Brain Dynein (MAP1C) Localizes on Both Anterogradely and Retrogradely Transported Membranous Organelles In Vivo

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Abstract. Brain dynein is a microtubule-activated ATPase considered to be a candidate to function as a molecular motor to transport membranous organelles retrogradely in the axon. To determine whether brain dynein really binds to retrogradely transported organelles in vivo and how it is transported to the nerve terminals, we studied the localization of brain dynein in axons after the ligation of peripheral nerves by light and electron microscopic immunocytochemistry using affinity-purified anti-brain dynein antibodies. Different classes of organelles preferentially accumulated at the regions proximal and distal to the ligated part. Interestingly, brain dynein accumulated both at the regions proximal and distal to the ligation sites and localized not only on retrogradely transported membranous organelles but also on anterogradely transported ones. This is the first evidence to show that brain dynein associates with retrogradely transported organelles in vivo and that brain dynein is transported to the nerve terminal by fast flow. This also suggests that there may be some mechanism that activates brain dynein only for retrograde transport.

The axons of nerve cells have served as a good model system for studying the mechanism of organelle transport. There is bidirectional movement of numerous classes of membranous organelles in the axon (Nakai, 1956; Lubinska and Niemierko, 1971; Tytell et al., 1981; Allen et al., 1982), and structural studies after the blockage of transport have revealed that different classes of organelles are preferentially transported by respective types of flow (Smith, 1980; Tsukita and Ishikawa, 1980). Several theories have been proposed to explain the underlying mechanisms (for review, see Grafstein and Forman, 1980; Schliwa, 1984). Electron microscopic studies of the neuronal cytoskeleton in vivo have suggested that microtubules (MTs) and cross-bridges between MTs and membranous organelles form the structural basis for this class of motility (Smith, 1971; Hirokawa, 1982; Miller and Lasek, 1985; Hirokawa and Yorifuji, 1986; Hirokawa et al., 1989a). In fact, observations of organelle movements in the isolated axoplasm by video-enhanced light microscopy have demonstrated that bidirectional organelle transport occurs along MTs (Brady et al., 1985; Schnapp et al., 1985; Vale et al., 1985a). Recently, candidates for the motor molecules of these transports, namely kinesin and brain dynein (MAP1C), have been identified (Brady, 1985; Vale et al., 1985b; Paschal and Vallee, 1987; Paschal et al., 1987; Schnapp and Reese, 1989). Brain dynein–like molecules were identified in Caenorhabditis elegans (Lye et al., 1987), Reticulomyxa (Euteneuer et al., 1988, 1989), and squid giant axons (Gilbert and Sloboda, 1986, 1989; Pratt, 1986) as well. Because MTs on cover glasses coated with brain dynein move from minus to plus ends, brain dynein is thought to function as a motor of retrograde transport (Paschal and Vallee, 1987). However, it has not been resolved whether brain dynein really associates with retrogradely transported membranous organelles and is a motor for retrograde transport in vivo. A related question is whether the retrograde motor is transported to the nerve terminals by fast or slow transport, and whether brain dynein attaches only to retrogradely transported organelles or if the motor binds to both anterogradely and retrogradely transported ones. If the latter were the case, some mechanism that activates brain dynein only for the retrograde transport would have to exist.

To address these issues, we studied the localization of brain dynein in the axons after ligation of peripheral nerves by light and electron microscopic immunocytochemistry using affinity-purified anti-brain dynein antibodies. Our study clearly demonstrated that brain dynein accumulates both anterogradely and retrogradely at regions both proximal and distal to the ligation and that it binds to both retrogradely and anterogradely transported membranous organelles. This suggests that brain dynein is really a motor for retrograde transport in vivo, that it is transported to the nerve terminal by fast flow, and that there may possibly exist some mechanism that activates brain dynein only for the retrograde transport.

