Overexpression of Tau in a Nonneuronal Cell Induces Long Cellular Processes

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Abstract. The ways in which the various microtubule-associated proteins (MAPs) contribute to cellular function are unknown beyond the ability of these proteins to modify microtubule dynamics. One member of the MAP family, tau protein, is restricted in its distribution to the axonal compartment of neurons, and has therefore prompted studies that attempt to relate tau function to the generation or maintenance of this structure. Sf9 cells from a moth ovary, when infected with a baculovirus containing a tau cDNA insert, elaborate very long processes. This single gene product expressed in a foreign host cell grossly alters the normal rounded morphology of these cells. The slender, relatively nonbranched appearance of these processes as well as their uniform caliber resembles the light-microscopic appearance of axons observed in several neuronal culture systems. Immunolabeling of the tau-expressing Sf9 cells demonstrated tau reactivity in the induced processes, and EM that microtubule bundles were present in the processes. Microtubule stabilization alone was insufficient to generate processes, since taxol treatment did not alter the overall cell shape, despite the induction of microtubule bundling within the cell body.

TAU is a microtubule-associated protein synthesized in neurons (Kosik et al., 1989) and abundant within the axonal compartment (Binder et al., 1985; Peng et al., 1986; Kowall and Kosik, 1987; Brion et al., 1988). The protein is the product of a single gene (Neve et al., 1986), which undergoes complex alternative splicing (Himmler, 1989). Studies concerning the function of this molecule have generally relied upon in vitro analyses of microtubules. These analyses have concluded that tau promotes the polymerization of tubulin (Weingarten et al., 1975). In the context of current models of dynamic instability (Mitchison and Kirschner, 1984), tau appears to decrease the frequency of transition between growing and shrinking phases of microtubules in vitro (Horio and Hotani, 1986), presumably by reducing microtubule instability (Bre and Karsenti, 1990). These authors also demonstrated that tau increased the average number of microtubules nucleated per centrosome. When microinjected into fibroblasts, tau stabilized the microtubules against depolymerization, but did not cause changes in cell morphology (Drubin and Kirschner, 1986).

The cloning and sequencing of tau cDNAs have suggested certain structure-function relationships. The most apparent was the finding of three imperfectly repeated sequences near the carboxy terminus of tau, which have the property of microtubule binding (Lee et al., 1989; Himmler et al., 1989). This finding derived from in vitro studies in which tau deletion constructs lacking portions of the carboxy terminus were expressed and assayed for their ability to bind to microtubules. Similar binding properties have been attributed to these sequences based upon the use of complementary synthetic peptides (Aizawa et al., 1989; Ennulat et al., 1989). To approximate more closely the in vivo behavior of tau, transfection of tau cDNAs in mammalian cells was used. Investigators who have expressed tau in L-cells (Kanai et al., 1989) and a variety of other cell types (Lewis et al., 1989), observed bundling of microtubules within the cytoplasm when certain tau sequences are transfected, but have not commented on changes in the overall shape of the cells. The particular properties of tau that are associated with bundle formation have been a matter of recent interest. Transient transfection of a four-repeat rat tau into L-cells was effective in bundling the microtubules (Kanai et al., 1989), whereas transfection of other tau constructs has not resulted in microtubule bundling.

In PC12 cells neurite outgrowth correlates with the induction of tau protein (Drubin et al., 1985) and tau mRNA (Drubin et al., 1988); therefore, tau has been considered to have a role in neurite elongation. Whether there is a special role for tau in polarized neurons is still unclear. While tau is selectively sorted to the axon in neuronal culture, it does not segregate until after a neurite attains an axonal morphology (Kosik and Finch, 1987). Although tau protein segregation did not herald axonal differentiation, abolition of tau expression by antisense administration in cerebellar macroneurons pointed toward a role for tau in the generation of an axon-like structure (Caceres and Kosik, 1990).

To pursue the relationship of tau expression to cell shape we have used a recombinant baculovirus to express high lev-

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els of tau in Sf9 insect cells. The normally rounded Sf9 cells, after infection with tau-expressing virus, elaborated very long processes that resembled an axonal shape and were densely packed with microtubules. This morphology could not be induced by the microtubule-stabilizing agent, taxol, and therefore reflects a function of tau expression other than the ability to stabilize microtubules.

Materials and Methods

Construction of Recombinant Baculovirus

A baculovirus transfer vector containing the three (pVL941-tau3)-repeat isoform of human tau was constructed. The repeat sequences are imperfectly repeated sequences of 31 amino acids near the carboxy terminus. The three-repeat human tau isoform (pl9tau; Lee et al., 1989) was digested with SalI, filled in with Klenow, and linked with BamHI linkers. It was then cut with BamHI and BclI. The fragment containing tau was gel isolated and cloned into the BamHI site of the baculovirus transfer/expression vector pVL941 (Luckow and Summers, 1989). This vector is designed to express high levels of nonfused proteins in the baculovirus system. A clone was selected in the correct orientation and named pVL941-tau3.

