The Isolation and In Situ Location of Adligin: the Microtubule Cross-linking Protein from Caenorhabditis elegans

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Abstract. Microtubules isolated from the nematode Caenorhabditis elegans contain long stretches of periodic cross-links formed by microtubule-associated proteins (MAPs). These cross-links are 5.7 nm long, 3 nm wide, and occur at one tubulin dimer (8-nm) intervals along the walls of microtubules (Aamodt, E., and J. Culotti, 1986. J. Cell Biol. 103:23–31). The structural protein of the cross-links was isolated from the MAPs by centrifugation and exclusion chromatography. The cross-links were formed exclusively from the most prevalent MAP, a 32,000 mol wt protein. We suggest the name adligin for this MAP. Adligin eluted from the exclusion column at 33,000 mol wt indicating that it was a monomer in solution. Antibodies were made against the purified adligin and affinity purified. The affinity-purified antibodies were used to locate adligin in situ and to determine its distribution relative to that of tubulin by the use of double label immunofluorescence. The anti-adligin antibodies labeled a fibrous network in the cytoplasm of most cells of C. elegans. Neurons were labeled especially well. This labeling pattern was similar to the labeling pattern obtained with antitubulin, but anti-adligin labeled some granules in the gut that were not labeled with antitubulin. These results suggest that adligin may be part of the interphase microtubule network in C. elegans.

Microtubules often appear in electron micrographs to be connected to each other, to other organelles, and to other cytoskeletal elements by thin cross-links (8). These cross-links probably serve to organize the microtubules into functional networks, and to mediate their interactions with other cellular components. We are interested in how microtubule-associated proteins (MAPs) are involved in microtubule-mediated functions, including those functions that require links between microtubules and between microtubules and other cellular components.

C. elegans is an excellent model system for studying microtubules and their role in development and nerve function. C. elegans contains at least three structurally distinct microtubule types. Most of the neuronal microtubules contain 11 protofilaments, the A subfiber of the sensory cilia contains 13 protofilaments, and the large microtubules in the mechanosensory neurons contain 15 protofilaments (7). Tubulin isolated from C. elegans contains at least 10 isoelectric variants, including 2 major α-tubulins, 2 major β-tubulins, and 6 minor isotypes (Siddiqui, S., E. Aamodt, F. Rastinijad, and J. Culotti, manuscript submitted for publication). In C. elegans there appear to be at least four α-tubulins and at least five β-tubulin genes or pseudogenes (9). Several mutants have been identified in C. elegans that show alteration of their microtubule cytoskeleton (2, 6, 10, 11).

We previously reported the isolation of microtubules and MAPs from C. elegans (1). C. elegans is a fairly rich source of microtubules, and it yields ~250 μg of microtubules and 33 μg of MAPs for each gram wet weight of C. elegans. The microtubules isolated from C. elegans have mainly 9–11 protofilaments, while most microtubules, including those isolated from bovine brain and assembled under similar buffer conditions, contain 13 protofilaments. The most prevalent MAP is 32,000 mol wt, but numerous other proteins are present in the MAP fraction.

One of the most interesting features of the microtubules isolated from C. elegans is that they are connected by stretches of periodic cross-links. These cross-links, which are 5.7 nm long and 3 nm wide, are located at one dimer (8-nm) intervals along the length of the microtubules. The cross-links must be composed of material in the MAP fraction since the cross-links are not present after the MAPs are eluted from the microtubules, and the cross-links reform when the microtubules and MAPs are recombined. At a low MAP-to-microtubule ratio, the cross-linking protein binds preferentially between microtubules linked in parallel, but, at a higher MAP to microtubule ratio, the cross-linking pro-
Figure 1. SDS polyacrylamide gels showing the polypeptide composition of the microtubule and MAP fractions that were tested for cross-links. (a) 20 µg of microtubules isolated from C. elegans. The major protein present in this preparation was tubulin, and the major MAP present in this preparation was adligin. (b) 20 µg of MAP-free microtubules; these microtubules contained nearly pure tubulin. (c) 8 µg of MAPs that were eluted from the microtubules with 0.4 M NaCl. A variety of proteins were present in this preparation, including a group of high molecular weight proteins and adligin. The MAPs were desalted and then centrifuged. (d) 1.0 µg of the pellet fraction. This fraction was highly enriched in the higher molecular weight MAPs. (e) 4.3 µg of the supernatant fraction. This fraction was highly enriched for adligin and contains only trace amounts of the other MAPs. (f) 400 ng of pure adligin. Lanes a–e were stained with Coomassie Blue and lane f was stained with silver as described in Materials and Methods.

