Interaction of the DYNLT (TCTEX1/RP3) Light Chains and the Intermediate Chains Reveals Novel Intersubunit Regulation during Assembly of the Dynein Complex

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The cytoplasmic dynein 1 cargo binding domain is formed by five subunits including the intermediate chain and the DYNLT, DYNLL, and DYNLRB light chain families. Six isoforms of the intermediate chain and two isoforms of each of the light chain families have been identified in mammals. There is evidence that different subunit isoforms are involved in regulating dynein function, in particular linking dynein to different cargoes. However, it is unclear how the subunit isoforms are assembled or if there is any specificity to their interactions. Co-immunoprecipitation using DYNLT-specific antibodies reveals that dynein complexes with DYNLT light chains also contain the DYNLL and DYNLRB light chains. The DYNLT light chains, but not DYNLL light chains, associate exclusively with the dynein complex. Yeast two-hybrid and co-immunoprecipitation assays demonstrate that both members of the DYNLT family are capable of forming homodimers and heterodimers. In addition, both homodimers of the DYNLT family bind all six intermediate chain isoforms. However, DYNLT heterodimers do not bind to the intermediate chain. Thus, whereas all combinations of DYNLT light chain dimers can be made, not all of the possible combinations of the isoforms are utilized during the assembly of the dynein complex.

Cytoplasmic dynein 1 is a microtubule-based molecular motor that functions to generate force for cargo transport to microtubule minus-ends (1–3). It is involved in numerous eukaryotic cell processes including the trafficking of membranous vesicles, viruses, and other intracellular particles. Cytoplasmic dynein 1 is a large multisubunit complex (~1.5 MDa) containing two copies of six subunits, the heavy chain (DYNC1H), the intermediate chain (DYNC1I), the light intermediate chain (DYNC1IL), and three distinct light chains, DYNLT (previously called Tctex1), DYNLRB (previously called roadblock), and DYNLL (previously called LC8) (2, 4–6). The motor domains of cytoplasmic dynein 1 are located in the C-terminal globular heads of the two identical heavy chains (7–9). The heavy chains dimerize via their N-terminal stalks, and the stalks also contain the light intermediate chain and intermediate chain binding sites (3, 10–13). The three light chains bind to different locations on the N terminus of the intermediate chain (14–16).

Whereas there is only a single heavy chain isoform, there are multiple isoforms of the five subunits that make up the cargo binding domain (2, 3, 17). In mammals, at least six intermediate chain isoforms are produced by the alternative splicing of two genes, and there are at least two genes for each of the other four subunits (2, 3, 5, 18). Assembly of individual subunit isoforms into the dynein complex creates different populations of the motor protein that are thought to be involved in specific cargo binding and regulation (2, 19–22). For example, pericentrin is transported to the centrosome exclusively by the dynein complexes that contain the light intermediate chain isoform DYNC1I-L1 (23). The DYNLT and DYNLL light chains have been shown to interact with numerous functionally unrelated proteins (3, 14, 15, 24–27).

The two members of the DYNLT family, DYNLT1 (previously called Tctex1)2 and DYNLT3 (previously called rp3) are found in all cultured cells and adult and fetal tissues so far examined (19, 28, 29). Unlike the DYNLT isoforms, the expression of the six intermediate chain isoforms is tissue and cell type-specific (18, 30–32). One intermediate chain isoform, DYNC1I-2C (IC-2C),3 is found in all cells and it is often the only isoform found in cultured cells (18). Most tissues express only

2 Cytoplasmic dynein 1 light chain and intermediate chain subunit nomenclature. There are three functionally distinct light chain families in the cytoplasmic dynein complex and each family has at least two members (or isoforms). The names of the light chains all begin with DYLN for dynein, followed by L for light chain, then additional letters that designate the families. The family names are based on the old common name of the first identified member of each family: DYNLT (T for the Tctex1 family); DYNLRB (RB for the Roadblock family); and DYNLL (L for the LC8 family). The different members (or isoforms) of the families are distinguished by adding numbers to the name, for example, DYNLT1 and DYNLT3 are the two members of the DYNLT light chain family. In the text, a light chain polypeptide subunit is identified at first mention with its formal name followed by the old common name in parentheses, for example DYNLT3 (previously called rp3). The intermediate chains are DYNCl1. The products of the two intermediate chain genes are distinguished by adding numbers to the name, and alternative splicing isoforms are referred to with letters. This nomenclature has been endorsed by the Human Genome Organization Nomenclature Committee (HGNC) and the International Committee on Standardized Nomenclature for Mice (5).

