# Association of Trk Neurotrophin Receptors with Components of the Cytoplasmic Dynein Motor

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Nerve growth factor (NGF) initiates its trophic effects by long-range signaling through binding, internalization, and transport of a ligand–receptor complex from the axon terminal to the cell body. However, the mechanism by which retrograde transport of NGF takes place has not been elucidated. Here we describe an interaction between the Trk receptor tyrosine kinase and a 14 kDa light chain of cytoplasmic dynein. After transfection in human embryonic kidney 293 cells, this 14 kDa dynein light chain was found to bind to TrkA, TrkB, and TrkC receptors. Mapping experiments indicated that the 14 kDa dynein light chain binds to the distal region of the TrkA juxtamembrane domain. Coimmunoprecipitation experiments *in vivo* indicate that Trk receptors are in a complex with the 14 kDa light chain and 74 kDa intermediate chain of dynein. Confirming the physiological relevance of this association, a marked accumulation of Trk with the 14 kDa and the 74 kDa dynein components was observed after ligation of the sciatic nerve. The association of Trk receptors with components of cytoplasmic dynein suggests that transport of neurotrophins during vesicular trafficking may occur through a direct interaction of the Trk receptor with the dynein motor machinery.

*Key words: NGF; Trk receptor; cytoplasmic dynein; retrograde transport; sciatic nerve; ligation*

The biological effects of neurotrophins require that signals are conveyed over long distances from the nerve terminal to the cell body. A central tenet of the neurotrophin hypothesis is that neuronal survival and differentiation depend on retrograde transport of trophic factors produced at the target tissue (Levi-Montalcini, 1966, 1987; McAllister et al., 1999). The neurotrophins, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and NT-4, bind to transmembrane receptors and undergo internalization and transport from axon terminals to neuronal cell bodies (Hendry et al., 1974; Stockel et al., 1975; DiStefano et al., 1992). Trafficking of neurotrophins is believed to be required not only for survival but also for modulatory effects on neuronal activity and synaptic function.

Each neurotrophin is capable of binding to the p75 receptor and a specific Trk tyrosine kinase receptor (Chao, 1992). Binding of neurotrophins to Trk receptors results in receptor autophosphorylation and association with adaptor proteins, such as Shc, FRS2, rAPS, and SH2-B (Kouhara et al., 1997; Qian et al., 1998). These interactions give rise to downstream phosphorylation cascades involving phosphoinositide lipid phosphorylation and activation of GTPases such as Ras and Rap1 (York et al., 1998).

Despite extensive information regarding the generation of intracellular signals by neurotrophin receptors, little is known about the mechanism or regulation of transport of neurotrophins and their receptors. Both Trk and p75 receptors undergo retrograde transport (Johnson et al., 1987; Curtis et al., 1995; Ehlers et al., 1995; Bhattacharyya et al., 1997; Senger and Campenot, 1997). Experiments conducted in PC12 cells have indicated that the NGF–TrkA complex could be found in clathrin-coated vesicles and endosomes associated with tyrosine kinase substrates, such as phospholipase C$\gamma$ (Grimes et al., 1997). Several tyrosine-phosphorylated proteins are associated with the TrkA receptor during transport, suggesting that signaling by neurotrophins persists after internalization of their receptors (Ehlers et al., 1995; Grimes et al., 1996; Senger and Campenot, 1997). For example, activation of the nuclear transcription factor cAMP response element-binding protein in sympathetic neurons depends on

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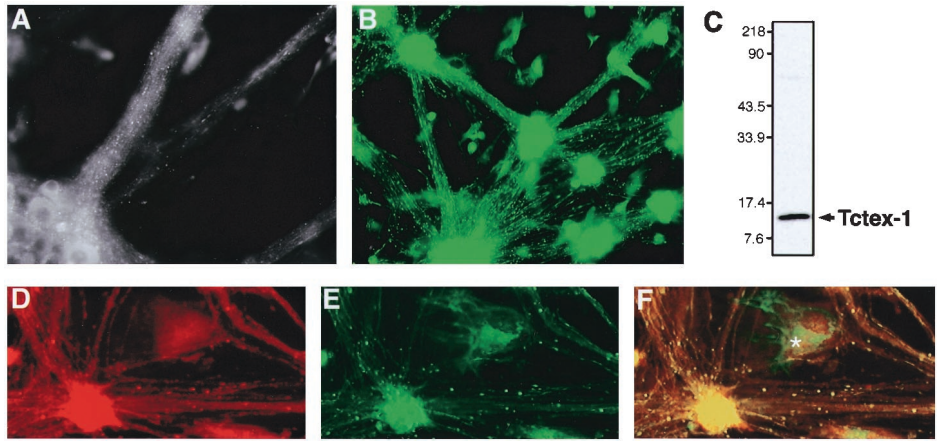


