate.

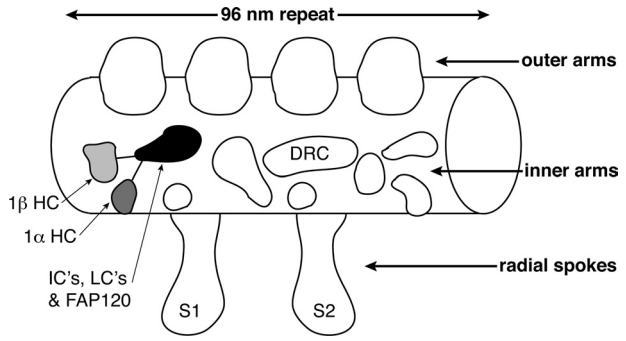

Figure 1. Proposed organizational structure of I1 dynein within 96-nm axoneme repeat. The schematic diagram shows the proposed location of the IC/LC complex based on previous studies of I1 dynein motor domain mutants (Myster *et al.*, 1998; Perrone *et al.*, 2000; modified from Porter and Sale, 2000).

rone *et al.*, 2000). Electron microscopy (EM) of isolated axonemes revealed the location of the globular motor domains within the trilobed structure of the I1 dynein and thereby indirectly suggested the location of the IC/LC complex at the base of I1 dynein (Myster *et al.*, 1999; Perrone *et al.*, 2000). One goal is to test this model for organization of the IC/LC domain in the axoneme (Figure 1).

Diverse evidence indicates that I1 dynein plays a key role in regulation of microtubule sliding by a mechanism involving phosphorylation of IC138 (reviewed in Porter and Sale, 2000; Smith and Yang, 2004; Wirschell *et al.*, 2007). Unlike other axonemal dyneins, the isolated I1 complex does not efficiently translocate microtubules in in vitro motility assays, possibly indicating a novel regulatory function (Smith and Sale, 1991; Kagami and Kamiya, 1992; Kotani *et al.*, 2007; Kikushima and Kamiya, 2008). Mutations in I1 assembly or IC138 phosphorylation result in altered axonemal bending and disrupted phototaxis, suggesting an important role in these processes (Brokaw and Kamiya, 1987; King and Dutcher, 1997; Hennessey *et al.*, 2002; Okita *et al.*, 2005). In addition, mutations in I1 subunits suppress paralysis in a central pair mutant, indicating a functional link between I1 activity and the central pair apparatus/radial spoke mechanism for control of microtubule sliding (Porter *et al.*, 1992; Smith, 2002). In vitro functional assays using isolated axonemes also have revealed an essential role for I1 dynein in the regulation of microtubule sliding through a mechanism that seems to involve the radial spokes and reversible phosphorylation of IC138 (Habermacher and Sale, 1997; King and Dutcher, 1997; Yang and Sale, 2000; Hendrickson *et al.*, 2004). Thus, we predict that assembly of IC138 is required for regulation of microtubule sliding by the radial spokes.

Here, we focus on IC138 and a null allele, *bop5-2*, obtained by insertional mutagenesis, that lacks IC138. Most I1 dynein subunits are present in *bop5-2* axonemes, but IC138, IC97, FAP120, and presumably LC7b are missing. We propose that these proteins form a regulatory unit, referred to as the "IC138 subcomplex." Analysis of *bop5-2* axonemes by EM and computer image averaging revealed a defect at the base of the I1 dynein, thus confirming the location of the IC138 subcomplex in the 96-nm axoneme repeat. Microtubule sliding velocities are also reduced in *bop5-2* axonemes. Transformation with the wild-type *IC138* gene restores assembly of the IC138 subcomplex and rescues microtubule sliding. We also analyzed microtubule sliding in a double mutant lacking the radial spoke heads (*pf17*) and IC138. As expected, treatment with protein kinase inhibitors increased sliding velocities in *pf17* axonemes, but not in *pf17 bop5-2* double mutants. Thus, the IC138 subcomplex is required for regulation of microtubule sliding by the central pair/radial spoke/phosphorylation pathway, but not for I1 assembly or targeting in the axoneme.

MATERIALS AND METHODS

Strains, Culture Conditions, and Genetic Analysis

Strains used in this study are summarized in Table 1. The *bop2-5* strain (6F5) was generated by transformation of the A54e18 strain (*nit1*, *ac17*, *mt plus*) with the plasmid pMN56 containing the nitrate reductase gene *NIT1* (Myster *et al.*, 1997). Cells were maintained on Tris acetate phosphate (TAP) medium (Harris, 1989). Cultures were resuspended in minimal medium or 10 mM HEPES, pH 7.6, and bubbled overnight to facilitate flagellar assembly and analysis of motility.

To determine whether the motility phenotype was linked to the *NIT1* plasmid, *bop5-2* was crossed to L8 (*nit1-305*, *apm1-19*, *mt minus*), and the resulting progeny of seven tetrads and additional random progeny were analyzed for their motility phenotypes and their ability to grow on selective media lacking ammonium. IC138 rescued strains were generated by transformation of *bop5-2* with a plasmid containing the wild-type *IC138* gene (Hendrickson *et al.*, 2004) and the selectable marker pSI103 containing the *aphVIII* gene (Sizova *et al.*, 2001) and plating cells on TAP medium containing 10 μg/ml paromomycin. Because transformation with *IC138* did not clearly rescue the motility defects, whole cell extracts of the transformants were screened on Western blots probed with the IC138 antibody. These and other experiments revealed the presence of a second, closely linked *mbo-like* mutation in the *bop5-2* strain in addition to the IC138 deletion. Dominance tests were performed by mating *bop5-2*, which also contains an *ac17* mutation, to an *arg7-2* strain and selecting for diploid cells on minimal medium. All diploids were mating type minus and displayed wild-type motility.

Southern Blot and Polymerase Chain Reaction (PCR) Analyses

Isolation of genomic DNA, restriction enzyme digests, agarose gels, and Southern blots were performed as described previously (Perrone *et al.*, 2000, 2003; Rupp *et al.*, 2001, 2003). PCR primers were designed using the sequence of the *IC138* gene (GenBank accession AY743342); the JGI *Chlamydomonas* genome database, versions 1.0, 2.0, and 3.0 (http://genome.jgi-psf.org//Chlre3/Chlre3.home.html); and the MacVector software package (MacVector, Cary, NC).

Isolation of Axonemes and Dynein Purification

Flagella were isolated by pH shock or dibucaine treatment and demembranated using 0.1–0.5% IGEPAL CA-630 (Sigma-Aldrich, St. Louis, MO) as described previously (Witman, 1986). Axonemes were resuspended in HMEEN (10 mM HEPES, pH 7.4, 5 mM $MgSO_4$, 1 mM EGTA, 0.1 mM EDTA, and 30 mM NaCl) plus 1 mM dithiothreitol and 0.1 μg/ml protease inhibitors (leupeptin, aprotinin, and pepstatin). Dynein extraction, dialysis, and sucrose gradient centrifugation were performed as described previously (Myster *et al.*, 1997, 1999; Perrone *et al.*, 1998, 2000).

