Overexpression of Tau Protein Inhibits Kinesin-dependent Trafficking of Vesicles, Mitochondria, and Endoplasmic Reticulum: Implications for Alzheimer’s Disease

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Abstract. The neuronal microtubule-associated protein tau plays an important role in establishing cell polarity by stabilizing axonal microtubules that serve as tracks for motor-protein–driven transport processes. To investigate the role of tau in intracellular transport, we studied the effects of tau expression in stably transfected CHO cells and differentiated neuroblastoma N2a cells. Tau causes a change in cell shape, retards cell growth, and dramatically alters the distribution of various organelles, known to be transported via microtubule-dependent motor proteins. Mitochondria fail to be transported to peripheral cell compartments and cluster in the vicinity of the microtubule-organizing center. The endoplasmic reticulum becomes less dense and no longer extends to the cell periphery. In differentiated N2a cells, the overexpression of tau leads to the disappearance of mitochondria from the neurites. These effects are caused by tau’s binding to microtubules and slowing down intracellular transport by preferential impairment of plus-end–directed transport mediated by kinesin-like motor proteins. Since in Alzheimer’s disease tau protein is elevated and mislocalized, these observations point to a possible cause for the gradual degeneration of neurons.

Key words: mitochondria • transport • endoplasmic reticulum • exocytosis • Alzheimer’s disease

The microtubule network plays an important role in maintaining cellular morphology, in mitotic processes, in intracellular trafficking, and in establishing cellular polarity and neurite outgrowth in differentiating neurons (Drubin and Nelson, 1996; Li and Black, 1996; Goodson et al., 1997). Microtubules serve as tracks for the movement of mitochondria (Nangaku et al., 1994; Morris and Hollenbeck, 1995; Rickard and Kreis, 1996), lysosomes (Hollenbeck and Swanson, 1990), peroxisomes (Wiener et al., 1997) and various other organelles such as endocytotic or exocytotic vesicles (Scales et al., 1997). Furthermore, they are important in establishing and maintaining the functionality of the endoplasmic reticulum (Waterman-Storer et al., 1995). Microtubule (MT)-dependent transport is achieved through motor proteins such as dynein or kinesin and their relatives (Waterman-Storer and Salmon, 1997; Hirokawa, 1998; Lippincott-Schwartz, 1998). Kinesin is a plus-end–directed motor, whereas dynein-related motor proteins move in the opposite direction (Brady, 1995; Vallee and Sheetz, 1996). The organelles are linked to the motors via membrane/organelle-bound adaptor proteins such as kinectin or dynactin (Kumar et al., 1995; Burkhart et al., 1997).

The microtubule tracks are stabilized by microtubule-associated proteins (MAPs) such as MAP2, tau, MAP4, and others. These are mostly filamentous proteins that bind to the microtubule surface and promote microtubule assembly (for reviews, see Hirokawa, 1994; Mandelkow and Mandelkow, 1995). The developmental regulation of MAPs, for instance in neuronal differentiation, suggests an important role in organizing the microtubule cytoskeleton (Matus, 1994). Tau is enriched in axons, MAP2 in the somatodendritic compartment of neurons (Binder et al., 1985), whereas MAP4 can be found in many types of tissues (Chapin and Bulinski, 1991; West et al., 1991). MAP2, tau, and MAP4 share a remarkable sequence homology in the conserved repeat regions located in the COOH-terminal microtubule-binding domain (Chapin and Bulinski, 1992). The repeated motifs are preceded by an NH\textsubscript{2}-terminal projection domain that has been suggested to serve as a spacer between microtubules (Chen et al., 1992) or as an anchor for cellular enzymes (Obar et al., 1989).
Since motor proteins and MAPs both interact with the microtubule surface, the question arises whether MAPs interfere with the movement of motor proteins. Several studies have suggested that this is the case for certain MAPs, especially those of higher molecular weight. Thus, MAP2 can inhibit the motility of dynein or kinesin in vitro (Paschal et al., 1989; Von Massow et al., 1989; Lopez and Sheetz, 1993; Hagiwara et al., 1994), while smaller fragments had only a weak effect. This could be accounted for in several ways: (a) MAPs could have a general steric blocking effect of the extended NH₂-terminal projection domain; (b) MAPs could compete with motors for common binding sites, probably on beta-tubulin (Hagiwara et al., 1994); and (c) MAPs could affect transport processes indirectly by altering microtubule dynamics (Waterman-Storer and Salmon, 1997).