*Figure 1.* Expression of 14 kDa DLC (*Tctex-1*) in sensory (*A*) and sympathetic (*B*) neurons. DRG and SCG neurons were cultured in the presence of NGF and subjected to indirect immunofluorescence with affinity-purified anti-14 kDa DLC (*Tctex-1*) C24/C25. *C*, Western blot analysis of SCG lysates with C24/C25 antibody. *D–F*, Colocalization of the 14 kDa DLC and 74 kDa DIC in SCG neurons. SCG neurons were immunostained with anti-14 kDa DLC C24/C25 and anti-74 kDa DIC. The punctate patterns of the 74 kDa DIC (*D*) and 14 kDa DLC (*E*) were overlapped along axons as shown in *F*. An *asterisk* in *F* designates a nonneuronal cell.

transport of the neurotrophin–Trk complex (Riccio et al., 1997; Watson et al., 1999).

NGF is directly transported in the axons of sympathetic and sensory neurons. Here we report an interaction between Trk receptors and a component of the dynein motor, the 14 kDa dynein light chain (DLC), called Tctex-1. Mapping experiments in transfected cells indicate that the 14 kDa DLC binds to the distal portion of the TrkA juxtamembrane region. The association of 14 kDa DLC with Trk receptor tyrosine kinase suggests a critical link between neurotrophin receptors and the dynein motor machinery.

## MATERIALS AND METHODS

*Yeast two-hybrid screen.* A two-hybrid interaction screen was performed with the TrkB juxtamembrane region (His458–Gly544). The bait plasmid was generated by PCR with rat TrkB cDNA as a template with a forward primer (5′-gaattcggtcgacattccaagtttg-3′) and a reverse primer (5′-ggtaccgccggcgttctccaagctccctct-3′). The amplified fragment was ligated into pGEM-T vector, sequenced, and subcloned into pEG202 at *Eco*RI–*Nco*I sites as an in-frame fusion with the LexA–DNA binding domain.

A cDNA library from postnatal day 1 (P1) dorsal root ganglia (DRG) was generated (Kong et al., 2001). The DRG library cDNAs were expressed as in-frame fusions with the Gal4 transcriptional activation domain. Approximately $5 \times 10^7$ yeast transformants were screened for β-galactosidase activity and growth in the absence of leucine.

*Cell culture.* Human embryonic kidney (HEK) 293 cells were grown as described previously (Yano et al., 2000). Superior cervical ganglion (SCG) neurons were prepared from P2 rats and cultured on collagen-coated coverslips in C-medium [minimum essential medium containing 10% fetal bovine serum (FBS) supplemented with 0.4% glucose and 2 mM L-glutamine] with 150 ng/ml 2.5 S NGF (Harlan, Madison, WI). To inhibit the growth of non-neuronal cells, 24.6 μg/ml 5-fluoro-2-deoxyuridine and 24.4 μg/ml uridine were included for the first 4 d. DRG neurons dissected from embryonic day 17 embryos were cultured in C-medium containing 50 ng/ml NGF.

*Antibodies.* Anti-pan-Trk antibodies, B-3 mouse IgG and C-14 rabbit IgG, and anti-phosphotyrosine antibody (PY99) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA); anti-74 kDa dynein intermediate chain (DIC) monoclonal antibodies and anti-68 kDa neurofilament polyclonal antibodies were provided by Chemicon (Temecula, CA); anti-Flag M2 monoclonal antibody was supplied by Sigma (St. Louis, MO); and anti-pan-Trk rabbit antiserum 44, raised against the C-terminal region of Trk, was obtained from B. Hempstead (Cornell University, New York, NY). Anti-TrkA rabbit antiserum (RTA) was provided by L. Reichardt (University of California, San Francisco, CA). C24 or C25 rabbit IgG were generated against bovine 14 kDa DLC. Another 14 kDa DLC (Tctex-1) polyclonal antibody, R5205, was from S. King (University of Connecticut Health Center, Farmington, CT).

*DNA transfection and immunoprecipitation/immunoblot analysis.* HEK 293 cells were transfected using a calcium phosphate procedure with expression plasmids encoding the 14 kDa DLC, TrkA, and/or the empty vector. Lysates were prepared as described previously (Yano et al., 2000) with phosphatase inhibitors 1 mM $Na_3VO_4$ and 10 mM NaF. Cell lysates from each transfection were incubated with anti-pan-Trk IgG (C-14) or anti-Flag M2 antibody conjugated to agarose beads (Sigma). The former was followed by incubation with protein A–Sepharose beads (Sigma). Immunocomplexes were analyzed by immunoblotting. Immunoreactive proteins were detected by enhanced chemiluminescence (Amersham Pharmacia Biotech, Uppsala, Sweden).