SDS-Polyacrylamide Gel Electrophoresis (PAGE) and Western Blot Analysis

Samples were analyzed on 5–15% polyacrylamide, 0–2.4 M glycerol gradient gels. Protein was transferred to Immobilon P (Millipore, Billerica, MA), and total protein on the blot was visualized with Blot FastStain (Millipore Bioscience Research Reagents, Temecula, CA). The following antibodies were used at the indicated dilutions: 1α DHC, 1:1000 (Myster *et al.*, 1997); IC140, 1:10,000 (Yang and Sale, 1998); IC138, 1:20,000 (Hendrickson *et al.*, 2004); IC97, 1:10,000 (Wirschell *et al.*, 2009); IC69, 1:10:000 (Sigma-Aldrich); FAP120, 1:10,000 (Ikeda *et al.*, 2009); move backwards only (MBO) 2, 1:10,000 (Tam and Lefebvre, 2002); tctex1, 1:50 (Harrison *et al.*, 1998); and tctex2b, 1:50 (DiBella *et al.*, 2004b). Immunoreactive bands were detected using alkaline phosphatase-conjugated secondary antibodies and a chemiluminescent detection system (Tropix, Bedford, MA).

EM and Image Analysis

Axonemes were prepared for EM (Porter *et al.*, 1992), and the methods for digitization and image averaging of thin sections were as described previously (Mastronarde *et al.*, 1992; O'Toole *et al.*, 1995).

Analysis of Flagellar Motility and Microtubule Sliding

The motility phenotypes of freely swimming cells were monitored using an Axioscope (Carl Zeiss, Thornwood, NY) equipped with phase contrast optics and a halogen light source (Porter *et al.*, 1992; Myster *et al.*, 1997, 1999). Selected fields were recorded using a Rolera-MGi EMCCD camera (Q-Imag-

Table 1. Strains used in this study

Strain name	Motility	Axoneme phenotype	References
Control strains			
137c (*nit1 nit2*) (CC-125)	Fast forward	Wild type	Harris (1989)
A54 e18 (*nit1-1 ac17 sr1*) (CC-2929)	Fast forward	Wild type	
L5 (*nit1, apm1-19, mt+*) (CC-4263)	Fast forward	Wild type	Tam and Lefebvre (1993)
L8 (*nit1, apm1-19, mt−*) (CC-4264)	Fast forward	Wild type	Tam and Lefebvre (1993)
arg7-8 (CC-1826)	Fast forward	Wild type	Harris (1989)
mbo2-1 (CC-2377)	Move backwards	Missing *mbo* proteins	Segal *et al*. (1984)
mbo3-1 (CC-3670)	Mixed motility	Reduced *mbo* proteins	Segal *et al*. (1984)
I1 related strains			
pf9-3 (CC-3913)	Slow forward	Missing I1 dynein	Myster *et al*. (1997)
bop5-1 (CC-4080)	Slow forward	Truncated IC138, loss of LC7b, FAP120	Dutcher *et al*. (1988); Hendrickson *et al*. (2004); Ikeda *et al*. (2009)
bop5-2 (CC-4284)	Mixed motility	Missing IC138 subcomplex, MBO2p	This study
bop5-2::IC138 (CC-4285)	Mixed motility, mostly backwards	Missing MBO2p	This study
pf17 (CC-262)	Paralyzed	Missing radial spokes	Harris (1989)
pf17 bop5-2	Paralyzed	Missing radial spokes and IC138 subcomplex	This study
pf17 bop5-2::IC138	Paralyzed	Missing radial spokes	This study
Diploid strains			
bop5-2, ac17, ARG7 / *BOP5, AC17, arg7-8*	Fast forward	Not tested	This study

ing, Tucson, AZ) and analyzed using the MetaMorph software package, version 7.1.7.0 (Molecular Devices, Sunnyvale, CA).

Microtubule sliding velocity was measured using the method of Okagaki and Kamiya (1986) and as described previously (Howard *et al*., 1994; Habermacher and Sale, 1996, 1997; Hendrickson *et al*., 2004). Briefly, isolated flagella were resuspended in buffer without protease inhibitors, demembranated with buffer containing 0.5% Nonidet-P-40, and added to perfusion chambers. Microtubule sliding was initiated by the addition of buffer containing 1 mM ATP and 3 μg/ml subtilisin A type VIII protease (Sigma-Aldrich). Sliding was recorded using an Axiovert 35 microscope (Carl Zeiss) equipped with dark field optics and a silicon intensified camera (VE-1000; Dage-MTI, Michigan City, IN). The video images were converted to a digital format using LabVIEW 7.1 software (National Instruments, Austin, TX). Sliding velocity was determined manually by measuring microtubule displacement on tracings calibrated with a micrometer.

RESULTS

Identification of a Null Mutation in IC138

To identify new alleles at the *IC138/BOP5* locus, we screened a collection of motility mutants generated by insertional mutagenesis with the nitrate reductase gene *NIT1*. This collection has been used previously to identify mutations in other I1 dynein subunits, central pair proteins, and the DRC (Myster *et al*., 1997; Perrone *et al*., 1998, 2000; Rupp *et al*., 2001; Rupp and Porter, 2003). Southern blots of genomic DNA isolated from >50 mutant strains were hybridized with a 9-kb fragment containing the complete *IC138* gene. One strain, 6F5, was associated with a significant rearrangement of the *IC138* gene (Supplemental Figure 1). PCR of wild-type and 6F5 DNA indicated that ~20 kb of genomic DNA has been deleted, including >90% of the *IC138* transcription unit (Figure 2). To confirm that the mutant motility phenotype is linked to the insertion of the *NIT1* plasmid and associated deletion, we backcrossed the 6F5 strain, now known as *bop5-2*, to a *nit1* strain with wild-type motility (L8). Analysis of tetrad progeny showed that the motility defects cosegregated with the ability to grow on ammonium-free medium and the absence of *IC138* (see *Materials and Methods*).

Loss of IC138 Disrupts the Assembly of a Subset of I1 Dynein Subunits

Null mutations in other I1 dynein subunits, such as the two dynein HCs and IC140, typically result in the failure to assemble the I1 dynein complex into the flagellar axoneme (Myster *et al*., 1997, 1999; Perrone *et al*., 1998, 2000). To determine whether the loss of IC138 has a similar phenotype, we analyzed axonemes from wild-type and mutant strains on Western blots probed with antibodies to dynein subunits (Figure 3). Consistent with previous reports, the 1α HC, IC140, and IC138 are missing or reduced in the *IC140* mutant *ida7-1*, and IC138 is truncated in *bop5-1* (Perrone *et al*., 1998; Hendrickson *et al*., 2004). However, although *bop5-2* axonemes lack IC138, both the 1α HC and IC140 are present at wild-type levels (Figure 3B). Recent studies have identified two other I1 dynein-associated proteins, IC97 (Wirschell *et al*. 2009) and the axoneme polypeptide FAP120 (Ikeda *et al*., 2009). Western blots probed with antibodies to IC97 and

Figure 2. Deletion of the *IC138* gene in the *bop5-2* strain. Shown here is a schematic diagram of the intron-exon structure of the *IC138* gene in wild-type and the deleted region in *bop5-2*. PCR with gene-specific primers demonstrated that only the 5′ end and first exon of the *IC138* gene is retained in *bop5-2*. Additional PCR reactions and Southern blots showed that the deletion extends ~15 kb beyond the 3′ end of the *IC138* gene.