Materials and Methods

Purification of Brain Dynein and Antibody Production

Brain dynein (MAP1C) was purified by the method of Paschal et al. (1987) with slight modifications as described previously (Yoshida et al., 1990).
Microtubules were prepared from bovine brain white matter and brain stem using taxol. After GTP extraction, MAPIC was extracted in 10 mM ATP solution from the MTs and purified by sucrose density gradient centrifugation and DEAE-Sepharose CL-6B column chromatography. The antigen to be used as a ligand for affinity purification was further purified by additional sucrose density gradient centrifugation.


to nitrocellulose, strips of which were stained with India ink (lane 1), anti-brain dynein antibody, or monoclonal antibodies against MAP1A (Shiomura and Hirokawa, 1989). The rest was electrophoretically blotted to nitrocellulose as described by Towbin et al. (1987).

**Immunoblotting Procedure**

Sciatic nerves and brains were dissected from adult mice and homogenized separately in extraction buffer (0.1 M Pipes, 1 mM MgCl2, 1 mM EGTA, 1 mM PMSE, 10 #g/ml leupeptin, 1 mM DTT, pH 6.6) and then centrifuged to yield the crude sciatic nerve and brain extracts. Taxol was added to the crude brain extracts to a final concentration of 20 #M (Vallee, 1982), and MT proteins were pelleted by centrifugation and rehomogenized in the extraction buffer. Cytoplasmic dyneins were purified from rat livers and mouse brains by a modification of the methods of Paschal et al. (1987), Bloom and Brasher (1989), and Collins and Vallee (1989). Briefly, dissected tissues were immediately homogenized at 4°C in PEM buffer (100 mM Pipes, 1 mM EGTA, 1 mM MgCl2, 0.1 mM DTT, 1 #g/ml leupeptin, 0.1 mM PMSE, 10 #g/ml tosyl arginyl methyl ester, 1 #g/ml pepstatin A [pH 6.8]) and centrifuged at 100,000 g for 20 min at 4°C. The supernatant was recovered and centrifuged for another 60 min. This supernatant was supplemented with 1 U/ml hexokinase, 50 mM glucose, 500 #g/ml phosphocellulose column-purified tubulin (PC tubulin), and 5 #M taxol. After incubation at 37°C for 5 min, the microtubules were sedimented at 4°C, washed by resuspension and centrifugation in PEM buffer alone, and then in PEM buffer containing 5 mM Mg GTP. Dyneins were extracted from microtubules using 10 mM Mg ATP and 400 mM KCl, and further fractionated by sucrose density gradient centrifugation.

Fractations containing brain dyneins were photoeluted by irradiation at 365 nm in the presence of 100 #M vanadate and 50 #M ATP for 30 min (Lee-Eiford et al., 1986). Crude sciatic nerve extracts, brain MT proteins, cytoplasmic dyneins purified from rat livers, fractions containing brain dyneins, and photoeluted brain dynein–containing fractions were respectively separated on polyacrylamide gels as described by Laemmli (1970). Liver cytoplasmic dyneins were bound to microtubules polymerized from PC tubulin in the presence of taxol. After centrifugation at 30°C for 30 min at 10,000 g, the resulting pellets were also separated by SDS gel electrophoresis. One part of the gel was stained with Coomassie brilliant blue and the rest was electrophoretically blotted to nitrocellulose as described by Towbin et al. (1979) and stained with India ink, anti-brain dynein antibody, or nonimmune rabbit IgG. Nitrocellulose sheets were incubated with 5% skim milk PBS before incubation with either affinity-purified anti-MAP1C antibodies, monoclonal antibodies against MAP1A (Shiomura and Hirokawa, 1987), MAP1B, MAP2 (Sato-Yoshitake et al., 1989), or nonimmune rab-
were ligated under anesthesia. 6-10 h later, the mice were
graphs from 3 samples for each portion (proximal and distal) were used
bules nitrocellulose sheets were incubated with horseradish peroxidase-
distal portions of the ligated nerves were processed for immu-
IgG (10-10 µg/ml) and rhodamine labeled anti-rabbit IgG (Cappel Laborato-
ties, Malvern, PA). Sections were preincubated for 1 h with 5% skim milk
microscopy, and cryoultrathin-section immunocytochemistry.