For endogenous interaction, P18 rat brain was homogenized and lysed in 10 mM Tris, pH 8, 150 mM NaCl, 1 mM EDTA, and 0.5% NP-40 with protease and phosphatase inhibitors. After centrifugation and preclearing with protein A–Sepharose beads, lysates were incubated with anti-pan-Trk antibody (B-3) with or without blocking peptide (Santa Cruz Biotechnology). The immunocomplex was precipitated using protein A–Sepharose beads. The bound proteins were eluted with RIPA buffer (50 mM Tris, pH 8, 150 mM NaCl, 1% NP-40, 0.5% deoxycholate, and 0.1% SDS) and analyzed by immunoblotting with anti-14 kDa DLC R5205 or anti-74 kDa DIC antibodies.

*Plasmid construction.* The rat TrkA cDNA was subcloned into pCMV5 vector (pCMV5-TrkA). pcDNA3 vectors containing rat TrkB and TrkC were provided by P. Weign (Cornell University, New York, NY). TrkA mutants in Shc binding site, pCMV5-TrkA-N487A, pCMV5-TrkA-Y490A, and the kinase-dead mutant (pCMV5-TrkA-K538A) were made by site-directed mutagenesis. The Shc binding site tyrosine residue is designated as amino acid (aa) 490, and other mutations are numbered relative to this. pCMV5-TrkA (1–513), pCMV5-TrkA (1–492), pCMV5-TrkA (1–484), and pCMV5-TrkA (1–443), which contain amino acids 1-G513, 1-S492, 1-I484, and 1-G443 of TrkA, respectively, have been described previously (Yano et al., 2000) as pCMV5-TrkA-Trunc12, pCMV5-TrkA-Trunc10, pCMV5-TrkA-Trunc15, and pCMV5-TrkA-Trunc. The TrkA (1–463) construct encoding amino acids 1-463 of human TrkA, also called pcDNA3-TrkA$^{\Delta INT}$ (Gargano et al., 1997), was provided by A. Levi (Istituto di Neurobiologia, Rome, Italy). Bovine Tctex-1 and N-terminal Flag-tagged bovine Tctex-1 cDNAs were subcloned in pRK5. The glutathione *S*-transferase (GST)–TrkA mutants pGEX-4T1-TrkA (439–513), pGEX-4T1-TrkA (439–503), pGEX-4T1-TrkA (439–463), pGEX-4T1-TrkA (464–503), and pGEX-4T1-TrkA (485–513) contain the rat TrkA cDNA encoding 75 aa (R439-G513), 65 aa (R439-D503), 25 aa (R439-M463), 40 aa (T464-D503), and 29 aa (M485-G513), respectively. These cDNA fragments were generated by PCR and subcloned in the pGEX-4T1 vector (Amersham Pharmacia Biotech).

*Preparation of GST fusion proteins and* in vitro *binding assays.* GST–TrkA juxtamembrane mutant proteins were immobilized on glutathione–Sepharose 4B beads (Amersham Pharmacia Biotech). HEK 293 cell lysates overexpressing the 14 kDa DLC were prepared as described above and incubated with immobilized GST–fusion proteins at 4°C. The beads were then washed and the bound proteins were analyzed by SDS-PAGE and immunoblotting with anti-14 kDa DLC antibodies (R5205).

*Sciatic nerve ligation.* Adult or P30 rats were anesthetized with ketamine and xylazine, and the sciatic nerve on one side was ligated using a 5-0 polypropylene monofilament. One day after ligation, the animal was killed, and the sciatic nerve was immediately frozen in liquid nitrogen. Fresh longitudinal frozen sections were prepared (20 μm). The contralat-

