Crystal Structure of Dynein Light Chain TcTex-1*

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sin to the apical surface in vivo, and using RP3, a TcTex-1 homolog (55% identity), to rescue the knock-out also failed (30).

Similarly, studies in polarized (Madin-Darby canine kidney cells showed that mutations linked to retinitis pigmentosa in the cytoplasmic tail of the rhodopsin receptor abolished TcTex-1 binding (13). Most interestingly, TcTex-1 and RP3 are temporally and spatially regulated (31). Other functions associated with TcTex-1 include non-Mendelian meiotic drive (32), which was recently (14) but again is only shared by a fraction of the reported TcTex-1 ligands. To better understand how TcTex-1 binding (13) most interestingly, TcTex-1 and RP3 are temporally and spatially regulated (31). Other functions asso-
ciated with TcTex-1 include non-Mendelian meiotic drive (32), which was recently (14) but again is only shared by a fraction of the reported TcTex-1 ligands. To better understand how TcTex-1 recognizes its ligands that share little sequence identity, we have solved the crystal structure of TcTex-1 as an initial step in defining the structural determinants of its target recognition.

**Materials and Methods**

**Cloning, Protein Purification, and Characterization**—Initially, we cloned the mouse TcTex-1 homolog, purified it, and crystallized it. X-ray diffraction experiments were carried out, but the diffraction was very weak, and the initial cell constants and space group suggested that there were 18–21 molecules per asymmetric unit. Consequently, we switched to the Drosophila homolog.

Full-length *Drosophila* TcTex-1 cDNA clones were obtained by PCR using EST-clones (LP04056 and LD36706) purchased from Research Genetics and placed into pET24d between the NcoI and XhoI sites (36). PCR primers were synthesized by the biopolymer core facility (Columbia University). The sequence of this construct was confirmed by DNA sequencing.

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**Characterization of Ternary Structure**—Size exclusion chromatography was carried out to determine the oligomerization state of TcTex-1. Moreover, because there was a question of a pH-dependent monomer-dimer equilibrium in LC8, the Superose 12 column (Amersham Biosciences) was equilibrated with two different buffers (38). The low pH buffer contained 50 mM MES, 100 mM NaCl, 1 mM EDTA, and 1 mM DTT, pH 5. The high pH buffer contained 50 mM CHES, 100 mM NaCl, 1 mM EDTA, and 1 mM DTT, pH 9. The protein applied to the column was in 10 mM Tris–HCl, pH 8.0, 100 mM NaCl, 5 mM EDTA, and 1 mM DTT, pH 9. The protein applied to the column was in 10 mM Tris, 100 mM NaCl, 1 mM EDTA, and 1 mM DTT, pH 8.0. The column was calibrated using bovine serum albumin (66 kDa), ovalbumin (45 kDa), chymotrypsin (25 kDa), and lysozyme (14 kDa) for both buffers. TcTex-1 (25 μl of 2 mg/ml solution) was applied to the column. The elution volume was measured and compared with the calibration graph as well as to each other.

**Crystallographic Analysis**—Purified protein was concentrated to ∼40 mg/ml in 10 mM Tris–HCl, pH 8.0, 100 mM NaCl, 5 mM EDTA, and 1 mM DTT. Crystals were grown from 100 mM CHES over a pH range of 9–10 and 2.2–2.5 mM (NH₄)₂SO₄, 0.1 M to 0.4 M Li₂SO₄, or 2 M K₂HPO₄ and 0.5 M NaH₂PO₄ or 1.6 M sodium citrate as a precipitating agent, each using the hanging drop method. Crystals could be obtained by using protein concentrations of 10–40 mg/ml. The largest crystals (∼100 × 50 × 50 μm³) were grown in 0.1 M CHES, pH 9–10, 2.4 mM (NH₄)₂SO₄, and 0.1 M Li₂SO₄ at protein concentration of 10–20 mg/ml. The SeMet TcTex-1 crystals were grown under same conditions and were generally larger (∼150 × 50 × 50 μm³).