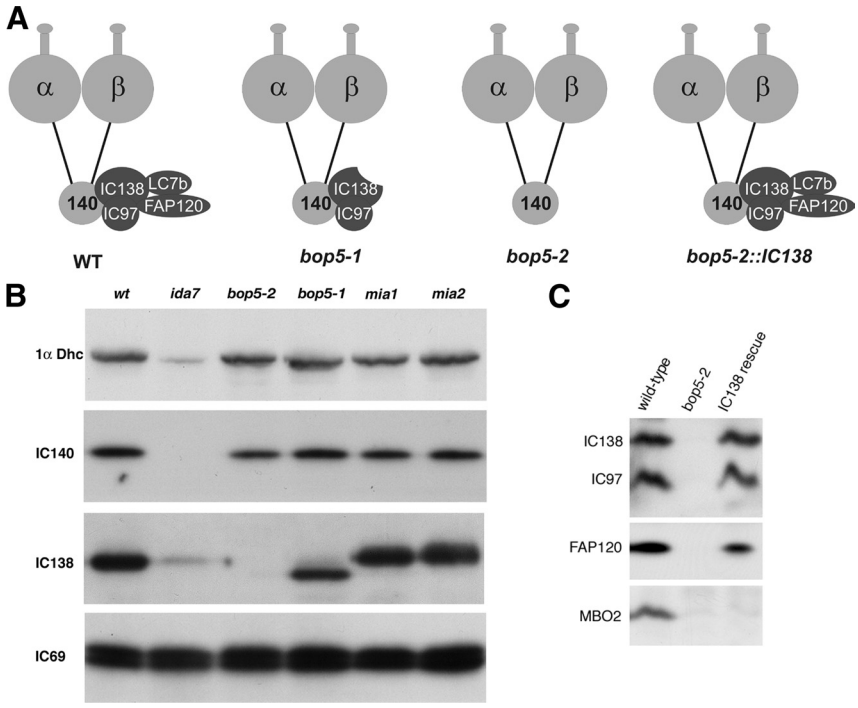

Figure 3. Identification of an IC138 subcomplex within the I1 dynein. (A) Schematic diagrams of the IC138 subcomplex in I1 dyneins from wild-type, *bop5-1*, *bop5-2*, and IC138 rescued (*bop5-2::IC138*) strains. The other I1 dynein LCs are not shown here. (B) Western blot of isolated axonemes from wild-type and mutant cells. Note the presence of IC140 and the 1α HC in the *bop5-2* axonemes, but the absence of IC138. As reported previously, IC138 is truncated in *bop5-1* and shifted by hyperphosphorylation in *mia1* and *mia2*. The outer arm subunit IC69 serves as a loading control. (C) Western blot of isolated axonemes from wild-type, *bop5-2*, and IC138 rescued cells. IC138, IC97 and FAP120 are missing in *bop5-2* axonemes, but all three polypeptides are restored in axonemes from the IC138 rescued strain. Note that the MBO2 protein is not part of the IC138 subcomplex, because it missing in axonemes from both *bop5-2* and the IC138 rescued strain.

FAP120 revealed that these two proteins are missing in *bop5-2* axonemes (Figure 3C). Because LC7b is a subunit of both I1 and outer arm dyneins (DiBella *et al*., 2004a), we did not analyze its assembly in *bop5-2*. However, previous studies of *bop5-1* have shown that the I1 dynein lacks LC7b when IC138 is truncated (Hendrickson *et al*., 2004). Thus, it is likely that I1 dynein lacks LC7b when IC138 is missing (Figure 3A).

To further characterize the effect of the loss of IC138 on I1 dynein, we prepared dynein extracts by incubation of isolated axonemes with 0.6 M NaCl and then fractionated the extracts on sucrose density gradients. Western blots of sucrose gradient fractions were probed with a series of antibodies against I1 dynein subunits. In wild-type extracts, all of the I1 dynein subunits cosediment at ~20–21 S (Figure 4A); but in *bop5-2* extracts, the I1 dynein is partially dissociated (Figure 4B). The 1α HC and IC140 cosediment across a broad region, with a peak at ~15–16 S, whereas two LCs, Tctex1 and Tctex2b, sediment more slowly near the top of the gradient. Thus, loss of the IC138 subcomplex does not impact the assembly of I1 dynein into the axoneme, but it does affect the stability of the I1 complex in high salt extracts.

Rescue of IC138 Assembly Defects

We previously transformed the *bop5-1* mutation with a wild-type copy of the *IC138* gene and observed rescue of the slow swimming motility phenotype and reassembly of the full-length IC138 polypeptide (Hendrickson *et al*., 2004). However, in initial efforts to rescue the *bop5-2* motility defect with *IC138*, we failed to recover transformants with wild-type motility. These observations suggested that there must be a second mutation in *bop5-2* that affects motility even though IC138 has reassembled into the axoneme (see below). We therefore analyzed whole cell extracts from 74 transformants on Western blots probed with an antibody specific for IC138 and identified three strains that expressed wild-type levels of the IC138 polypeptide in their cytoplasm. Western blots of isolated axonemes confirmed that re-expression of IC138 was accompanied by the reassembly of IC138, IC97, and FAP120 (Figure 3C). These results demonstrate that the assembly of IC97 and FAP120 is dependent on the presence of IC138 and that IC138, IC97, LC7b, and FAP120 form a subcomplex within the I1-dynein.

Localization of the IC138 Subcomplex within the Structure of the I1 Dynein

Previous work has shown that the I1 dynein forms a trilobed structure located at the proximal end of the 96-nm axoneme repeat (Goodenough and Heuser, 1985a,b; Piperno and Ramanis, 1991; Mastronarde *et al*., 1992; Nicastro *et al*., 2006; Figure 1). We also analyzed several strains in which constructs encoding the amino-terminal portions of the two I1 HCs were used to restore the assembly of I1 dyneins lacking one or the other I1 motor domain (Myster *et al*., 1999; Perrone *et al*., 2000). Analysis of isolated axonemes by thin section EM and image averaging identified the position of the two motor domains within the structure of the I1 dynein. These studies suggested that the multiple ICs and LCs are located within the third lobe of the I1 dynein, at a strategic position between the radial spokes and the outer dynein arms (Figure 1).

To directly determine the location of the I1 dynein ICs, we prepared isolated axonemes from wild-type, *bop5-2*, and *IC138* rescued strains for thin section EM. Longitudinal sections with clear views of the 96-nm repeat were processed by computer image averaging (O'Toole *et al*., 1995). Comparison of all three strains indicated that some of the densities associated with the I1 dynein are reduced in *bop5-2* axonemes and restored in axonemes from the *IC138* rescued strain (*bop5-2::IC138*) (Figure 5). Difference plots demonstrated that the defect in the assembly of the IC138 subcomplex is associated with a statistically significant decrease in the density of the third lobe of I1 dynein. This third lobe corresponds to the base of the I1 dynein, which is also responsible for interaction of I1 with the doublet microtu-

