The Mouse t-Complex-encoded Protein Tctex-1 Is a Light Chain of Brain Cytoplasmic Dynein

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Stephen M. King‡§, James F. Dillman III¶, Sharon E. Benashski‡, R. John Lye¶, Ramila S. Patel-King§, and K. Kevin Pfister¶

From the ‡Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut 06032-3305 and the ¶Department of Cell Biology, University of Virginia Health Science Center, Charlottesville, Virginia 22908-0439

Mammalian brain cytoplasmic dynein contains three light chains of $M_r = 8,000, 14,000$, and 22,000 (King, S. M., Barbarese, E., Dillman, J. F., III, Patel-King, R. S., Car- son, J. H., and Pfister, K. K. (1996) J. Biol. Chem. 271, 19358–19366). Peptide sequence data (16/16 residues correct) implicate the $M_r = 14,000$ polypeptide as Tctex-1, a protein encoded within the mouse t-complex. Tctex-1 cosediments with microtubules and is eluted with ATP or salt but not with GTP as expected for a dynein subunit. The ATP-eluted protein precisely cosediments with known cytoplasmic dynein proteins in sucrose density gradients. Tctex-1 also is immunoprecipitated from brain and other tissue homogenates by a monoclonal antibody raised against the 74-kDa cytoplasmic dynein intermediate chain. Quantitative densitometry indicates that Tctex-1 is a stoichiometric component of the dynein complex. As Tctex-1 is a candidate for involvement in the transmission ratio distortion (meiotic drive) of mouse $t$-haplotypes, these results suggest that cytoplasmic dynein dysfunction may play an important role in non-mendelian chromosome segregation.

Dyneins are microtubule-based molecular motors. Axonemal dyneins are responsible for generation and propagation of ciliary/flagellar bends (see Ref. 1), whereas cytoplasmic dynein is involved in a wide range of intracellular motile events including retrograde vesicle transport in axons, membrane trafficking, nuclear migration, and both the positioning and anaphase movement of the mitotic spindle (2–9). Although involved in diverse motile systems, recent molecular analyses have revealed that the flagellar and cytoplasmic dynein isozymes are related both in terms of their component polypeptides and in their overall structural organization (for review, see Refs. 10 and 11).

Cytoplasmic dynein is a multimeric complex containing two dynein heavy chains (DHCs)1 ($\approx 530$ kDa) that exhibit both ATPase and motor activities; these components form the globular heads and stems seen in the soluble particle (reviewed in Ref. 10). A recent report suggests that there are at least three cytoplasmic DHC isoforms in mammals that are associated with discrete intracellular structures (12). Thus, different cytoplasmic DHC-containing isoforms may have distinct functions, as do their flagellar counterparts (reviewed in Refs. 11 and 13).

Associated with these large motor subunits are 2–3 copies of a 74-kDa intermediate chain (IC74) (14) that contains five repeats of the WD motif (15) and is therefore likely a member of the $\beta$-propeller family of proteins (16). IC74 apparently is involved in attaching the cytoplasmic dynein motor to its cargo by mediating the interaction between dynein and dynactin (17, 18). Multiple alternatively spliced and phosphorylated variants of this component have been identified (14, 19–21), and these may be involved in targeting the motor to various intracellular cargoes. The cytoplasmic ICs are related both structurally and functionally to their counterpart proteins found within flagellar dyneins (reviewed in Ref. 11). For example, the $M_r = 78,000$ IC (IC78) of *Chlamydomonas* outer arm dynein is also a WD-repeat protein that mediates the ATP-insensitive (cargo) attachment of that enzyme to the flagellar doublet microtubules (22–24). The cytoplasmic dynein complex also includes several light intermediate chains (LICs) (50–60 kDa) that contain an ATP-binding loop motif related to that found in the ABC transporter family of ATPases (25, 26).

Recently, we found that cytoplasmic dynein also contains three light chains (LICs) of $M_r = 8,000, 14,000$, and 22,000 (27). Interestingly, the $M_r = 8,000$ protein (actual mass = 10.3 kDa) is essentially identical to an LC of axonemal outer arm dynein that was originally identified in *Chlamydomonas* flagella (28). This protein has now also been found as a stoichiometric component of brain myosin V (60) indicating that both actin- and microtubule-based molecular motors share some common structural or functional feature. The function of this $M_r = 8,000$ polypeptide within dynein remains unknown although its presence in two distinct classes of motor enzyme raises the possibility of a common docking or targeting role. However, it is clear from the phenotypes observed in *Drosophila* and Aspergillus mutants that this protein is very important for motor function. In *Drosophila*, partial loss of function results in female sterility and a wide variety of morphogenetic defects; total loss induces apoptosis and leads to embryonic lethality (29). In Aspergillus, mutation at the nudG locus, which encodes the homologous protein (–75% sequence identity with the *Chlamydomonas* $M_r = 8,000$ LC), results in defective nuclear migration (62), a process for which cytoplasmic dynein is known to be essential (9).