3 The abbreviations used are: IC, intermediate chain; GST, glutathione S-transferase; HA, hemagglutinin.

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the IC-2C and IC-2B isoforms. All six isoforms are found only in brain, where their expression levels are regulated during development, and changes in the expression levels of the IC-2C and IC-2B isoforms and the light intermediate chains are also observed upon nerve growth factor-induced PC12 cell differentiation (18, 30–32). The intermediate chain and light intermediate chain isoforms define distinct pools of dynein in axons (18, 33). However, much remains to be learned about the subunit organization of the dynein cargo binding domain.

To investigate the contributions of different subunit isoforms to the structural organization of the dynein complex, we investigated the interactions of two members of the DYNL light chain family, DYNL1 and DYNL3, with one another and with other subunits of the dynein cargo binding domain. The DYNL light chain family was chosen for this study because the two isoforms bind to different proteins and mediate their transport by dynein (20, 27, 34). Also, structural studies indicate that the DYNL1 and DYNL3 light chains contain unique domains that are predicted to be utilized for specific cargo binding, whereas many of the proteins that bind to DYNL1 and DYNL2 compete with the intermediate chain for binding to the light chains (35–38). We found that dynein with the DYNL light chain also contained the DYNL light chain, and that whereas all the DYNL isoforms co-purified with the dynein complex, most of the DYNL polypeptides did not. Using yeast two-hybrid and co-immunoprecipitation assays, we found that DYNL1 and DYNL3 form homodimers and heterodimers, and that the homodimers bound to all six of the intermediate chain isoforms in vivo. Most importantly, we found that the DYNL1–DYNL3 heterodimer did not bind to the intermediate chain. These results demonstrate that binding to the intermediate chain regulates the assembly of light chain dimers into the dynein cargo binding domain.

**EXPERIMENTAL PROCEDURES**

Plasmids—Human DYNL1 (NM_006519) and rat DYNL3 (XP_343770) were cloned by PCR into pGEX-KG, (39), pGEX-CT, (40), and pMal-c2X, (New England Biolabs). The six intermediate chain isoforms were individually inserted into the yeast expression vector, pGADT7 (Clontech). DYNL1 (NM_053319), DYNL2 (NM_080697), DYNL1, and DYNL3 were individually cloned into the pCMV-Myc and pCMV-HA mammalian expression vectors (Clontech), and the yeast expression vectors pGBK7 (Clontech) and pGADT7 as described previously (34). The Myc-DYNC1L1-2C (Myc-2C) (U39046) construct is described in Ref. 41, and DYNL3 was cloned into the pcDNA3.1/His mammalian expression vector (Invitrogen) to add the His8 and Xpress epitope tags. The nucleotide sequences and correct reading frames were verified for all constructs.

Antibodies—The antibodies used were 74.1, a mouse monoclonal antibody that reacts with all isoforms of the intermediate chain (42); CT199, a rabbit polyclonal antibody that reacts with all the DYNL family members, and R4058, a rabbit polyclonal antibody that reacts with all the DYNL family members (43, 44); HC8, a rabbit polyclonal antibody that reacts with the cytoplasmic dynein heavy chain; and R1B2, a rabbit polyclonal antibody that reacts with the isoforms of the LIC subunit, both from Dr. R. Vallee (23); and anti-HA (12C5) and anti-Myc (9E10) antibodies obtained from the Lymphocyte Culture Center, University of Virginia.

**Yeast Two-hybrid Studies**—Pairwise yeast two-hybrid analysis was performed as described previously with slight modifications (16, 34). Briefly, AH109 yeast strains were co-transformed with the constructs as indicated in the figures according to the manufacturer’s instructions (Clontech). The transformed yeast cells were resuspended in 100 μl of sterile water and 20 μl were dropped onto both −2 and −3 plates. The −2 plates, lacking both leucine (Leu) and tryptophan (Trp), showed co-transformation. The −3 plates, lacking Leu, Trp, and histidine (His), demonstrated protein-protein interactions. To eliminate false positives arising from “leaky” HIS3 expression, 3 mm 3-aminopyridine, 1, a competitive inhibitor of histidine synthase, was added to the −3 plates. The plates were incubated at 30 °C for 5 days. Positive interactions were confirmed with a β-galactosidase assay that screened for the independent expression of the lacZ reporter gene. Co-transformation with the pGBK-p53 and pGAD-T-antigen vectors was used as a control for positive interaction.