When MAP4 is overexpressed in mouse Ltk− cells, it interferes with motor-protein–dependent organelle motility and trafficking in vivo (Bulinski et al., 1997). Furthermore, overexpression of tau or MAP2c in COS-fibroblasts indicates that both MAPs might function as phosphorylation-dependent regulators of organelle transport (Sato-Harada et al., 1996). The phosphorylation and dephosphorylation of microtubule-associated protein tau (Wischik et al., 1995; Mandell and Banker, 1996; Sato-Harada et al., 1996; Drewes et al., 1997). Indeed, MAPs isolated from cells are phosphorylated and it is well documented that this phosphorylation interferes with binding to microtubules in vitro and in vivo (Drechsel et al., 1992; Biernat et al., 1993; Illenberger et al., 1996).

In axonal growth, where a dynamic microtubule array is indispensable for differentiation, the phosphorylation and distribution of tau are correlated (Mandell and Banker, 1996; Li and Black, 1996), suggesting a regulated balance between kinases and phosphatases, as well as a finely tuned gradient of tau concentration. In Alzheimer’s disease (AD), this balance between kinases and phosphatases seems to be disturbed (Trojanowski and Lee, 1995; Mandelkow et al., 1995). Neurofibrillary tangles, one of the hallmarks of this disease, consist of paired helical filaments, which are mainly composed of hyperphosphorylated microtubule-associated protein tau (Wischik et al., 1988). Furthermore, tau in AD mislocalizes to the somatodendritic compartment of neurons (Braak et al., 1994) and tau concentration has been reported to be elevated in AD brain to a significant extent (Khatoon et al., 1992). This further strengthens the importance of proper control of posttranslational modifications, localization, and intracellular concentration of microtubule-associated proteins in vivo.

These observations lead us to investigate whether the overexpression of tau protein had an effect on organelle and vesicle trafficking in cultured cells. This approach had been useful for studying the distribution of exogenous MAPs (Barlow et al., 1994; Olson et al., 1995; Bulinski et al., 1997; Kaech et al., 1996). In previous studies, we had shown that CHO and neuroblastoma N2a cells can serve as models for the state of MAP phosphorylation during the cell cycle, and that the phosphorylation of tau by the kinase MARK leads to microtubule breakdown (Preuss et al., 1995; Drewes et al., 1997; Illenberger et al., 1998). Here we demonstrate that the overexpression of tau leads to a change in cell shape, loss of polarization, and the retardation of cell growth. One of the most visible consequences is the dramatically altered distribution of mitochondria. Instead of being dispersed throughout the cytoplasm, the mitochondria become clustered near the nucleus at the microtubule organizing center (MTOC). In differentiated neuroblastoma cells, the neurites become nearly devoid of mitochondria. The endoplasmic reticulum becomes less dense and retracts from the cell periphery or from neurites. Transferrin endocytosed by clathrin-coated vesicles accumulates near the MTOC and recycling is significantly slowed down. These effects can be explained by assuming that tau interferes with MT-driven transport, in the case of mitochondria preferentially with the plus-end–directed transport (by kinesin-like motors) so that the minus-end–directed transport (by dynein) predominates. This results in a net inward transport of mitochondria and other organelles such as peroxisomes towards the MTOC, where the microtubule minus-ends are organized. The perturbations of the balance in microtubule-dependent transport, especially in neurites, would be consistent with the observed impairment of axonal traffic in Alzheimer’s disease neurons, their loss of polarity, and their subsequent degeneration.

