Identification of the \( t \) Complex–encoded Cytoplasmic Dynein Light Chain Tctex1 in Inner Arm I1 Supports the Involvement of Flagellar Dyneins in Meiotic Drive

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Abstract. The cytoplasmic dynein light chain Tctex1 is a candidate for one of the distorter products involved in the non-Mendelian transmission of mouse \( t \) haplotypes. It has been unclear, however, how the \( t \)-specific mutations in this protein, which is found associated with cytoplasmic dynein in many tissues, could result in a male germ cell–specific phenotype. Here, we demonstrate that Tctex1 is not only a cytoplasmic dynein component, but is also present both in mouse sperm and Chlamydomonas flagella. Genetic and biochemical dissection of the Chlamydomonas flagellum reveal that Tctex1 is a previously undescribed component of inner dynein arm I1. Combined with the recent identification of another putative \( t \) complex distorter, Tctex2, within the outer dynein arm, these results support the hypothesis that transmission ratio distortion (meiotic drive) of mouse \( r \) haplotypes involves dysfunction of both flagellar inner and outer dynein arms but does not require the cytoplasmic isozyme.

Dyneins are complex, microtubule-dependent molecular motors. These enzymes may be classified into four structurally and functionally distinct groups: cytoplasmic, outer arm, inner arm I1, and inner arms I2/3 (Witman et al., 1994). The flagellar dyneins generate motive force by causing interdoublet microtubule sliding that is ultimately converted to an axonomal bend (Warner et al., 1989). The outer arms provide most of the power for flagellar beating, whereas the heterogeneous inner arm system appears responsible for the initiation of a flagellar bend and for the shear amplitude of the propagated wave (Kamiya et al., 1989). Cytoplasmic dyneins exhibit a wide range of functions that include axonal vesicle transport, membrane trafficking, nuclear migration, and the positioning and anaphase movement of the mitotic spindle (Paschal and Vallee, 1987; Schroer et al., 1989; Cortés-Theulaz et al., 1992; Li et al., 1993; Xiang et al., 1994; Cottingham and Hoyt, 1997). Despite these wide differences in function, flagellar and cytoplasmic dyneins exhibit some striking similarities in both their polypeptide composition and overall morphology (for reviews see Holzbaur et al., 1994; Mitchell, 1994).

The major isoform of cytoplasmic dynein and inner arm I1 both contain two heavy chains (HCs)\(^1\) (Vallee et al., 1988; Piperno et al., 1990). Flagellar outer arm contains either two or three HCs, depending on the source (Bell et al., 1979; Piperno and Luck, 1979; Pfister et al., 1982; Porter and Johnson, 1983), and each of the multiple versions of arms I2/3 contain a single HC (Piperno et al., 1990; Kagaami and Kamiya, 1992; Porter et al., 1996). Each HC consists of a globular head domain that is responsible for ATP hydrolysis and force production and an NH\(_2\)-terminal stem domain that, in the multimeric dyneins, connects the HCs to a common base. In addition to the ATP hydrolytic site, all HCs sequenced to date contain additional phosphate-binding loops, several of which also are involved in nucleotide binding and may function in allosteric regulation (Wilkerson, C.G., and G.B. Witman. 1995. Mol. Biol. Cell. 6:33a; Mocz and Gibbons, 1996).

Each dynein also contains a distinct complement of smaller components associated with the HCs. Cytoplasmic dynein has four light intermediate chains (LICs [53–59 kD]; Gill et al., 1994; Hughes et al., 1995), which have no known flagellar homologues, and two copies of a 74-kD intermediate chain (IC74; Paschal et al., 1992), which is a WD repeat protein related to the \( b \) subunit of heterotrimeric G proteins (Wilkerson et al., 1995; Sondek et al., 1996). Both

1. Abbreviations used in this paper: HC, heavy chain; IC, intermediate chain; LIC, light intermediate chain; PVDF, polyvinylidene difluoride.
outer arm dynein and inner arm II also contain members of the WD repeat family (Mitchell and Kang, 1991; Ogawa et al., 1995; Wilkerson et al., 1995; Yang, P., and W.S. Sale. 1996. Mol. Biol. Cell. 7:568a). These proteins are located at the base of the soluble dynein particle (King and Witman, 1990; Steffen et al., 1996) and appear to be important for attachment of the motor to its cargo (King et al., 1991, 1995; Karki and Holzbaur, 1995).