**Cell Culture, Transfection, and Co-immunoprecipitations**—293T cells were maintained in Dulbecco’s modified Eagle’s medium and transfected as described previously (41). For the co-immunoprecipitation assays, cells were resuspended in lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 1 mm phenylmethylsulfonyl fluoride, 1 μg/ml pepstatin A, 1 μg/ml leupeptin), and spun in a microcentrifuge for 10 min at 4 °C, and lysates were incubated 3 h at 4 °C with anti-HA antibody (10 μg) pre-bound to Protein A beads (Zymed Laboratories Inc.). The beads were then washed extensively with 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA. The co-immunoprecipitates were analyzed by SDS-PAGE and immunoblotting with anti-Myc antibody as described previously (41, 45). To study the equilibrium association of the members in DYNL and DYNL families, 293T cells were individually transfected with the HA- or Myc-tagged light chains and equal volumes of the resulting lysates with homodimers of the light chains were mixed together for 3 h at 4 °C. The lysates were then incubated for an additional 3 h at 4 °C with 10 μg of anti-HA antibody prebound to Protein A beads, and the co-immunoprecipitating proteins were analyzed by SDS-PAGE and immunoblotting.

**Dynein Purification**—Cytoplasmic dynein was immunoprecipitated from rat brain lysates with antibodies to the dynein intermediate chain and the light chains as described previously (33, 46) except that the wash buffer was as described above. Rat brain and 293T cytosols were fractionated on 5–20% sucrose density gradients as described previously for microtubule affinity purified dynein (19, 42). Microtubule pellets were prepared as described previously (42, 47).

**DYNL Heterodimer Binding to the Intermediate Chain**—To determine whether DYNL1–DYNL3 light chain heterodimers were competent to bind to the intermediate chain, 293T cells were co-transfected with His-DYNL3 and Myc-DYNL1 light chains. Cell lysates were prepared as described above, except that EDTA was omitted from the buffer. The
lysates were incubated with Ni²⁺-nitrilotriacetic acid beads (Novagen) for 1 h at 4 °C. Protein complexes bound to the beads were eluted with 0.25 M imidazole buffer, 0.1% bovine serum albumin, pH 8.0, and the eluates were then incubated with cytosol from cells expressing Myc-tagged intermediate chain albumin, pH 8.0, and the eluates were then incubated with preciptated proteins were eluted with 0.1M glycine, 0.1% acetic acid. The beads were washed extensively and immuno-collected and concentrated by precipitation with 10% trichloroacetic acid. The supernatant and unbound proteins remaining in the supernatant were 74.1 antibody bound to Protein A beads. The beads were pelleted and the supernatant was then incubated for an additional 3 h at 4 °C with 74.1 antibody bound to Protein A beads. The beads were pelleted and the supernatant was then incubated for an additional 3 h at 4 °C with 74.1 antibody bound to Protein A beads. The beads were pelleted and the supernatant was then incubated for an additional 3 h at 4 °C with 74.1 antibody bound to Protein A beads.

**Preparation and Characterization of Monoclonal Antibodies to DYNLT1 and DYNLT3**—The two light chain–myosin basic protein fusion proteins were expressed in bacteria and purified with amylose column chromatography following the manufacturer’s instructions as described previously (19). The purified fusion proteins were injected into separate mice and monoclonal antibodies were prepared by the University of Virginia Lymphocyte Culture Center as described previously (48, 49). Positive hybridoma colonies producing antibodies specific for each light chain were identified by enzyme-linked immunosorbent assay screening of tissue culture supernatants against purified DYNLT1- and DYNLT3-GST fusion proteins. Antibodies from positive colonies were screened in immunoprecipitation and immunoblot assays with lysates of the bacteria expressing the GST fusion proteins, as described previously (34, 42), and positive hybridomas were cloned.