Materials and Methods

Cell Culture

CHO cells were grown in HAM’s F12 medium supplemented with 10% fetal calf serum and 2 mM glutamine (Biochrom, Berlin, Germany). For immunofluorescence, cells were seeded onto coverslips at a density of 2 × 10⁴ cells/cm² in 24-well culture dishes and grown overnight at 37°C with 5% CO₂. N2a neuroblastoma cells were grown in MEM Earle supplemented with 10% fetal calf serum, 2 mM glutamine (Biochrom) and 1% nonessential amino acids (Sigma Chemical Co., Deisenhofen, Germany). Differentiation of N2a cells was achieved by addition of 1 µM retinoic acid (Sigma Chemical Co.) for 24–48 h in the presence of 0.1% fetal calf serum. For stable transfection, plasmids derived from pcDNA3 (Invitrogen Corp., Leek, The Netherlands) coding for the longest human tau isoform htau40 under the control of the cytomegalovirus promoter were introduced into the cells with the DOTAP-method (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer’s instructions. Stable transfectants were selected in the presence of 800 µg/ml (CHO) or 1 mg/ml geneticin (N2a) as described (Preuss et al., 1995), and subsequently recloned.

Antibodies and Dyes

The following antibodies were used: rat monoclonal antitubulin antibodies YLI/2 and mouse monoclonal antibody DM1A were purchased from Serotec (Oxford, UK) and Sigma Chemical Co., respectively. Polyclonal rabbit anti-tau antibody K9A was from DAKOPATTS (Hamburg, Germany), polyclonal rabbit antibody used as an ER marker was a gift from Dr. D. Louvard (Institut Curie, Paris; Louvard et al., 1982) and polyclonal anticatalase antibody was from Dr. W. Just (University of Heidelberg, Heidelberg, Germany). Antisense antibody V9 was from Boehringer Mannheim. All fluorescently (TRITC, FITC, and AMCA) labeled secondary antibodies were from Dianova (Hamburg, Germany). Fluorescent dyes DiOC₃(3), MitoTracker™ Green, MitoTracker™ Red, and rhodamine-labeled transferrin were purchased from Molecular Probes, Inc. (Eugene, OR). DiOC₃(3) was used as a stain for in vivo observation of the ER at 2.5 µg/ml.
Cells were fixed with 2% paraformaldehyde or methanol, and incubation with antibodies was performed essentially as described (Preuss et al., 1995). Tau isoforms tightly bound to microtubules can be visualized by methanol fixation (which allows unbound protein to diffuse out, see Fig. 2 c). By contrast, weakly bound constructs (e.g., K10, K12) cannot be visualized by methanol fixation (since they would diffuse away). Instead, it is necessary to use paraformaldehyde, which fixes all cellular proteins but tends to dissociate tau from microtubules (see Fig. 3, for details see Illenberger et al., 1998). To visualize mitochondria, MitoTracker™ Green was added at a concentration of 400 nM 30 min before fixation. CHO cells stably transfected with human tau40 or mock-transfected cells were incubated for 1 h with 5 μM nocodazole (Sigma Chemical Co.) or 6 h with 1–10 μM taxotere (Flamme-Poulenc Rorer, Vert, France) before fixation at a concentration of 200 nM, cells were finally fixed with paraformaldehyde at given time points and prepared for immunofluorescence. For quantitative analysis of the intracellular concentration of tau protein in the tau40-stable cell line, 1.9, 0.95, and 0.38 mg/ml purified recombinant tau protein, as well as buffer only, were microinjected into CHO cells. Cells were then fixed in parallel with the tau-stable cell line and stained with a polyclonal anti-tau antibody. The fluorescence of the tau stain was then quantified using the CCD camera and the MetaMorph® software package and normalized to the cell dimensions. A minimum of 80 cells were analyzed per microinjection and at least 100 tau-stable cells were scored. Regression analysis of the normalized fluorescence intensities of microinjected cells was performed with the program Sigma Plot (R = 0.9995), and the mean intracellular tau concentration of the stable cell line was calculated.