A variety of light chain (LC) components also are present in these enzymes. For example, inner arms 1/2 contain both a 28-kD protein, which is essential for arm assembly, and the Ca$^{2+}$-binding protein centrin (LeDizet and Piperno, 1995). Likewise, multiple LCs have been found within outer arm dynein, including a novel calmodulin homologue (King and Patel-King, 1995a) and two thioredoxins (Patel-King et al., 1996). The outer arm also contains a highly conserved 8,000-Mr LC (King and Patel-King, 1995b) that has been subsequently identified in several other enzymes, including cytoplasmic dynein (King et al., 1996a), myosin V (Espindola, F.S., R.E. Cheney, S.M. King, D.M. Suter, and M.S. Mooseker. 1996. Mol. Biol. Cell. 7:372a), and neuronal nitric oxide synthase (Jaffrey and Snyder, 1996), where it apparently acts to regulate synthase activity by converting the active dimer to an inactive monomeric form. To date, the 8,000-Mr LC is the only component that is reportedly shared by both flagellar and cytoplasmic dynein isoenzymes, and in Drosophila, it is essential for viability (Dick et al., 1996a). Null mutants in both yeast and Chlamydomonas, however, are viable. The yeast mutant has no overt phenotype (Dick et al., 1996b), whereas in Chlamydomonas, a wide variety of flagellar defects in the assembly of axonemal components and in retrograde intraflagellar transport are evident (Pazour, G.J., G.G. Wilkerson, and G.B. Witman. 1997. Mol. Biol. Cell. 8:162a).

Also associated with cytoplasmic dynein is a family of 14,000-Mr LCs that includes the protein Tctex1 (King et al., 1996b), which in mice is encoded within a 30–40-Mb region of chromosome 17 known as the t complex. Variant forms of this region termed haplotypes exist, which exhibit several fascinating properties (for reviews see Silver, 1993; Olds-Clarke, 1997), including the non-Mendelian transmission of the t haplotype to almost all the progeny of heterozygous males. This phenomenon of transmission ratio distortion, a form of meiotic drive through an effect on a flagellar inner dynein arm rather than (or perhaps in addition to) having consequences for cytoplasmic dynein function. Combined with our previous identification of the Tctex2 protein as an outer arm dynein LC (Patel-King et al., 1997), these results support a model whereby the differential incorporation of dysfunctional flagellar dyneins during spermiogenesis contributes to the meiotic drive of mouse t haplotypes.

Materials and Methods

Preparation of Mouse Sperm

Sperm were prepared from congeneric +/+, r1/r1, and r1/p2 mice using the conditions described in Olds-Clarke et al. (1996). Whole sperm proteins were separated by electrophoresis in 15% acrylamide gels before immunoblot analysis.

Chlamydomonas Axoneme Isolation and Dynein Purification

Flagellar axonemes were prepared from Chlamydomonas reinhardtii using standard protocols (Witman, 1986; King, 1995). Dynein was extracted with 0.6 M NaCl and purified by centrifugation in a 5–20% sucrose density gradient (King et al., 1986). Gradient fractions were concentrated in a 5–15% acrylamide gradient gel, treated, separated by electrophoresis in a 5–15% acrylamide gel before immunoblot analysis.

Peptide Sequencing

Sucrose gradient fractions containing the 14,000-Mr protein were concentrated, separated by electrophoresis in a 5–15% acrylamide gradient gel, and blotted to polyvinylidene difluoride (PVDF) membrane (Immobilon P; Millipore Corp., Bedford, MA) in 10 mM NaHCO₃, 3 mM Na₂CO₃, 0.01% SDS, and 20% methanol. The blot was stained with 0.2% Ponceau S, and a thin strip was probed for the 14,000-Mr protein. The immunoreactive band was then identified on the unprobed strip, excised, and treated with trypsin. Peptides eluting from the membrane were purified by reverse-phase chromatography on a C₄ column. Two peptides were sequenced (model 492A sequencer; Applied Biosystems Inc., Foster City, CA) at the Protein Chemistry Facility at the Worcester Foundation for Biomedical Research (Shrewsbury, MA). For one peptide, a 12-h cycle of treatment with TFA vapor at 60°C was required to remove a blocked NH₂ terminus via an Asp-Pro cleavage. Peptide masses were determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using a Linear Voyager Biospectrometry workstation (PerSeptive Biosystems, Framingham, MA).
Electrophoresis and Immunoblotting

All Chlamydomonas samples were electrophoresed in 5–15% polyacrylamide gradient gels and either stained with Coomassie brilliant blue or blotted to nitrocellulose (0.2 μm pore size; Schleicher & Schuell, Keene, NH) in 10 mM NaHCO₃, 3 mM Na₂CO₃, 0.01% SDS, and 20% methanol. For immunoblotting, the nitrocellulose was blocked in 5% dried milk, and 0.1% Tween-20 in TBS before being probed with blot-purified primary antibody followed by a peroxidase-conjugated secondary antibody (King et al., 1996a). After washing, the antibody signal was visualized using an enhanced chemiluminescent system (ECL; Amersham Corp., Arlington Heights, IL) and Rx film (Fuji Photo Film Co. Ltd., Tokyo, Japan). Total protein was subsequently detected using 0.2% Ponceau S.