To construct a baculovirus transfer vector containing the four-repeat form of tau (pB4tl), we obtained the fourth repeat by the polymerase chain reaction (PCR)[1] from human hippocampal polyA+ mRNA (Clontech, Palo Alto, CA). cDNA was made from the polyA$^+$ RNA using a primer specific to the 3' end of tau mRNA and AMV reverse transcriptase (Molecular Genetic Resources). PCR was performed on this cDNA using primers that spanned the region of the inserted repeat of the four repeat tau isoform (nucleotides 541–873 of the four-repeat tau described by Goedert et al., 1989). This PCR product was cloned into M13 and sequenced to confirm its identity, and double-stranded replicative form DNA was prepared from the M13 phage. The HindIII to PstI fragment containing the fourth repeat sequence from this DNA was ligated to the 585-bp SalI and PstI, and the 297-bp HindIII to BclI tau fragments from ptau19. These were all cloned into the SalI BamHI sites of pUC19 to form plasmid pT2. Restriction digests confirmed that the above fragments combined in pT2 so that the complete coding sequence for the four-repeat form of human tau was obtained. The tau sequences were cut out of pT2 with SalI and EcoRI, filled in with Klenow, and blunt cloned into the BamHI site of pVL941. A clone was selected in the correct orientation and named pB4tl.

Cells and Viruses

The wild type baculovirus used was the E2 strain of the Autographa californica nuclear polyhedrosis virus (AcMNPV) provided by Max Summers (Texas A & M University) (Smith and Summers, 1978). *Spodoptera frugiperda* (Sf9) cells were obtained from the American Type Culture Collection (ATCC #CRL 1711; Rockville, MD), and were used to propagate wild type and recombinant baculoviruses. Sf9 cells were grown in suspension or as a monolayer culture in Ex-Cell 400 (J. R. Scientific, Woodland, CA) with the addition of 10% heat-inactivated FCS at 28°C. Depending upon the experimental requirements cells were grown either directly on the plastic dish or on glass coverslips.

Foreign DNA was inserted into the genome of AcMNPV at the polyhedrin gene locus by homologous recombination using the pVL941 (Luckow and Summers, 1989) transfer vector. The transfer plasmids containing tau sequences were cotransfected with wild type baculovirus DNA onto Sf9 cells by the calcium phosphate procedure. Viruses released by the transfected cells were plated out for plaque assay and the recombinant viruses were identified by visually screening in the presence of neutral red for polyhedrin occlusion negative plaques. Due to insertional inactivation of the nonessential polyhedrin gene, the occlusion negative plaques have a distinct appearance. Transfection of Sf9 cells, selection and screening of recombinant baculoviruses, and propagation of AcMNPV in SF9 cells were as described (Smith and Summers, 1987), except that 0.01% neutral red was included in the agarose overlay during plaque identification to assist in the visualization of recombinant plaques. Viral cultures were further screened for their ability to produce tau protein in infected cells by immunoblots.

Viral stocks were made by infecting attached cell cultures at a low multiplicity of infection (MOI) and harvesting supernatants 2–4 d after infection. To produce recombinant proteins, suspension cultures of Sf9 cells at 10^6 cells/ml were mixed with viral stocks at a MOI of 5 to 20, and cells and supernatants harvested at an appropriate time after infection. Cells over-expressing tau were harvested and analyzed by SDS-PAGE and by immunoblots. The wild type baculovirus, the β-galactosidase (β-gal) expressing baculovirus, and the transfer vector pVL941 were generously provided by Drs. Luckow and Summers (Texas A & M University).

Immunocytochemistry

Tau was immunocytochemically visualized with mAb 5E2 or tau 1, both of which have defined epitopes within the tau sequence (Kosik et al., 1988). Tubulin was visualized with mAb N.35 (Amersham Corp., Arlington Heights, IL) which is directed against the beta-tubulin subunit. For tubulin immunocytochemistry the cells were fixed for 5 min in −20°C methanol and rinsed in PBS. For tau immunocytochemistry, the cells were fixed in periodate-lysine-paraformaldehyde (McLean and Nakane, 1974) or first extracted in 0.1% triton for 10 min and then fixed. Fixed cells were blocked by incubation in 10% goat and 10% calf serum in PBS, incubated in the primary antibody, washed three times in PBS, incubated with goat antimouse FITC conjugated antibody, washed again, mounted in citifluor (Citifluor Ltd., UK), and visualized by fluorescence microscopy. Cells were also immunolabeled with the Vector Red alkaline phosphatase substrate kit (Vector Laboratories, Burlingame, CA) according to the package insert with the addition of levamisole (Vector Laboratories) to inhibit endogenous alkaline phosphatase.

EM

Cells were fixed for EM as follows. The media were removed and attached cells were washed gently in either serum-free medium or PBS. Freshly prepared fixation buffer (2.5% glutaraldehyde in 0.1 M Na-phosphate, pH 7.3) was added and the cells were incubated for 30 min at room temperature. Fixed cells were subsequently washed in PBS, postfixed with 1% osmium tetroxide in PBS, washed, and dehydrated through a graded ethanol series. After infiltration with LX-112 resin (Ladd Research, VT), cells were embedded on the culture dish, an appropriate sized block face trimmed, and 70-nm ultrathin sections cut with a Diatome diamond knife in an MT-5000 ultramicrotome (Sorvall Instruments Div., Newton, CT). Sections were picked up on parlodian-coated copper EM grids, stained with uranyl acetate and lead citrate, and examined in a JEOL 100 CX-II electron microscope at 80 kV.

Chemical Treatment

For some experiments, uninfected cells or wild type infected cells were treated with cytochalasin D (5 μg/ml) for 15 h or taxol (20 μM) for 15–20 h.