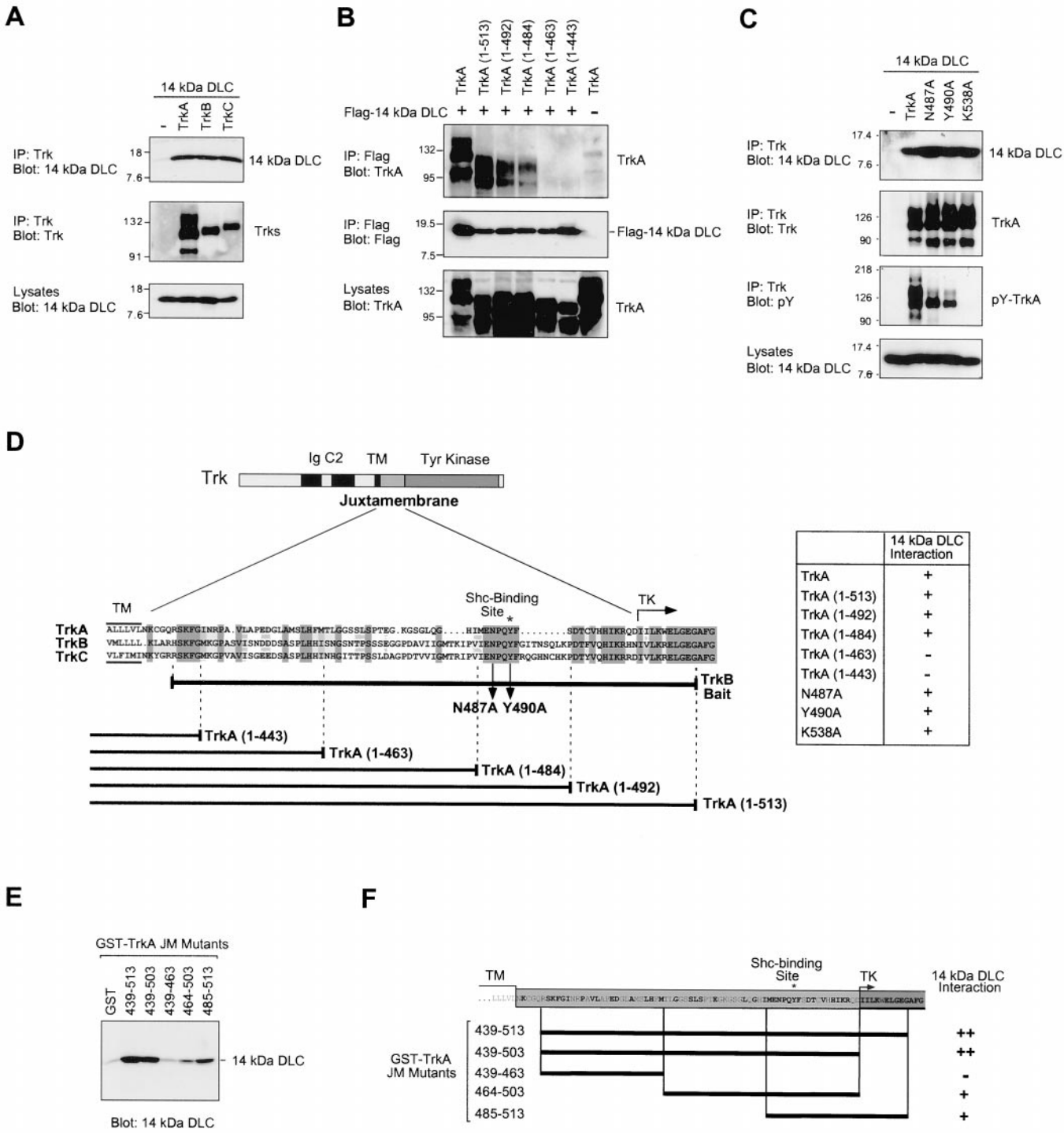


*Figure 2.* *A*, Coimmunoprecipitation of 14 kDa DLC with TrkA, TrkB, and TrkC. HEK 293 cells were cotransfected with cDNAs encoding the 14 kDa DLC and TrkA, TrkB, TrkC, or empty vector (−). Cell lysates were immunoprecipitated with anti-pan-Trk C-14 and immunoblotted with anti-14 kDa DLC (*Tctex-1*) R5205 (*top panel*). Immunoprecipitation of Trk receptors was confirmed by immunoblotting with anti-pan-Trk antibody (*middle panel*). Crude lysates were immunoblotted with R5205 antibody to confirm equivalent expression level (*bottom panel*). *B*, Mapping the interaction of the 14 kDa DLC with TrkA mutants. HEK 293 cells were cotransfected with Flag-tagged 14 kDa DLC cDNA and either full-length TrkA or TrkA truncation mutants. Lysates were immunoprecipitated with anti-Flag M2-agarose and immunoblotted with anti-TrkA RTA (*top panel*). Immunoprecipitation of Flag-14 kDa DLC was confirmed by immunoblotting with anti-Flag M2 (*middle panel*); Trk expression was verified with anti-TrkA RTA (*bottom panel*). *C*, Tyrosine kinase activity and the Shc site are not required for TrkA–14 kDa DLC association. The cDNA encoding the 14 kDa DLC and TrkA mutants (N487A, Y490A, or K538A) were cotransfected in HEK 293 cells, and their association was analyzed by immunoprecipitation with C-14 and immunoblotting with anti-14 kDa DLC R5205 (*top panel*). TrkA was confirmed by immunoblotting with anti-Trk and anti-pY (*middle two panels*). Expression of 14 kDa DLC was assessed with R5205 antibody (*bottom panel*). N487A and Y490A, TrkA mutants in the Shc-binding region; K538A, kinase-inactive TrkA. *D*, Comparison of juxtamembrane sequences among rat TrkA, TrkB, and TrkC. The TrkA mutants used in *B* and *C* and a summary of 14 kDa DLC interactions with the mutants are provided. *E*, *F*, *In vitro* binding assay. GST–TrkA fusion proteins were incubated with lysates from HEK 293 cells overexpressing the 14 kDa DLC. *E*, Bound 14 kDa DLC was detected by immunoblotting with R5205 antibody. *F*, Schematic representation of GST–TrkA fusion proteins and summary of binding data. Identical or conserved amino acids among rat TrkA, TrkB, and TrkC are shown in *bold*. *JM*, Juxtamembrane.

eral sciatic nerve was ligated immediately after killing, and sections were prepared in parallel as a control.