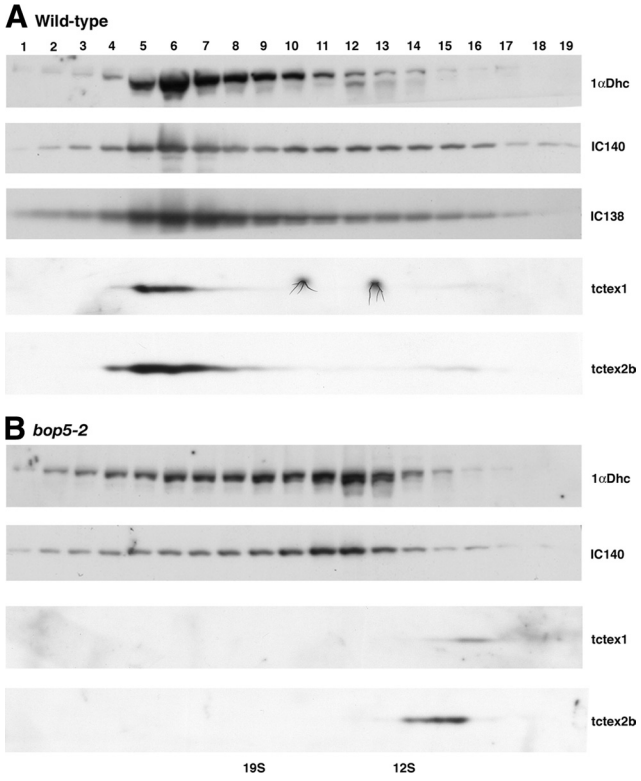

Figure 4. Dissociation of the I1 dynein complex in *bop5-2* dynein extracts. (A) Western blot of sucrose gradient fractions from a wild-type dynein extract probed with antibodies against several I1 dynein subunits. The I1 dynein subunits cosediment and peak at >20 S. FAP120 does not copurify with I1 dynein after salt extraction of wild-type axonemes (Ikeda *et al*., 2009) and thus was not analyzed here. (B) Western blot of sucrose gradient fractions from a *bop5-2* dynein extract probed with antibodies against I1 dynein subunits. IC140 and the 1α DHC cosediment at >12 S, but the I1 dynein LCs Tctex1 and Tctex2b dissociate and sediment near the top of the sucrose gradient.

bule. Such a position is also consistent with the hypothesis that IC138 is a target for regulated phosphorylation by the radial spokes due to its proximity to radial spoke 1 (Porter and Sale, 2000; Smith and Yang, 2004; Gaillard *et al*., 2006; Wirschell *et al*., 2007). The position of the IC138 subcomplex also may facilitate interactions between the inner and outer dynein arms to coordinate their activity (reviewed in Brokaw, 1994; Kamiya, 2002; King and Kamiya, 2009).

The IC138 Subcomplex Alters Flagellar Motility and Dynein-driven Microtubule Sliding

Although transformation with *IC138* restores assembly of I1 dynein, we did not observe complete rescue of the motility defects. Both the original *bop5-2* mutant and IC138 rescued cells swim poorly, with a high percentage of cells that move backward or spin in place (Supplemental Figure S2). The motility phenotypes are similar to that described previously for the *move backward only* (*mbo*) mutations (Segal *et al*., 1984). *mbo* mutants are associated with defects in the assembly of six axonemal polypeptides (Segal *et al*., 1984). To determine whether there might be any *mbo*-related deficiencies in the *bop5-2* strain, we probed Western blots of isolated axonemes with an antibody against MBO2p (Tam and Lefebvre, 2002). MBO2p is missing or reduced in axonemes from both the *bop5-2* strain and the IC138 rescued strain (Figure 3C), even though MBO2p could be detected in whole cell extracts (VanderWaal and Porter, unpublished results). These observations are consistent with the presence of a second, *mbo*-like mutation that affects motility in the *bop5-2* strain (see *Discussion*). Given this complexity, we needed an alternative method to analyze the function of the IC138 subcomplex.

To test the hypothesis that the IC138 subcomplex plays a regulatory role in control of microtubule sliding, we used a microtubule sliding disintegration assay to measure sliding velocities in isolated axonemes (Okagaki and Kamiya, 1986). This assay has proven to be a reliable method to assess dynein activity, or regulation of dynein activity, in axonemes that are paralyzed or otherwise impaired for motility (Witman *et al*., 1978; Smith and Sale, 2001; Smith, 2002). Our prediction was that IC138 plays a fundamental role in I1 dynein function; that its assembly is required for normal microtubule sliding; and that sliding velocities would be reduced in *bop5-2* axonemes, similar to the reduced sliding velocities characteristic of I1 dynein mutants (Smith and Sale, 1991; Habermacher and Sale, 1997).

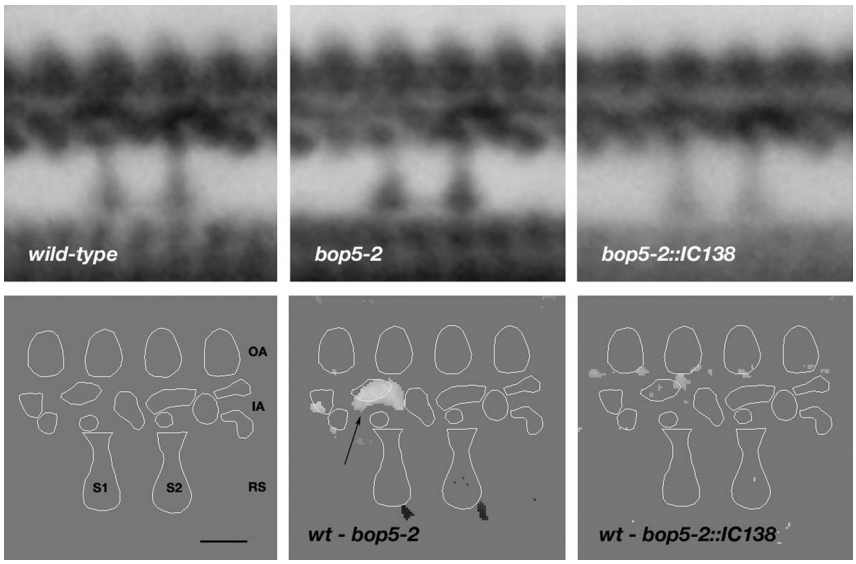

Figure 5. Defects in I1 structure in *bop5-2* axonemes. Top row, averages of the 96-nm axoneme repeat from wild-type, *bop5-2*, and IC138 rescued (*bop5-2::IC138*) cells, based on six, six, and nine individual axonemes and 61, 63, and 93 repeating units, respectively. The proximal end of the repeat is on the left, the outer arms (OA) are shown on the top, and the two radial spokes (S1 and S2) are shown on the bottom. The I1 dynein is the trilobed structure at the proximal end of the repeat. The density of the third lobe near the base of S1 is reduced in *bop5-2* and restored in the IC138 rescued (*bop5::IC138*) strain. Bottom row, diagram of densities within the 96-nm repeat and difference plots showing a statistically significant difference in the third lobe of the I1 dynein in *bop5-2* (see arrow).

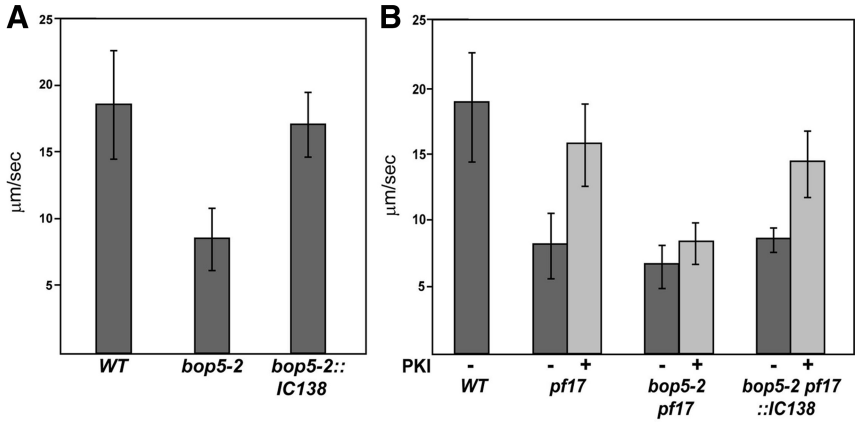

Figure 6. IC138 is required for regulated microtubule sliding. (A) Microtubule sliding disintegration assays indicate that *bop5-2* axonemes display slow microtubule sliding velocities relative to wild type. Sliding velocities increase to wild-type levels in the IC138 rescued strains (*bop5-2::IC138*). (B) Pretreatment of radial spoke mutants with kinase inhibitors increases microtubule sliding velocities only in presence of the IC138 subcomplex (*pf17* and *pf17 bop5-2::IC138*). Kinase inhibitors have no effect in the absence of the IC138 subcomplex (*pf17 bop5-2*). Microtubule sliding velocities are expressed as micrometers per second. The average microtubule sliding velocity was calculated from three independent experiments, each with a sample size of at least 70 axonemes. Values shown are means and standard deviations.