Molecular Cloning

A gene-specific primer (5′-CCGTGACCTCGAGACGACGAG-TAC-3′) was designed based on the peptide sequence LQNOQ and incorporated the Chlamydomonas codon bias (Harris, 1989). The primer also incorporated an EcoRI site and a GC clamp at the 5′ end. The reverse primer was the standard oligo (dT) adapter primer (5′-CCGCGGTGAACAGCTCTAC-3′) that contains SalI and XhoI sites at the 5′ end. 100–μl PCRs were performed in 10 mM Tris-Cl, pH 8.85, 25 mM KCl, 5 mM (NH₄)₂SO₄, 2 mM MgSO₄, and 0.2 mM dNTP, and they contained 1 μg of each primer. The template used was first-strand cDNA made from mRNA isolated from cells actively regenerating their flagella. Samples were heated at 95°C for 5 min, cooled on ice, and 2.5 U of Pfu DNA polymerase (Stratagene, La Jolla, CA) were added. Samples were subjected to 35 rounds of the following program: 96°C for 1 min, 35°C for 1 min, and 75°C for 2 min. Products were subcloned into pBluescript II SK− (Stratagene) and sequenced using Sequenase v2.0 and a 7-deaza-dGTP.

Results

Tctex1 Is Present in the Flagellar Axoneme

We recently identified the mouse t complex–encoded protein Tctex1 as one of the 14,000-Mr LCs associated with cytoplasmic dynein isolated from mammalian brain, kidney, liver, spleen, and testis (King et al., 1996b). Intriguingly, a previous report had suggested that Tctex1 may also be present in the flagellum of mammalian sperm (O’Neill and Artzt, 1995). To confirm this observation, we examined sperm derived from congenic +/+, r⁵/+, and r⁵/ps² mice for the presence of Tctex1 using the specific R5205 antibody described previously (King et al., 1996b). In all samples, an immunoreactive band of ~14,000-Mr was observed (Fig. 1a, upper panel). To ensure that the reaction was specific, the blot-purified R5205 antibody was probed against recombinant Tctex1 before probing a companion blot; this yielded no signal (Fig. 1a, lower panel). Thus, Tctex1 is indeed a component of mammalian sperm.

To further investigate the role of Tctex1 in the flagellum, axonemes were prepared from Chlamydomonas and probed with the R5205 antibody raised against human Tctex1. A single immunoreactive band migrating at ~14,000 Mr was observed (Fig. 1b), indicating that a Tctex1-like protein exists in Chlamydomonas and that it is a component of the flagellar axoneme. After treatment of these axonemes with 0.6 M NaCl, ~90% of the immunoreactive band was found in the high salt supernatant (Fig. 2), as would be expected for a component of axonemal dynein. Analysis of membrane/matrix fractions also revealed a very small pool of this protein (not shown). This pool does not appear to be distinct from the major axonemal fraction (see below).

Tctex1 Is a Component of Inner Arm I

To further define the intraflagellar associations of the Chlamydomonas Tctex1-like protein, axoneme samples were prepared from mutants lacking various axonemal structures, including the outer arm (oda9), inner arm II (ida1, ida2, and ida3), inner arms 12/13 (ida4), the radial spokes (p/f14), and the central pair complex (p/f18). When
these samples were probed with an antibody (R5391) against LC2 of the outer arm, which is a homologue of the putative t complex distorter Tctex2, this protein was found to be missing only in axonemes prepared from the oda9 mutant, as described previously (Patel-King et al., 1997). Interestingly, the minor 15,000-Mr protein recognized by this antibody was present in all axoneme samples, indicating that it is not a component of the dynein arms, radial spokes, or central pair complex. In contrast, analysis of the same samples with the R5205 antibody revealed that the Tctex1-like protein was missing specifically in those strains (ida1–ida3) unable to assemble inner arm I1 (Fig. 3). This result strongly suggests that in Chlamydomonas, the Tctex1 protein is an integral component of the inner arm I1 complex.

To further confirm the association deduced from genetic dissection, axonemes were prepared from the oda9 mutant and subjected to high salt extraction. The extract (which contained no outer arm components) was then sedimented through a 5–20% sucrose gradient. Electrophoretic and immunological analysis of the resulting fractions revealed that the Tctex1-like protein sedimented at ~18 S and indeed precisely comigrated with bona fide inner arm I1 components (Fig. 4). This result confirmed that Tctex1 is an inner arm I1 polypeptide. The small membrane pool of Tctex1 was missing from ida1 flagella, suggesting that it represents inner arm I1 that either dissociated during detergent extraction or had not yet been assembled into the axonemal superstructure (not shown).