**Monitoring Mitochondrial Distribution and Endoplasmic Reticulum In Vivo**

For the visualization of mitochondria in vivo, cells were seeded onto LabTek chambered cover glass (NUNC, Inc., Naperville IL.) at 70% confluence. 30 min before immunofluorescence examination, MitoTracker™ Green or Red was added to cells at a final concentration of 200 nM; cells were then rinsed once with medium. For monitoring mitochondrial redistribution tau-stable CHO cells were incubated for 1 h with 5 μM nocodazole and 200 nM MitoTracker™ Green. Disassembly of the microtubule network was confirmed by fixation and tubulin staining in immunofluorescence microscopy. Nocodazole was then removed and cells were stained with the cooled CCD camera on an inverted microscope (Axiovert). The fraction of the cell area containing mitochondria was quantified as follows: after fixation and immunofluorescence labeling, the pictures were recorded and the areas containing mitochondria were circumscribed manually (omitting the nucleus) and calculated. The same was done with the total cell area visible in the microtubule stain. A minimum of 30 cells were measured per experiment and the ratios plotted (e.g., see Fig. 11).

**Transferrin Uptake and Recycling**

Cells were grown in 35-mm six-well tissue culture plates for 2 d to ~80% confluency at 37°C and 5% CO₂. Supernatant was then replaced by serum-free medium, and 2 h later tetramethylrhodamine (TMR)-labeled transferrin was added to a final concentration of 380 nM. Cells were further incubated for 1 h to achieve maximal transferrin uptake. Medium containing excess or recycled transferrin was removed and new medium containing 60 μg/ml partially Fe⁺⁺-saturated transferrin (Sigma Chemical Co.) was added. Recycling was then monitored by again removing medium at given time points and quantitating fluorescence of TMR-labeled transferrin in a Fluoro Max spectrophuorometer (Spx Industries Inc., Edison, NJ) at 575 nm emission and 552 nm excitation wavelength. Cells were then trypsinized, lysed, and protein concentration was determined to normalize fluorescent signals to total protein present in the culture dishes, representing cell density. At least five independent experiments were performed with tau-stable and mock-transfected CHO cells to ensure statistical significance. To quantitate residual intracellular transferrin, cells were seeded onto coverslips, grown overnight, and subsequently saturated with TMR transferrin (see above). Cells were then fixed in methanol either immediately or 7 min after removal of TMR transferrin. Residual transferrin fluorescence was quantitated as above using standard filters for rhodamine fluorescence. Pictures were corrected by background subtraction and shadowing correction provided with the MetaMorph® software package (VisiRon). At least 300 different cells per time point and cell line were quantified by thresholding.
stably transfected cells, corresponding to movement towards the cell periphery (centrifugal; denoted as tripetal; denoted as were grouped into three categories: movement towards the nucleus (centripetal; denoted as -)).

**Inhibition of Dynein-dependent Transport by Overexpression of Dynamin**

Dynamin (p50) expression plasmid was transfected into tau-stable CHO cells with the DOTAP-method. 6 h after transfection, cells were fixed in paraformaldehyde as described (above). Mitochondria were stained with MitoTracker™ Red (Molecular Probes, Inc.), tubulin with rat monoclonal antitubulin antibody (YL1/2; Serotec) and transfected p50-dynamin with mouse monoclonal antidynamin 50-I. Immunofluorescence analysis was performed as described above. The p50 expression plasmid and antibody were generous gifts from C. Echeverri and R. Vallee (Worcester Foundation, Shrewsbury, MA) (Burkhardt et al., 1997).