Materials and Methods

Materials

Taxol was kindly provided by the Natural Products Branch, Division of Cancer Treatment, National Cancer Institute (Frederick, MD). GTP (type I), MgSO₄, EGTA, Pipes, mannitol, succrose, leupeptin, pepstatin, Nαp-tosyl-L-arginine methyl ester, glycine, dithioerythritol, sodium phosphate, Tween-20, NP-40, Triton X-100, sodium azide, H₂O₂, chloronaphthol, isopropyl ß-D-thiogalactopyranoside, Amido black, ß-propyl gallate, and NaCl were from Sigma Chemical Co. (St. Louis, MO). Acetone, acetic acid, and methanol were from Mallinkrodt Inc. (St. Louis, MO). Nitrocellose was from Schleicher & Schuell (Keene, NH). SDS, acrylamide, biacrylamide, ammonium persulfate, Coomassie Blue R-250, and TEMED were from Bio-Rad Laboratories (Richmond, CA). Tris (hydroxymethyl) amino methane was from Aldrich Chemical Co. (Milwaukee, WI).

The buffers used in this study were PEMMI (50 mM Pipes, 1.0 mM EGTA, 1.0 mM MgSO₄, 0.5 M mannitol, 80 µg/ml leupeptin, 80 µg/ml pepstatin, 1.0 mg/ml Nαp-tosyl-L-arginine methyl ester, and 2.0 mM diethylenetriamine, pH 6.6), PEM (100 mM Pipes, 1.0 mM EGTA, 1.0 mM MgSO₄, pH 6.6), PBS (100 mM sodium phosphate, 150 mM NaCl, pH 7.4), TBS (10 mM tris, 150 mM NaCl, pH 8.0), milk-TBS (TBS, 3% nonfat dry milk, 0.1% Nonidet P-40), C-buffer (20 mM sodium phosphate, 1.0 mM MgSO₄, 1.0 mM EGTA, 0.2 g/liter sodium azide, pH 7.0), and M9 buffer (15).

General Methods

C. elegans were grown and used to prepare microtubules as previously described (1, 17). Negative staining of microtubules and electron microscopy was also as described (1). Protein concentrations were determined by the method of Bradford (4) with BSA as a standard. SDS-gel electrophoresis was done in 10% polyacrylamide gels (12). Gels were either stained with Coomassie Blue R-250, or with silver by the method of Wray et al. (21) with the following modification: after the gel was rinsed in 50% reagent grade methanol, it was treated with 10% gluteraldehyde for 40 min, and then rinsed twice with glass-distilled water for 40 min each time. Sepharcy S-300 chromatography was carried out on a 1.6 cm × 62 cm column equilibrated with C-buffer at a flow rate of 35 ml/h. The sample volume was 3% of the column volume.

Western Blotting

Electrophoretic transfer of proteins to nitrocellulose from SDS gels was done in tris-glycine electrophoresis buffer (12) containing 20% methanol (16) at 0.2 A for 20 h at 5°C. A portion of the blot was stained for protein with 0.1% Amido black in 5% methanol, and 10% acetic acid, and then de-stained in the same solution without Amido black. The remaining portion of the blot was blocked for 2 h in PBS containing 5% nonfat milk, rinsed for 30 min in PBS containing 0.05% Tween-20, and then dried and stored in the dark.

To locate proteins on the blot that bound antibody, we cut vertical strips from the dried blot, rehydrated them in milk-TBS for ~1 h, and incubated them overnight in an appropriate dilution of the antibody (usually 10 µg/ml IgG in milk-TBS). The strips were rinsed four times over a 30-min period in milk-TBS containing 3% milk, and then incubated in an appropriate dilution (usually 1:1000) of hors eradish peroxidase-conjugated second antibody (Bio-Rad Laboratories) in TBS containing 3% milk for 2-16 h. The strips were rinsed with TBS four times over a 30-min period and developed in 0.05% 4-chloronaphthol and 0.01% hydrogen peroxide in TBS.