To further analyze the LC complement of this dynein, the I1-containing fractions from several gradients were pooled and concentrated in a Centricon 30 ultrafiltration unit. After electrophoresis and staining with Coomassie blue, three distinct bands were observed in the low molecular weight region (Fig. 4, inset). Immunological analysis revealed that the slowest migrating band was Tctex1. The fastest migrating band was recognized by antibody R4058 (King and Patel-King, 1995b) and, thus, is likely to be identical to the 8,000-Mr LC previously found in both outer arm and cytoplasmic dyneins, as well as in several other enzyme systems (Benashski et al., 1997). Intrigu-

![Figure 2. Distribution of Tctex1 in Chlamydomonas axonemes.](image)

125 µg of axonemes were treated with 0.6 M NaCl. Equivalent amounts of axonemes (Ax), extracted axonemes (EA), and the high salt extract (HSE) were electrophoresed in 5–15% acrylamide gradient gels. One portion of the gel was stained with Coomassie blue (upper panel) while the other was blotted to nitrocellulose and probed with R5205 antibody (lower panel). The locations of the relative molecular mass markers and the dye front (DF) are indicated on the left. Approximately 90% of the Tctex1 band was extracted with 0.6 M NaCl, suggesting that it may be a component of axonemal dynein.

![Figure 3. Intra-axonemal localization of Tctex1.](image)

Intra-axonemal localization of Tctex1. Axonemes were prepared from wild-type Chlamydomonas (WT) and from strains lacking the outer arm (oda9), inner arm I1 (ida1, ida2, and ida3), inner arms 12/13 (ida4), radial spokes (pf14), and central pair complex (pf18). 100 µg of each preparation was electrophoresed in a 5–15% acrylamide gradient gel and either stained with Coomassie blue (upper panel) or blotted to nitrocellulose (lower panels) and probed with R5205 and R5391 antisera to detect Tctex1 and LC2 (Tctex2) of outer arm dynein, respectively. LC2 is missing only in the oda9 mutant, which lacks the outer arm (the smaller immunoreactive band is present in all strains tested as described previously [Patel-King et al., 1997]). Tctex1 is specifically absent in those strains that fail to assemble inner arm I1, suggesting that it may be a component of that complex.

Quantitative densitometry of Coomassie blue–stained gels was used to determine the stoichiometry of the Tctex1 and 8,000-Mr LCs within the I1 dynein (Table I). This analysis revealed that there are two copies of Tctex1 and suggested the presence of 8–12 copies of the 8,000-Mr LC per I1 arm (this latter value is likely an overestimate; see Discussion). Furthermore, based on the presence of two HCs (Iα and Iβ), the data indicate that the I1 particle contains one to two copies of IC140, a single IC138, and one to two copies of IC97. A 34,000-Mr protein has also been suggested to be part of the I1 complex (King and Dutcher, 1997). Although a band of that relative molecular mass is present in the appropriate region of the sucrose gradients, the 34,000-Mr polypeptide peak is offset from that of I1 dynein by one fraction and, instead, it appears to comigrate with 100,000- and 106,000-Mr proteins.

**Molecular Analysis of Chlamydomonas Tctex1**

To conclusively identify the 14,000-Mr protein as the Chlamydomonas homologue of Tctex1, it was essential to compare the sequences of the algal and mammalian proteins. Therefore, the sucrose gradient–purified inner arm I1 from the oda9 mutant was concentrated in a Centricon...
Figure 4. Tetex1 is a component of inner arm dynein II. Proteins extracted from *oda9* axonemes (which completely lack the outer arm) by high salt were separated by sucrose density gradient centrifugation. Equal volumes of each fraction were electrophoresed in 5–15% gradient gels. Three bands are evident. The upper band was recognized by the R5205 antibody and is therefore Tctex1. The lower band was de

Table I. Stoichiometry of Components within Inner
Dynein Arm II*

| Component† | Relative stoichiometry§ | Copies per dynein arm |
|------------|-------------------------|-----------------------|
| HC         | 1.00                    | 21                   |
| IC140      | 0.71 0.53 0.63 (0.5–1.0)| 1–2                  |
| IC138      | 0.53 (0.5)              | 1                    |
| IC97       | 0.95 0.67 0.56 (0.5–1.0)| 1–2                  |
| Tctex1     | 1.28 1.12 0.71 (1.0)    | 2                    |
| 8,000-Mr LC| 5.50 4.00 (4.0–5.5)     | 8–12†                |

†Determined by quantitative densitometry of Coomassie blue–stained gels.