* See Ref. 52.

| TABLE I | Diffraction data |
|---------|------------------|
| λ₁ (low remote) | 0.991870 |
| λ₂ (inflexion) | 0.97949 |
| λ₃ (peak) | 0.97896 |
| λ₄ (high remote) | 0.96672 |
| λ₅ (native) | 0.99187 |

| TABLE II | MAD diffraction difference ratios (20 > d > 2) |
|----------|------------------|
| λ₁ | 0.034 |
| λ₂ | 0.070 |
| λ₃ | 0.060 |
| λ₄ | 0.035 |

| TABLE III | Refinement statistics |
|------------|----------------------|
| Bragg spacings (Å) | 6–1.7 |
| R_Work | 19.3% |
| R_Free | 23.5% |
| No. reflections used in refinement | 10,140 |
| No. reflections used in refinement | 501 |
| Completeness of data | 94% |
| Total | 97% |
| Last shell | 98% |
| No. protein atoms | 813 |
| No. waters | 160 |
| r.m.s.d. from bond length | 0.014 |
| Ideal stereochemistry, bond angle | 2.7 |
| r.m.s.d. B-factors | 1.762 |
| Main chain bond/angles | 3.09/6.5 |
| Side chain bond/angles | 24.9 |
| Mean B-factor for main chain | 27.5 |
| Mean B-factor for side chains | 50.9 |
| Ramachandran dihedral angles* | 96% |
| Residues in favored regions | 100% |

* Outer shell of the data (2.0–2.07 Å for MAD data set; 1.7–1.76 Å for native data set).
**FIG. 1. TcTex-1 structure.**

A, the overall structure of TcTex-1 is a dimer consisting of two α-helices followed by four β-strands, where the second β-strand is swapped with the crystallographic protomer (one protomer in blue and the other in yellow). In green are residues 8–13 from an adjacent crystallographic symmetry mate that form an anti-parallel β-strand. B, 90° rotation of TcTex-1 as shown in A about an axis that runs horizontal in the page. This view illustrates how the N terminus is splayed away from the “compact” structure. The ordered N-terminal amino acids, 8–13, from crystallographic symmetry mates have been added and are shown in green. C, stereoview of Cα trace of a TcTex1 protomer as shown in A rotated 90° about an axis that runs vertical in the page (every 10th residue is labeled). The electron density covering residues 8–13 of the crystallographic symmetry shown in stereo indicates that the N-terminal tail is well defined. D, stereoview approximately along the 3-fold screw axis. Each dimer is individually colored. Note that the N terminus from each is splayed away and bound to a crystallographic partner.
Drosophila TcTex-1 crystals diffract to Bragg spacings beyond 1.5 Å. MAD data were collected at four wavelengths: λ1 (0.99187 Å) at the low energy side remote from the absorption edge; λ2 (0.97950 Å) at the inflection point; λ3 (0.97897 Å) at the peak of selenium absorption curve; and λ4 (0.96672 Å) at the high energy side remote from the absorption edge. One MAD data set was collected to a limit of 2.0 Å spacing, and another set was collected to 1.5 Å using a different SeMet crystal. For the 2.0 Å data set, 100 images (15 s exposure deg-1) were collected per wavelength for each of the direct beam and inverse beams, whereas 65 images (60 s exposure deg-1) were collected for the 1.5 Å data set.

Structure Determination and Refinement—The data were scaled and reduced using HKL (39). The crystal belongs to the space group P3221 with cell dimensions of a = b = 60.60 Å and c = 48.49 Å (Tables I and II). The selenium sites were determined using Shake-n-Bake (40), and the protein phases were calculated using SHARP (41). The low resolution phases (2.0 Å) were extended to 1.7 Å using DM of CCP4 suite (42). The initial model was generated using ARP-wARP (43) and completed using O NO (44); the model was then refined using CNS (45) and ARF-wARP. The final refined model contains residues 8–111 of Drosophila TcTex-1, which correspond to residues 10–113 of human TcTex-1. The N-terminal 7 residues have no electron density in the maps, and thus they are disordered in the crystals. A summary of data collection and refinement statistics is given in Table III.