Figure 2. Electron micrographs of negatively stained microtubule and MAP mixtures. (a) Microtubules isolated from C. elegans contained periodic cross-links. (b) After the MAPs were extracted from the microtubules with 0.4 M NaCl, the cross-links were gone. (c) The cross-links reformed when desalted MAPs were combined with the microtubules at a ratio of 0.4 mg MAPs/mg microtubules. (d) No cross-links formed when the proteins in the MAP pellet fraction were combined with the microtubules at a ratio of 0.4 mg MAPs/mg microtubules. (e) The cross-links reformed when the MAP supernatant fraction was combined with the microtubules at a ratio of 0.4 mg of MAPs/mg microtubules. (f) The cross-links reformed when the purified adligin was combined with the microtubules at a ratio of 0.08 mg adligin/mg microtubules. Bar, 100 nm.
Figure 3. Chromatogram from the Sephacryl S-300 column on which the supernatant MAPs were chromatographed. The first peak corresponds to the void volume of the column. The higher molecular weight proteins were eluted in the void peak. The major peak corresponds to the elution of adligin. Adligin eluted at 33,000 mol wt which indicates that it was a monomer under the chromatography conditions. The standards were soybean trypsin inhibitor, ovalbumin, and BSA.

Production of Antibodies

We used a white New Zealand rabbit to produce antibodies against adligin. The rabbit was immunized with 36 μg of column purified adligin emulsified in an equal volume (0.4 ml) of complete Freund’s adjuvant. The immunogen was injected subcutaneously at eight sites along the rabbit’s back. The rabbit was boosted after 3 wk in the same way it was originally immunized, and it was bled weekly for 6 wk after the boost.

Affinity Purification of Antibodies

To affinity purify the polyclonal antibodies, we first used adligin, which was isolated by SDS-gel electrophoresis and transferred to nitrocellulose (13). In later experiments, we purified the antibody with two β-galactosidase fusion proteins from a modified Agt11 expression vector (20) called XRB1. The cytological staining was the same for antibodies purified by both methods. Because the use of fusion proteins allowed the isolation of larger quantities of pure antibody, all of the cytological stain results shown were done with antibody affinity purified with the fusion proteins.

Immunofluorescent Staining of C. elegans

Immunocytochemical staining was done with the use of anti-adligin antibodies described above in combination with a mouse antibitin monoclonal antibody E6B6 (kindly provided by T. Arai, University of Tsukuba) that was previously found to bind strongly to β-tubulins and to most, if not all, microtubules in C. elegans. A developmentally mixed population of C. elegans was washed from a petri plate with M9 buffer, and then rinsed three to five times with M9. The animals were suspended in PBS containing 1.5% paraformaldehyde and 0.1% glutaraldehyde (14) for 10 min. Partially fixed C. elegans were placed on a gelatin subbed slide and then quickly covered with a subbed coverslip. The worms were squashed between the coverslip and the slide with 15–20 pounds of pressure and then quickly frozen in liquid nitrogen. The coverslip was popped off with a razor blade and the slide, containing frozen and fractured C. elegans, was placed in methanol at −20°C for 10 min, and then into acetone at −20°C for 10 min. The animals were postfixed in PBS containing 1.5% paraformaldehyde and 0.1% glutaraldehyde for 30 min, and then rinsed three times in PBS over a 30-min period. The slide was left overnight at 4°C in a blocking solution of 3% BSA, 0.02% sodium azide, and 0.1% NP-40 to which 10% horse serum was added. The primary antibodies were applied for 4–16 h at 4°C, the slide was rinsed in PBS three times over a 30-min period, and the secondary antibodies (Texas red-conjugated sheep anti-mouse Ig, and fluorescein-conjugated donkey anti-rabbit Ig from Amersham Corp., Arlington Heights, IL) were applied for 2–4 h. The slide was rinsed in PBS three times over a 30-min period, excess PBS was blotted from the slide, and the stained animals were mounted in 76% glycerol, 20% 0.1 M tris-HCl, pH 8.0, and 4% N-propyl-galate.

Microscopy

A Nikon Optiphot microscope was used. Kodak 2415 technical pan film was exposed at ASA 100 for fluorescein and ASA 25 rhodamine. The film was developed in HC-110 dilution F.