LICs of flagellar outer arm dyneins have been found to exhibit a variety of intriguing and unexpected functional attributes, including cAMP-dependent phosphorylation (30), Ca$^{2+}$ binding (31), and sulfhydryl oxidoreductase activity (32). Accordingly, we have initiated an analysis of the low molecular weight

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§ To whom correspondence should be addressed: Dept. of Biochemistry, University of Connecticut Health Center, 263 Farmington Ave., Farmington, CT 06032-3305. Tel.: 860-679-3347; Fax: 860-679-3408; E-mail: king@panda.uchc.edu.

1 The abbreviations used are: DHC, dynein heavy chain; LC, intermediate chain; LIC, light intermediate chain; IC, light chain.
polypeptides observed in cytoplasmic dynein preparations. In this report, we identify the M* = 14,000 protein as Tctex-1, and we demonstrate that it is indeed a bona fide LC of this cytoplasmic microtubule motor enzyme. In mice, Tctex-1 is encoded within the t-complex, a large region of chromosome 17 containing multiple inversions. This region represents the most extreme vertebrate example of meiotic drive or transmission ratio distortion (for review, see Ref. 33). Heterozygous males pass the t-haplotype-bearing chromosome to 99% or more of their progeny; homozygous males containing two complementing t-haplotypes are completely sterile. Based on mapping and expression studies, Tctex-1 has been implicated in the multigene phenomenon of transmission ratio distortion and possibly also in the male sterility phenotype (34). Consequently, our results imply that this LC is of considerable importance for dynein motor activity and that cytoplasmic dynein dysfunction plays a role in non-mendelian chromosome segregation in mammals.

EXPERIMENTAL PROCEDURES

Cytoplasmic Dynein Purification—Cytoplasmic dynein was purified from bovine and rat brain homogenates by ATP-sensitive microtubule affinity. Following homogenization and high speed centrifugation, microtubules were polymerized from the resulting supernatant at 37 °C and stabilized by the addition of taxol. The microtubules were pelleted, and concentrated solubilized samples were sequentially eluted with buffers containing GTP, 5 mM ATP, and 1 M NaCl. The dynein in the ATP eluate was further purified by centrifugation in 5–20% sucrose density gradients. This procedure represents a minor modification of the standard protocol detailed by Paschal et al. (35).

In a purification scheme based on properties distinct from those detailed above, cytoplasmic dynein, kinesin, and dynactin were obtained directly from a rat brain homogenate by immunoprecipitation (as described in Ref. 19) using monoclonal antibodies 74–1 (19), H-2 (36), and 50–1 (37), which recognize IC74 of cytoplasmic dynein, the kinesin heavy chain, and p50 of dynactin, respectively. Control immunoprecipitations contained beads but no primary antibody.

The above immunoprecipitation procedure also was used to isolate cytoplasmic dynein from liver, spleen, kidney, brain, and testis homogenates to assess the tissue distribution of Tctex-1.

Peptide Sequencing—Cytoplasmic dynein purified by ATP-dependent microtubule affinity and sucrose density gradient centrifugation was concentrated by ultrafiltration in Centricon 30 units (Amicon, Danvers, MA) that had previously been treated with 5% Tween 20 to reduce nonspecific protein binding. The concentrated protein was electrophoresed in a 5–15% acrylamide gradient gel, blotted to polyvinylidene difluoride membrane (Immobilon P™, Millipore, Woburn, MA) in 10 mM NaHCO3, 3 mM Na2CO3, 0.1% SDS, 20% methanol, and stained with Amido Black. The membrane was treated with 5% Tween 20 to reduce nonspecific protein binding. The concentrated protein was electrophoresed in a 5–15% acrylamide gradient gel, blotted to polyvinylidene difluoride membrane (Immobilon P™, Millipore, Woburn, MA) in 10 mM NaHCO3, 3 mM Na2CO3, 0.1% SDS, 20% methanol, and stained with Amido Black. The M* = 14,000 protein band was excised, washed, and incubated with trypsin. Peptides eluting from the membrane were purified by reverse phase chromatography on a C18 column. Peptide sequencing was performed using an Applied Biosystems model 492A sequencer in the Protein Chemistry facility at the Worcester Foundation for Biomedical Research (Shrewsbury, MA).

Computational Methods—Searches of the Genbank™ and Expressed Sequence Tag data bases were performed using BLAST (38). Pairwise sequence comparisons were made using GAP (39), and multiple sequence alignments were generated with CLUSTALW (40).

