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 I, 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 axonemal 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 propagating 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-Thullaz 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; Kagami 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 NH2-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 β subunit of heterotrimeric G proteins (Wilkerson et al., 1995; Sondek et al., 1996).

1. Abbreviations used in this paper: HC, heavy chain; IC, intermediate chain; LC, light 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 12/3 contain both a 28-kd protein, which is essential for arm assembly, and the Ca2+−binding protein centrin (LeDizet and Pipperno, 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 enzyme systems, including cytoplasmic dynein (King et al., 1996a), myosin V (Espinola, 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., C.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, is thought to derive from the action of mutant “distorter” and “responder” proteins (all encoded within the t haplotype) during spermiogenesis that lead to the inability of those sperm carrying the wildtype t complex to fertilize an oocyte. The Tctex1 protein has been of interest because it is a candidate for one of the distorter protein products responsible for the meiotic drive effect (Lader et al., 1989; O’Neill and Artzt, 1995). Intriguingly, we recently found that a Chlamydomonas outer arm dynein LC (Patel-King et al., 1997) was homologous to a second putative distorter termed Tctex2 (Huw et al., 1995).

This observation raised the possibility that transmission ratio distortion might derive from the dysfunction of both cytoplasmic and flagellar dyneins in the testis (Patel-King et al., 1997). Although there is some evidence for the presence of Tctex1 in sperm (O’Neill and Artzt, 1995), it has remained unclear how a defect in a cytoplasmic dynein component found in many tissues could result in a testis-specific phenotype.

In this report, we describe the further characterization of Tctex1 and conclusively demonstrate that this cytoplasmic dynein component is present in mouse sperm and in the flagella of Chlamydomonas. Genetic dissection of the Chlamydomonas axoneme revealed that flagellar Tctex1 is specifically located in inner dynein arm 11. This same dynein was also found to contain the 8,000-Mr LC and, thus, to closely resemble the cytoplasmic isozyme in terms of LC content. The data presented here suggest that the t-specific mutations in Tctex1 might contribute to 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 congenic +/+, r1v+/+, and r1v[ps2] 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 Centricon 30 unit (Amicon Corp., Danvers, MA). Nonspecific protein binding was minimized by preincubating the unit for 48 h with 5% Tween-20.

For the initial identification of the 14,000-Mr protein, the wild-type strain cc124-w5 was used. A mutant lacking the outer arm, oda9, was used for all subsequent purifications. Axonemes from mutants lacking inner arm components (ida1, ida2, ida3, and ida4), radial spokes (pfl4), and the central pair microtubule complex (pfl8) were used to localize the 14,000-Mr protein.

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 (Immobilin 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 C8 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).

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Electrophoresis and Immunoblotting  
All *Chlamydomonas* samples were electrophoresed in 5-15% polyacryl-
amide 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., 1996e). After washing, the antibody signal was visualized using an 
enhanced chemiluminescent system (ECL; Amersham Corp., Arlington 
Heights, IL) and X-ray film (Fuji Photo Film Co., Ltd., Tokyo, Japan). Total protein was subsequently detected using 0.2% Ponceau S. Antibodies 
used were R4058 vs. the Chlamydomonas 8,000-M₉, LC (King and Patel-
King, 1995b), R5205 vs. human Tctex1 (King et al., 1996b), and R5391 vs. 
the *Chlamydomonas* Tctex2 homologue LC2 (Patel-King et al., 1997).
Quantitation of Coomassie blue–stained gels was performed using an 
IS1000 digital imaging system (Alpha Innotech, San Leandro, CA).

**Molecular Cloning**

A gene-specific primer (5’-GCGGAATTCTTGCGACGACGGAGCAG-
TAC-3’) was designed based on the peptide sequence LQNOYY and in-
corporated 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’-CGTCTACGAC-
TCGAGTCACTGG-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 2 min, cooled on ice, and 2.5 U of Phusion DNA pol-
ymerase (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 
(Sambrook et al., 1989) and probed using the conditions described in King and Patel-King (1995b).

The PCR product corresponding to the 14,000-M₉ protein was used to 
screen a λZapII cDNA library made from mRNA derived from cells ac-
tively regenerating flagella (Wilkersen et al., 1995). Phagemids were res-
queed using helper phage, and the longest clone was sequenced on both 
strands using a double-stranded DNA template. Northern and Southern 
blots were prepared by standard methods (Sambrook et al., 1989) and probed using the conditions described in King and Patel-King (1995b).
The probes used were the 14,000-M₉ protein full-length cDNA, a full-
length calmodulin cDNA that had been isolated during a screen of the 
λZapII library for the calmodulin-like LC4 protein.