As described previously, in 1 mM MgATP, microtubule sliding is very rapid in isolated wild-type axonemes (Figure 6A) (Howard *et al*., 1994; Habermacher and Sale, 1997; Smith, 2002). As predicted, microtubules slide at greatly reduced velocities in *bop5-2* axonemes. Moreover, microtubule sliding is increased when *bop5-2* cells are transformed with *IC138* (Figure 6A). The same result was observed in all of the *bop5-2::IC138* transformants. Treatment with the kinase inhibitor protein kinase inhibitor (PKI) had no effect on sliding velocities in axonemes from wild type or *bop5-2* and *bop5-2* transformants (data not shown). The results indicate that assembly of the IC138 subcomplex is required for I1 dynein activity and normal microtubule sliding.

The IC138 Subcomplex Is Required for Regulation of Microtubule Sliding by the Radial Spoke–Phosphorylation Pathway

Diverse evidence indicates that assembly of I1 dynein is required for regulation of microtubule sliding by a regulatory pathway that involves the central pair apparatus, radial spokes, and axonemal kinases and phosphatases (reviewed in Porter and Sale, 2000; Smith and Yang, 2004; Wirschell *et al*., 2007). The regulatory pathway was revealed by functional and pharmacological analysis of microtubule sliding in paralyzed axonemes from central pair or radial spoke mutants (Habermacher and Sale, 1996, 1997; Yang and Sale, 2000; Smith and Sale, 2001; Hendrickson et al., 2004; Howard *et al*., 2004). For example, dynein-driven microtubule is globally inhibited in isolated, paralyzed axonemes from radial spoke mutants such as *pf14* or *pf17*, and normal microtubule sliding velocity can be rescued by pretreating the axonemes with kinase inhibitors. Rescue of microtubule sliding requires assembly of the I1 dynein, indicating that I1 plays an essential role in this pathway (Habermacher and Sale, 1997; Yang and Sale, 2000). The mechanism of inhibition and the rescue of microtubule sliding correlate with phosphorylation and dephosphorylation of IC138 (Habermacher and Sale, 1997; Porter and Sale, 2000; Smith and Yang, 2004; Wirschell *et al*., 2007). Because IC138 is the primary phosphoprotein in the I1 dynein (Habermacher and Sale, 1997; King and Dutcher, 1997; Yang and Sale, 2000; Hendrickson *et al*., 2004), the results suggest that IC138 is the key substrate required for phosphoregulation of microtubule sliding.

To further test the regulatory role of the IC138 subcomplex, we crossed *bop5-2* with the paralyzed radial spoke mutant *pf17* to recover the double mutant *pf17 bop5-2*. We also transformed *pf17 bop5-2* with the *IC138* gene to recover several IC138 rescued strains (*pf17 bop5-2::IC138*). Expression of *IC138* in the transformants was confirmed by PCR and Western blotting (our unpublished data). We then measured microtubule sliding velocities of disintegrating axonemes in the absence or presence of the kinase inhibitor PKI. We predicted that rescue of microtubule sliding in *pf* mutants with PKI would require the assembly of IC138. As described previously (Porter and Sale, 2000; Smith and Yang, 2004; Wirschell *et al*., 2007), microtubule sliding is greatly reduced in axonemes from *pf17*, and the addition of PKI restores microtubule sliding to wild-type levels (Figure 6B). Similarly, sliding velocities are greatly reduced in the double mutant, *pf17 bop5-2* and the transformant *pf17 bop5-2::IC138*. However, PKI treatment only increases sliding velocities in *pf17 bop5-2::IC138* (Figure 6B). Thus, assembly of the IC138 subcomplex (IC138, IC97, FAP120, and LC7b) is necessary for regulation of I1-dynein mediated microtubule sliding by the radial spoke–phosphorylation pathway.

DISCUSSION

IC138 Is Not Required for Assembly of the I1 Dynein

To better understand the role of IC138 in the control of dynein activity, we identified a null mutation, *bop5-2*, associated with the deletion of >90% of the *IC138* gene. Surprisingly, loss of IC138 did not impact assembly of I1 dynein into the axoneme; both I1 HCs, IC140, Tctex1, and Tctex2b are still present (Figures 3 and 4). Mutations in either IC1 or IC2 usually block assembly of the outer arm dynein (Mitchell and Kang, 1991; Wilkerson *et al*., 1995), and mutations in IC140 are associated with defects in assembly of the I1 dynein (Perrone *et al*., 1998; Figure 3B). However, IC138 is part of a distinct subcomplex, which is not required for either I1 dynein assembly or targeting into the axoneme.

The IC138 Subcomplex: IC138 Is Closely Associated with LC7b, IC97, and FAP120

Previous studies of *bop5-1* have shown that the I1 dynein lacks LC7b when IC138 is truncated (Hendrickson *et al*., 2004; Figure 3A). Because LC7b is also an outer dynein subunit (DiBella *et al*., 2004a), we did not directly analyze its presence in *bop5-2*. However, we can reasonably infer that the I1 dynein lacks LC7b when IC138 is missing.

IC97 is a novel I1 subunit that shares homology with axonemal proteins in several organisms, including the murine lung adenoma susceptibility 1 protein. Several biochemical assays indicate that IC97 interacts directly with tubulin subunits (Wirschell *et al*., 2009). We show here that assembly