Fusion Protein Preparation and Antibody Production—The coding sequence for the human Tctex-1 protein was obtained by the polymerase chain reaction using the I.M.A.G.E consortium clone 128643 from a human fetal liver/spleen cDNA library as template. The primers were designed such that the product had a blunt 5′-end and an XbaI site at the 3′-end following the stop codon. This was subcloned across the XbaI and XhoI sites of pMalc-2 (New England Biolabs, Beverly, MA) and resulted in the C-terminal fusion of Tctex-1 to maltose-binding protein via a short linker segment that contains a Factor Xa protease site immediately N-terminal to the first Met residue of Tctex-1. Fusion protein production and purification was performed as described in (31).

A rabbit polyclonal antiserum was obtained using the entire fusion protein as immunogen. Subsequently, an anti-Tctex-1 antibody fraction was isolated by blot purification (41). Whole serum (diluted approximately 1:25) was incubated overnight with a nitrocellulose strip containing electrotheropically purified recombinant Tctex-1. Following multiple washes, antibody was eluted with 0.2 M glycine, pH 2.15, for 1–2 min and immediately neutralized by the addition of 1.5 M Tris–Cl, pH 8.8. Blot-purified antibody preparations were routinely diluted 1:50 to 1:1000.

Electrophoresis and Immunoblotting—All samples were electrophoresed in 5–15 or 4–16% acrylamide gradient gels and either stained with Coomassie Brilliant Blue or blotted to nitrocellulose using the buffer conditions described above. For immunoblotting, the nitrocellulose was blocked with 5% dry milk, 0.1% Tween 20 in Tris-buffered saline and then probed with purified anti-Tctex-1 antibody (R5205) or the 74–1 monoclonal antibody followed by the appropriate peroxidase-conjugated secondary antibody. Antibody reactivity was detected using an enhanced chemiluminescent procedure (ECL, Amersham) and Fuji RX film. Subsequently, blots were stained with Amido Black to reveal total protein.

Quantitation of Coomassie Blue-stained gels was performed using an IS-1000 digital imaging system (Alpha Innotech, San Leandro, CA) or a Molecular Dynamics personal densitometer and ImageQuant software.

RESULTS

Electrophoretic analysis of cytoplasmic dynein purified both by microtubule affinity-sucrose gradient centrifugation and by immunoprecipitation with a specific monoclonal antibody revealed the presence of three LC components of M* = 8,000, 14,000, and 22,000 (27) (see Fig. 1, inset). To identify the M* = 14,000 protein, bovine brain cytoplasmic dynein polypeptides were separated in a 5–15% acrylamide gradient gel and blotted to polyvinylidene difluoride membrane. The M* = 14,000 band was excised and digested with trypsin in situ. Eluted peptides were purified by reverse phase chromatography (Fig. 1) and one peptide was sequenced. The sequence obtained was 16 residues in length (Fig. 1) and, as it derived from a tryptic digestion, is presumably preceded by either an Arg or Lys residue.

Examination of the Genbank™ and Expressed Sequence Tag data bases using BLAST revealed that this peptide (including the presumptive N-terminal basic residue) was 100% identical with both the mouse (A32995; 34) and human (H11202; 42) Tctex-1 proteins (Fig. 2). The probability of this match occurring by chance (P(n)) is 8.1 × 10−5 (calculated by BLAST) and no other statistically significant matches were identified in the non-redundant data base at National Center for Biotechnology Information. The mouse Tctex-1 protein contains 113 residues and has a calculated mass of 12.5 kDa, in good agreement with the electrophoretically determined size of the cytoplasmic dynein-associated protein. This result suggested that the M* = 14,000 polypeptide is Tctex-1.

To investigate the hypothesis that the M* = 14,000 protein is indeed Tctex-1, the human protein was prepared as a C-terminal fusion with maltose binding protein (Fig. 3A), and the entire recombinant molecule was used to raise a high affinity polyclonal antiserum (R5205). Following digestion of the fusion protein with Factor Xa, antibody against Tctex-1 was obtained by blot purification versus electrophoretically purified recombinant Tctex-1. When used to probe total rat brain oligodendrocyte protein, this antibody preparation was found to be highly specific (Fig. 3B). Only a single major band of M* = −14,000 was observed in these samples. There was also a very minor reaction with a band migrating between Tctex-1 and the dye front. This additional band may represent a proteolytic fragment of Tctex-1 or it may be due to cross reaction of the antibody preparation with some other small cellular protein. It is important to note however that this minor band was readily distinguishable from Tctex-1 following gradient gel electrophoresis.