*Immunofluorescence analysis.* SCG neurons cultured for 7–10 d were fixed with 4% paraformaldehyde and blocked with PBS containing 0.075% saponin and 10% FBS or normal goat serum (NGS). Cells were incubated with anti-14 kDa DLC rabbit IgG C24 or C25 (0.1–0.3 μg/ml) with or without anti-74 kDa DIC mouse IgG (0.5 μg/ml). Primary antibodies were visualized using fluorescence-conjugated secondary antibodies [Alexa 488-conjugated anti-rabbit IgG (Molecular Probes, Eugene, OR), FITC-conjugated goat anti-rabbit IgG (The Jackson Laboratory, Bar Harbor, ME), rhodamine Red-X-conjugated goat anti-mouse IgG (The Jackson Laboratory), or a combination of the above]. Images were collected on a confocal microscope from Leica (Nussloch, Germany) or Bio-Rad (Richmond, CA).

For analysis of the sciatic nerve, fresh-frozen sections were incubated with blocking solution (PBS containing 10% bovine serum albumin and 10% NGS) and then incubated for 1–2 hr with a combination of the following antibodies in dilution buffer (PBS containing 10% lamb serum and 10% NGS): anti-pan-Trk IgGs (C-14 and B-3) (2 μg/ml), anti-74 kDa DIC IgG (2 μg/ml), and anti-14 kDa DLC rabbit IgG (C24 or C25) (~0.5 μg/ml). Primary antibodies were visualized by Cy3-conjugated goat anti-rabbit IgG (The Jackson Laboratory) (1:200) and biotin-conjugated anti-mouse IgG (The Jackson Laboratory) (1:500) followed by avidin-conjugated FITC (Vector Laboratories, Burlingame, CA) (1:500).

## RESULTS

To identify proteins that interact directly with Trk receptors, a yeast two-hybrid screen was performed with a postnatal DRG cDNA library using the TrkB juxtamembrane region as a bait. The juxtamembrane region contained sequences between the transmembrane region and the tyrosine kinase domain. Three positive clones encoding a 14 kDa rat DLC called Tctex-1 (GenBank accession number AB010119), were identified among 75 positive clones. Tctex-1 was originally identified as a mouse *t* haplotype gene (Lader et al., 1989). Subsequent studies indicated that this protein represented a light chain component in the dynein complex (King et al., 1996; Harrison et al., 1998). We will refer to Tctex-1 as the 14 kDa DLC.

To determine whether the 14 kDa DLC is appropriately expressed in neurotrophin-responsive cells, primary cultures from SCG and DRG were immunostained with an antibody made against the 14 kDa DLC Tctex-1. This antibody recognizes only the 14 kDa DLC and not other dynein proteins (Fig. 1*C*), including the rp3 dynein light chain. Using this antibody, punctate staining was observed in the processes of both sympathetic and sensory neurons (Fig. 1*A,B*). In addition, immunoreactivity for the 14 kDa DLC was colocalized in axons of sympathetic neurons with the 74 kDa DIC (Fig. 1*D–F*). These results indicate that two components of the cytoplasmic dynein complex are expressed and localized to axons of sympathetic neurons, consistent with their function in retrograde transport.

### Mapping the TrkA-14 kDa DLC interaction

Using the yeast two-hybrid assay, we observed binding of the 14 kDa DLC to the juxtamembrane region of TrkA as well as TrkB receptors (data not shown). To investigate whether the Trk receptors interact with the 14 kDa DLC in a mammalian cell environment, we expressed full-length versions of TrkA, TrkB, and TrkC receptors in HEK 293 cells. After immunoprecipitation of each Trk receptor, an association was observed with the DLC, as assessed by Western blotting with anti-14 kDa DLC antibody (Fig. 2*A*). Each Trk receptor was capable of associating with the 14 kDa DLC. A complex between the 14 kDa DLC and the TrkA receptor was also detected after immunoprecipitation of the 14 kDa DLC and immunoblot with anti-TrkA antibodies (Fig. 2*B*). Different species of Trk receptors were observed because of differential glycosylation.