Results

Tau Expression in Sf9 Cells

Fig. 1 shows the time course of tau expression in Sf9 cells after infection with recombinant baculovirus containing either three- or four-repeat forms of human tau. At an MOI of 5, expression of tau is detectable at 21 h after infection and is maximal at 48 h after infection by immunoblots. Large amounts of tau protein are produced. By densitometric comparison against standards on Coomassie-stained gels there is ~20 μg of tau protein in 10^6 cells. Although the virus contains the sequence for only a single tau cDNA, the expressed protein migrates as several indistinct bands on an immunoblot (Fig. 1). These bands range from 52 to 58 kD for the three-repeat tau, and from 56 to 64 kD for the four-repeat tau. The tau bands corresponding to the three-repeat–expressed protein migrate similarly to the lower molecular mass tau bands prepared from bovine brain. The tau bands derived from the four-repeat–expressed protein migrate similarly to the higher molecular mass tau bands from bovine brain. These results indicate that the tau heterogeneity ob-

1. *Abbreviations used in this paper*: MOI, multiplicity of infection; PCR, polymerase chain reaction; Sf9, *Spodoptera frugiperda* 9.

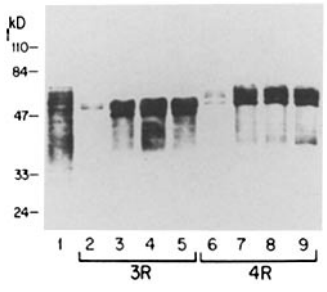


Figure 1. Time course of tau expression in baculovirus-infected Sf9 cells. Lanes *2–5* represent expression of the three-repeat isoform in cells harvested at 21, 29, 45, 53 h after infection, respectively. Lanes *6–9* represent expression of the four-repeat isoform harvested at the same time points. All cells were infected at an MOI of 5. Lane *1* is bovine brain tau prepared by the perchloric method. The samples were run by SDS-PAGE and immunoblotted with the tau mAb 5E2. Molecular mass standards are indicated on the left. The less immunoreactive, more rapidly migrating bands represent proteolytic fragments.

served by gel electrophoresis can arise from both splicing, as recently shown by Goedert and Jakes (1990), and by post-translational modifications.

Tau expression on immunoblots correlated temporally with process formation. At an MOI of 5, tau was readily detected at 21 h after infection by immunoblots (Fig. 1), coincident with the onset of process formation. Long unipolar and occasionally bipolar processes were present in most of the infected cells before 32 h (Fig. 2). Bipolar processes often, but not invariably, emanated from opposite sides of the cell. In rare instances it was possible to observe a cell with three processes. Processes were variable in length, sometimes extending 250–300 μm. The processes branched very infrequently, and processes arising from different cells did not fasciculate as axons often do in primary neuronal culture. They had a nearly uniform caliber of 2.4–3.1 μm and did not taper. Immediately at the point of departure from the cell body, tau-induced processes achieved a narrow caliber that was maintained over the entire length. Occasionally a slight flattening was observed at the process tip. The processes were indistinguishable between cells expressing three- or four-repeat tau. There were no obvious differences in the appearance of the processes with the following substrata: a glass coverslip, a plastic dish, a laminin-coated surface, or even free in suspension. When the time course of tau expression was delayed by infecting cells with an MOI of less than one, the elaboration of processes and immunoblot detection of tau was also delayed. Cells infected with wild type virus, or recombinant viruses expressing β-galactosidase or a variety of unrelated proteins did not show these processes (Fig. 2). Uninfected cells were also rounded, however, there were occasional cells that tapered slightly at one or both ends.

To determine the composition of the induced processes, the cells were reacted with tubulin and tau antibodies. The processes were immunoreactive with both antibodies (Fig. 3). Extraction of the cells with the nonionic detergent, triton, did not diminish the tau immunoreactivity, suggesting that the tau protein was bound to microtubules. Immunoblots of triton-extracted infected cells (Duerr et al., 1981) revealed tau immunoreactivity in the insoluble pellet, although a large portion was in the supernatant (data not shown). This suggests that some of the tau protein is bound to cytoskeletal elements and some is soluble. The tubulin and the tau antibody staining was present throughout the length of the process and double labeling revealed that tau and tubulin codistributed.

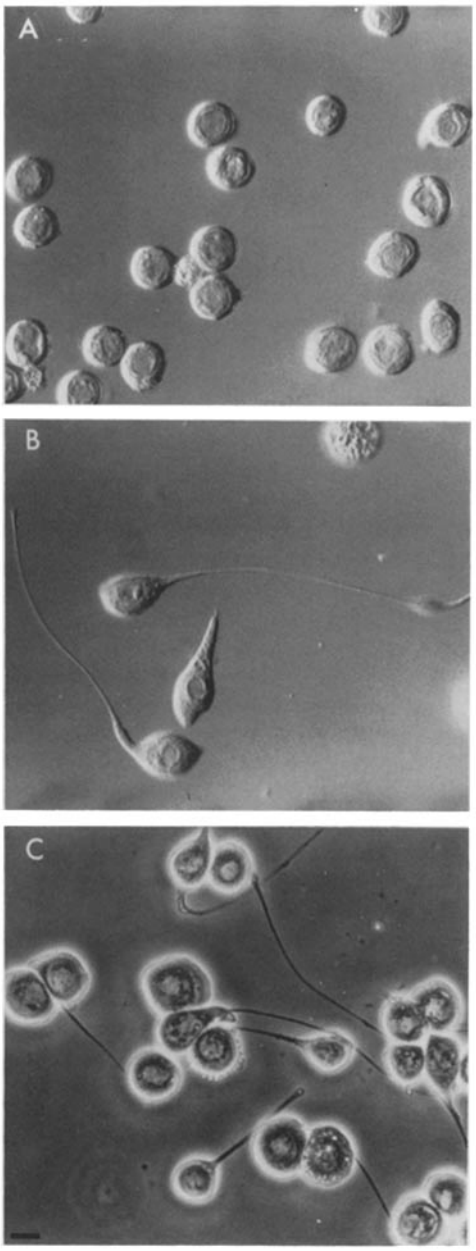


Figure 2. Light microscopy of Sf9 cells, 30–35 h after infection. (*A*) Control Sf9 cells infected with a baculovirus expressing β-galactosidase observed by Nomarski optics. (*B*) Sf9 cells infected with baculovirus expressing three-repeat tau protein by Nomarski optics. Occasionally a bulge in the process is observed as is seen on the right. (*C*) Sf9 cells with baculovirus expressing three-repeat tau protein by phase microscopy. Bar, 10 μm.