To further test whether Tctex-1 is an LC of cytoplasmic dynein, the distribution of this molecule and cytoplasmic dynein in the supernatants and pellets from a standard microtubule-associated protein purification scheme was examined (Fig. 4). Approximately 90% of both IC74 from cytoplasmic dynein and Tctex-1 were found to cosediment with microtubules po-
lymerized from a brain homogenate by incubation at 37°C in the presence of taxol. Both proteins remained microtubule-associated following a buffer wash and subsequent incubation with 5 mM GTP. Upon elution of the microtubule pellet with 5 mM ATP,

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\frac{75}{50}\% \text{ of IC74 and Tctex-1 were solubilized.}
\]

Those fractions of both proteins that remained microtubule-associated following ATP elution were completely solubilized with 1M NaCl. Interestingly, in the whole brain homogenate, considerably more of the minor band detected in the oligodendrocyte sample by the R5205 antibody (see Fig. 3B) was evident; this protein did not cosediment with microtubules and remained in the first supernatant (not shown).

Proteins eluted from microtubules with ATP were further fractionated by centrifugation through a 5–20% sucrose density gradient (Fig. 5, upper panel). Immunoblot analysis revealed that Tctex-1 precisely cosedimented with the DHC and IC74 of cytoplasmic dynein at \(-20\) S in fractions 6–9 (Fig. 5, lower panel). It was not observed in kinesin-containing fractions (fractions 10–13) nor did its sedimentation profile coincide completely with dynactin, which was found in all fractions from the dynein peak to the bottom of the gradient (fractions 1–9). Thus, sucrose density gradient analysis indicates that Tctex-1 cosediments with cytoplasmic dynein, but not with kinesin or dynactin, as predicted for a dynein subunit.

To obtain further evidence for the association of Tctex-1 with dynein, cytoplasmic dynein, dynactin and kinesin were each purified using a methodology biochemically distinct from that detailed above. Each complex was obtained by immunoprecipitation directly from a rat brain homogenate using previously characterized specific monoclonal antibodies. Electrophoretic analysis of the immunoprecipitates following staining with Coomassie Blue is shown in Fig. 6 (upper panel). Each antibody specifically precipitated the appropriate complex. Identical samples were probed with blot-purified R5205 and 74–1 antibodies (lower panels). This revealed that Tctex-1 and IC74 were present only in the cytoplasmic dynein sample. This strongly supports the hypothesis that Tctex-1 is a dynein LC and demonstrates that this molecule is not a component of either kinesin or dynactin.

Neither Tctex-1 nor cytoplasmic dynein were specifically im-

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**Fig. 1.** Peptide purification from the M, = 14,000 cytoplasmic dynein LC. Electrophoretic analysis of the three LCs of cytoplasmic dynein following staining with Coomassie Blue is shown in the inset. The electrophoretically isolated bovine M, = 14,000 LC was blotted to polyvinylidene difluoride and digested in situ with trypsin. Peptides eluting from the membrane were purified by reverse phase chromatography on an Aquapore RP-300 (C18) column. The 16-residue sequence shown was obtained from the peak marked with an asterisk.

**Fig. 2.** Comparison of the M, = 14,000 LC peptide sequence with Tctex-1. Sequence comparison between the 16-residue peptide obtained from the electrophoretically purified bovine M, = 14,000 LC and the mouse (A32995) (34) and human (H11202) (42) sequences for the protein Tctex-1. The alignment was generated by pairwise comparisons with the program GAP using the default parameters. The symbols (\(\), \(\), \(\)) represent identity and conservative and semi-conservative substitutions, respectively. The 100% 16-residue peptide match to Tctex-1 gives \(P(n) = 8.1 \times 10^{-5}\) (calculated by BLAST (Altschul et al., 1990)).
munoprecipitated from rat brain homogenates by either the blot-purified or crude R5205 Tctex-1 antiserum, suggesting that the epitopes recognized are not accessible in the native complex (not shown).

An important criterion for a bona fide dynein component is that it be found in stoichiometric amounts within the purified complex. Previously, we demonstrated that the $M_r = 8,000$ LC is present within cytoplasmic dynein at a stoichiometry of 1 per IC74 (27). Densitometry of Coomassie Blue-stained gels of dynein samples isolated by both methodologies indicates that Tctex-1 also is present in similar amounts relative to IC74 (Table I). Thus, as there are 2–3 copies of the IC per particle, Tctex-1 is indeed a stoichiometric component of the cytoplasmic dynein complex. Interestingly, the $M_r = 22,000$ protein, which also appears to copurify with cytoplasmic dynein, is present in lesser amounts such that there is probably only one copy per dynein particle (Table I).