Immunofluorescence Microscopy
The nerves were sectioned on a cryostat (4-5-µm sections) and stained with
affinity-purified anti-brain dynein antibodies (20-30 µg/ml), anti-tubulin anti-
bodies (10-10 µg/ml) (Polysciences, Inc., Warrington, PA) or normal rabbit
IgG (20-30 µg/ml) and rhodamine labeled anti-rabbit IgG (Cappel Laborato-
yes, Malvern, PA). Sections were preincubated for 1 h with 5% skim milk
in PBS and stained as described previously (Hirokawa et al., 1984, 1985).
Nonligated nerves were also stained similarly.

Immunocytochemistry of Brain Dynein Using
Ultrathin Cryosections
Procedures for immunolabeling of ultrathin cryosections were performed
as described previously (Tokuyasu, 1980; Hirokawa et al., 1989b) with
some modifications. After cryoprotection in graded series of sucrose PBS,
the nerves were embedded in 2% gelatin and frozen with liquid Freon 22.
We used affinity purified anti-brain dynein IgG (20-30 µg/ml) in PBS or nor-
mal rabbit IgG (20-30 µg/ml) in PBS as the first antibodies, and 10 nm gold-
labeled anti-rabbit IgG in PBS as the second antibodies. The antibodies
were centrifuged at 5,000 rpm for 15 min at 4°C before being used for
removal of the aggregates.

EM and Measurement
The ultrathin sections were observed with a JEOL 2000 EX electron micro-
scope at 100 kV. For measurement, the negatives were printed at a mag-
nification of 200,000. To determine the localization of gold particles in the
axon, the transverse sections, the numbers of gold particles were counted in four
regions from six proximal nerve sections with a total area of 23-49 µm² for each region and in four regions from six distal nerve sections with a total
area of 20-45 µm² for each region at the axoplasm relatively removed from
the ligation. The four different types of areas in the sections examined were:
(a) regions where membranous organelles had accumulated; (b) cytoskel-
eton-enriched areas; (c) myelin sheath; and (d) extracellular space. In each
of these regions, the average number of gold particles per unit area was de-
termined. Furthermore, the distance between gold particles and membranes
of membrane organelles was measured with a magnifying glass in areas
where membranous organelles were relatively sparsely localized. 10 micro-
graphs from 3 samples for each portion (proximal and distal) were used
for measurement.

Results

Brain Dynein Accumulates at Axoplasm Both
Proximal and Distal to the Ligation
Peripheral nerves (saphenous nerves) of female albino mice
were ligated under anesthesia. 6-10 h later, the mice were
fixed and the nerves were dissected out. The proximal and
distal portions of the ligated nerves were processed for immu-
nofluorescence microscopy, electron microscopy and electron
microscopic immunocytochemistry by cryoultramicroscopy.
Brain dynein was purified from bovine brains as described previously (Paschal et al., 1987; Yoshida et al., 1990) (Fig. 1 A). Polyclonal antibodies against bovine brain dynein were
raised against rabbits. The antiseraum was purified by affinity
columns using purified cytoplasmic dynein and absorbed with purified MAP2. In the crude extracts of mouse peripheral
nerves, this affinity-purified IgG specifically recognized a high-
molecular mass band and low-molecular mass bands corresponding to the heavy chain and light chains of brain
dyneins, respectively (Fig. 1 B, lanes 1, 2, and 3). Whereas
it recognized both heavy and light chains of brain dynein, the
reaction was more intense with the light chains (Fig. 1, B and
C). It stained the same bands in mouse brain MT proteins
(data not shown). The specificity of this antibody was con-
Firmned by immunoblotting data, indicating that it reacted
with cytoplasmic dyneins purified from rat liver (Fig. 1 B,
lanes 4, 5, and 6) and mouse brain dynein (Fig. 1 B, lanes
7, 8, 9), and also that it recognized brain dynein heavy
chains after UV light-induced cleavage in the presence of
vanadate and ATP (Fig. 1 B, lanes 10, 11, and 12). As shown
in Fig. 1 D, it did not react with MAP1A, MAP1B, and
MAP2. This antibody also faintly stained the 150-kD band
of liver cytoplasmic dynein, which is possibly an intermedi-
ate chain (Fig. 1 C) (Collins and Vallee, 1989). Fig. 1 C
demonstrates liver MAPIC bound to tubulin in the presence
of taxol (lane 1). We can identify the heavy chain, interme-
diate 150-kD chain, and light chains. In this sample anti-
MAPIC antibody clearly stained the heavy chain (arrow-
head), light chains (arrows), and the 150-kD intermediate
chain (lane 2). These immunoblotting data collectively indi-
cated that this antibody specifically recognizes the heavy
chain and light chains of brain dyneins.