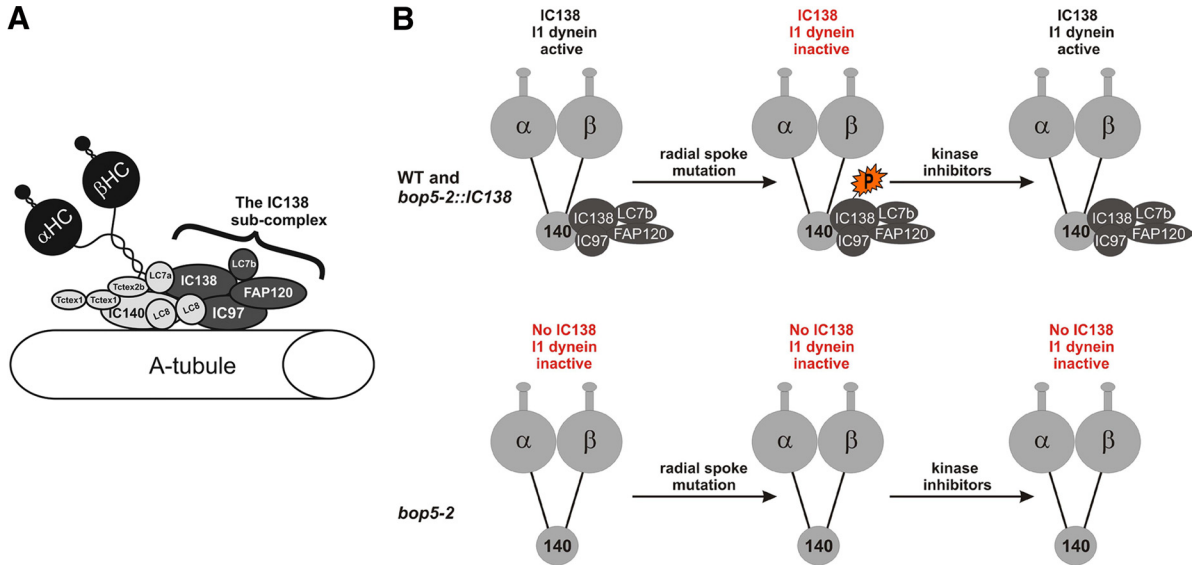

Figure 7. Model of the IC138 subcomplex and its role in regulating I1 dynein activity. (A) Shown here is a schematic diagram of the I1-dynein subunits on the A-tubule of the outer doublet. IC138 forms a regulatory subcomplex with LC7b, FAP120, and IC97. Attachment of the IC138 subcomplex to the outer doublet may be facilitated by IC97, which also interacts with tubulin and LC8 (Wirschell *et al*., 2009). Attachment of the remaining I1 subunits to the A-tubule may be facilitated by IC140 (Perrone *et al*., 1998; Yang *et al*., 1998). The precise locations of the other LC subunits are unknown. (B) Diagram showing the role of the IC138 subcomplex in regulating I1 dynein activity in response to signals from the radial spoke complex. In wild-type and IC138 rescued cells, the I1 dynein is active and microtubule sliding is fast. Radial spoke mutations result in hyperphosphorylation of IC138, inhibition of I1 activity, and reduced microtubule sliding velocities. Pretreatment of axonemes with kinase inhibitors results in dephosphorylation of IC138 by endogenous phosphatases, stimulation of I1 activity, and increased microtubule sliding. In the absence of the IC138 subcomplex, the I1 dynein is inactive under all conditions.

of IC97 into the axoneme depends on the presence of IC138, because it is missing in *bop5-2* axonemes but restored in the IC138 rescued strain (Figure 3C).

IC138 also influences the assembly of FAP120, a novel ankyrin-related protein recently identified as an I1-associated protein (Ikeda *et al*., 2009). We show here that FAP120 is missing in *bop5-2* and restored in the *bop5-2::IC138* rescued strain, which confirms the close association between this polypeptide and IC138 (Figure 3C). However, the interaction between FAP120 and IC138 may be mediated indirectly through LC7b, because FAP120 is also missing in *bop5-1*, which assembles a truncated version of IC138 but lacks LC7b (Ikeda *et al*., 2009; Figure 3A).

Together, these observations demonstrate that IC138, LC7b, IC97, and FAP120 form a distinct subcomplex on the A-microtubule (Figure 7A). The presence of multiple subcomplexes within the I1 dynein is consistent with the phenotype of a *tctex2b* mutation, which blocks assembly of Tctex2b but does not prevent assembly of other I1 subunits into the axoneme (DiBella *et al*., 2004b).

The IC138 Subcomplex Is Located at the Base of the I1 Dynein

Our previous studies of I1 dynein HC mutants identified the positions of the two motor domains, and by implication, suggested the position of the IC/LC complex (Figure 1). Here, we show directly by EM image averaging of *bop5-2* axonemes that the IC138 subcomplex is located at the base of the I1 dynein, between radial spoke 1 and the outer dynein arms (Figures 5 and 7A). However, the N-terminal regions of the dynein HCs, IC140, and other I1 LCs are probably still present in this region. More detailed insight into the unique position of each subunit and their respective interactions within the IC138 subcomplex will require cryo-electron tomography of I1 mutant axonemes with less severe structural defects (e.g., *bop5-1*, *ida7::IDA7 5a*, *tctex2b*, *fla14-3*), as well as the development of alignment and averaging procedures that can accurately determine differences in three dimensions. Even so, the analysis of *bop5-2* clearly locates IC138, which is the only known I1 phosphoprotein, in a position to be regulated by the radial spoke phosphorylation machinery.

The IC138 Subcomplex Is Required for I1 Dynein-mediated Microtubule Sliding

The phenotypes of *bop5-2* and the *IC138* rescued strains indicate that there are two closely linked mutations that affect flagellar motility in *bop5-2*, one mutation associated with the loss of the IC138 subcomplex, and a second mutation associated with defects in assembly of MBO2p. Transformation with a wild-type copy of *IC138* altered motility but did not correct the MBO2p-associated defects (Figures 3C and Supplemental Figure S2). The locus of the *mbo* mutation is unknown but currently under investigation.

Given this complexity, and to more directly assess the effect of the *bop5-2* mutation on I1 activity, we measured microtubule sliding velocities during sliding disintegration in vitro, an assay useful for assessing dynein activity in axonemes that are otherwise paralyzed or defective in motility (Witman *et al*., 1978; Okagaki and Kamiya, 1986; Smith and Sale, 1991). Interestingly, loss of the IC138 subcomplex was correlated with a decrease in sliding velocity similar in magnitude to that observed with the loss of the entire I1 dynein complex (Habermacher and Sale, 1997). Reassembly of the IC138 subcomplex restored sliding velocities to wild-type levels (Figure 6A). Thus, the IC138 subcomplex is required to couple the activity of I1 motor domains to microtubule sliding.

Defects in radial spokes disrupt the signaling pathway that regulates I1-dynein activity, resulting in hyperphospho-

rylated forms of IC138 and decreased sliding velocities (Habermacher and Sale, 1997; King and Dutcher, 1997; Yang and Sale, 2000; Hendrickson *et al*., 2004). Inhibition of dynein activity can be overcome by treatment with kinase inhibitors; axoneme-associated phosphatases are thought to dephosphorylate IC138 and increase sliding velocities to wild-type levels (Porter and Sale, 2000; Gaillard *et al*., 2006; Wirschell *et al*., 2008). To demonstrate that IC138 is the critical substrate for the axonemal phosphatases, we analyzed microtubule sliding of *bop5-2* in a radial spoke mutant (Figure 6B). As predicted, treatment with kinase inhibitors only increased microtubule sliding when the IC138 subcomplex was present, consistent with a model in which assembly of I1 dynein, IC138, and possibly other subcomplex proteins, is required for regulation by the radial spoke–phosphorylation pathway (Figure 7B).

Within the IC138 subcomplex, FAP120 and LC7b do not seem to be required for regulation of I1 activity by the radial spoke pathway. *bop5-1* axonemes lack LC7b and FAP120, but microtubule sliding in the double mutant *pf17 bop5-1* can be rescued with kinase inhibitors, indicating that LC7b and FAP120 are not necessary for regulation of microtubule sliding by the radial spokes (Hendrickson *et al*., 2004; Ikeda *et al*., 2009). However, the *bop5-1* strain does display altered motility and can partially suppress the motility defects observed in *pf10* (Dutcher *et al*., 1988), which indicates that LC7b and FAP120 must play some role in modifying I1 activity. The precise functions of LC7b and FAP120 await further study but probably include a role for I1 dynein in control of axonemal bending not revealed by microtubule sliding assays.