Effects of Taxol and Cytochalasin D on Process Formation

Because process outgrowth may have been a function strictly of the microtubule-stabilizing property of tau, infected cells were treated with taxol to see if similar process outgrowth could be replicated. Volkmann and Zaal (1990) had not observed morphological changes after taxol treatment of uninfected IPLB-Sf-21 cells, a cell line closely related to Sf9, although microtubule bundles were induced. To exclude the possibility that infection might be a concomitant or prior requirement for taxol induction of process outgrowth, Sf9 cells

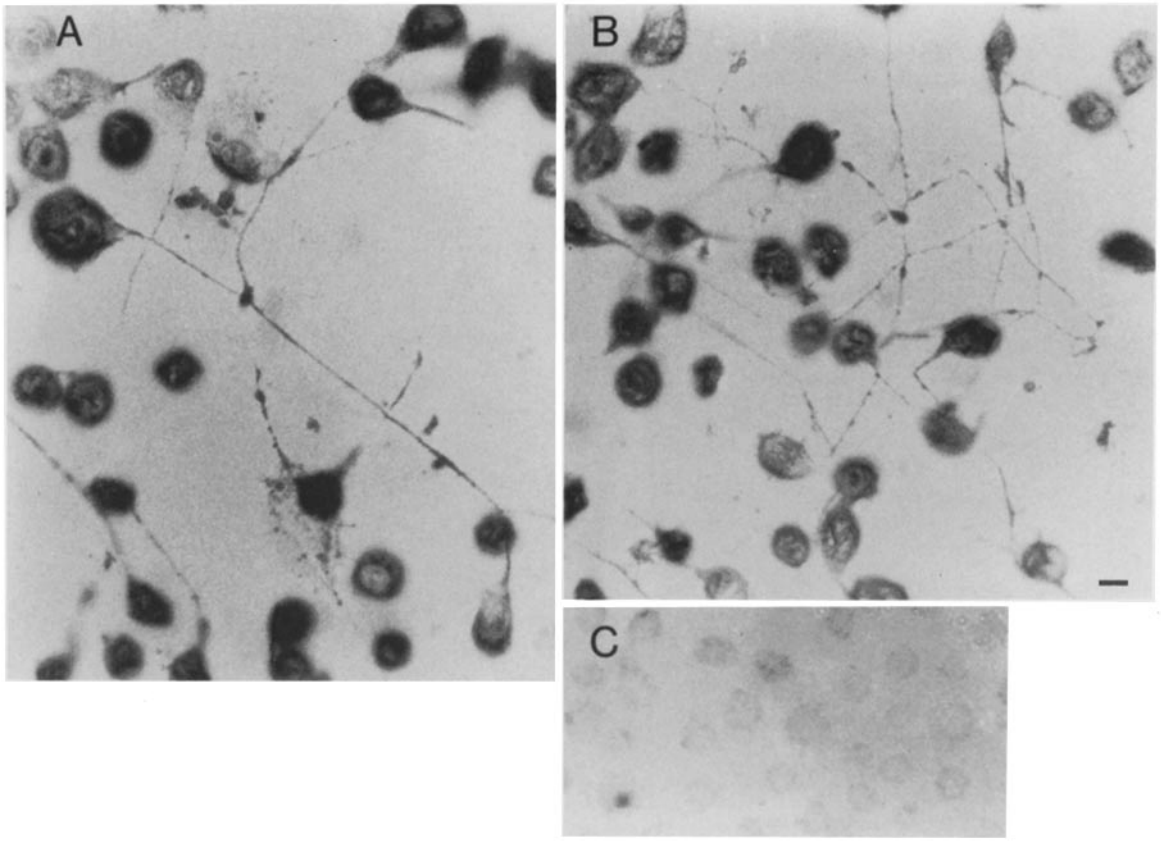


Figure 3. Sf9 cells infected with tau-expressing baculovirus and immunostained at 30 h after infection by tau mAb 5E2. (*A* and *B*) A network of long processes is reactive with the antibody as are the cell bodies. Similar staining was seen with the tubulin antibodies. (*C*) Control infected cells do not react with the antibody. Bar, 10 μm.

were first infected with virus expressing β-galactosidase. At 20 h after infection, when tau synthesis would normally become detectable in cells infected with tau-expressing virus, 20 μM taxol was added. 15 h later, when tau-expressing control cells displayed processes, the taxol-treated cells remained rounded (Fig. 4). Taxol-treated cells were stained with tubulin to demonstrate that the taxol treatment was effective in bundling the microtubules (Fig. 4). Control in-