‡The IC140, IC138, and IC97 proteins in I1 samples are present at a stoichiometry of one per particle. This polypeptide, however, has not yet been formally demonstrated to be a dynein LC.

§The values are quoted relative to the HCs. The most likely stoichiometry is in parentheses.

Previous published results indicate that there are two HCs (Iα and Iβ) per particle (Piperno et al., 1990).

18S-Labeling of *Chlamydomonas* proteins in vivo suggests this value may be an overestimate by approximately twofold (Wilkinson, C.G., and G.B. Witman, personal communication).
gene for this protein exists within Chlamydomonas. Two messages for this protein, however, were detected on Northern blots (Fig. 7b). The larger message of \( \sim 1.35 \) kb was observed in total RNA samples from nondeflagellated cells, but it was not detected in samples from cells that were undergoing flagellar regeneration. In contrast, the smaller \( \sim 1.30 \) -kb mRNA was greatly upregulated in cells actively regrowing flagella. This differential regulation of both message levels was very apparent when compared with the levels of the single messages for two other proteins (Fig. 7c). The mRNA for LC4 of the outer arm was essentially undetectable in nondeflagellated cells, but it was highly upregulated during regeneration (King and Patel-King, 1995a). Calmodulin mRNA exhibited a different pattern and was readily detectable in both samples because the same protein is used constitutively in the cytoplasm but is also upregulated and required for flagellar assembly and/or function (Gitelman and Witman, 1980; Zimmer et al., 1988).

The secondary structure for Chlamydomonas Tctex1 was predicted using PHD (Fig. 8a; Rost and Sander, 1993). The NH\(_2\)-terminal half of the molecule has two segments that have a high probability of being helical. Significant portions of both segments (Fig. 8a, i and ii) are predicted to be highly amphiphilic and may therefore be involved in protein–protein interactions. The COOH-terminal region consists of a series of extended sheet structures.

Homology of the Chlamydomonas and Murine Tctex1 Proteins

Examination of the GenBank and Expressed Sequence Tag databases using BLAST revealed that the Tctex1 protein from Chlamydomonas flagella is closely related to mammalian Tctex1. A comparison between the Chlamydomonas and human proteins generated by GAP using the default parameters is shown in Fig. 8b. These proteins share 62% identity (68% similarity) with a smallest Poisson probability \( P_{(n)} = 6.3 \times 10^{-45} \) (calculated by BLAST), indicating that the match is highly significant. In addition...
to human Tctex1, the *Chlamydomonas* protein is closely related to murine Tctex1 (60% identity; \( P_{(n)} = 1.2 \times 10^{-45} \)) and *Drosophila* Tctex1 (56% identity; \( P_{(n)} = 1.9 \times 10^{-39} \)). *Chlamydomonas* Tctex1 is more distantly related to the human protein rp3 (46% identity, \( P_{(n)} = 2.1 \times 10^{-35} \); Roux et al., 1994), which we have recently shown to be an additional LC of cytoplasmic dynein (King, S.M., E. Barbar ese, J.F. Dillman III, S.E. Benashski, K.T. Do, R.S. Patel-King, and K.K. Pfister. 1997. *Mol. Biol. Cell.* 8:163a). These proteins are also distantly related to another *t* complex distorter candidate (Tctex2; Huw et al., 1995) and its *Chlamydomonas* homologue, which is an LC of outer arm dynein (Patel-King et al., 1997), as well as to several mammalian, nematode, and trypanosome Tctex1 homologues present in the Expressed Sequence Tag database (not shown). All of these proteins are significantly less related to Tctex1 than is the *Chlamydomonas* LC and, indeed, several of them group with the much more diverse Tctex2 branch of this family.

**Implications of a Flagellar Form of Tctex1 for the Mechanism of Meiotic Drive**

Meiotic drive or transmission ratio distortion is thought to derive from defects in spermiogenesis, where sperm bearing the *t* haplotype–containing chromosome are normal, but those with the wild-type *t* complex are dysfunctional and unable to fertilize an oocyte (Olds-Clarke and Peitz, 1985). This phenotype could be caused by aberrant flagellar motility because sperm from *t/+* males exhibit subtle defects in motility that have been shown to affect their ability to reach the oocytes both in vivo and in vitro (see Olds-Clarke [1997] for recent review). Genetic analysis suggests that meiotic drive derives from the interaction of three to four distorter proteins with a responder that is expressed after meiosis (Lyon, 1984, 1986). To achieve distortion, the responder must be kept in close association with the nucleus that encoded it. We have recently suggested that distortion might be achieved if the responder acts as the gatekeeper or sorting mechanism for flagella.