Although IC138 is the only phosphoprotein in the I1 dynein and the primary target for regulation by the radial spoke–phosphorylation pathway, recent studies suggest that changes in the phosphorylation state of IC138 must be communicated through other axoneme components to effectively regulate motility. Indeed, in vitro microtubule gliding assays of the isolated I1 complex have failed to detect any changes in motor activity in response to kinase or phosphatase treatment (Sakakibara, personal communication). Moreover, microtubule sliding assays with I1 dyneins lacking one or the other motor domain have indicated that the 1β DHC is the primary motor domain that responds to changes in the phosphorylation state of IC138 (Fox, Tritschler, Porter, and Sale, unpublished data; Toba *et al*., 2008). Our recent studies on IC97 further suggest that this subunit plays a critical role in communicating changes in the phosphorylation state of IC138 to other components within the axoneme (Wirschell *et al*., 2009). A better understanding of the mechanism by which the IC138 subcomplex regulates I1 activity and microtubule sliding will require additional high resolution structural analysis of wild-type and mutant axonemes (Nicastro *et al*., 2006; Bui *et al*., 2008), biophysical studies of I1 dyneins with altered subunit composition (Kotani *et al*., 2007), and identification and mutation of key phosphoresidues in IC138.

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REFERENCES

Brokaw, C. J. (1994). Control of flagellar bending: a new agenda based on dynein diversity. Cell Motil. Cytoskeleton *28*, 199–204.

Brokaw, C. J. (2008). Thinking about flagellar oscillation. Cell Motil. Cytoskeleton (*in press*).

Brokaw, C. J., and Kamiya, R. (1987). Bending patterns of *Chlamydomonas* flagella: IV. Mutants with defects in inner and outer dynein arms indicate differences in dynein arm function. Cell Motil. Cytoskeleton *8*, 68–75.

Bui, K. H., Sakakibara, H., Movassagh, T., Oiwa, K., and Ishikawa, T. (2008). Molecular architecture of inner dynein arms in situ in *Chlamydomonas reinhardtii* flagella. J. Cell Biol. *183*, 923–932.

DiBella, L. M., Sakato, M., Patel-King, R. S., Pazour, G. J., and King, S. M. (2004a). The LC7 light chains of *Chlamydomonas* flagellar dyneins interact with components required for both motor assembly and regulation. Mol. Biol. Cell *10*, 4633–4646.

DiBella, L. M., Smith, E. F., Patel-King, R. S., Wakabayashi, K., and King, S. M. (2004b). A novel Tctex2-related light chain is required for stability of inner dynein arm I1 and motor function in the *Chlamydomonas* flagellum. J. Biol. Chem. *279*, 21666–21676.

Dutcher, S. K., Gibbons, W., and Inwood, W. B. (1988). A genetic analysis of suppressors of the *PF10* mutation in *Chlamydomonas reinhardtii*. Genetics *120*, 965–976.

Gaillard, A. R., Fox, L. A., Rhea, J. M., Craige, B., and Sale, W. S. (2006). Disruption of the A-kinase anchoring domain in flagellar radial spoke protein 3 results in unregulated axonemal cAMP-dependent protein kinase activity and abnormal flagellar motility. Mol. Biol. Cell. *17*, 2626–2635.

Goodenough, U. W., and Heuser, J. E. (1985a). Outer and inner dynein arms of cilia and flagella. Cell. *41*, 341–342.

Goodenough, U. W., and Heuser, J. E. (1985b). Substructure of inner dynein arms, radial spokes, and the central pair/projection complex of cilia and flagella. J. Cell Biol. *100*, 2008–2018.

Habermacher, G., and Sale, W. S. (1996). Regulation of flagellar dynein by an axonemal type-1 phosphatase in *Chlamydomonas*. J. Cell Sci. *109*, 1899–1907.

Habermacher, G., and Sale, W. S. (1997). Regulation of flagellar dynein by phosphorylation of a 138-kD inner arm dynein intermediate chain. J. Cell Biol. *136*, 167–176.

Harris, E. H. (1989). The *Chlamydomonas* Sourcebook: A Comprehensive Guide to Biology and Laboratory Use, San Diego, CA: Academic Press.

Harrison, A., Olds-Clarke, P., and King, S. M. (1998). Identification of the t complex-encoded cytoplasmic dynein light chain tctex1 in inner arm I1 supports the involvement of flagellar dyneins in meiotic drive. J. Cell Biol. *140*, 1137–1147.

Hendrickson, T. W., Perrone, C. A., Griffin, P., Wuichet, K., Mueller, J., Yang, P., Porter, M. E., and Sale, W. S. (2004). IC138 is a WD-repeat dynein intermediate chain required for light chain assembly and regulation of flagellar bending. Mol. Biol. Cell *15*, 5431–5442.

Hennessey, T. M., Kim, D. Y., Oberski, D. J., Hard, R., Rankin, S. A., and Pennock, D. G. (2002). Inner arm dynein 1 is essential for Ca++-dependent ciliary reversals in *Tetrahymena thermophila*. Cell Motil. Cytoskeleton *53*, 281–288.

Howard, D. R., Habermacher, G., Glass, D. B., Smith, E. F., and Sale, W. S. (1994). Regulation of *Chlamydomonas* flagellar dynein by an axonemal protein kinase. J. Cell Biol. *127*, 1683–1692.

Ikeda, K., Yamamoto, R., Wirschell, M., Yagi, T., Bower, R., Porter, M. E., Sale, W. S., and Kamiya, R. (2009). A novel ankyrin-repeat protein interacts with the regulatory proteins of inner arm dynein f (I1) of *Chlamydomonas reinhardtii*. Cell Motil. Cytoskeleton *66*, (10.1002/cm. 20324).

Kagami, O., and Kamiya, R. (1992). Translocation and rotation of microtubules caused by multiple species of *Chlamydomonas* inner-arm dynein. J. Cell Sci. *103*, 653–664.

Kamiya, R. (2002). Functional diversity of axonemal dyneins as studied in *Chlamydomonas* mutants. Int. Rev. Cytol. *219*, 115–155.

Kikushima, K., and Kamiya, R. (2008). Clockwise translocation of microtubules by flagellar inner-arm dyneins *in vitro*. Biophys J. *94*, 4014–4019.

King, S. J., and Dutcher, S. K. (1997). Phosphoregulation of an inner dynein arm complex in *Chlamydomonas reinhardtii* is altered in phototactic mutant strains. J. Cell Biol. *136*, 177–191.

King, S. M., and Kamiya, R. (2009). Axonemal dyneins: assembly, structure, and force generation. In: The *Chlamydomonas* Sourcebook: Cell Motility and Behavior. Vol. 3, ed. G. B. Witman, Oxford: Academic Press, 131–208.

Kotani, N., Sakakibara, H., Burgess, S. A., Kojima, H., and Oiwa, K. (2007). Mechanical properties of inner-arm dynein-f (dynein I1) studied with *in vitro* motility assays. Biophys. J. *93*, 886–894.

Mastronarde, D. N., O'Toole, E. T., McDonald, K. L., McIntosh, J. R., and Porter, M. E. (1992). Arrangement of inner dynein arms in wild-type and mutant flagella of *Chlamydomonas*. J. Cell Biol. *118*, 1145–1162.

Mitchell, D. R., and Kang, Y. (1991). Identification of *oda*6 as a *Chlamydomonas* dynein mutant by rescue with the wild-type gene. J. Cell Biol. *113*, 835–842.