Searches of the Genbank™ and Expressed Sequence Tag data bases using BLAST revealed a series of proteins homologous to the mouse Tctex-1 protein. These include the human Tctex-1 protein (94% sequence identity; see Fig. 2) and a second human protein (rp3) (43) that was a candidate for involvement in X-linked retinitis pigmentosa type 3 prior to the recent identification of the retinitis pigmentosa GTPase regulator as the RP3 gene (44). The rp3 candidate protein shares 55% identity and 74% similarity with Tctex-1 (Table I) and give $P(n)$ scores of $9.8 \times 10^{-5}$ to $4.5 \times 10^{-10}$ An alignment of Tctex-1, rp3, and a C. elegans open reading frame encoded within cosmid T05C12 (T05C12-5; $P(n) = 4.5 \times 10^{-10}$) generated by CLUSTALW is shown in Fig. 7. A number of intriguing structural features are evident including several conserved Cys residues and a Trp-Asp dipeptide located in the more highly related C-terminal portion of these molecules. Also identified in the data base search was another t-complex protein (Tctex-2)
FIG. 6. Immunoprecipitated cytoplasmic dynein contains Tctex-1. Cytoplasmic dynein, kinesin, and dynactin were immunoprecipitated from brain homogenates using the monoclonal antibodies 74–1, H-2, and 50–1, respectively. Samples (and a bead control) were electrophoresed in a 5–15% acrylamide gradient gel and either stained with Coomassie Blue (upper panel) or blotted to nitrocellulose and probed sequentially with the R5205 and 74–1 antibodies (lower panels). Both IC74 and Tctex-1 were found exclusively in the cytoplasmic dynein sample. The bands migrating at Mₗ = ~50,000 and 30,000 are due to antibody heavy and light chains.

| Component | Relative stoichiometry⁴ | Copies per dynein particle |
|-----------|------------------------|---------------------------|
| IC74      | 1.00                   | 2–3⁵                      |
| Mₗ 22,000 LC | 0.70, 0.35, 0.20, (0.3–0.5) | 1                         |
| Mₗ 14,000 LC | 1.21, 0.93, 0.64 (1) | 2–3                      |
| Mₗ 8,000 LC | 1.20, 1.40, 0.76 (1) | 2–3                      |

|= Determined by quantitative densitometry of Coomassie Blue stained gels.

⁴ The actual values obtained relative to IC74 are shown with the most likely stoichiometry in parentheses. The first value for each LC is from sucrose gradient purified bovine brain dynein; the others are from rat brain samples isolated by immunoprecipitation.

⁵ Previously published values indicate that there are 2 DHCs and 2–3 copies of IC74 per particle (58, 59).

(45)² that is apparently very distantly related to Tctex-1 (P<sub>in</sub> = 2.6 × 10⁻³). Again however, it is the C-terminal region that shows the highest degree of conservation (~23% identity, 45% similarity) including the Trp-Asp dipeptide noted above (not shown).

Previous Northern blot analysis indicated that the Tctex-1 message was expressed at a much higher level in testis than in other adult tissues (34). To further assess the apparent differential expression of this protein, cytoplasmic dynein was purified from rat brain, kidney, liver, spleen, and testis by immunoprecipitation with the 74–1 monoclonal antibody. These samples subsequently were probed with blot-purified antibody against Tctex-1 (Fig. 8). This revealed that cytoplasmic dyneins obtained from kidney, spleen, and testis contain significantly more Tctex-1 than do the brain or liver forms of this motor complex. These data support the hypothesis that Tctex-1 is differentially expressed in different tissues and suggest the existence of distinct dynein subtypes based on LC complement.

DISCUSSION

In this report, we provide evidence that the mouse t-complex-encoded protein Tctex-1 is a stoichiometric component of brain cytoplasmic dynein. Data from several complementary experiments provide support for this statement. First, sequencing of a 16-residue peptide from the Mₗ = 14,000 protein associated with bovine cytoplasmic dynein revealed a match with both mouse and human Tctex-1 proteins (16/16 residues correct). Second, biochemical and immunological analysis of the various fractions from a brain homogenate indicate that Tctex-1 is a microtubule-associated protein. Third, microtubule-bound Tctex-1 may be eluted with ATP or salt but not with GTP as expected for a cytoplasmic dynein subunit. Fourth, the ATP-eluted Tctex-1 copurifies with cytoplasmic dynein in sucrose density gradients. Fifth, Tctex-1 is specifically found in cytoplasmic dynein samples purified from multiple tissues by immunoprecipitation with a monoclonal antibody specific for Tctex-1 (Fig. 8). This revealed that cytoplasmic dyneins obtained from kidney, spleen, and testis contain significantly more Tctex-1 than do the brain or liver forms of this motor complex. These data support the hypothesis that Tctex-1 is differentially expressed in different tissues and suggest the existence of distinct dynein subtypes based on LC complement.