**The Chlamydomonas 14,000-Mr, Protein Is Closely Related to Murine Tctex1**

The Tctex1 protein family consists of two major branches exemplified by Tctex1 (shown in Fig. 8c) and by murine Tctex2 and its *Chlamydomonas* homologue LC2 (Patel-King et al., 1997). Molecular cloning of the *Chlamydomonas* 14,000- Mr protein described here revealed that it shares 62% identity with mammalian Tctex1. The closest known mammalian homologue of Tctex1 is the rp3 polypeptide that was originally cloned by Roux et al. (1994) and is ~55% identical with Tctex1. The rp3 protein has recently been demonstrated to be an additional cytoplasmic dynein LC that is most prevalent in cells and tissues distinct from those expressing Tctex1 (King, S.M., E. Barbar ese, J.F. Dillman III, S.E. Benashski, K.T. Do, R.S. Patel-King, and K.K. Pfister. 1997. *Mol. Biol. Cell.* 8:163a). Improtantly then, the dendrogram clearly distinguishes rp3 from the *Chlamydomonas*, insect, and mammalian Tctex1 proteins. Thus, phylogenetic analysis strongly supports the identification of the 14,000-Mr protein as the *Chlamydomonas* version of murine Tctex1. Furthermore, there are several partial and complete sequences of additional mammalian, nematode, and trypanosome Tctex1 homologues present in the Expressed Sequence Tag database (not shown). All of these proteins are significantly less related to Tctex1 than is the *Chlamydomonas* LC and, indeed, several of them group with the much more diverse Tctex2 branch of this family.

**Discussion**

In this report, we demonstrate that the *t* complex–encoded cytoplasmic dynein LC Tctex1 is also an integral stoichiometric component of flagellar inner dynein arm II. This conclusion is supported by several observations. First, a 14,000-Mr protein in both mouse sperm and *Chlamydomo nas* flagella is specifically recognized by an antibody raised against human Tctex1. Second, the *Chlamydomonas* protein copurifies with inner arm II and is missing only in those strains that fail to assemble this particular dynein complex. Third, there are two messages for this protein in *Chlamydomonas*; one is expressed constitutively, as expected for a cytoplasmic dynein component, while a second is subject to upregulation after deflagellation, as predicted for an axonemal protein. Finally, molecular analysis revealed that the *Chlamydomonas* protein shares considerable sequence identity with murine Tctex1.
and insect Tetex1 than it is to human rp3, which is an additional cytoplasmic dynein LC sharing 55% identity with Tetex1 (King, S.M., E. Barbarese, J.F. Dillman III, S.E. Benashski, K.T. Do, R.S. Patel-King, and K.K. Pfister. 1997. Mol. Biol. Cell. 8:163a).

assembly located at or near the basal body (Patel-King et al., 1997). The wild-type responder is thought to interact with both wild-type and t mutant distorter proteins, whereas the mutant responder may offer a protective effect by interacting poorly with mutant distorters and thereby incorporating mainly the wild-type versions (Lyon, 1984, 1986; Cebra-Thomas et al., 1991). The consequences of this model for incorporation of Tetex1, Tetex2, and the Tcd2 distorter into + and t sperm are illustrated in Fig. 9. Simultaneous analysis of the speed and path shape of sperm populations from t/+ males shows two distinct peaks, one of which resembles that of wild-type sperm and one that is similar to sperm from mice carrying two t haplotypes (Olds-Clarke and Johnson, 1993). These data support the idea that t/+ males produce two subpopulations of sperm that differ in their motility characteristics.

Tetex1 and Tetex2 are located in the appropriate regions of the t haplotype to be candidates for distorter factors Tcd1 and Tcd3, respectively (Lader et al., 1989; Rappold et al., 1987; Huw et al., 1995). The t haplotype forms of both proteins contain mutations that are likely to affect dynein function. In Tetex1, the mutation Q41H disrupts a conserved hydrophilic tripeptide sequence that has been completely conserved in all Tctex1 proteins (except for Tcd2) and is more closely related to mammalian dynein function and, thus, for the motile properties of flagellar Tctex1 and human Tetex1 (D50663). The alignment was generated by GAP using the default parameters. These proteins share 62% identity (68% similarity) with the smallest Poisson probability \( P(n) = 6.3 \times 10^{-45} \) (calculated by BLAST).