**RESULTS**

**Specificity of DYNLT1 and DYNLT3 Antibodies**—The specificities of four antibodies to the DYNLT isoforms were demonstrated by immunoprecipitation or probing Western blots of bacterial cell extracts expressing GST-light chain fusion proteins (Fig. 1). Two antibodies, R1 and T1, reacted specifically with the DYNLT3 and DYNLT1 fusion protein bands, respectively (Fig. 1A). These antibodies also detected single bands of the appropriate size from brain and tissue culture cell lysates and microtubule pellets prepared from rat brain (Fig. 1B). The T1 antibody has a higher affinity for human DYNLT1 than the rat DYNLT1 (not shown). Because, the two proteins differ in only two conserved amino acid substitutions at the N terminus of the polypeptide; it is likely that the antibody is directed toward the DYNLT1 N terminus. Two antibodies, R2 and R3, immunoprecipitated both the DYNLT3 and DYNLT1 GST-light chain fusion proteins (Fig. 1C). Thus, these antibodies recognize both members of the DYNLT light chain family. The R1 antibody also immunoprecipitated recombinant DYNLT3. However, R1 did not immunoprecipitate the dynein complex (Fig. 6) suggesting that the epitope is masked when DYNLT3 is incorporated into the dynein complex.

**Homodimer and Heterodimer Formation by Members of the DYNLT Light Chain Family in Eukaryotic Cells**—Two different approaches were used to determine whether DYNLT1 and DYNLT3 exist as heterodimers or homodimers in eukaryotic cells: a pairwise yeast two-hybrid assay and co-immunoprecipitation assay. In the yeast two-hybrid analyses, positive results were obtained when either DYNLT light chain isoform was present in both the pGBK7T7 and pGAD7T vectors (Fig. 2A). Transformation with individual light chain constructs did not activate the His gene. The specificity of the interaction was confirmed with the β-galactosidase assay (data not shown).
DYNLT Light Chain Isoform Interactions within Cytoplasmic Dynein

These data indicated that members of the DYNLT family can associate in all possible combinations of homodimers and heterodimers. The co-immunoprecipitation assay further demonstrated that both homodimers and heterodimers are formed between members of the DYNLT family in cultured mammalian cells (Fig. 2B). Cells were co-transfected with two light chains, each marked with a different epitope tag. Immunoprecipitation with antibodies to one tag invariably co-immunoprecipitated the light chain with the second tag. To determine whether the heterodimers detected in the co-immunoprecipitation assay were formed after cell lysis, cytosols prepared from cells expressing either DYNLT1 or DYNLT3 homodimers were mixed. After incubation, no co-immunoprecipitation of the two isoforms was detected (Fig. 2C). The absence of heterodimer formation in the mixed cytosols demonstrates that there is no exchange of subunits between the DYNLT1 and DYNLT3 homodimers.

Homodimers of the DYNLT Light Chain Family Interact with All Intermediate Chain Isoforms in Vivo—To determine whether the two DYNLT light chain family members bind to specific intermediate chains or to all the intermediate chain isoforms, a pairwise yeast two-hybrid assay was used. Yeast cells were co-transformed with DYNLT1 or DYNLT3 in pGBK7 and one of the six intermediate chains, IC-1A, -1B, -1C, -2A, -2B, and -2C, in pGADT7. Both DYNLT1 and DYNLT3 interacted with all of the intermediate chain isoforms (Fig. 3). The interactions were further confirmed with β-galactosidase assays (data not shown). With the yeast two-hybrid and mammalian expression data showing that the DYNLT light chains form homodimers (Fig. 2), and the DYNLT1 structural data derived from bacterially expressed proteins (35, 36, 50), we conclude that homodimers of both members of the DYNLT light chain family are capable of directly interacting with all six intermediate chain isoforms.

DYNLT1–DYNLT3 Heterodimers Do Not Bind the Intermediate Chain—We next sought to determine whether the DYNLT1–DYNLT3 heterodimers bind to intermediate chains. When light chains with different tags are co-transfected into cultured cells, three populations of dimers are created: DYNLT1 homodimers, DYNLT3 homodimers, and DYNLT1–DYNLT3 heterodimers. To separate the heterodimers and the DYNLT3 homodimers from the DYNLT1 homodimers, we expressed His-DYNLT3 and Myc-DYNLT1 in cultured cells and purified the His-DYNLT3 homodimers and His-DYNLT3–Myc-DYNLT1 heterodimers on nickel beads. As a control, the nickel bead binding confirmed that the His tag did not interfere with the formation of His-DYNLT3–Myc-DYNLT1 heterodimers (Fig. 4) or His-DYNLT3–Myc-DYNLT3 homodimers (data not shown). After the His-DYNLT3 homodimers and His-DYNLT3–Myc-DYNLT1 heterodimers were eluted from the nickel beads, they were incubated with Myc-tagged intermediate chains. When the intermediate chains were immunoprecipitated, DYNLT3 was found in the immunoprecipitate, but DYNLT1 was not (Fig. 4). This demonstrated that DYNLT3 homodimers but not DYNLT3–DYNLT1 heterodimers bound to the intermediate chain. Myc-DYNLT1 and His-DYNLT3