Results

Isolation of Adligin

Microtubule proteins were isolated from a developmentally
mixed population of *C. elegans* by the use of taxol (1, 17). The MAPs were stripped from the microtubules with 0.4 M NaCl, and then fractionated by sedimentation and gel exclusion chromatography. Each fraction was mixed with MAP-free microtubules and tested for the presence of cross-links by EM. Fig. 1 shows the polypeptide composition of each of the microtubule protein fractions, as resolved by SDS-PAGE, and Fig. 2 shows electron micrographs of the negatively stained microtubule and MAP mixtures. Fig. 1 a shows the protein composition of microtubules isolated from *C. elegans*. In addition to tubulin (55,000 mol wt) these microtubules contain a variety of MAPs. The principal MAP (adligin) is 32,000 mol wt. Fig. 2 a shows a micrograph of these microtubules. As shown previously (1), these microtubules are extensively cross-connected by 5.7-nm-long periodic cross-links.

Fig. 1 b shows the protein composition of microtubules that were extracted with 0.4 M NaCl to remove the MAPs. Fig. 2 b shows that the MAP-free microtubules do not contain cross-links. The polypeptide composition of the MAPs that were extracted from the microtubules are shown in Fig. 1 c. When these proteins were desalted by dialysis into PEM buffer, and then recombined with the microtubules at a ratio of 0.4 mg MAPs/mg microtubules, the cross-links reformed (Fig. 2 c). This shows that the material that forms the cross-links is present in the MAP fraction, and it demonstrates that EM provides a useful method to assay for the cross-link protein.

The desalted MAPs were centrifuged at 22,500 g for 30 min at 4°C. The MAP pellet, which contained paracrystals (see below), was enriched for five major proteins of ~100,000, 105,000, 110,000, 180,000, 200,000 mol wt, and had very little adligin (Fig. 1 d). When the pelleted MAPs were recombined with the microtubules at a ratio of 0.4 mg MAPs/mg microtubules, no cross-links were seen (Fig. 2 d). Interestingly, the paracrystals, which were present in this fraction before the microtubules were added, were no longer found after the microtubules were added.

The MAP supernatant fraction was enriched in adligin (Fig. 1 e). When these MAPs were recombined with microtubules, at a ratio of 0.4 mg MAPs/mg microtubules, the microtubules became extensively cross-linked (Fig. 2 e).

The supernatant MAPs were chromatographed on a Sepharose S-300 column. Fig. 3 shows the chromatogram. The major peak on the chromatogram corresponds to the elution of adligin. This MAP eluted from the column at 33,000 mol wt, which indicates that it was a monomer under these chromatography conditions. Adligin from the column appeared to be pure even on an SDS-gel stained with silver (Fig. 1 f). When pure adligin was recombined with microtubules at a ratio of 0.08 mg adligin/mg microtubules the cross-links reformed (Fig. 2 f).

### MAP Paracrystals

When the MAPs were desalted into PEM buffer they became turbid, and some of the MAPs associated into paracrystals, as determined by EM. The structure of the MAP paracrystals depended on how the salt was removed. Fig. 4 shows electron micrographs of the paracrystals negatively stained with uranyl acetate. When a centrifuge column containing G-50 fine was used to rapidly desalt the MAPs, the paracrystals had a striation pattern of a broad band followed by two narrow bands followed by another broad band (Fig. 4 a). The spacing between the broad bands was 78.0 nm ± 1.1 nm (n = 12), while the distance from a broad band to the closest narrow band was 31.7 nm ± 0.6 nm (n = 9), and the closest distance between narrow bands was 14.6 nm ± 0.4 nm (n = 14). When the MAPs were slowly desalted by dialysis, the paracrystals appeared much more compact. They were of variable length, and tapered to points on their ends (Fig. 4 b). As measured over 34 striations, the distance between striations was 14.5 nm ± 0.2 nm. The paracrystals were sedimented and the proteins in the paracrystal fraction are shown in Fig. 1 d.

### Antibodies against Adligin

Antibodies were made against the column-purified adligin and affinity purified as described in Materials and Methods. Fig. 5 shows immunoblots of these antibodies. The affinity-purified antibodies bound specifically to a single 32,000 mol wt protein in soluble *C. elegans* proteins, and they bound specifically to adligin on blots of *C. elegans* MAPs.
**Distribution of Adligin In Situ**