Figure 8. Sequence analysis of Chlamydomonas flagellar Tetex1. (a) The secondary structure of Tetex1 was predicted using PHD (Rost and Sander, 1993). E, extended sheet; H, helix. Helical stretches (i) and (ii) are amphiphilic and displayed using HELICALWHEEL. Hydrophobic and hydrophilic residues cluster to opposite sides of the helix. (b) Sequence comparison between Chlamydomonas flagellar Tetex1 and human Tetex1 (D50663). The alignment was generated using PHD (Rost and Sander, 1993). E, extended sheet; H, helix. Hydrophilic residues cluster to opposite sides of the helix. (c) Phylogenetic analysis of the members of the Tetex1 protein family. The relationship was calculated with DISTANCES and plotted with GROWTREE (UPGMA option). Chlamydomonas flagellar Tetex1 is more closely related to mammalian dyneins than it is to human rp3, which is an additional cytoplasmic dynein LC sharing 55% identity with Tetex1 (King, S.M., E. Barbarese, J.F. Dillman III, S.E. Benashski, K.T. Do, R.S. Patel-King, and K.K. Pfister. 1997. Mol. Biol. Cell. 8:163a).

Figure 9. A mechanism for flagellar dynein–mediated meiotic drive. In this model, the responder is hypothesized to be a "gatekeeper" or sorting mechanism that determines what may enter the growing flagellum during spermiogenesis. Because t mutations in both putative distorters Tetex1 and Tetex2 could lead to flagellar dynein dysfunction, their incorporation into + sperm by the wild-type responder (Tcr+) might result in defective motility. In contrast, the t mutant responder (Tcr−) is thought to protect the t sperm with which it associates by being unable to interact with (or having a lower affinity for) the mutant distorters. In the heterozygous case, this would lead to incorporation of only wild-type dyneins into t haplotype–bearing sperm and thus to normal motility. The molecular identities of all the distorters are unknown at the present time. In this model, it is hypothesized that Tetex1 and Tetex2 are the Tcd1 and Tcd3 distorters, respectively.

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sperm. In our model, distortion arises because the t responder interacts only weakly with the t distorters and, therefore, preferentially incorporates the wild-type forms into the growing sperm tail. Sperm from f/t<f/t mice are known to contain both Tctex1 and Tctex2 (Hu et al., 1995; O’Neill and Artzt, 1995; this study). All sperm from these mice, however, exhibit defective motility and are completely nonprogressive (Olds-Clarke and Johnson, 1993). This observation is also predicted by our model (Table II). The f/t<f/t homozygous animals represent the sole occasion on which the t forms of both the distorters and responder are the only versions present within the cell. We suggest that in the absence of the competing “high affinity” + distorters, the “low affinity” t forms would interact with the t responder and become incorporated by default. Thus, in the absence of competition with the high affinity distorters, low affinity interactions could suffice for the insertion of the t mutant distorters. The predicted variation in motile properties between + and t sperm from heterozygotes may then reflect a qualitative difference in the type of distorter protein (+ or t) incorporated into the two sperm classes.

Tctex1 and Tctex2 are candidates for the proximal Tcd1 and Tcd3 distorters. Analysis of rare partial haplotypes has revealed, however, that the strongest distorter (Tcd2) is located in the distal portion of the t complex (see Fig. 1 in Pilder et al., 1993). The molecular identity of this protein, which may be encoded at the Hybrid Sterility-6 locus (Pilder et al., 1993), is unknown at the present time. Sperm from the Hst6 mutant exhibit an abnormal flagellar curvature as well as poor motility. This phenotype is only seen in motile gametes, raising the possibility that it derives from the misregulation of flagellar beating, perhaps through direct effects on the dynein arm and thus potentially on Tctex1 and/or Tctex2) or radial spoke systems. Intriguingly, a presumptive axonemal dynein HC has recently been mapped to the distal region of the t complex (Dnahe8; Vaughan et al., 1996). Although the precise location of the Dnahe8 gene relative to Hst6 is not yet clear, this does raise the possibility that the distal distorter could be a dynein component. Such a scenario might then explain how Tcd2 greatly enhances the effects due to the other distorters.