FIGURE 2. The DYNLT isoforms form stable homodimers and heterodimers in vivo. A, yeast two-hybrid assay. Yeast strain AH109 transformed with various combinations of binding and activation domain vectors (left panel) were plated on −2 and −3 plates. Yeast carrying a DYNLT isoform in both vectors (spots 1, 2, 5, and 8) were able to grow on the −3 plate, indicating that DYNLT1 and DYNLT3 are capable of forming homodimers and heterodimers in vivo. Neither the original vectors (spot 9), nor individual DYNLT isoforms (spots 3, 4, 6, and 7), were capable of self-activation in the absence of the light chain partner. B, co-immunoprecipitation assay. 293T cells were co-transfected with the pairs of DYNLT light chain isoforms tagged with the HA and Myc epitopes indicated by the plus sign (+). The HA-tagged light chain isoforms in the cell lysates were immunoprecipitated with anti-HA antibody. The lysates (Myc Inputs) and immunoprecipitates (Anti-HA IP) were analyzed by SDS-PAGE and immunoblotting, probed with anti-Myc antibody. Lysates are shown to verify expression of DYNLT proteins. Control immunoprecipitations were performed without transfacting HA-tagged light chain and in these no Myc-tagged light chains were immunoprecipitated by the HA antibody. As in the yeast two-hybrid assay, all combinations of DYNLT isoform homodimers and heterodimers were detected in the co-immunoprecipitation assay. C, no exchange of DYNLT isoforms. Human 293T cells were individually transfected with Myc-tagged DYNLT1 and HA-tagged DYNLT3. The resulting lysates (inputs) were mixed together at 4 °C for 3 h. The HA-DYNLT3 was immunoprecipitated with anti-HA antibody and the immunoprecipitate was analyzed by SDS-PAGE and the immunoblot was probed with anti-Myc antibody. No Myc-tagged DYNLT1 (IB) was co-immunoprecipitated with HA-tagged DYNLT3.

FIGURE 3. The DYNLT1 and DYNLT3 homodimers interact with both gene 1 and gene 2 intermediate chain isoforms in a yeast two-hybrid assay. Yeast strain AH109 containing various combinations of binding and activation domain vectors (left panel) were plated on −2 co-transformation plate and −3 interaction plate. Both DYNLT1 (A) and DYNLT3 (B) interacted with all intermediate chain isoforms (spots 1–6). Spot 9 is the positive control for the −2 transformation plate and a negative control for the −3 interaction plate. Spot 8 was the positive control for the −3 interaction plate. There was no interaction when the individual light chains (spot 7) were co-transfected with the empty partner vector.
were found in the supernatant after the immunoprecipitation, confirming that the heterodimer was not immunoprecipitated (Fig. 4). Therefore, whereas DYNLT1 and DYNLT3 form heterodimers in vivo, the heterodimers do not bind intermediate chains and are thus not incorporated into dynein complexes.

Because the DYNLT1–DYNLT3 heterodimer was unable to bind to the intermediate chain, we sought to determine the size of the endogenous pools of DYNLT1 and DYNLT3 subunits that were not incorporated into dynein complexes. Cytosol from rat brain or cultured human cells was applied directly to sucrose density gradients without prior selection of intact dynein complexes. In order to assay the presence of either DYNLT1 or DYNLT3 in rat brain cytosol or cultured human 293T cells, we homogenized brains or human 293T cells were homogenized in dynein purification buffer (42, 60). The cytosol was fractionated by centrifugation on a 5–20% sucrose gradient and the fractions were analyzed by SDS-PAGE and Western blotting. As a control to demonstrate that intact dynein complexes were immunoprecipitated, we confirmed the presence of the heavy and intermediate chain subunits (Fig. 6). In addition, whereas R1 immunoprecipitated recombinant DYNLT3 (Fig. 1), it did not immunoprecipitate DYNLT3 from cytosol (Fig. 6). This provides further evidence that there is no free pool of DYNLT3 in cytosol.