**Monitoring Transport of Individual Mitochondria After Nocodazole Removal**

Cells were seeded onto LabTek chambered cover glass (NUNC, Inc.) at 70% confluency. Nocodazole was then added at a final concentration of 1 μM for 2 h and subsequently removed. 30 min before removal, MitoTracker™ Red was added at a concentration of 200 nM to visualize mitochondria. After another 30 min to allow recovery of the microtubule cytoskeleton, reaggregation of the organelles was monitored in vivo on an inverted microscope with a cooled CCD camera (see above) by recording pictures every second for 2 min with the following constraint: only mitochondria translocated with a minimum distance of 1 μm and a velocity of 0.5 μm/s were taken into consideration to exclude diffusional processes. By using the nucleus as a reference, transported mitochondria were grouped into three categories: movement towards the nucleus (centripetal; denoted as - in Fig. 7) was regarded as minus-end directed, movement towards the cell periphery (centrifugal; denoted as +) as plus-end directed and all other movements as circumferential (denoted as -/+).

**Results**

**Cell Shape and Mitochondrial Distribution Is Strongly Altered in Tau-overexpressing CHO Cells**

To investigate the effects of human tau expression in vivo, we established CHO cells stably transfected with tau40, an isoform containing four repeats (Preuss et al., 1995, and this report). Immunofluorescent staining of microtubules showed that their network was normal and contained bound tau (Fig. 2), consistent with similar results on MAP4-transfected cells (Olson et al., 1995; Nguyen et al., 1997). In particular, no bundling of microtubules was seen, contrary to experiments with transient transfection (Kanai et al., 1989; Lee and Rook, 1992) or with microinjection (Preuss et al., 1997), where higher cellular concentrations of MAPs are obtained. To calibrate the intracellular tau concentration, we microinjected different amounts of purified, recombinant tau protein into CHO cells, and prepared them for immunofluorescence analysis in parallel with the tau-stable cells (see Materials and Methods). This yielded an average concentration of 0.04 μg/ml tau in the stably transfected cells, corresponding to ~1 μM. Since the cellular tubulin concentration is ~40 μM (Hiller and Weber, 1978), the ratio of tau to tubulin remained low, ~1:40, similar to that reported for PC12 (Drubin et al., 1985) or HeLa cells (Bulinsky and Borisy, 1979). Furthermore, stable expression of tau in PC12 cells under control of the cytomegalovirus promoter (which was used in our experiments) resulted in only a twofold increase of intracellular tau-protein level (Brandt et al., 1995). Taken together, these data show that the concentration of tau in the stably transfected CHO and N2a cells is in the range of endogenous MAP levels in the control cells and increases the amount of total MAP only about two- to threefold.

In spite of the low cellular concentration, the expression of tau leads to conspicuous changes in cell shape and growth behavior. Whereas control cells were elongated, tau-transfected CHO cells rounded up (compare Fig. 2, b vs. c). This phenotype can be evoked in several ways, but one possibility is the inhibition of the motor protein kinesin as described by Rodionov et al. (1993), and a similar interpretation is appropriate in our case as well (see below). A second noticeable effect was that overexpression of tau in CHO cells slowed down proliferation approximately twofold (data not shown), similar to observations with MAP4-transfected cells (Nguyen et al., 1997).

We wanted to investigate whether the observed changes in shape and proliferation were indeed related to microtubule-dependent transport and therefore analyzed the cells with several markers for organelles. This revealed a remarkable clustering of mitochondria in tau40-expressing CHO cells near the nucleus in the vicinity of the microtubule organizing center (Fig. 2, a and c). This effect was reproduced in two independently generated tau40-stable cell lines, thus ruling out recloning artifacts. In contrast, mock-transfected CHO cells showed that mitochondria were dispersed throughout the entire cytoplasm (Fig. 2 d), frequently aligned with microtubules, suggesting a microtubule-dependent organization of mitochondria. The fraction of the cell area containing mitochondria decreased from 66.1% in mock-transfected cells to 27.7% in the tau-stable cell line (see Fig. 10). To exclude the possibility of a fixation artifact, we established CHO cells stably transfected with an enhanced green fluorescent protein (EGFP)–tau fusion protein that allowed us to monitor tau’s effect on mitochondrial distribution in living cells. EGFP tau was tightly associated with microtubules (as seen by EGFP fluorescence) and led to the same effects on mitochondrial clustering (data not shown).