A Generic Requirement for Tctex1 Family LCs in Dyneins Containing Multiple HCs

Tctex1 is part of a diverse protein family, members of which have now been identified in the major cytoplasmic dynein isoform (Tctex1 [King et al., 1996b] and rp3 [King, S.M., E. Barbarese, J.F. Dillman III, S.E. Benashski, K.T. Do, R.S. Patel-King, and K.K. Pfister. 1997. Mol. Biol. Cell. 8:163a], outer arm dynein (Tctex2; Patel-King et al., 1997), and inner arm I1 (Tctex1; this study). At least in the outer arm, the Tctex2 LC is an essential component and null mutants fail to assemble the entire structure (Pazour, G.J., A. Koutoulis, H. Sheng, R.S. Patel-King, S.M. King, and G.B. Witman, unpublished data). This LC family, however, is not apparently represented in the inner arm I2/I3 complexes, all the components of which are now known (LeDizet and Piperno, 1995). Thus, Tctex1 family–containing dyneins are distinct in that they all include two or more HCs and at least one IC that is a member of the WD repeat protein family. In outer arm and cytoplasmic dynein, the Tctex1 family LCs are known to interact with the ICs and form part of the basal IC/LC complex (Mitchell and Rosenbaum, 1986; King and Witman, 1990; for review see Witman et al., 1991; King, S.M., E. Barbarese, J.F. Dillman III, S.E. Benashski, K.T. Do, R.S. Patel-King, and K.K. Pfister. 1997. Mol. Biol. Cell. 8:163a). By analogy, a similar IC-associated location is likely in inner arm I1 and suggests that the Tctex1 homologues play a generic and essential role in maintaining the integrity of these multi-HC complexes.

The Highly Conserved 8,000-M, LC Is also Present in Inner Arm I1

Electrophoretic analysis of inner arm I1 revealed the presence of three distinct LC components. The smallest LC was recognized by antibody R4058 and is, therefore, the highly conserved 8,000-M, LC that was first identified as a component of Chlamydomonas outer arm dynein (King and Patel-King, 1995). Since then, this dimeric protein has been found as an integral component of several other enzyme systems, including mammalian cytoplasmic dynein (King et al., 1996a), the unconventional actin-based motor myosin V (Espindola, F.S., R.E. Cheney, S.M. King, D.M. Suter, and M.S. Mooseker. 1996. Mol. Biol. Cell. 7:372a), and neuronal nitric oxide synthase (Jaffrey and Snyder, 1996), where it apparently acts to control synthase activity. These studies have led to the suggestion that this 8,000-M, protein acts as a generalized regulatory element, perhaps in a manner analogous to calmodulin (discussed in Benashski et al., 1997). Unsurprisingly then, this LC is essential in multicellular organisms. In Drosophila, partial loss of function leads to morphogenetic defects, female sterility, and alterations in axonal guidance; total loss of function results in apoptosis and embryonic lethality (Dick et al., 1996a; Phillis et al., 1996). In both Chlamydomonas and Saccharomyces cerevisiae, null mutants have essentially no effect on viability (Dick et al., 1996b; Pazour, G., C.G. Wilkerson, and G.B. Witman. 1997. Mol. Biol. Cell. 8:162a). The Chlamydomonas mutant, however, does exhibit defects in both outer and inner arm dyneins, intradoublet microtubule projections, radial spokes, and in retrograde intraflagellar transport that is likely caused by the dysfunction of a cytoplasmic dynein (Pazour, G., C.G. Wilkerson, and G.B. Witman. 1997. Mol. Biol. Cell. 8:162a).

Identification of the 8,000-M, LC in inner arm I1 suggests that this protein is also a ubiquitous component of all dynein classes that contain two or more HCs. Stoichiome-

| Sperm genotype | Spermatocyte genotype | Predicted incorporation of + and t dyneins | Predicted motility |
|----------------|-----------------------|------------------------------------------|-------------------|
| +/+ | +/+ | Normal |
| +/t | t+ | + and t | Defective |
| t/t | t/t | t | Very poor |

Table II. Predicted Sperm Motility Phenotypes

Identification of the 8,000-M, LC in inner arm I1 suggests that this protein is also a ubiquitous component of all dynein classes that contain two or more HCs. Stoichiome-
try calculations based on dye binding suggest that inner arm II contains 8–12 copies of this LC per particle. A similarly high number was obtained for outer arm dynein (for review see King and Witman, 1989). However, based on recent studies measuring 35S incorporation into outer arm components in vivo (Wilkerson, C.G., and G.B. Witman, personal communication), it is likely that these numbers represent a significant overestimate (by approximately two-fold), presumably because the Chlamydomonas version of this LC has an unusually high affinity for the dye. Thus, it seems most likely that inner arm I, like the outer arm, contains four 8,000-M, LCs (i.e., two dimers) per particle.

In conclusion, we describe here a set of LCs from flagellar inner arm II that are apparently identical to recently identified components of cytoplasmic dynein. Because one of these LCs is the t complex-encoded protein Tctex1, these observations provide a mechanism by which the t haplotype mutations in murine Tctex1 might result in a testis-specific phenotype. Combined with our previous identification of another putative distorter, Tctex2 within the outer arm, this supports the hypothesis that transmission ratio distortion of t haplotypes involves the dysfunction of flagellar dyneins. Further structural and functional analyses of this intriguing class of dynein components will likely provide additional insight into this example of the fascinating phenomenon of meiotic drive.

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