The t-complex is a large region of mouse chromosome 17 that contains four inversions that suppress recombination, and therefore, t-haplotypes are inherited as a single unit. These variant chromosomes have evolved over the last ~3 × 10⁹ years and now represent the most extreme vertebrate example of the phenomenon of transmission ratio distortion or meiotic drive (see Ref. 33 for a recent discussion of the molecular genetics and ancestry of t-haplotypes). Heterozygous males transmit the t-bearing chromosome to ~99% of their progeny, a ratio that obviously violates the rules of standard mendelian chromosome segregation. In genetic terms, the distortion phenomenon results from the combined action of 3 or 4 distorter genes interacting with a single post-meiotically expressed responder locus; all of these loci are within the t-complex (46). During spermiogenesis, it appears that the meiotic partners of t-haploype-bearing spermatids are “poisoned” such that they become inca-
Intriguingly, males containing two complementing haplotypes are completely and unconditionally sterile. This phenomenon is thought to be a consequence of homozygosity for the genes responsible for the transmission ratio distortion phenotype.

Tctex-1 was originally cloned because the message is greatly overexpressed in t-haplotype versus wild-type testis (34); intriguingly, this abundant RNA is not translated and testicular protein levels are equivalent. Tctex-1 represents a small gene family (there are 4 copies of this gene in both wild-type and t-bearing mice), and mapping of partial t-haplotypes makes it a strong candidate for the t-complex distorter gene Tcd-1 (34). All wild-type Tctex-1 genes encode identical proteins. The t-complex-encoded genes are either translationally inactive (they lack an appropriate start codon) or contain three t-specific mutations. These three single nucleotide differences between the wild-type and t-complex Tctex-1 coding sequences result in three amino acid substitutions, namely, Q41H, L47I, and R59K (47). The residues at positions 47 and 59 are not identical in mouse and human Tctex-1 proteins. However, Ghn-41 is conserved in both mammalian Tctex-1 polypeptides and in the related protein rp3 (43) (see below). For this reason, it has been suggested that the Q41H transition might cause Tctex-1 dysfunction (47).

The identification of Tctex-1 as a LC of cytoplasmic dynein raises many questions concerning the function of this molecule and the role dynein may play in the phenomenon of meiotic drive. First, unlike the $M_r = 8,000$ cytoplasmic dynein LC (see Ref. 27), there appears to be only a single pool of Tctex-1 protein within mammalian brain, all of which is associated with cytoplasmic dynein. Thus, any function ascribed to Tctex-1 presumably must involve dynein. As meiotic drive is due to the action of several distorters upon a single responder (46), identification of Tctex-1 as a subunit of cytoplasmic dynein suggests that the responder might be a specific cargo for, or perhaps a regulator of, this molecular motor. Another possibility is that the responder locus might encode a subunit of dynein or dynactin.

In testis, cytoplasmic dynein is present in large quantities within Sertoli cells, which are the support cells of the seminiferous epithelium (48). This enzyme also has been localized to the manchette microtubule array and to the spermatid nuclear envelope during spermatogenesis in rats where it may participate in the protrusion of the spermatid nucleus from the cytoplasm (49). An attractive hypothesis is that dysfunction of this testicular motor due to mutation of the Tctex-1 LC results in defective spermatozoa and hence the infertility phenotype observed in t-complex homozygotes.

In our experiments, essentially all of the brain Tctex-1 protein was microtubule-associated and copurified with cytoplasmic dynein. However, immunohistochemistry of mouse testis and ovary using a Tctex-1 antisera different from that used here showed staining of both oocyte cytoplasm and sperm tails (47). This suggests that Tctex-1, and therefore cytoplasmic dynein, may be present in the developing sperm tail. Unfortunately, this conclusion must be considered preliminary as immunoblot analysis of whole testis protein using this antisera revealed several immunoreactive bands in addition to Tctex-1 (see Fig. 1 in Ref. 47). Furthermore, previous immunohistochemical analysis using the 74–1 monoclonal antibody found no evidence for the presence of IC74 in mammalian cilia and flagella, although a related molecule of significantly lower $M_r = 19,000$ was detected (14).

The locations of the various LCs within the cytoplasmic dynein complex have yet to be defined. In flagellar outer arm dynein, two classes of LCs are found. Several distinct LCs interact directly with the ICs and are, therefore, presumably located at the base of the soluble particle (22, 50–52). In addition, each outer arm DHC has at least one LC to which it is tightly bound (53, 54). In Chlamydomonas, the $M_r = 19,000$ LC is known to interact with the N-terminal third of the $\beta$ outer arm DHC (55) and is, therefore, likely in the stem or at the base of the complex. Previously we suggested, by analogy with flagellar outer arm dynein, that the $M_r = 8,000$ LC is associated with IC74 at the base of the soluble particle (27). We have recently found that the Chlamydomonas $M_r = 19,000$ LC mentioned above is a homologue of the t-complex protein Tctex-2 (62) and is also related to Tctex-1. Thus, it seems probable that Tctex-1 interacts directly with the N-terminal region of the cytoplasmic DHC as does the $M_r = 19,000$ LC with the outer arm $\beta$ DHC.