**Computational Methods**

Sequence assembly and analysis was performed using the GCG suite of 
software (Devereux et al., 1984). Searches of the GenBank database were 
made using BLAST (Altschul et al., 1990). Pairwise sequence comparisons 
were generated using GAP (Devereux et al., 1984), and multiple align-
ments were constructed with CLUSTALW (Thompson et al., 1994).
The secondary structure prediction was made using the PHD program (Rost 
and Sander, 1993), and the helical segments were analyzed with HELI-
CALWHEEL (Devereux et al., 1984). The phylogenetic tree was calcu-
lated with DISTANCES and plotted with GROWTREE.

**Results**

**Tctex1 Is Present in the Flagellar Axoneme**

We recently identified the mouse t complex–encoded protein 
Tctex1 as one of the 14,000-M₉ LCs associated with 

cytoplasmic dynein isolated from mammalian brain, kid-
ney, liver, spleen, and testis (King et al., 1996b). Intrigu-
ingly, 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 ++/+, t⁵⁺/+, and 

a⁵/⁻⁻ 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-M₉ 
was observed (Fig. 1, upper panel). To ensure that the re-
action was specific, the blot-purified R5205 antibody was 
preadsorbed against recombinant Tctex1 before probing a 
companion blot; this yielded no signal (Fig. 1, lower 
panel). Thus, Tctex1 is indeed a component of mammalian 
sperm.

To further investigate the role of Tctex1 in the flagel-
um, axonemes were prepared from *Chlamydomonas* and 
probed with the R5205 antibody raised against human 
Tctex1. A single immunoreactive band migrating at 
~14,000-M₉ was observed (Fig. 1, b), 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 immuno-
reactive band was found in the high salt supernatant (Fig. 2), 
as would be expected for a component of axonemal dy-
nein. 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 11**

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 ida7), inner arms I2/I3 (ida4), the radial 
spokes (pH4), and the central pair complex (pP18). When

![Figure 1. Tctex1 is present in both mouse sperm and *Chlamydo-
omonas* axonemes. (a) Whole-cell extracts of equal numbers of sperm from ++/+, t⁵⁺/+, and t⁵/⁻⁻ mice were electrophoresed in a 15% acrylamide gel, blotted to Immobilon-P membranes, and probed with R5205 antibody (upper panel) or R5205 antibody that had been preadsorbed against recombinant Tctex1 (lower panel). Tctex1 is present in sperm from all genotypes. (b) 150 μg of *Chlamydomonas* axonemes were electrophoresed in a 5–15% acrylamide gradient gel and either stained with Coomassie blue (CBB) or blotted to nitrocellulose and probed with R5205 anti-
tbody. The locations of the relative molecular mass markers and the dye front (DF) are indicated on the left. A single 14,000-M₉ band was observed.
these samples were probed with an antibody (R5391) against LC2 of the outer arm, which is a homologue of the putative \( \tau \) complex distorter Tctex2, this protein was found to be missing only in axonemes prepared from the \( \text{oda}\) mutant, as described previously (Patel-King et al., 1997). Interestingly, the minor 15,000-\( M_r \) 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 \((\text{ida}1–\text{ida}3)\) unable to assemble inner arm I1 (Fig. 3). This result strongly suggests that in \textit{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 \( \text{oda}\) 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 \( \sim 18 \) 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 \( \text{ida} \) 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-\( M_r \) LC previously found in both outer arm and cytoplasmic dyneins, as well as in several other enzyme systems (Benashski et al., 1997). Intriguingly, a third band (\( \sim 12,000-\text{M}_r \)) was also observed in arm I1; at the present time, no further information is available concerning the identity of this protein.

Quantitative densitometry of Coomassie blue–stained gels was used to determine the stoichiometry of the Tctex1 and 8,000-\( M_r \) 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-\( M_r \) LC per I1 arm (this latter value is likely an overestimate; see Discussion). Furthermore, based on the presence of two HCs (I\( \alpha \) and I\( \beta \)), 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-\( M_r \) 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-\( M_r \) polypeptide peak is offset from that of I1 dynein by one fraction and, instead, it appears to comigrate with 100,000- and 106,000-\( M_r \) proteins.