Since there are several reports (e.g., Summerhayes et al., 1983) linking the distribution of mitochondria to the integrity of the intermediate filament system, we investigated whether the vimentin network was altered through tau overexpression. In tau40-stable cells, the extension of this filamentous network is significantly reduced and becomes located in a perinuclear area (Trinczek, B., A. Ebnet, E.-M. Mandelkow, and E. Mandelkow, manuscript in preparation). Thus, one might speculate that tau’s effects on the intermediate filament system may account for the aggregation of mitochondria at the MTOC. However, when treating cells with nocodazole to disrupt microtubules, mitochondria began to disperse again (see below), whereas the extension of the intermediate filament network was not altered in the tau-stable CHO cells despite disruption of microtubules. It has been shown that the disruption of microtubules leads to a collapse of the intermediate filament system (Klymkowski et al., 1989). Removal of nocodazole again did not affect the extension of the vimentin network (data not shown), but led to a reclustering of mitochondria, which indicates that the integrity of the microtubule system, not the intermediate filament system, is responsible for the observed phenotype of mitochondrial clustering in tau40-stable cells.
Interestingly, inhibition of kinesin motors by microinjection of an inhibitory antibody into fibroblasts led to a strikingly similar phenotype of a perinuclear localization of the vimentin system (Gyoeva and Gelfand, 1991). Mitochondria are organelles that can disappear from cellular regions with lowered metabolic demand (e.g., Morris and Hollenbeck, 1993). This raises the possibility that clustering of mitochondria was not due to an impairment of the cell’s transport, but to a change in the metabolic activity in the periphery due to the overexpression of tau. For this reason, we looked for other organelles known to be transported via microtubule-dependent motors and asked if they were clustered, too. This was indeed the case. When staining tau-stable cells with an antibody raised against catalase, an enzyme present in the matrix of peroxisomes, the organelles were significantly enriched in the perinuclear region, whereas in control cells they were evenly distributed (data not shown). This argues that organelle clustering near the MTOC is caused by a general influence of tau on microtubule-dependent plus-end-directed transport, rather than a selective retraction of mitochondria in response to altered metabolic demands.

To identify the domains of tau responsible for the clustering of mitochondria, we studied CHO cells stably transfected with several tau isoforms or constructs whose behavior had already been characterized in vitro (Gustke et al., 1994; Fig. 1): human tau40 (the longest isoform in the central nervous system), tau23 (the shortest isoform, found predominantly in embryonic brain), K12 (a tau construct containing only the three repeats plus the COOH-terminal flanking region), K10 (containing three repeats plus the whole COOH-terminal rest of the molecule), K35 (containing three repeats and both flanking regions), and K23 (a variant of tau23 without the repeats). The isoforms
tau40, tau23, and K35 induced pronounced mitochondrial clustering around the MTOC (Figs. 2 and 3, a–d, and data not shown), whereas K10 (data not shown), K12, and K23 did not show the effect (Fig. 3, e–h). This correlates well with the strengths of binding (dissociation constants $\approx 1$–$2 \mu$M for tau40, tau23, and K35, compared with $\approx 7$–$20 \mu$M.

**Figure 3.** Effects of different tau constructs on mitochondrial distribution in CHO cells. Mitochondrial distribution in CHO cells stably transfected with different tau constructs was examined after paraformaldehyde fixation. This allows the visualization of total tau in the cells, but tends to dissociate tau from the microtubules (see Materials and Methods). Human tau23, the shortest tau isoform (a and b), as well as K35 (c and d), a tau construct lacking nearly the entire projection domain (see Fig. 1) both led to the phenotype of mitochondrial clustering near the MTOC as seen in tau40-stable cells. On the other hand, K23 (e and f), a tau construct lacking the microtubule-binding region and K12 (g and h), consisting mainly of this MT-binding region (Fig. 1), did not alter the distribution of mitochondria. This argues for a tight binding of tau to microtubules as a prerequisite for impairment of plus-end-directed transport of these organelles and excludes the possibility that tau might inhibit kinesin by directly interacting with the motor protein. Mitochondria were stained with Mitotracker™ Red (a, c, e, and g) and transfected tau with polyclonal anti-tau antibody K9JA (b, d, f, and h). Bar, 20 $\mu$m.