Data base searches revealed that Tctex-1 is highly related (~55% identity) to the previously cloned protein rp3 (43). This molecule is of interest because it is encoded by the sole reading frame identified inside a 170-kilobase region of the human X chromosome (within the interval Xp21.1) to which Musarella et al. (56) mapped a gene for X-linked retinitis pigmentosa type 3. Identification of this region was based on both linkage analysis and the physical mapping of deletions present in several patients exhibiting this progressive retinal dystrophy. Obviously, considering the high homology between Tctex-1 and the candidate rp3 protein, it is quite possible that the latter represents a variant cytoplasmic dynein LC that is differentially expressed in certain tissues. Recent studies have indicated that an RP3 gene (RPRGR) encodes a guanine nucleotide exchange factor and is located centromeric to the region containing the Tctex-1 homologue (44). However, the RPRGR gene is both outside of the large deletion present in the patient (B.B.) used by Musarella et al. (56) for physical mapping analysis and, moreover, is not mutated. Thus, it is unclear why this patient exhibits retinitis pigmentosa. It remains quite possible that one or more additional RP genes tightly linked to RPRGR exist at the Xp21.1 interval within or near the region mapped by Musarella et al. (44, and see Ref. 57 for commentary).

In conclusion, we demonstrate here that the $M_r = 14,000$ polypeptide associated with brain cytoplasmic dynein is the t-complex protein Tctex-1. This result implies a causal effect of cytoplasmic dynein dysfunction in non-mendelian chromosome
segregation. Further detailed analysis of this LC protein both in vivo and in vitro will allow us to define the intriguing role it plays in dynein activity.