**Dynein Complexes with the DYNLT Light Chains Contain the DYNLL and DYNLRB Light Chains**—The DYNLT, DYNLL, and DYNLRB light chains bind to separate regions of the intermediate chain N terminus (14, 15, 51). Whereas these data suggested that the light chains bind to the intermediate chain simultaneously, that fact has not yet been demonstrated with purified dynein. To determine whether dynein with the DYNLT family members also contained either the DYNLL or DYNLRB light chains, endogenous dynein complexes were immunoprecipitated from rat brain lysates using antibodies that recognize both members of the DYNLT family (Figs. 1 and 6). We found that both DYNLL and DYNLRB light chains co-immunoprecipitated with the antibodies to the DYNLT family. As a control to demonstrate that intact dynein complexes were immunoprecipitated, we confirmed the presence of the heavy and intermediate chain subunits (Fig. 6). In addition, whereas R1 immunoprecipitated recombinant DYNLT3 (Fig. 1), it did not immunoprecipitate DYNLT3 from cytosol (Fig. 6). This provides further evidence that there is no free pool of DYNLT3 in cytosol.

**DYNLT Isoform Dimerization and Intermediate Chain Isoform Binding**—Using the approaches described in the previous sections to characterize the DYNLT family, we found that the DYNLT isoforms formed both homodimers and heterodimers (Fig. 7, A and B). Interestingly, when cytosols from cells expressing DYNLL homodimers were mixed, the two isoforms co-immunoprecipitated (Fig. 7C). Thus, in contrast to the two DYNLT homodimers, there was exchange of subunits between the two DYNLL homodimers. We further found that both DYNLL isoforms bound to all intermediate chain isoforms (Fig. 7D). However, we were unable to determine whether the DYNLT heterodimers bound to intermediate chains, in part due to nonspecific binding of an overexpressed protein to the purification matrix.

**DISCUSSION**

To better understand the functional significance of different subunit isoforms to the dynein complex, we characterized the interactions of the DYNLT family members with the dynein complex.
DYNLT Light Chain Isoform Interactions within Cytoplasmic Dynein

FIGURE 6. Cytoplasmic dynein complexes have both the DYNLT and DYNLL light chain family members. Rat brain cytoplasmic dynein was immunoprecipitated with antibodies to the intermediate chains (74.1) and the DYNLT light chain family (R1, R2, and R3). The proteins in the immunoprecipitates were resolved by SDS-PAGE and transferred to polyvinylidene fluoride and analyzed by staining with Coomassie Blue and antibodies (Ab) to the dynein intermediate chain and light chain families. When dynein was immunoprecipitated with the R2 and R3 antibodies that react with both DYNLT light chains, all the dynein subunits are found in the immunoprecipitates including the DYNLL and DYNLRB light chains. The R1 antibody does not immunoprecipitate the dynein complex. Top panel, the antibody heavy chain (Ab HC) and the dynein heavy chain and intermediate chain are seen on the stained gel. Bottom panels, strips of the blots probed with the antibodies indicated on the left side of the strips that detect the polypeptides indicated on the right side of the strips.