**Molecular Analysis of Chlamydomonas Tctex1**

To conclusively identify the 14,000-\( M_r \) protein as the \textit{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 \( \text{oda}\) mutant was concentrated in a Centricon
Table I. Stoichiometry of Components within Inner Dynein Arm II*  

<table>
<thead>
<tr>
<th>Component1</th>
<th>Relative stoichiometry1</th>
<th>Copies per dynein arm</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC</td>
<td>1.00</td>
<td>2†</td>
</tr>
<tr>
<td>IC140</td>
<td>0.71 0.53 0.63 (0.5–1.0)</td>
<td>1–2</td>
</tr>
<tr>
<td>IC138</td>
<td>0.53 (0.5)</td>
<td>1</td>
</tr>
<tr>
<td>IC97</td>
<td>0.95 0.67 0.56 (0.5–1.0)</td>
<td>1–2</td>
</tr>
<tr>
<td>Tctex1</td>
<td>1.28 1.12 0.71 (1.0)</td>
<td>2</td>
</tr>
<tr>
<td>8,000- M1 LC</td>
<td>5.50 4.00 (4.0–5.5)</td>
<td>8–12†</td>
</tr>
</tbody>
</table>

* Determined by quantitative densitometry of Coomassie blue–stained gels.
† The ~12,000-M1 protein observed in I1 samples is 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.
§ Previously published results indicate that there are two HCs (Ia and Ib) 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).

Figure 4. Tctex1 is a component of inner arm dynein I1. 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. One gel was stained with Coomassie blue (upper panel), while the other was blotted to nitrocellulose and probed with R5205 antibody (lower panel). The bottom of the gradient is at the left. After immunoblots, the nitrocellulose was restained with Ponceau S to reveal the location of individual lanes and the relative molecular mass markers. Tctex1 comigrates with inner arm I1 components (IC140 and IC138) at ~18 S, but it is clearly distinct from arms I2/3, which contain actin, p28, and centrin. (inset, upper panel) The I1 dynein–containing fractions from two gradients were pooled and concentrated in a Centricon-30 ultrafiltration unit and electrophoresed in a 5–15% acrylamide gel. The LC region of the Coomassie blue–stained gel is shown. Three bands are evident. The upper band was recognized by the R5205 antibody and is therefore Tctex1. The lower band was detected by the R4058 antibody, indicating that it represents an additional pool of the 8,000-M1 LC (King and Patel-King, 1995b). A third LC band of unknown origin is also present.

30, and the components were separated by electrophoresis and blotted to a PVDF membrane. The 14,000-M1 band was identified using blot-purified R5205 antibody, excised from the PVDF blot, and digested in situ with trypsin. Eluted peptides were purified by reverse-phase chromatography (Fig. 5, a). Mass spectrometry revealed that peak (i) contained a single peptide with a mass (M+H+) of 1,825 D (Fig. 5, b). This peptide subsequently yielded the sequence ESIDAVLONQOYSEAK, which has a calculated molecular weight of 1,823 D. Analysis of peak (Fig. 5 a, ii) revealed a major peptide of 2,463 D and a minor one of 2,479 D (Fig. 5, c). As the mass difference between these two peptides is 16 D, the latter very likely represents a methionine oxidation product of the former. Intriguingly, the actual mass of the blocked NH2-terminal peptide was 42-D greater than calculated from the encoded sequence. This mass difference strongly suggests that the modification resulting in NH2-terminal blockage was caused by acetylation of the initial methionine residue.

Southern blot analysis of Chlamydomonas genomic DNA revealed a single band after digestion with BamHI, PstI, PvuII, and SmaI (Fig. 7 a), suggesting that a single
gene for this protein exists within *Chlamydomonas*. Two messages for this protein, however, were detected on Northern blots (Fig. 7b). The larger message of \(1.35\) 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 \(1.30\)-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 NH2-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 \(P_{(n)} = 6.3 \times 10^{-15}\) (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; $P_\text{(m)} = 1.2 \times 10^{-40}$) and *Drosophila* Tctex1 (56% identity; $P_\text{(m)} = 1.9 \times 10^{-39}$). *Chlamydomonas* Tctex1 is more distantly related to the human protein rp3 (46% identity, $P_\text{(m)} = 2.1 \times 10^{-35}$; Roux et al., 1994), which we have recently shown to be an additional LC of cytoplasmic dynein (King, S.M., E. 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). Importantly 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.