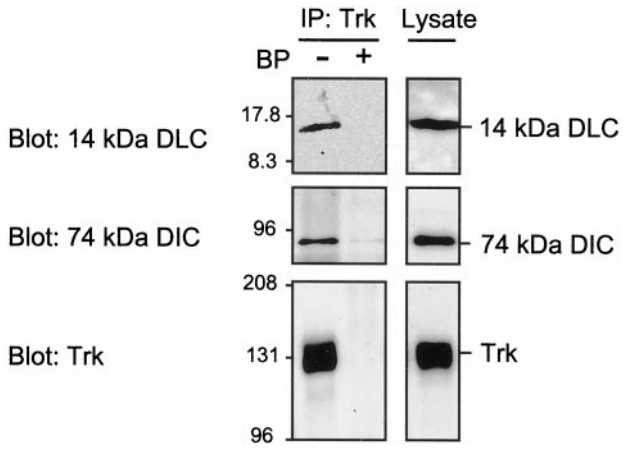


*Figure 3.* Endogenous association of Trk receptors with dynein subunits. P18 whole-brain lysates were subjected to immunoprecipitation with anti-pan-Trk B-3 in the presence or absence of a blocking peptide (*BP*) for this antibody. The immune protein complex was then eluted, and the 14 kDa and 74 kDa dynein components and Trk receptors were detected by immunoblotting. *Right panels* reflect protein expression in the original lysate.

Additional coprecipitation experiments were performed with a series of deletions of the TrkA receptor that eliminated the tyrosine kinase domain and sequences in the juxtamembrane regions (Fig. 2*D*). Whereas the entire TrkA juxtamembrane region [TrkA (1–513)] was capable of binding to the 14 kDa DLC, removal of 50 aa [TrkA (1–463)] abolished this interaction. A deletion of 29 aa [TrkA (1–484)] still retained the interaction. These coprecipitation results suggested that TrkA association with DLC involves sequences between amino acid 464 and amino acid 484.

To confirm the region of the TrkA receptor responsible for binding the 14 kDa DLC, *in vitro* binding studies were also performed. GST–fusion proteins carrying different segments of the TrkA juxtamembrane domain were immobilized on glutathione beads and incubated with HEK 293 cell lysates overexpressing the 14 kDa DLC (Fig. 2*E,F*). Strong binding of DLC was observed with GST–TrkA fusion proteins that spanned the entire juxtamembrane domain [(439–513) and (439–503)] but not with shorter regions (439–463), consistent with the deletion analysis shown in Figure 2*B*. Constructs representing the more distal regions of the juxtamembrane region interacted with the 14 kDa DLC. Together, these results suggest that a 40 aa segment of the distal region of the juxtamembrane domain is involved in the association with the 14 kDa DLC.

To determine whether the interaction of the 14 kDa DLC with Trk receptors is dependent on kinase activity, HEK 293 cells were cotransfected with a kinase-inactive form of TrkA (K538A) and DLC. Although transfection of wild-type TrkA frequently results in constitutive autophosphorylation, expression of the K538A mutant did not produce a tyrosine-phosphorylated species. After immunoprecipitation of TrkA receptors, the 14 kDa DLC was found to associate with the K538A receptor (Fig. 2*C*). Likewise, an association was observed between 14 kDa DLC and TrkA receptors with mutations in the Shc-binding site (N487A and Y490A) located in the juxtamembrane region. These results suggested that the 14 kDa DLC interaction can occur in the absence of TrkA kinase activity.