Fig. 2, A, B, and C display the immunofluorescence micro-
graphs of the proximal (Fig. 2, A and C) and distal por-
tions (Fig. 2 B) of ligated nerves. Interestingly, anti-brain
dynein antibodies strongly stained not only the distal por-
tions but also the proximal portions. We also noticed that
the staining in both the proximal and distal portions followed a
gradational pattern, tending to be much brighter at the
regions closer to the ligated portions, despite the ligated
points having been somewhat damaged (Fig. 2, A and B).
Furthermore, the diameter of the nerves tended to become
wider at regions closer to the ligated parts (Fig. 2 A and B).
As indicated in Fig. 2 C (higher magnification), the staining
pattern tended to appear as dots at the more distant regions
from the ligation. This was observed even more clearly when
nonligated control peripheral nerves were stained with this
antibody. Anti-brain dynein antibody tended to stain normal
axons (nonligated) in the form of dots on the faint background,
and much less intensely than in the ligated nerves (Fig. 2 E).
These results suggest that brain dynein accumulates both an-
terogradely and retrogradely at the ligated portion, and cer-
tain parts become localized on the discontinuous small dotty
structures. In control sections we did not find any positive
staining (Fig. 2 F).

Anti-tubulin antibodies stained axonemes at both prox-
imal and distal parts of the ligated nerves diffusely, and the
above gradational patterns of staining were not observed.
The intensity of staining with the anti-tubulin antibodies at
the ligated regions was similar to that at the nonligated ax-
plasm (data not shown; see Fig. 8 in Hirokawa et al., 1985).
Figure 2. (A–F) Immunofluorescence micrographs of ligated (A, B, C, and F) and nonligated peripheral nerves (saphenous nerves) (E) stained with anti-brain dynein (MAP1C) antibodies. (A) Axons proximal to the ligated region (right). Ligated portion is between A and B. (B) Axons distal to the ligated region (left). Note that axons in A and B are wider at regions closer to the ligated parts, where staining is very bright. (arrows). (C) Higher magnification of axons proximal to the ligated region (right). Arrows indicate punctate staining. (D) Nomarski micrograph of the same region as C. (E) Nonligated axons stained with the same antibodies. Note the punctate staining pattern (arrows). (F) Axons proximal to the ligation stained with normal rabbit IgG as with the first antibody. Bars: (A and B) 50 μm; (C, E, and F) 100 μm.
Brain Dynein Associates with Both Anterogradely and Retrogradely Transported Membranous Organelles

We further analyzed the localization of brain dynein at the electron microscopic level. We observed that different classes of organelles preferentially accumulated at the regions both proximal and distal to the ligated portion (Fig. 3). As previously reported (Smith, 1980; Tsukita and Ishikawa, 1980), tubulovesicular membranous structures and mitochondria increased in number in the axons proximal to the ligated part (Fig. 3 A), whereas membranous organelles such as lysosomes, multivesicular bodies and mitochondria accumulated at the regions distal to the ligated part (Fig. 3 B). We could identify three distinct regions in the axoplasms of the ligated nerves relatively distant from the ligature. They were the anterogradely transported membranous organelle-rich regions on the proximal side, the retrogradely transported membranous organelle-rich regions on the distal side, and cytoskeleton-enriched regions on both sides of the ligature. At the axoplasm very close to the ligature, only organelle-enriched regions were recognizable, while those removed from the ligature we could identify both membranous organelle-enriched regions and cytoskeleton-enriched region in the same area (Figs. 4 A and 5).