RESULTS AND DISCUSSION

Overall Structure of TcTex-1—The crystal structure has one molecule per asymmetric unit; however, each is paired by crystallographic dyad axes indicating that TcTex-1 is homodimeric. Size exclusion chromatography further confirms its dimeric state. Also, we could not produce a monomer from the dimeric form by changing the pH (data not shown) as found for LC8 studies (38). Each protomer core contains two α-helices followed by four β-strands (Fig. 1A). The N terminus of TcTex-1 is splayed away from the folded core and bound to an adjacent crystallographic “dimer” as an additional β-strand (β0) (Fig. 1B). The second β-strand (β2) is domain swapped and makes extensive main chain hydrogen bonds and side chain interactions with the other protomer. Sequence alignment shows that TcTex-1 is highly conserved across a number of species, particularly in β-strands (Fig. 2).

TcTex-1 Shares the Same Fold as PIN/LC8—Despite the lack of sequence identity, TcTex-1 has the same fold as PIN/LC8. As shown in Fig. 3A, the α-helices, β-strands, and loops are generally longer in TcTex-1 compared with PIN/LC8, but mostly to the N-terminal half of the protein (based on the orientation of the bound N-terminal tail). The r.m.s.d.1 between common residues is less than 1.6 Å. A structure-based sequence alignment of TcTex-1 and LC8 has zero sequence identity (Fig. 2). An alignment based solely on sequence, on the other hand, produced 11% identity, but this was achieved by overlapping the C terminus of LC8 with the N terminus of TcTex-1.

TcTex-1 Dimerization Interface—The interface between each TcTex-1 protomer is extensive and predominantly hydrophobic (supplemental Fig. 1). In our calculations, each protomer buries 1532 Å2 of surface area, whereas each protomer in PIN/LC8 buries 760 Å2. It is interesting to note that there is a solvent channel running through the center of the TcTex-1 dimer (Fig. 3B), whereas the PIN/LC8 structure does not have such a feature. The function, if any, of this solvent channel is unclear. It does, however, produce a dimer interface with two hydrophobic patches. These patches include residues Met-66, Met-68, Leu-103, Leu-75, and the methyl of Thr-77 for the N-terminal half of the molecule and residues Leu-49, Thr-53, Ile-62, Cys-81, for the C-terminal half (nonprimed and prime residues denote individual protomers).

Several polar interactions also participate in and possibly stabilize the dimeric state. One that stands out is between residues Met-66, Met-68, Leu-103, Leu-75, and the methyl of Thr-77 for the N-terminal half of the molecule and residues Leu-49, Thr-53, Ile-62, Cys-81, for the C-terminal half (nonprimed and prime residues denote individual protomers).

The abbreviations used are: r.m.s.d., root mean square deviation; nNOS, neuronal nitric-oxide synthase; DTT, dithiothreitol; CHES, 2-(cyclohexylamino)ethanesulfonic acid; MES, 4-morpholineethanesulfonic acid; SeMet, selenomethionyl.
highly conserved His-76’ (Asn in Caenorhabditis elegans), part of the domain swapped β-strand, and completely conserved Tyr-34 in the α1–α2 loop and the highly conserved Asn-38 in the α-helix 2 (Ser in Chlamydomonas sp.). Because the molecule is symmetric, each interaction listed occurs twice.

Potential TcTex-1 Target-binding Site—TcTex-1 is similar in tertiary fold to LC8. Based on how it binds the N-terminal tail from a neighboring molecule in the crystal lattice, it is likely to bind its targets in the same fashion as LC8.

Peptides derived from putative LC8 cargo bind as an extended anti-parallel strand to swapped β-strand 2 of LC8, making eight backbone hydrogen bonds. This strand is surrounded by α-helix 2 on one side and β-strands 3 and 4 on the other.