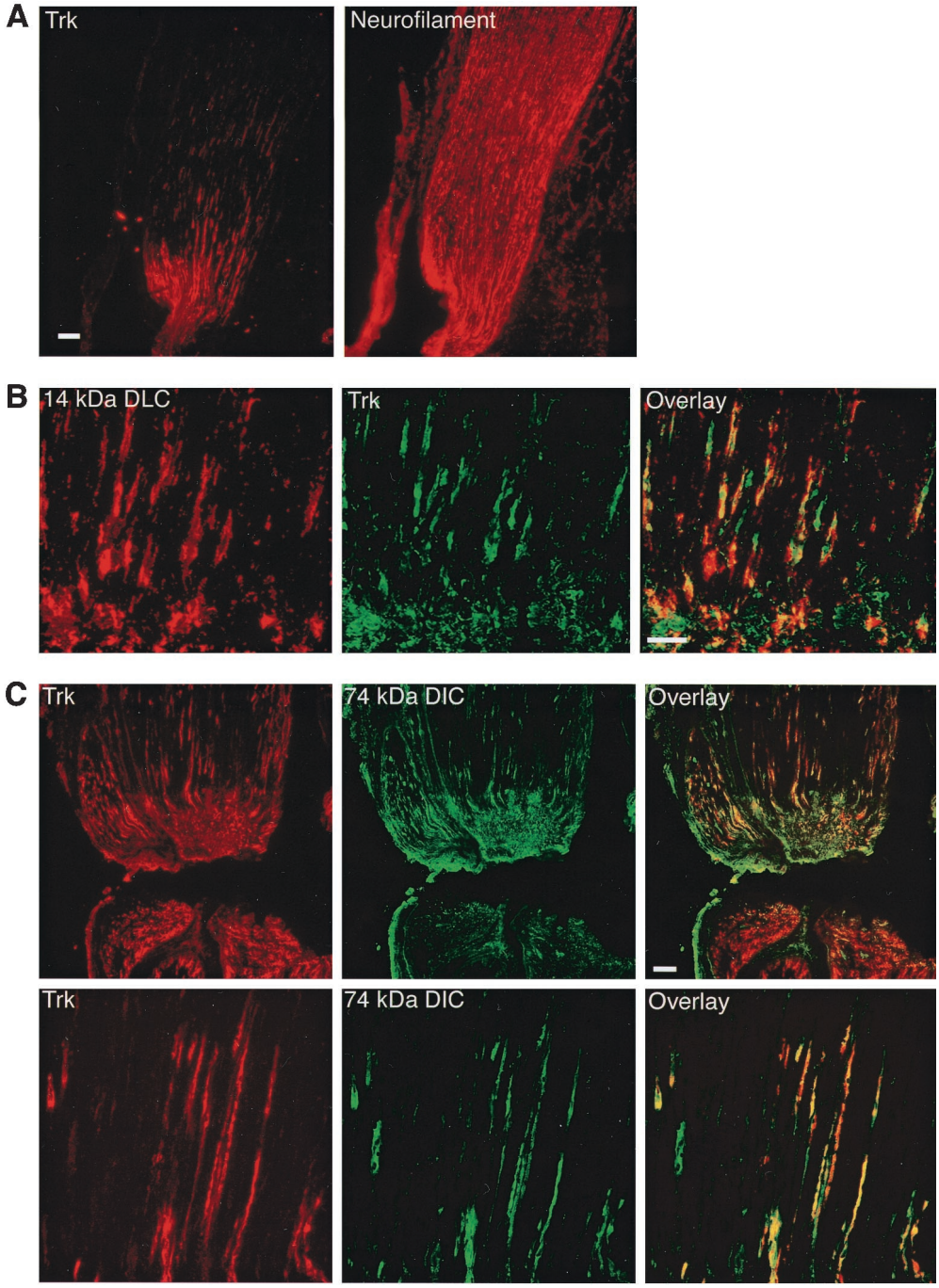


*Figure 4.* Accumulation of Trk receptors and dynein motor components after ligation of the sciatic nerve. *A*, The sciatic nerve from a P30 rat was ligated for 1 d. Longitudinal sections were immunostained with either anti-pan-Trk C-14 (*left*) or anti-68 kDa neurofilament (*right*) antibody. Images were taken from the distal side of a ligature. Scale bar, 100 μm. *B, C*, Colocalization of Trk with dynein motor components, 14 kDa DLC, and 74 kDa DIC analyzed by confocal microscopy. *B*, Longitudinal sections from a ligated sciatic nerve were double-immunostained with anti-14 kDa DLC C24/C25 and anti-pan-Trk B-3. *C*, Sections were stained with anti-pan-Trk C-14 and anti-74 kDa DIC antibodies. Images in *B* display the distal side of a ligature. In *C*, the distal portion is at the *top* and the proximal end of the ligation is at the *bottom*. The *bottom panels* in *C* are higher magnifications of the distal side of the ligated nerve. Colocalization of Trk with 14 kDa DLC or 74 kDa DIC is indicated in *yellow* (*right panels*). Scale bars: *B*, 50 μm; *C*, 100 μm.

### Endogenous interaction of Trk receptors with dynein components

The association between Trk receptors and the 14 kDa light chain was based on cotransfection in HEK 293 cells and yeast two-hybrid interactions. To examine whether this interaction occurs under physiological conditions, we performed additional coimmunoprecipitation experiments of endogenous Trk and dynein proteins from brain lysates. Lysates from P18 rat brain were immunoprecipitated with anti-pan-Trk antibody, and the immunocomplex was analyzed by immunoblotting with anti-14 kDa DLC antibody. As shown in Figure 3, the 14 kDa DLC protein coimmunoprecipitated with Trk receptors. In addition, the same immunocomplex was found to contain the 74 kDa DIC component. This result indicates that an endogenous association of Trk receptors could be detected with cytoplasmic dynein components in brain tissues. An association between the 14 kDa DLC and the 74 kDa DIC was also observed after immunoprecipitation in brain lysates (data not shown) and in immunofluorescence studies of cultured sympathetic neurons (Fig. 1), confirming that these two dynein motor components are in the same protein complex. These observations indicate that Trk receptors and components of the dynein motor complex display an endogenous association in cells responsive to neurotrophins.

### Colocalization of Trk receptors with dynein components

To test the hypothesis that retrograde transport of Trk receptors is associated with 14 kDa DLC *in vivo*, we performed sciatic nerve

ligation to follow the localization of these proteins. Adult or P30 rats were anesthetized, and the sciatic nerve was ligated for 1 d (Korsching and Thoenen, 1983). The nerve was removed, sectioned longitudinally, and processed for immunohistochemistry.