Antibody-labeling experiments using immunogold-labeled second antibodies indicated that gold particles tended to accumulate at the regions where membranous organelles accumulated and, further, that the gold labels were closely associated with the membranous organelles at the regions both proximal and distal to the ligated portion, although some gold particles were also observed at some distance from the membranous organelles (Figs. 4 and 5). Fig. 4 A shows an axoplasm proximal and a little bit away from the ligation, in an area where regions enriched in membrane-bound organelles were readily distinguished from cytoskeletal domains. Proteins associated with membrane-bound organelles should be restricted to the membrane-enriched regions, whereas freely diffusible proteins would be expected to equilibrate between membrane-enriched regions and cytoskeleton-enriched regions. Gold particles were preferentially localized in the membrane-enriched regions and were very closely associated with the membranous organelles (Fig. 4 A). In an axoplasm away from ligation gold particles tended to be closely associated with the sparsely distributed membranous organelles (Fig. 4 B).

In the axoplasm proximal to the ligation, gold labels were associated with both tubulovesicular membranous organelles and mitochondria (Fig. 4). We calculated the relative density of gold particles for the membrane-enriched areas, cytoskeleton-enriched areas, the myelin sheath, and the extracellular space. For the calculations we chose areas away from the ligation in which we could identify both membranous organelle-enriched regions and cytoskeleton-enriched regions such as shown in Figs. 4 and 5 C. As Table I illustrates, gold particles were primarily localized in the membrane-enriched areas.

Fig. 5 displays axoplasms distal to and away from the ligation where membranous organelles accumulated between, or aside from, the cytoplasms filled with cytoskeletons. Gold labels tended to be localized mainly in the membrane-enriched area. At these regions distal to the ligation, we identified gold particles close to presumptive lysosomes, multivesicular bodies, and mitochondria (Fig. 5). We also calculated the relative density of gold particles for the membrane-enriched area, cytoskeleton-enriched area, the myelin sheath, and the extracellular space at the axoplasm distal to the ligation. Gold particles were primarily localized at the membrane-enriched areas (Table I). In control sections only a few, randomly dispersed gold particles were observed (Fig.
Figure 5. (A–C) Cryoultrathin sections of axoplasms distal to the ligation stained with anti-brain dynein antibodies and 10 nm gold-labeled second antibodies. These electron micrographs show axoplasms where membranous organelles have accumulated between, or aside from, cytoskeletons. Many gold particles are associated with membranous organelles such as multivesicular bodies, lysosomes and mitochondria. Insets in A show gold particles associated with lysosomes and multivesicular bodies. Bars: (A–C) 200 nm.

We noticed in both proximal and distal portions that gold particles were associated with the surface of membranous organelles in a sporadic manner rather than densely covering the entire surface of membranous organelles. This could reflect the number of brain dynein molecules binding to the membranous organelle.

We measured the relationship of the distance between the surface membranes of organelles and gold particles. For this

Figure 4. Cryoultrathin sections of axoplasms proximal to the ligation stained with anti-brain dynein antibodies and 10 nm gold-labeled second antibodies. (A) This particular section is relatively thick. The regions where membranous organelles (MO) (tubulovesicular membranous organelles and mitochondria) have accumulated are separated from the region where cytoskeletons (C) are enriched. Note gold particles are mostly located at the membranous organelles–enriched axoplasm. (Inset) Thinner section showing accumulation of gold particles in a membrane organelle enriched region. (B) An axoplasm away from the ligature. Membranous organelles are sparsely distributed and gold labels are closely associated with membranous organelles (arrows). Bars: (A and B) 200 nm.
measurement we chose areas where membrane organelles were relatively sparse, such as the areas shown in Figs. 4 B and 5. Fig. 7 indicates the results in a histogram. As can be seen, most of the gold particles (74% in the proximal portion, 70% in the distal portion) existed <60 nm from the surface of the membranous organelles. Together, these data strongly suggest that brain dynein binds to both anterogradely and retrogradely transported membranous organelles in vivo.

**Discussion**

In this study we identified three different domains in the axoplasm of ligated nerves. They are the anterogradely transported membranous organelle-enriched region on the proximal side, the retrogradely transported membranous-organelle enriched region on the distal side, and cytoskeleton-enriched regions on both sides of the ligation. At the axoplasm very close to the ligature, only organelle-enriched regions were recognizable, whereas at those removed from the ligature we could identify both membranous organelle-enriched regions and cytoskeleton-enriched region in the same area. Our measurements of the relative density of gold particles for the membranous-enriched area, cytoskeleton-enriched area, myelin sheath, and extracellular space in the area away from the ligature indicated that a greater number of particles tended to localize in both the anterogradely transported membranous organelle-enriched and retrogradely transported membranous organelle-enriched regions.