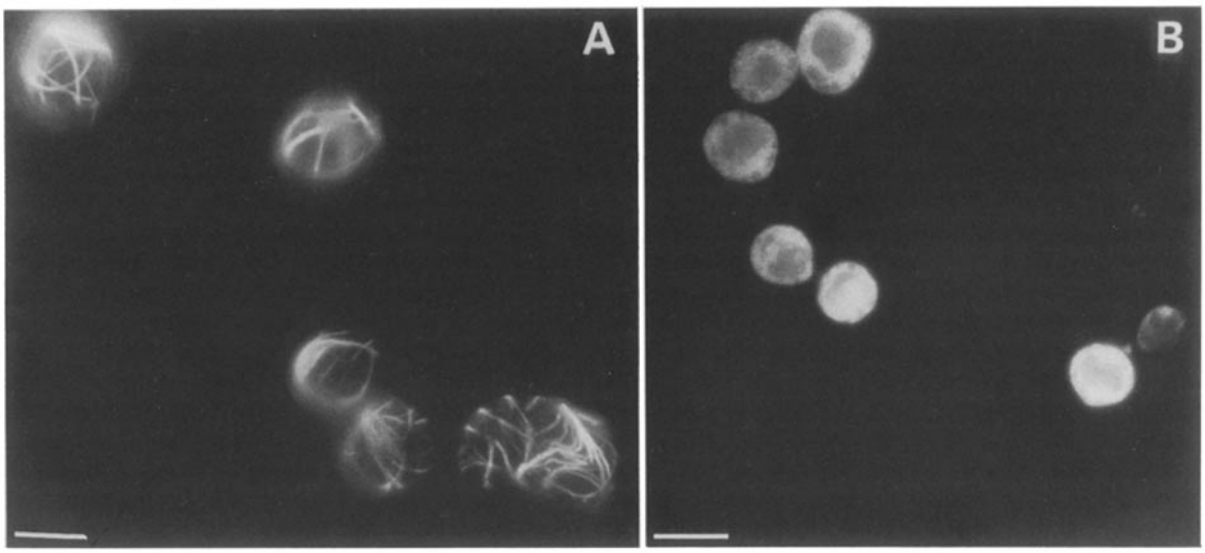


Figure 4. Taxol treatment of Sf9 cells. Sf9 cells were infected at an MOI of 5 with the β-galactosidase expressing baculovirus and 20 h after infection they were treated with 20 μM taxol. 15 h later they were processed for immunostaining with antitubulin antibodies. (*A*) After taxol administration immunoreactive bundles are readily apparent. (*B*) Without taxol treatment tubulin stains the Sf9 cells diffusely. Bars: (*A*) 12 μm; (*B*) 16 μm.

fected cells without taxol labeled diffusely with the tubulin antibody, and did not reveal bundles (Fig. 4). The inability of taxol to form similar processes indicates that the bundling or stabilization of microtubules alone is not sufficient to cause process outgrowth.

Hess et al. (1989) have reported that treatment of IPLB-Sf-21 cells with cytochalasin D results in the formation of retraction processes. To compare these processes to those induced by tau expression, we treated Sf9 cells with cytochalasin D under conditions identical to those described by the above authors. The resulting retraction processes had a very different light microscopic appearance from tau-induced processes (Fig. 5, *A* and *B*). After ~4–5 h cytochalasin D induced multiple spike-like processes from a single cell rather than long polar processes seen with tau infection. Furthermore, cytochalasin-induced processes are relatively short, do not taper at their point of exit, and arise from a lamellopodial-like veil of cytoplasm surrounding the cell body that is not present in tau-infected cells. The longer of these retraction processes stained with tubulin antibody, while the shorter processes sometimes did not (Fig. 5 *B*). The lamellopodial-like veil also failed to stain with the tubulin antibody. In contrast to tau-reactive processes (see below), few microtubules were observed by electron microscopic examination of cytochalasin D–induced retraction processes (Fig. 5 *C*).

Ultrastructure of Tau-induced Processes

The ultrastructure of the tau-induced processes revealed a highly regular organization of microtubules. The microtubules were axially aligned and densely packed; many appeared contiguous over several microns (Figs. 6 *A*). Microtubule cross-sections were not observed in sections cut along the process axis, implying that the microtubules were aligned parallel to the length of the tau-induced extensions. Single isolated microtubules were not observed. Instead, tau-induced microtubules were packed into bundles that often lay in proximity (<20 nm) to the plasma membrane. Cross-linking between axially aligned microtubules was suggested, since a constant intertubule distance of ~15 nm was maintained along the length of the process. Control cells, both infected and uninfected, contained only a few individual microtubules within their somata and no microtubule bundles were apparent (data not shown).

Along the central portion of some processes was an electron-dense core of fibrous material. Although most processes had this central core, there were also processes without it, suggesting that it was not necessary for process formation. This electron-dense material was also spatially separated from the microtubules within the process (Fig. 6 *C*). Similar structures were observed within the cell bodies of all control cells infected either with wild type virus or beta-galactosidase inserts. It looked very similar to the fibrous virally encoded p10 protein present in the cell bodies of the infected cells (Van Der Wilk et al., 1987), with the exception that the material in the processes was kept within straight boundaries. It is therefore possible that the electron-dense structure represents the p10 viral gene product.