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REFERENCES

1. Warner, F. D., Satri, P., and Gibbons, I. R. (1989) Cell Movement Volume 1: The Dynein ATPases. Alan R. Liss, Inc., New York
2. Corthe´sy-Theulaz, I., Pauloin, A., and Pfeffer, S. R. (1992) J. Cell Biol. 118, 1333–1345
3. Li, Y.-Y., Yeh, E., Hays, T., and Bloom, K. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 10986–10990
4. Pattee, K. K., M. Bar, R. B. (1987) Nature 330, 181–183
5. Pfarr, C. M., Coue, M., Grissom, P. M., Hays, T. S., Porter, M. E., and McIntosh, J. R. (1994) J. Cell Biol. 128, 617–624
6. Sondek, J., Bohm, A., Lambricht, D. G., Hamm, H. E., and Sigler, P. B. (1994) Nature 370, 297–300
7. Kaufman, S., and Hulbou, E. L. F. (1995) J. Biol. Chem. 270, 28806–28811
8. Vaughan, K. T., and Valley, R. B. (1995) J. Cell Biol. 121, 1557–1568
9. Dlavan, S., III, and Pfister, K. K. (1994) J. Cell Biol. 127, 1671–1681
10. Pfister, K. K., Salata, M. W., Dillman, J. F., III, Torre, E., and Lye, J. R. (1996) Mol. Biol. Cell 7, 331–343
11. Pfister, K. K., Salata, M. W., Dillman, J. F., III, Vaughan, K. T., Valley, R. B., Torre, E., and Lye, J. R. (1996) J. Biol. Chem. 271, 1687–1694
12. King, S. M., Wilkerson, C. G., and Witman, G. B. (1991) J. Biol. Chem. 266, 8401–8407
13. King, S. M., Patel-King, R. S., Wilkerson, C. G., and Witman, G. B. (1995) J. Cell Biol. 131, 399–409
14. Wilkerson, C. G., King, S. M., Kondualis, A., Pazoie, G. J., and Witman, G. B. (1995) J. Cell Biol. 129, 169–178
15. Gill, S. R., Cleveland, D. W., and Schroer, T. A. (1994) Mol. Biol. Cell 5, 645–654
16. Hughes, S. M., Vaughan, K. T., Herskovits, J. S., and Valley, R. B. (1995) J. Cell Sci. 108, 17–24
17. King, S. M., and Patel-King, R. S. (1995) J. Biol. Chem. 270, 11445–11452
18. Dick, T., Ray, K., Salz, H. K., and Chia, W. (1996) Mol. Cell. Biol. 16, 1966–1977
19. Barklow, K., Hamasaki, T., and Satri, P. (1994) J. Cell Biol. 120, 737–735
20. King, S. M., and Patel-King, R. S. (1995) J. Cell Sci. 108, 3757–3764
21. Patel-King, R. S., Benashski, S. E., Harrison, A., and King, S. M. (1996) J. Biol. Chem. 271, 6283–6291
22. Silver, L. M. (1993) Trends Genet. 9, 250–254
23. Lader, E., Ha, H. S., O’Neill, M., Artzt, K., and Bennett, D. (1989) Cell 58, 969–979
24. Paschal, B. M., Shpetner, H. S., and Valley, R. B. (1991) Methods Enzymol. 196, 181–191
25. Pfister, K. K., Wagner, M. C., Stettenheim, D. L., Brady, S. T., and Bloom, G. S. (1989) J. Cell Biol. 108, 1453–1465
26. Paschal, B. M., Holzbaehr, E. L. F., Pfister, K. K., Clark, S., Meyer, D. L., and Valley, R. B. (1993) J. Biol. Chem. 268, 1518–1523
27. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 403–410
28. Dever, J., Haeberli, P., and Smithies, O. (1984) Nucleic Acids Res. 12, 387–395
29. Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1984) Nucleic Acids Res. 22, 4673–4680
30. Olmsted, J. B. (1986) Methods Enzymol. 134, 467–472
31. Hillier, L., Clark, N., Dubusque, T., Elliston, K., Hawkins, M., Holman, M., Hultman, M., Kucaba, T., Le, M., Lennon, G., Marra, M., Parsons, J., Riffkin, L., Rohlifing, T., Soares, M., Tan, F., Trevaskas, E., Waterston, R., Williamson, A., Wohldmann, P., and Wilson, R. (1995) The WashU-Merck EST Project
32. Roux, A.-F., Rommens, J., McDowell, C., Anon-Cartwright, L., Bell, S., Schappter, K., Fishman, G. A., and Musarella, M. (1994) Hum. Mol. Genet. 3, 257–263
33. Meindl, A., Dry, K., Herrmann, K., Manon, F., Ciccodicila, A., Edgar, A., Carvalho, M. R. S., Achatz, H., Hellebrand, H., Lennon, A., Migliaccio, C., Porter, K., Zrenner, E., Bird, A., Jay, M., Wittmer, B., D’Urso, M., Mezinger, T., and Wright, A. (1996) Nat. Genet. 13, 55–62
34. Huw, L.-Y., Goldsborough, A. S., Willson, K., and Artzt, K. (1995) Dev. Biol. 170, 183–194
35. Lyon, M. F. (1984) Cell 44, 357–363
36. O’Neill, M. J., and Artzt, K. (1995) Development 121, 561–568
37. Neely, M. D., and Boekelheide, K. (1988) J. Cell Biol. 107, 1767–1776
38. Yoshida, T., Ioshii, S. O., Imanaka-Yoshida, K., and Izutsu, K. (1994) J. Biol. Chem. 269, 1687–1694
39. King, S. M., and Witman, G. B. (1990) J. Biol. Chem. 265, 19807–19811
40. Mitchell, D. R., and Rosenbaum, J. L. (1986) Cell Motil. Cytoskeleton 6, 510–520
41. Sale, W. S., Goodenough, U. W., and Heuser, J. E. (1985) J. Cell Biol. 101, 1409–1412
42. Pfister, K. K., and Witman, G. B. (1984) J. Biol. Chem. 259, 12072–12080
43. Pfister, K. K., Fay, B. R., and Witman, G. B. (1982) Cell Motil. Cytoskeleton 2, 525–547
44. Sakakibara, H., Takada, S., King, S. M., Witman, G. B., and Kamiya, R. (1993) J. Cell Biol. 123, 653–661
45. Musarella, M. A., Anon-Cartwright, C. L., McDowell, C., Burghes, A. H. M., Coulson, S. E., Worton, R. G., and Rommens, J. M. (1991) Genomics 11, 263–272
46. Egan, S. M., and McIntosh, R. (1998) Nature 391, 194–195
47. Paschal, B. M., Shpetner, H. S., and Valley, R. B. (1987) J. Cell Biol. 105, 1273–1282
48. Valley, R. B., Wall, J. S., Paschal, B. M., and Shpetner, H. S. (1988) Nature 322, 561–563
49. Espindola, F. S., Cheney, R. E., King, S. M., Suter, D. M., and Mooseker, M. S. (1996) Mol. Biol. Cell 7, in press (abstr.)
50. Patel-King, R. S., Benashski, S. E., and King, S. M. (1996) Mol. Biol. Cell, in press (abstr.)
51. Beckwith, S. M., and Morris, N. R. (1995) Mol. Biol. Cell 6, 5(abstr.)