A marked accumulation of Trk receptors was observed in the distal portion of the sciatic nerve after ligation (Fig. 4*A*). The Trk immunoreactivity was axonal in nature, because positive staining was observed in adjacent sections with neurofilament antibodies. When expression of the 14 kDa DLC was examined in the same sections, a pattern similar to the Trk receptors was observed in the distal side of the ligated nerve (Fig. 4*B*). Likewise, antibodies against the 74 kDa DIC revealed a discrete pattern of accumulation in the nerve that closely matched the distribution of Trk receptors (Fig. 4*C*).

Several observations support the possibility that the staining of these proteins was specific and a reflection of axonal transport. First, other axonal proteins, such as neurofilament, did not give the same pattern of specific accumulation as the dynein proteins and Trk receptors (Fig. 4*A*). Therefore, not all axonal proteins accumulate in the same manner after ligation. Second, more robust staining of the 14 kDa DLC and the 74 kDa DIC was observed on the distal side of the ligation, consistent with a retrograde movement of these proteins in the sciatic nerve (Fig. 4*C*). However, Trk receptors were detected on both the proximal and distal sides of the ligature, consistent with their ability to undergo retrograde and anterograde transport (Fig. 4*C*). Finally, antibodies against the dynein subunits and the Trk receptor were highly specific for each protein, as assessed by Western blot analysis (Figs. 1, 2). Therefore, colocalization of the Trk receptor with the 14 kDa DLC and the 74 kDa DIC components after sciatic nerve ligature provides more evidence for their *in vivo* interaction.

## DISCUSSION

Retrograde axonal transport of neurotrophins requires binding of ligands to their receptors, internalization through a clathrin-coated mechanism, and routing into transport vesicles or to degradative or recycling pathways (Grimes et al., 1997). The sorting mechanisms that direct these different processes are currently unknown. Presumably, neurotrophin–Trk receptor vesicles are formed and directed toward a microtubule- or actin-based motor system. Given the existence of many proteins that undergo axonal transport (Johanson et al., 1995; Senger and Campenot, 1997), it is plausible that proteins bound to neurotrophin–receptor complexes dictate their directionality, distribution, and transport in neurons.

Cytoplasmic dynein is a negative end-directed motor that transports organelles along microtubules in a retrograde direction. Each cytoplasmic dynein complex consists of at least two heavy chains of 500 kDa, two intermediate chains, two 14 kDa light chains, and two 8 kDa light chains, with several additional accessory light intermediate chains (Hirokawa, 1998). The existence of several different dynein light chains has given rise to the hypothesis that these subunits may be involved in specific cargo attachment (King, 2000).

Here we have demonstrated a discrete association of cytoplasmic dynein components with Trk receptors. These results imply that dynein motor proteins play a direct role in retrograde transport of neurotrophins and their receptors. Despite decades of evidence for transport of neurotrophins, there has been little information regarding how neurotrophins and their receptors are linked to the transport machinery. Cytoplasmic dynein is responsible for retrograde transport in axons, but how this motor complex is involved in neurotrophin transport has not been established. The association of the 14 kDa DLC with Trk receptors suggests that there is a targeting mechanism to direct neurotrophin-bearing vesicles toward axonal transport. This mechanism has yet to be rigorously proven, but components of cytoplasmic dynein may provide one level of regulation for axonal transport.

Recent studies have indicated that the 14 kDa DLC can bind to several proteins, including the Fyn tyrosine kinase (Campbell et al., 1998), Doc2 (Nagano et al., 1998), and rhodopsin (Tai et al., 1999). An important question concerning the role of cytoplasmic dynein during transport is how each cargo is recognized in a specific manner. The specificity of TrkA–dynein interaction is supported by the appropriate localization of 14 kDa DLC with Trk and the 74 kDa DIC in axonal cellular compartments and by the binding of 14 kDa DLC to a discrete domain in the juxtamembrane region of the TrkA receptor. Moreover, a complex was detected between endogenous Trk receptors and the 14 kDa and 74 kDa dynein proteins. Future studies will need to interfere with these interactions and examine the functional consequences.

Another important question is whether intracellular second messenger signals are transmitted retrogradely to the cell body. Such a mechanism is supported by the production of neurotrophins in target tissues and by the internalization and transport of Trk receptors with their respective neurotrophins (Ehlers et al., 1995; Bhattacharyya et al., 1997; Riccio et al., 1997; Senger and Campenot, 1997; Tsui-Pierchala and Ginty, 1999; Watson et al., 1999; Kuruvilla et al., 2000). Proteins associated with Trk receptors, such as Shc, phosphatidylinositol 3-kinase, phospholipase C$\gamma$, and the p75 neurotrophin receptor, or the dynein motor complex, may serve to activate or enhance downstream signal transduction pathways. Additional characterization of Trk–dynein interaction will provide further clues as to how the transmission of trophic signals is regulated by retrograde transport.

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