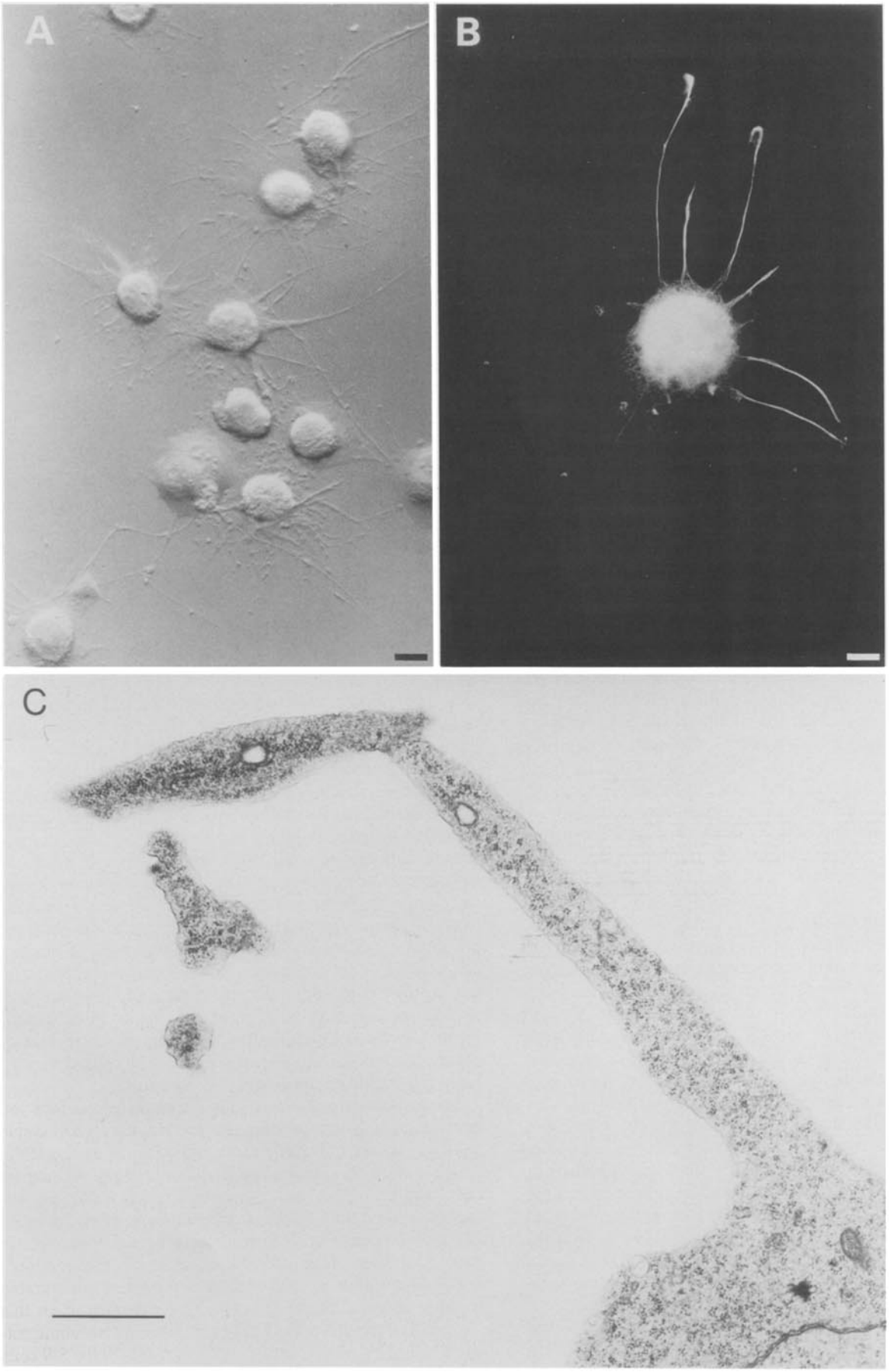
Tau protein is a component of the Alzheimer paired helical filaments in neurofibrillary tangles. Although one of the original motivations for utilizing this high expression system was to determine whether such filaments might form, there was no electron microscopic evidence of any filamentous structures, either paired helical or straight.

Discussion

Infection of Sf9 cells with a baculovirus expressing human tau markedly alters the morphology of the host cells. Tau-expressing infected cells elaborated exceedingly long processes not seen under the control conditions (Fig. 2). These processes were reactive with both tubulin and tau antibodies (Fig. 3). Cells infected with either the three- or four-repeat tau cDNA appeared identical. The elaboration of these processes was independent of the substratum and even occurred in suspension. This observation suggests a "push" mechanism of elongation that has been associated with anterograde transport of the cytoskeleton and associated organelles, as opposed to the "pull" exerted by the growth cone (Letourneau et al., 1987). By EM, infected cells contained densely packed arrays of microtubules oriented parallel to the direction of the process. By light microscopy, these processes resembled the axon-like neurites observed in some neuronal culture systems such as those from the embryonic hippocampus (Bartlett and Banker, 1984) or the primordial cerebellum (Ferreira and Caceres, 1989). Both are long, relatively unbranched, and have a uniform caliber over their entire length. In the neuronal cultures the uniform caliber of neurites was sufficient to predict the ultrastructural identity of a neurite as an axon. This resemblance does not indicate that tau-induced processes in Sf9 cells resemble axons in other respects. Many characteristics of axons such as synaptic vesicles and the tendency of the processes to fasciculate (reviewed in Jessell, 1988) were not observed. Tau may have a role in the generation of a single axonal property shape.

Previous studies utilized tau antisense techniques in neuronal cultures to eliminate tau expression specifically. These experiments generated a phenotype that failed to develop an axon-like structure (Caceres and Kosik, 1990) and, in more mature cultures, resulted in the specific loss of axon-like structures (Caceres et al., 1991). While these studies implicated tau in the generation of axons, they are negative studies in which secondary effects could contribute to the observed phenotype. The results here report the addition of tau to a cell where it is not normally expressed, and coincident with tau expression the Sf9 cells transform their rounded morphology to one with long processes. While these experiments also do not exclude the possibility that a secondary effect accounts for the phenotype, the Sf9 cells described here also implicate tau in process elongation.