Superposition of the common α-helices and β-strands of the core region of TcTex-1 and LC8 shows that the N-terminal tail and the nNOS peptide have similar backbone hydrogen bonding patterns (supplemental Fig. 2, A and B). Moreover, residues Ser-8 to Val-12 of the TcTex-1 tail based on this superposition align well with Gly-8 to Asp-12 of the nNOS peptide (r.m.s.d. = 0.95 Å). Residues in TcTex-1 equivalent to the residues that line the LC8 groove include His-34’, Asn-38’, and Glu-46’ of α-helix 2; Ser-90, Thr-92, and Arg-94 of β-strand 3; and Tyr-101, Ile-103, and Ser-105 of β-strand 4.

All LC8 targets encode an invariant glutamine that caps the α-helix 2. The residues that precede and succeed this glutamine bind to hydrophobic sites derived from residues in the β-strands 3 and 4. Similarly, in TcTex-1, the residues directly before (Gln-9) and after (Ile-11) Phe-10, which corresponds to Gln-10 of LC8, are both buried in hydrophobic cavities.

A major difference between the binding groove of LC8 and the putative TcTex-1 groove is that the site on LC8 that interacts with the invariant glutamine in LC8 targets (Fig. 3, B and C). In TcTex-1, α-helix 2 is extended by an additional N-terminal turn that presents conserved residues His-34 and Asn-38 to the site.

The mechanism used by LC8 and TcTex-1, as we presume, to bind their respective targets provides a possible explanation of the low sequence identity observed among the LC8 target peptides and among the TcTex-1 target peptides. Arguments like those that explain sequence promiscuity in subtilisin substrate (46) and major histocompatibility complex-antigen complexes (47) also apply here; backbone hydrogen bonds and hydrophobic interactions do not place absolute restrictions on the sequence identity.

Our prediction for the importance of hydrophobic peptide residues on the binding targets to TcTex-1 differs from the basic motif, (R/K)2(R/K)(R/K)2(R/K), proposed by Mok et al. (9). Glutathione S-transferase pull-down studies showed that a peptide derived from the dynein intermediate chain, LGRRNLKGLVSKVTQVDF, could bind TcTex-1. Titration of a truncated peptide (underlined) produced chemical shifts that map to the N-terminal half of the TcTex-1 structure (supplemental Fig. 2C) (9); however, this truncated positively charged peptide could not pull-down TcTex-1 (9). The chemical shift differences coincide with the negative charged surface of TcTex-1 (Fig. 3B) and likely reflect adventitious electrostatic binding.

A Potential Second Binding Site—It was shown recently that exchanging a single residue that differs between the two LC8
isofoms (98% identity) altered partitioning of the isoform from myosin V to the dynein intermediate chain (48). This position maps to a-helix 2, away from the nNOS- and Bim-binding site (48). Most interestingly, a point mutation in TcTex-1 that is associated with T-specific overexpression of TcTex-1, a phenotype associated with transmission ratio distortion or meiotic association with TcTex-1, a phenotype associated with transmission ratio distortion or meiotic

Post-translational Modification of TcTex-1 Should Not Affect Ligand Binding—Campbell et al. (18) and Mou et al. (20) found that TcTex-1 is tyrosine-phosphorylated. There are four tyrosines in human TcTex-1 and three are conserved throughout the family (Fig. 2). Moreover, the conserved three are either buried or involved in extensive side chain interaction. However, there is an incompletely conserved tyrosine, Tyr-4, in the human, bovine, and the torpedo sequences, whereas it is a phenylalanine in mouse and rat and valine in Chlamydomonas. This position corresponds to the flexible region that is splayed away from the dimer core and, thus, potentially available for post-translational modification. However, Campbell et al. (18) showed that phosphorylation of TcTex-1 did not affect the binding of Fyn.

Conclusions—We have shown that TcTex-1 is similar to LC8 in tertiary and quaternary structure despite no sequence identity. Based on this similarity and common modes of peptide binding, we suggest that TcTex-1 binds its targets in a similar manner.

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