The distribution of adligin in *C. elegans* at various stages of development was determined by the use of double label immunofluorescence microscopy with an antibody against β-tubulin and the affinity-purified antibody against adligin (Fig. 6). Fig. 6, A and B, shows an adult and four embryos double labeled with antitubulin (6 A) and anti-adligin (6 B). Both antibodies labeled a fibrous network in the cytoplasm of most cells. Particularly bright labeling was observed in the nerve ring area of the head and the preanal ganglion of the tail. These are the major nerve ganglia in *C. elegans*. The only structures labeled by anti-adligin antibodies that were not labeled by antitubulin were some round spots in the cytoplasm of gut cells. Fig. 6 C shows an isolated gonad stained with antibody against adligin. Figs. 6, D–F shows embryos stained for adligin. The embryonic cells were evenly stained up to about the time of gastrulation. After gastrulation the posterior of the embryo stained more brightly. The area of bright stain contains the gut precursor cells so this increased staining was probably related to the gut stain seen in adults. This hypothesis is supported by the fact that the gut precursor cell stain was sometimes punctate (Fig. 6 F). Fig. 6, G and H shows the head region of an animal double labeled with antitubulin (6 G) and anti-adligin (6 H).

The focal plane is near the top of the animal. The bright line labeled ALM is the nerve process from one of the anterior lateral microtubule neurons. Fig. 6, I and J, shows gut and gonad isolated from *C. elegans* and double labeled with antitubulin (6 I) and anti-adligin (6 J). The filamentous microtubule network was easier to see in the isolated tissues because the background fluorescence was reduced. All of the structures in these tissues labeled by antitubulin also appeared to be labeled by anti-adligin. This suggests that adligin is associated with microtubules in situ.

**Discussion**

**Isolation of Adligin**

Adligin was easily isolated from *C. elegans*, and ~100 μg of adligin was isolated from 5 g wet wt of worms. The only problem with this procedure was that adligin was isolated at a low dilution, and it was a particularly difficult protein to concentrate. The methods we tried were ammonium sulfate precipitation, concentrating membranes, lyophilization, and ion exchange chromatography. Pure adligin was precipitated only at 90% ammonium sulfate, and it was then insoluble. It adhered to the concentrating membranes we tried; it did
not resolubilize after lyophilization; and it did not bind to the
ion exchange resins we tried. Adligin could be concentrated
by cosedimentation with microtubules, and it could then be
eluted from the microtubules with 0.4 M NaCl. However, the
adligin concentrated in this way sometimes contained some
tubulin contamination.

From the Sephacryl S-300 chromatography results, it
seems very likely that adligin is a monomer in C-buffer. This
does not necessarily imply, however, that adligin is a mono-
mer when it cross-links microtubules. It may be a dimer or
larger oligomer in microtubule assembly buffer (PEM), or it
may dimerize after binding to the microtubules.

Because the adligin and microtubule preparations used in
these experiments were very pure it seems likely that the
cross-links are formed exclusively from adligin, and that no
other factors (with the possible exception of the buffer com-
ponents) are required to form the cross-links. Taxol was
not necessary for cross-link formation since microtubules
formed from purified bovine brain tubulin, which was as-
sembled into microtubules in the presence of glycerol (but
in the absence of taxol), were cross-linked by adligin. Free
GTP or guanosine 5'-diphosphate were probably also not
required since taxol stabilized microtubules, which were resus-
pended in PEM without GTP or taxol, formed cross-links
when combined with MAPs that were thoroughly dialyzed
in PEM. The residual free GTP and taxol concentrations in
this experiment were no higher than 1.0 μM GTP and 20 nM
taxol.

MAP Paracrystals

The paracrystals that formed in the desalted MAP prepara-
tions seem to be formed from MAPs, and were not merely
isolated under the same conditions as the microtubules, since
neither the paracrystals nor the 100,000-110,000 mol wt pro-
teins were present in control preparations made without the
addition of taxol. The paracrystals did not contain adligin,
and the 200,000- and 180,000-mol-wt proteins are probably
not included in the paracrystal structure since they were
present in control preparations made without taxol. The
paracrystals were never seen in the presence of microtubules,
including experiments where preparations which contained
paracrystals were combined with microtubules (Fig. 2 d).
The dissociation of the paracrystals in this experiment did
not result from dilution since buffer alone had no effect.
Thus, the paracrystal proteins may have a higher affinity for
microtubules than they have for each other, or the microtu-
bules may in some way prevent the paracrystals from sticking
to the electron microscope grids.