Several laboratories have either transfected various tau constructs or microinjected tau protein into mammalian cells that do not ordinarily express tau (Kanai et al., 1989; Lewis et al., 1989; Drubin and Kirschner, 1986). Among the cells studied in this way, tau bound to microtubules, enhanced microtubule stability, and, in some cases, induced microtubule bundling. However, there were few changes in the overall shape of the cells after transfection or microinjection, and certainly no elaboration of processes comparable to those observed in the Sf9 cells. One explanation for this difference is the presence of only a minimal microtubule network in the Sf9 cells. Control infected or uninfected Sf9 cells are diffusely reactive with tubulin antibodies (Fig. 4 *B*) and


A
B
C

their ultrastructure reveals a paucity of microtubules. In contrast, the mammalian cells used for tau transfection and microinjection experiments display an extensive network of microtubules (Lewis et al., 1989; Kanai et al., 1989; Drubin and Kirschner, 1986). After infection with the baculovirus expressing tau, process formation includes the coordinate assembly of microtubules. Perhaps the minimal endogenous microtubule network of Sf9 cells presents a more plastic cytoskeletal environment which allows tau to exert effects that it is incapable of in the presence of preexisting microtubules.

An alternative explanation for the ability of tau to induce process formation only in the baculovirus system could be related to the vast overexpression of tau in this system, much in excess of that in mammalian host cells. This abundance of tau could overwhelm modification systems such that there is a critical amount of undermodified tau in the baculovirus system. When polyoma middle T antigen was overexpressed in the baculovirus system, some of it was normally phosphorylated, but a large fraction of it was not, presumably due to the vast excess of middle T antigen over a specific kinase (Forstova et al., 1989). The presence of a critical mass of underphosphorylated, or otherwise undermodified tau in the baculovirus system may lead to the formation of a process.

There is precedence for an association between stabilization of microtubules and morphogenic changes. In MDCK II cells the onset of polarity involves the stabilization of microtubules (Bre et al., 1990), and during myogenic differentiation specific microtubules are temporally and spatially stabilized coincident with cell elongation and alignment (Gundersen et al., 1989). A similar situation in which local microtubule populations are selectively stabilized may also exist in differentiating neurons in culture (Ferreira and Caceres, 1989). The regulation of tau binding to microtubule subsets and the consequent stabilization of these microtubules may provide a mechanism for axonal elongation.

Is the ability of tau to stabilize microtubules involved in the generation of these tau-induced processes? The absence of process outgrowth after taxol treatment suggests that microtubule stabilization and bundling may be necessary, but are not sufficient to induce processes. The interaction of tau with microtubules that leads to process elongation is more complex than its role in stabilizing or bundling microtubules. One distinction between taxol administration and virally directed tau expression is the rapid rate of bundling induced by taxol and the more gradual time course over which tau is expressed in the infected cells. A controlled rate of tau synthesis at specific sites within the cell may permit a more regulated stabilization of specific microtubule populations and process formation. Alternatively, some function of tau protein other than its ability to stabilize microtubules may be responsible for the formation of these structures. Expression of various subregions of tau protein in the baculovirus system could help disect what properties of tau are required for process formation.

Although microtubules are discontinuous in axons (Chalfie and Thompson, 1979; Bray and Bunge, 1981; Tsukita and Ishikawa, 1981), they maintain their uniform orientation throughout the axon (Heidemann et al., 1981; Burton and Paige, 1981; Filliatreau and DiGiamberdino, 1981; Baas et al., 1988). The uniform microtubule polarity orientation is maintained in the axon by limiting new microtubule assembly to the elongation of existing stable microtubules (Baas and Heidemann, 1986). In this regard, stable microtubules act as microtubule nucleating structures in the axon (Baas and Black, 1990). This contrasts with the situation in nonneuronal cells, in which stable microtubules are apparently capped against assembly at both ends (Webster et al., 1987). The stable microtubules of the axon may be uniquely able to serve as nucleating structures for microtubule assembly and may be of fundamental importance during the elongation of an axon (Baas and Black, 1990). If the microtubules within tau-induced Sf9 cell processes are formed by a mechanism similar to that of axons, a testable prediction is that the microtubules will have an axonal type polarity orientation.

Treatment of the Sf9 cells with cytochalasin resulted in the presence of retraction processes which are believed to result from a collapse of the actin network around the nucleus. The dome of the cell collapses first, leaving a lamellopodial-like veil that may be stabilized by adhesion plaques. Also remaining are cytoplasmic rays of microtubules, unaffected by cytochalasin, that form multiple short spike-like processes. These do not usually extend beyond the original periphery of the cell, and are therefore quite distinct from the elongated processes observed in the presence of high tau expression. Within the cytochalasin-induced spikes are occasional single microtubules. In the tau-induced processes are densely packed microtubule bundles in arrays as close as 15 nm. The organization of microtubules into bundles is considered a function of tau. In tau-transfected mammalian cells, Kanai et al. (1989) and Lewis et al. (1989) described microtubule packing densities as close as 20 nm. Purified tubulin polymerized with tau and observed by the quick-freeze deep-etch method exhibits microtubule crossbridges of 18.7 nm (Hirokawa et al., 1988).