Furthermore we measured the distance between the gold particles and surfaces of the membranous organelles. Because the lengths of IgG and brain dynein are ~20 and ~50 nm, respectively (Vallee et al., 1988), the maximum distances between gold particles and the surfaces of membrane could be 50 nm (brain dynein) + 20 nm x 2 (IgG x 2) = 90 nm at the outside of the surface membranes and 20 nm

| Region                  | No. of gold particles/μm² | Proximal | Distal |
|-------------------------|---------------------------|----------|--------|
| Extracellular space     | 2.4                       | 1.8      |
| Myelin sheath           | 3.2                       | 1.6      |
| Cytoskeleton            | 3.8                       | 2.8      |
| Membrane-bounded organelles | 17.3                  | 19.3     |

**Figure 7.** A histogram of the relationship of the distance between the surface membranes of organelles and gold particles. Minus and plus mean gold particles located inside and outside the membranous organelles, respectively. 453 and 471 gold particles were examined at proximal and distal regions, respectively.

**Figure 6.** Axoplasm proximal to the ligation stained with normal rabbit IgG as with the first antibody. Bar, 200 nm.
Figure 8. Schematic drawing of the conclusions obtained from our studies (this study, and Hirokawa, N., R. Sato-Yoshitake, N. Kobayashi, K. K. Pfister, G. S. Bloom, and S. T. Brady, manuscript in preparation). Brain dynein molecules are associated with both anterogradely and retrogradely transported organelles. Although kinesin is associated with small percentages of retrogradely transported membranous organelles, it is primarily bound to anterogradely transported organelles. Some mechanism is thought to activate brain dynein for the retrograde flow.

× 2 (IgG × 2) = 40 nm at the inside of the surface membranes. Our data indicated that ~85% of the gold particles were located within 90 nm outside and 40 nm inside of the organelle membranes. Together, these results mean that many brain dynein molecules are associated with both anterogradely and retrogradely transported organelles. This conclusion is supported by an in vitro study showing that a substantial amount of a high–molecular mass ATPase that comigrated with a flagellar dynein heavy chain associated with vesicles from squid giant axons (Pratt, 1986, 1989), as well as a recent study demonstrating that antibodies against a brain dynein-like high molecular weight protein in squid giant axons immunofluorescently stained membrane vesicle–like particles in the dissociated squid axoplasm (Gilbert and Sloboda, 1989). Our study, however, does not exclude the possibility that some brain dynein molecules exist in the cytoplasm freely or in association with other organelles such as cytoskeletal structures, and in fact rather suggests that although it is a small population, some brain dynein molecules are apparently located at some distance from the membranous organelles as well.

In this study we did not find any membranous organelles with their surfaces covered heavily with gold particles. Of course we should bear in mind the sensitivity of immunogold cytochemistry using cryoultrathin sections; our data also indicate that the number of brain dynein molecules associated with a single membranous organelle are not many. This is consistent with our previous findings that the cross-bridges between an MT and a membranous organelle in the axon are small in number (Hirokawa, 1982; Hirokawa et al., 1985; Hirokawa and Yorifuji, 1986; Hirokawa et al., 1989a).

Previous studies have shown that kinesin translocates polystyrene beads along MTs from minus ends to plus ends and moves MTs on cover glasses from plus ends to minus ends (Vale et al., 1985a, b; Porter et al., 1987; Saxton et al., 1988). On the other hand, brain dynein translocates microtubules on cover glasses from minus ends to plus ends (Paschal et al., 1987) and membranous organelles on MTs from plus ends to minus ends in vitro (Schroer et al., 1989; Schnapp and Reese, 1989). Most MTs in axons are aligned with their plus ends toward the periphery (Heidemann et al., 1984). Therefore, kinesin and dynein appear to represent ideal candidates for anterograde and retrograde translocators, respectively.