Although we do not know the origin of the paracrystal pro-
teins, there are at least two interesting possibilities. It is pos-
sible that they are paramyosin since paramyosin also forms
paracrystals that are morphologically very similar to the
MAP paracrystals seen here (19). Paracrystals from both
sources have a form with a 14-nm repeat. Paramyosin had
a second form with a repeat of ~70 nm, while the MAP
paracrystals have a second form with a repeat of 78 nm.

Another interesting possibility is that the MAP paracrystal
stabilized microtubules. In the published micrographs of Chalfie and
these specialized cells. Chalfie and Thomson (7) have shown that the axons of
the touch neurons contain large bundles of 15-protofilament
microtubules. In the published micrographs of Chalfie and
Thomson (see Fig. 6 in reference 7), these microtubules ap-
pear to be connected by thin cross-links that are ~6 nm long and
3 nm wide. These are the dimensions of the cross-links
formed from adligin in vitro. The microtubules in the touch
sectioned striated rootlet from C. elegans (for illustration see reference 18) is also ~78 nm.

Antibodies against Adligin

Antibodies against adligin are useful for three types of ex-
periments. They were used in this study to locate adligin in
situ. They should also allow us to isolate the adligin gene,
and to see if antigenically similar proteins are present in
other species.

One can never be absolutely certain, in this type of study,
that every location of the antigen in the animal has been de-
tected, or that all of the fluorescence results from the antigen
of interest. However, in this study we are reasonably certain
that all of the fluorescence we detected in situ was due to the
presence of adligin. No fluorescence was detected if no pri-
mary antibody was used, if preimmune antibody was used for
the primary antibody, or if preimmune antibody taken
through the affinity purification procedure was used for the
primary antibody. Because the adligin that was used as an an-
tigen appeared to be completely pure on silver-stained SDS
gels it is unlikely that antibodies against other worm proteins
were induced. And finally, because this antibody was affinity
purified, and appeared to only bind adligin on Western blots,
it is unlikely that antibodies against other epitopes were
present.

Adligin appeared to be located in the cytoplasm of most,
perhaps all, cells in C. elegans. The fact that adligin is ubiq-
uitous in the cytoplasm of C. elegans suggests that it has a
general cytoplasmic role. We doubt that this role is limited
to the cross-linking of microtubules since most of the
microtubules in the cytoplasm of C. elegans appear, in elec-
tron micrographs, to be well separated rather than connected
or bundled together. Perhaps adligin not only connects micro-
tubules to other microtubules but also connects microtubules
to other structures in the cell.

Because of the small size and rounded morphology of C.
elegans cells, it was difficult to resolve individual microtu-
bules. Thus, while the adligin staining pattern was obviously
related to the tubulin staining pattern, the congruity of these
proteins at the level of individual microtubules remains to be
determined.

The round structures in the cytoplasm of gut cells that were
stained by antibodies against adligin are interesting. While
we do not yet know what these structures are, they do not seem to be the autofluorescent granules that are present in
the gut cells of C. elegans (3). The granules that stain for ad-
ligin were not autofluorescent, and the autofluorescent gran-
ules did not stain for adligin.

The ubiquity of adligin in C. elegans is not surprising in
light of the large amounts of adligin that are isolated. Adligin
is overwhelmingly the most prevalent MAP, and it is ~10% of
the protein in the microtubule preparation.

The presence of adligin in the axons of the touch neurons
suggests a biological role for adligin in these specialized
cells. Chalfie and Thomson (7) have shown that the axons of
the touch neurons contain large bundles of 15-protofilament
microtubules. In the published micrographs of Chalfie and
Thomson (see Fig. 6 in reference 7), these microtubules ap-
pear to be connected by thin cross-links that are ~6 nm long and
3 nm wide. These are the dimensions of the cross-links
formed from adligin in vitro. The microtubules in the touch

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neurons appear to provide a stiff bundle that resists deformation, and, therefore, allows the axon to transduce a light touch into a nerve signal. The cross-links probably serve to stiffen the microtubules by bundling them together. Thus, in this limited case, a function for adligin is strongly suggested.

Recently, Bramblett et al. (5) reported finding a protein similar to adligin in trypanosomatids. If this protein turns out to be closely related to adligin, then similar proteins may be present in a large cross-section of species.

In summary, adligin is a 32,000 mol wt MAP isolated from C. elegans that forms short periodic cross-links between microtubules. Adligin appeared to be bound to the interphase microtubules in most cells of C. elegans. This suggests that adligin is involved in one or more roles common to the function of microtubules throughout the animal.

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