Whether tau overexpression in this setting elicits coordinate expression of other microtubule-associated proteins or tubulin is as yet undetermined. Since free tubulin levels control the synthesis of β-tubulin by translational regulation of the mRNA stability (Yen et al., 1988), an increase in tubulin assembly brought about by the presence of tau would be predicted to increase tubulin synthesis. In addition to the assembly of microtubules concomitant with process formation, membranous and cytoplasmic components necessary for processes probably also need to be coordinately synthesized and assembled. How the reorganization of cellular architecture derives from the overexpression of a single protein could be addressed by examining coincident levels of cytoskeletal protein expression during Sf9 process induction.

Figure 5. Cytochalasin D treatment. Uninfected Sf9 cells were treated with 5 μg/ml cytochalasin D and 15 h later retraction processes were observed by (*A*) phase microscopy and by (*B*) immunofluorescence utilizing an antitubulin antibody. Their very thin appearance here is due to the fact that the lamellopia surrounding these cells (see *A*) labels less intensely with the tubulin antibody. (*C*) A retraction processes by EM. Bars: (*A*) 10 μm; (*B*) 5 μm; (*C*) 1 μm.

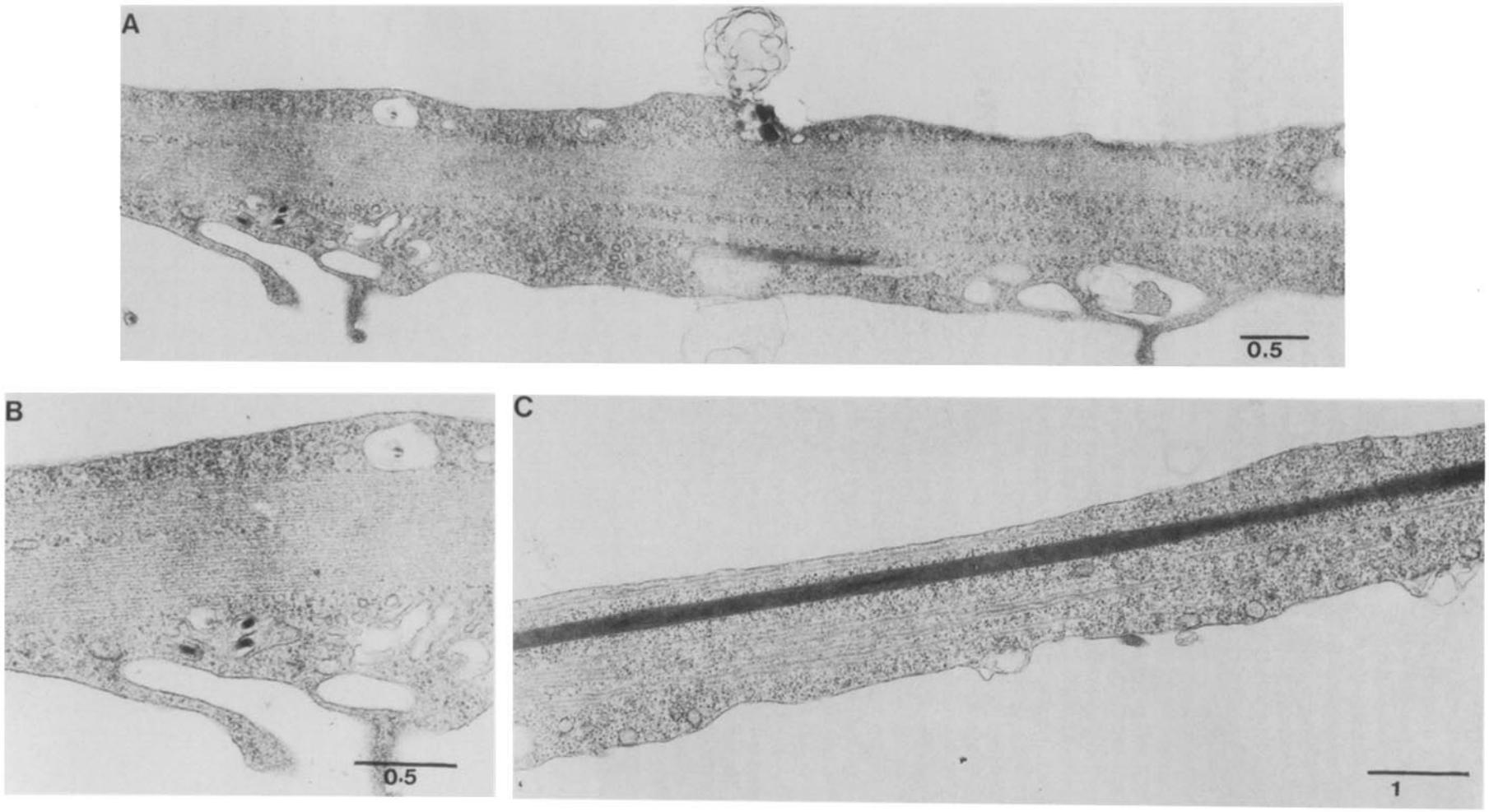


Figure 6. Electron micrograph of tau-induced processes in Sf9 cells infected with a baculovirus containing the four-repeat isoform. (*A*) Microtubule bundles coursing through the center of the process. (*B*) High magnification of the process in *A* to demonstrate the close packing density of the microtubules. (*C*) Another tau-induced process containing an electron-dense band in the central region, which is similar in appearance to the viral gene product p10. p10 inclusions are abundantly present in control infected cell bodies. The microtubules in this process lie in close apposition to the plasma membrane. The scale bars are in microns.

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