Myster, S. H., Knott, J. A., O'Toole, E., and Porter, M. E. (1997). The *Chlamydomonas Dhc1* gene encodes a dynein heavy chain subunit required for assembly of the I1 inner arm complex. Mol. Biol. Cell *8*, 607–620.

Myster, S. H., Knott, J. A., Wysocki, K. M., O'Toole, E., and Porter, M. E. (1999). Domains in the 1-alpha dynein heavy chain required for inner arm assembly and flagellar motility in *Chlamydomonas*. J. Cell Biol. *146*, 801–818.

Nicastro, D., Schwartz, C., Pierson, J., Gaudette, R., Porter, M. E., and McIntosh, J. R. (2006). The molecular architecture of axonemes revealed by cryoelectron tomography. Science *313*, 944–948.

Okagaki, T., and Kamiya, R. (1986). Microtubule sliding in mutant *Chlamydomonas* axonemes devoid of outer or inner dynein arms. J. Cell Biol. *103*, 1895–1902.

Okita, N., Isogai, N., Hirono, M., Kamiya, R., and Yoshimura, K. (2005). Phototactic activity in *Chlamydomonas* 'non-phototactic' mutants deficient in Ca2+-dependent control of flagellar dominance or in inner-arm dynein. J. Cell Sci. *118*, 529–537.

O'Toole, E., Mastronarde, D., McIntosh, J. R., and Porter, M. E. (1995). Computer-assisted analysis of flagellar structure. Methods Cell Biol. *47*, 183–191.

Perrone, C. A., Myster, S. H., Bower, R., O'Toole, E. T., and Porter, M. E. (2000). Insights into the structural organization of the I1 inner arm dynein from a domain analysis of the 1-beta dynein heavy chain. Mol. Biol. Cell *11*, 2297–2313.

Perrone, C. A., Tritschler, D., Taulman, P., Bower, R., Yoder, B. K., and Porter, M. E. (2003). A novel Dynein light intermediate chain colocalizes with the retrograde motor for intraflagellar transport at sites of axoneme assembly in *Chlamydomonas* and mammalian cells. Mol. Biol. Cell *14*, 2041–2056.

Perrone, C. A., Yang, P., O'Toole, E., Sale, W. S., and Porter, M. E. (1998). The *Chlamydomonas IDA7* locus encodes a 140-kDa dynein intermediate chain required to assemble the I1 inner arm complex. Mol. Biol. Cell *9*, 3351–3365.

Piperno, G., and Ramanis, Z. (1991). The proximal portion of *Chlamydomonas* flagella contains a distinct set of inner dynein arms. J. Cell Biol. *112*, 701–709.

Piperno, G., Ramanis, Z., Smith, E. F., and Sale, W. S. (1990). Three distinct inner dynein arms in *Chlamydomonas* flagella: molecular composition and location in the axoneme. J. Cell Biol. *110*, 379–389.

Porter, M. E., Knott, J. A., Myster, S. H., and Farlow, S. J. (1996). The dynein gene family in *Chlamydomonas reinhardtii*. Genetics *144*, 569–585.

Porter, M. E., Power, J., and Dutcher, S. K. (1992). Extragenic suppressors of paralyzed flagellar mutations in *Chlamydomonas reinhardtii* identify loci that alter the inner dynein arms. J. Cell Biol. *118*, 1163–1176.

Porter, M. E., and Sale, W. S. (2000). The 9 + 2 axoneme anchors multiple inner arm dyneins and a network of kinases and phosphatases that control motility. J. Cell Biol. *151*, F37–F42.

Rupp, G., O'Toole, E., and Porter, M. E. (2001). The *Chlamydomonas PF6* locus encodes a large alanine/proline-rich polypeptide that is required for assembly of a central pair projection and regulates flagellar motility. Mol. Biol. Cell *12*, 739–751.

Rupp, G., and Porter, M. E. (2003). A subunit of the dynein regulatory complex in *Chlamydomonas* is a homologue of a growth arrest-specific gene product. J. Cell Biol. *162*, 47–57.

Segal, R. A., Huang, B., Ramanis, Z., and Luck, D. J. (1984). Mutant strains of *Chlamydomonas reinhardtii* that move backwards only. J. Cell Biol. *98*, 2026–2034.

Sizova, I., Fuhrmann, M., and Hegemann, P. (2001). A *streptomyces rimosus aphVIII* gene coding for a new type phosphotransferase provides stable antibiotic resistance to *Chlamydomonas reinhardtii*. Gene *277*, 221–229.

Smith, E. F. (2002). Regulation of flagellar dynein by the axonemal central apparatus. Cell Motil. Cytoskeleton *52*, 33–42.

Smith, E. F., and Sale, W. S. (1991). Microtubule binding and translocation by inner dynein arm subtype I1. Cell Motil. Cytoskeleton *18*, 258–268.

Smith, E. F., and Sale, W. S. (1992). Regulation of dynein-driven microtubule sliding by the radial spokes in flagella. Science *257*, 1557–1559.

Smith, E. F., and Yang, P. (2004). The radial spokes and central apparatus: mechano-chemical transducers that regulate flagellar motility. Cell Motil. Cytoskeleton *57*, 8–17.

Tam, L. W., and Lefebvre, P. A. (2002). The *Chlamydomonas MBO*2 locus encodes a conserved coiled-coil protein important for flagellar waveform conversion. Cell Motil. Cytoskeleton *51*, 197–212.

Toba, S., Fox, L. A., Sakakibara, H., Porter, M. E., Sale, W. S., and Oiwa, K. (2008). Distinct roles of 1α and 1β heavy chains of the I1 inner arm dynein of *Chlamydomonas* flagella. Mol. Biol. Cell *19* (Suppl), abstract 1787/B249. (CD-ROM).

Wilkerson, C. G., King, S. M., Koutoulis, A., Pazour, G. J., and Witman, G. B. (1995). The 78,000 M(r) intermediate chain of *Chlamydomonas* outer arm dynein is a WD-repeat protein required for arm assembly. J. Cell Biol. *129*, 169–178.

Wirschell, M., Hendrickson, T., and Sale, W. S. (2007). Keeping an eye on I *1*, I1 dynein as a model for flagellar dynein assembly and regulation. Cell Motil. Cytoskeleton *64*, 569–579.

Wirschell, M., Yang, C., Yang, P., Fox, L., Yanigasawa, H., Kamiya, R., Witman, G. B., Porter, M. E., and Sale, W. S. (2009). IC97 is a novel intermediate chain of I1 dynein that interacts with tubulin and regulates interdoublet sliding. Mol. Biol. Cell *20*, 3044–3054.

Witman, G. B. (1986). Isolation of *Chlamydomonas* flagella and flagellar axonemes. Methods Enzymol. *134*, 280–290.

Witman, G. B., Plummer, J., and Sander, G. (1978). *Chlamydomonas* flagellar mutants lacking radial spokes and central tubules. Structure, composition, and function of specific axonemal components. J. Cell Biol. *76*, 729–747.

Yang, P., and Sale, W. S. (1998). The Mr 140,000 Intermediate chain of *Chlamydomonas* flagellar inner arm dynein is a WD-repeat protein implicated in dynein arm anchoring. Mol. Biol. Cell *9*, 3335–3349.

Yang, P., and Sale, W. S. (2000). Casein kinase I is anchored on axonemal doublet microtubules and regulates flagellar dynein phosphorylation and activity. J. Biol. Chem. *275*, 18905–18912.