FIGURE 7. Interactions of the DYNLL isoforms. A, yeast two-hybrid assay: DYNLL isoforms form heterodimers. Yeast strains containing various combinations of binding and activation domain vectors (left panel) were plated on -2 co-transformation and -3 interaction plates. Only yeast strains carrying a DYNLL isoform in both vectors (spots 1, 2, 5, and 8) were able to grow on the -3 plate, indicating that members in DYNLL family are capable of forming homodimers and heterodimers in vivo. There was no interaction when the light chains were co-transformed with their respective empty partner plasmids (spots 3, 4, 6, and 7), B, co-immunoprecipitation assay. 293T cells were co-transfected with the pairs of DYNLL light chain isoforms tagged with the HA and Myc epitopes indicated by the plus sign (+). The HA-tagged light chain isoforms in the cell lysates were immunoprecipitated with anti-HA antibody. The lysates (Myc Inputs) and immunoprecipitates (Anti-HA IP) were analyzed by SDS-PAGE and immunoblotting, probed with anti-Myc antibody. Lysates are shown to verify expression of DYNLL proteins. Control immunoprecipitations were performed without transfecting HA-tagged light chain and in these no Myc-tagged light chains were immunoprecipitated by the HA antibody. Consistent with the yeast two-hybrid assay, all combinations of DYNLL homodimers and heterodimers were detected in the co-immunoprecipitation assay. C, DYNLL heterodimer formation by exchange of subunits between homodimers. 293T cells were co-transfected individually with HA-DYNLL1 and HA-DYNLL2. The Myc-DYNLL1 containing lysates (input) were then mixed with HA-DYNLL2 containing lysates (input) for 3 h at 4 °C. The HA-DYNLL2 was immunoprecipitated with anti-HA antibody and immunoprecipitate was analyzed by SDS-PAGE and the immunoblot (1B) was probed with anti-Myc antibody. D, interactions of DYNLL homodimers with the intermediate chain isoforms. Yeast strains containing various combinations of vectors (left panel) were plated on -2 co-transformation and -3 interaction plates. Both members in the DYNLL family interacted with all intermediate chain isoforms (spots 1–6). Co-transformation with pGBK7 and pGAD77 (spot 9) was used as a positive control for the -2 co-transformation plate and a negative control for the -3 interaction plate. Co-transformation with p53 and T-antigen vectors (spot 8) was used as a positive control for the -3 interaction plate. There was no interaction when the light chains (spot 7) were co-transfected with the empty partner vector.

organization and assembly interactions of the DYNLT isoforms with other subunits in the dynein cargo binding domain. We found that the two isoforms of DYNLT light chain family, DYNLT1 and DYNLT3, form all possible combinations of homodimers and heterodimers in yeast and cultured mammalian cells (Fig. 2). The structure of DYNLT1 has been the subject of considerable study. The NMR and crystal structures have been solved and show that two monomers associate across a strand-switched β-sheet interface to form a symmetrical dimer (35, 36). Using bacterially expressed protein, the Barbar group (50) was unable to identify conditions to detect structured monomers, and they calculated that the dimer exists at concentrations greater or equal to 10−15 M. This is consistent with our finding that there is no exchange of DYNLT subunits between homodimers in cytosol and together these data suggest that DYNLT dimer formation may be coupled to assembly into the dynein complex. DYNLT1 and DYNLT3 share 74% sequence similarity (55% identity) and sequence analysis shows that the regions that make the dimerization interface are highly conserved. The only non-conservative amino acid difference between DYNLT1 and DYNLT3 is at the periphery of the inter-

face (36). Thus, there is no structural impediment to the formation of the DYNLT1–DYNLT3 heterodimers or the DYNLT3 homodimers that we observed in yeast and tissue culture cells.
The two members of the second light chain family, DYNLL1 and DYNLL2, also formed both homodimers and heterodimers in the yeast and mammalian expression systems (Fig. 7). However, in contrast to the DYNLT light chain homodimers, we observed an exchange of subunits between the DYNLL homodimers. It has previously been observed that, unlike the DYNLT dimers, the DYNLL dimers undergo a pH-dependent shift to the monomer (14, 35, 52, 53). The ability of the DYNLL light chains to form a structured monomer may facilitate the exchange of subunits we observed between the DYNLL homodimers.

We further found that the DYNLT1 and DYNLT3 isoforms bind all six intermediate chain isoforms in a yeast two-hybrid binding assay (Fig. 3). Having shown that both the DYNLT light chains form homodimers in yeast two-hybrid and mammalian expression assays (Fig. 2), and with the structural data from bacterially expressed proteins discussed above, we concluded that homodimers of both members of the DYNLT light chain family were capable of directly interacting with all six intermediate chain isoforms. However, as shown is Fig. 4, the DYNLT1–DYNLT3 heterodimers were incapable of binding to the intermediate chain and thus only the homodimers assemble into the dynein complex. This is the first report that only a subset of the light chain dimers is utilized during the assembly of the dynein complex. Whereas it has previously been shown that the two DYNCL1II (light intermediate chain) subunits also incorporate into dynein as homodimers, not heterodimers, the light intermediate chains did not form heterodimers in vivo, in contrast to the DYNLT light chains (13). Our data are also in agreement with the report that the DYNLT1–DYNLT3 heterodimer was not found when dynein was purified from brain (54).