**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 distorser 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.
and insect Tctex1 than it is to human rp3, which is an additional cytoplasmic dynein LC sharing 55% identity with Tctex1 (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 8. Sequence analysis of Chlamydomonas flagellar Tctex1. (a) The secondary structure of Tctex1 was predicted using PHD (Rost and Sander, 1993). E, extended sheet; H, helix. Helical stretches (i) and (ii) are amphiphilic and displayed using HELICALWHEEL. Hydrophilic and hydrophobic residues cluster to opposite sides of the helix. (b) Sequence comparison between Chlamydomonas flagellar Tctex1 and human Tctex1 (D50663). The alignment was generated with 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). (c) Phylogenetic analysis of the members of the Tctex1 protein family. The relationship was calculated with DISTANCES and plotted with GROWTREE (UPGMA option). Chlamydomonas flagellar Tctex1 is more closely related to mammalian 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 Tctex1, Tctex2, and the Tcd2 distorter into $+t$ and $t+$ sperm are illustrated in Fig. 9. Simultaneous analysis of the speed and path shape of sperm populations from $t+/t+$ 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 $t+/t+$ males produce two subpopulations of sperm that differ in their motility characteristics.

Tctex1 and Tctex2 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 Tctex1, the mutation Q41H disrupts a conserved tripeptide sequence that has been completely conserved in both proteins contain mutations that are likely to affect dynein function. In Tctex1, the mutation Q41H disrupts a conserved tripeptide sequence that has been completely conserved in all Tctex1 proteins (except for Drosophila, which contains Asn at the equivalent position) and in the closely related rp3. Similarly, the $t$ form of Tctex2 contains a three-residue deletion and a proline insertion within a predicted helical segment. As Tctex2 is an essential outer arm component, it is relatively easy to see how its dysfunction might affect sperm motility. However, identification of Tctex1 as a cytoplasmic dynein LC was more problematic, because this protein is present in many tissues but heterozygous $t$ haplotype–bearing mice show an abnormal phenotype only in male germ cells. If Tctex1 is a component of an inner arm in mouse sperm flagella, this would provide a mechanism by which the $t$ haplotype allele could lead to alterations in sperm activity.

The action of the distorter proteins is cumulative such that the degree of ratio distortion is directly related to the particular alleles present in a given haplotype (Lyon, 1984, 1986). These allele-specific effects can be readily understood through increasingly severe consequences for flagellar dynein function and, thus, for the motile properties of

Figure 9. A mechanism for flagellar dynein–mediated meiotic drive. In this model, the responder is hypothesized to be a "gate-keeper" or sorting mechanism that determines what may enter the growing flagellum during spermiogenesis. Because $t$ mutations in both putative distorters Tctex1 and Tctex2 could lead to flagellar dynein dysfunction, their incorporation into $+t$ 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 Tctex1 and Tctex2 are the $Tcd1$ and $Tcd3$ distorters, respectively.
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 r/r mice\(^3\) 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 nonprogressive (Olds-Clarke and Johnson, 1993). This observation is also predicted by our model (Table II). The t/r 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 distortor 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 haploptypes 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 (Dnahc8; Vaughan et al., 1996). Although the precise location of this HC has not yet been determined, it may also interact with the Tctex1 family LCs 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 distortor protein (+ or t) incorporated into the two sperm classes.

**Table II. Predicted Sperm Motility Phenotypes**

<table>
<thead>
<tr>
<th>Sperm genotype</th>
<th>Spermatocyte genotype</th>
<th>Predicted incorporation of + and t dynemes</th>
<th>Predicted motility</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ +</td>
<td>t/t</td>
<td>+ and t</td>
<td>Normal</td>
</tr>
<tr>
<td>+ t</td>
<td>t/t</td>
<td>t</td>
<td>Defective</td>
</tr>
<tr>
<td>t t</td>
<td>+ t +</td>
<td>+</td>
<td>Normal</td>
</tr>
<tr>
<td>t t</td>
<td>t</td>
<td>t</td>
<td>Very poor</td>
</tr>
</tbody>
</table>

Tctex1 and Tctex2 are candidates for the proximal Tcd1 and Tcd3 distorters. Analysis of rare partial haploptypes 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 (Dnahc8; Vaughan et al., 1996). Although the precise location of this HC has not yet been determined, it may also interact with the Tctex1 family LCs 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 distortor protein (+ or t) incorporated into the two sperm classes.

**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, LC 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-

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\(^3\) r/r indicates mice that carry r haplotypes with different embryonic lethal factors that do not affect sperm function (Silver, 1993).

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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 I2/3 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.
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 $^{35}$S 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 twofold), presumably because the Chlamydomonas version of this LC has an unusually high affinity for the dye. Thus, it seems most likely that inner arm II, like the outer arm, contains four 8,000-M$_{r}$ 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 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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