There are three main possibilities concerning the mechanism of bidirectional transport of organelles. One is that organelles transported anterogradely bind only to the anterograde transporter, kinesin, and those transported retrogradely bind only to the retrograde translocator, brain dynein. The other possibilities are first, that both translocators are localized on all organelles transported by fast flow, and the respective translocators are activated for the specific flow (anterograde kinesin, retrograde dynein) or, second, that one translocator binds to specific organelles, whereas the other translocator is localized on all organelles and will be activated for the specific flow. However, the precise localization of these translocators in vivo was not known and the question as to whether these molecules are really associated with membranous organelles has not been answered.

Recent immunofluorescence and quantitative immunoblotting studies using anti-kinesin antibodies have suggested that in fibroblasts a certain percentage of kinesin molecules is bound to membranous organelles (Pfister et al., 1989; Hollenbeck, 1989). Very recently, using cryoultramicrotomy of ligated nerves, we found that kinesin is primarily associated with anterogradely transported membrane organelles (Hirokawa, N., R. Sato-Yoshitake, N. Kobayashi, K. K. Pfister, G. S. Bloom, and S. T. Brady, manuscript in preparation). This study demonstrated for the first time that brain dynein is associated with membranous organelles transported both anterogradely and retrogradely in the axons. Schliwa's group identified a dynein-like ATPase in the giant ameba, Reticulomyxa. Because sucrose density fractions that contained this protein as a major ATPase caused bidirectional movements of beads along MTs this protein was considered to be a bidirectional motor (Koonce and Schliwa, 1985, 1986; Euteneuer et al., 1988, 1989). We found in this study that brain dynein associates with both anterogradely and retrogradely transported membranous organelles. From these results it can be supposed that brain dynein also could function as a bidirectional motor in the mammalian axons, like the high–molecular weight ATPase in the Reticulomyxa. However, because brain dynein moves membranous organelles or beads only from plus to minus ends along MTs in vitro (Schroer et al., 1989; Schnapp and Reese, 1989), and because most MTs in the axons are aligned with their plus ends toward the periphery (Heidemann et al., 1984), it is more likely that brain dynein is transported to the nerve terminal in an inactive form to be activated there to work as a retrograde motor by some unknown mechanism. This may be related to the additional cytosolic factors necessary for producing retrograde organelle motility in vitro as suggested recently by Schroer et al. (1989), or may be related to some other mechanisms such as phosphorylation.

Still to be resolved was how the retrograde motors (brain dynein) are transported to the nerve terminals, by fast or slow transport. Here we presented evidence to show that the retrograde motors are conveyed to the nerve terminals at least by fast flow because brain dyneins accumulated at the proximal portions of ligated nerves and mainly associated with the membranous organelles that were conveyed by fast transport, whereas slowly transported tubulin was not accu-
mulated in large amounts at the proximal portions during the period passed between the operation and killing. We studied the localization of kinesin in the same system and found it to bind primarily to anterogradely transported organelles, although kinesin was associated with certain percentages (13.5%) of retrogradely transported membranous organelles (Hirokawa, N., R. Sato-Yoshitake, N. Kobayashi, K. K. Pfister, G. S. Bloom, and S. T. Brady, manuscript in preparation). Fig. 8 shows these conclusions in a schematic manner. Kinesin works as a motor for the anterograde transport which conveys mitochondria and tubulovesicular membranous organelles, possible precursors of synaptic vesicles, and detaches from the vesicles at the nerve terminal, possibly being mostly degraded, whereas some kinesin may associate with small percentage of retrogradely transported membranous organelles in an inactive form (Hirokawa, N., R. Sato-Yoshitake, N. Kobayashi, K. K. Pfister, G. S. Bloom, and S. T. Brady, manuscript in preparation). On the other hand, brain dynaein could be conveyed to the nerve terminals anterogradely as a membrane bound inactive form, detach from the membrane organelles at the nerve terminal, and reassociate with the retrogradely transported membranous organelles in an activated form. This conclusion agrees with the observation that some organelles switch their direction of movement rapidly (Breuer et al., 1975; Freed and Lebowitz, 1970; Forman et al., 1983, 1987; Hirokawa and Yorifuji, 1986). Probably this is mainly because the activation of inactive brain dynaein would happen accidentally. The mechanism for the activation of brain dynaein for only retrograde transport is a very important issue, indeed, and will need to be resolved in the near future.

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