Crystal and NMR structural studies have shown that the two intermediate chain binding domains of the symmetrical DYNLT dimer are formed by contributions from both monomers, thus the DYNLT1 dimer is required for intermediate chain binding (36, 38). From the crystal structure of the DYNLT1 dimer bound to an intermediate chain peptide, two DYNLT1 amino acids, His34 and Asn38, were identified that were important for intermediate chain binding (38). Whereas sequence analysis shows that both of these amino acids are conserved in DYNLT3, several of the amino acids near them are not conserved (36, 38). This suggests that the differences in the neighboring amino acids may produce an asymmetric environment in the DYNLT1–DYNLT3 heterodimer that does not favor intermediate chain binding.

The DYNLT and DYNLL light chains bind near one another on the N terminus of the intermediate chain, and the structures of the DYNLL1 and DYNLL1 homodimers are very similar (15, 35, 36, 55). This suggested that their presence in dynein might be redundant and that dynein complexes would incorporate only one or the other of these light chains (15). Supporting this hypothesis were genetic studies showing that DYNLT1 is not an essential cytoplasmic dynein component in Drosophila (56). In addition, the Schroer group (4) isolated a part of the dynein complex whose mass indicated the presence of two intermediate chains and only four light chains. Previously, when dynein was purified either by immunopurification with antibodies to the intermediate chain, or by utilizing microtubule binding by the heavy chain, it was not possible to demonstrate that the same dynein complexes contained both the DYNLT and DYNLL light chains (44, 46). Our data, obtained by immunopurification with antibodies to the DYNLT isoforms (Fig. 6), shows that purified mammalian dynein contains the different light chain families. Thus, the DYNLT and DYNLL light chains are present in the same complex. Whereas we were unable to detect a pool of free soluble DYNLT1 or DYNLT3 isoforms (Figs. 1, 5, and 6), we cannot rule out the possibility that they were present in the insoluble fraction or in a very small soluble pool that we could not detect.

When the sedimentation profiles of all the subunits of cytosolic dynein were compared, the fractionation pattern of the DYNLT subunit was atypical (Fig. 5). Consistent with our previous report, most of the endogenous DYNLL did not copurify with dynein (44). These data further support the hypothesis that DYNLL has many roles independent of the dynein complex (22, 37, 57, 58). In particular, it has been suggested that DYNLL is necessary for the dimerization of various proteins (57). Our finding that both DYNLL isoforms bind to all the intermediate chain isoforms is consistent with our finding that both DYNLL isoforms are present when dynein is immunoprecipitated with antibodies to the intermediate chain, but does not support the hypothesis that one DYNLL isoform is associated with dynein and the other with myosin V (46, 59).

Assembly of individual subunit isoforms into the dynein complex creates different populations of the motor protein. Given two genes for each of the five cargo binding subunits, and a total of six intermediate chain alternative splice variants, and assuming that only homodimers are assembled into the complex, then 96 variants of the dynein complexes are possible. One possible functional role for the different populations is to allow specific dynein regulation or cargo binding (2, 19–22). Our finding that the DYNLT1 and DYNLT3 heterodimers, but not the heterodimer, bind to all the intermediate chains has important implications for models of dynein regulation or cargo binding. Because dynein complexes will not contain both light chain isoforms, the light chain homodimers define two different dynein complexes. Many cells express only one intermediate chain isoform, IC-2C, so the ability of both DYNLT homodimers to bind this intermediate chain doubles the number of different dynein complexes in such cells. Whereas there is no specificity in the interactions of the DYNLT homodimers with the intermediate chains, functional specificity of the intermediate chain-defined dynein complexes may be obtained through the limited expression patterns of subunit isoforms. For example, whereas DYNLT1 and DYNLT3 are found in almost all cells and tissues, the expression of the intermediate chain isoforms is cell- and tissue-specific. Intermediate chains encoded by gene 1 are expressed only in neurons (or testis) (18, 30, 31). Similarly, the DYNLNR2 light chain is not expressed in brain and the light intermediate chains are also not uniformly expressed in all cells and tissues (32, 43, 46).

In conclusion, we have shown that dynein complexes have multiple light chain families, that the DYNLT and DYNLL isoforms can form all combinations of homodimers and heterodimers, and that all the homodimers bind the six interme-


DYNLT Light Chain Isoform Interactions within Cytoplasmic Dynein

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36878 JOURNAL OF BIOLOGICAL CHEMISTRY

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