**Figure 4.** Microinjection of recombinant tau protein leads to mitochondrial clustering near the MTOC. Wild-type CHO cells were microinjected with 1.3 mg/ml ($\approx 1.2 \mu$M) of purified recombinant tau protein and incubated for 4 h. After addition of Mitotracker™ Red (a and b), cells were fixed with paraformaldehyde and stained with antibodies against tubulin (c and d) and tau (e and f). Mitochondrial clustering is visible even within 4 h after microinjection. Note that the microtubule network retains its normal appearance and that mitochondria in microinjected cells similar as in the tau-stable CHO and N2a cells tend to cluster at one side of the nucleus in the vicinity of the MTOC. The cell periphery of microinjected cells is outlined for clarity. Bar, 10 $\mu$m.
for the other constructs; Gustke et al., 1994). K35 binds tightly but lacks parts of the NH₂-terminal projection domain so that this domain appears to be of lesser importance for mitochondrial transport. Conversely, K10 and K12 contain the repeats but lack the NH₂-terminal projection and flanking domains. Thus, their binding to microtubules is weak and this explains why they have no effect on mitochondrial distribution. K23 is a special case because it contains the projection domain, the two flanking domains, but not the repeats. It binds to microtubules with reasonable affinity ($K_d \approx 7 \mu M$), but still does not influence microtubule-based traffic. These observations argue that a high affinity of tau to microtubules is the major determinant for observing the effects of tau overexpression and that it is highly unlikely that tau inhibits plus-end–directed transport by directly binding to kinesins.

**Microinjection of Tau into CHO Cells Leads to Mitochondrial Clustering at the MT OC**

Next we asked whether the elevation of tau protein was directly responsible for the phenotype of mitochondrial clustering at the MT OC, or whether it was due to other factors not related to the protein level. Recombinant tau40 was microinjected into mock-transfected CHO cells to a final concentration of $\sim 1.2 \mu M$ where the microtubule network is not affected (Preuss et al., 1997). Nevertheless, mitochondria began to accumulate near the nucleus within 4 h (Fig. 4). Microinjection of mouse serum at the same concentration as a control on the other hand had no effect on mitochondrial distribution (data not shown). Thus, the phenotype can be generated without affecting the microtubule cytoskeleton, suggesting that tau protein has a direct effect on the redistribution of mitochondria, presumably by retarding their plus-end directed movement (see below).

**Nocodazole and Taxotere Reverse the Effect of Tau on Mitochondrial Distribution**

To investigate whether the organization and integrity of microtubules was important for the clustering of mitochondria, we applied two microtubule drugs, nocodazole and taxotere. Nocodazole causes the disassembly of microtubules, taxotere (a taxol derivative) causes their assembly and stabilization (Jordan and Wilson, 1998). In the presence of taxotere, new microtubules are nucleated randomly in the cytoplasm, independently of the MT OC, so...
that the defined polarity and orientation is lost (McNiven and Porter, 1986; Weisshaar et al., 1992). If microtubules mediated the relocation of mitochondria towards the cell center, one would expect that disruption of the microtubule network in the tau-overexpressing cells might lead to a wild-type distribution. This was indeed observed. Fig. 5 shows the disruption of microtubules in tau-stable CHO cells treated with nocodazole, which resulted in an increased cytosolic background staining arising from disassembled tubulin dimers. Within 1 h after nocodazole treatment, the mitochondria were redistributed to peripheral regions of the cell. The mitochondria-containing area thereby increases from 27.7 to 63.9%, which reaches the value of control cells within the error margin (see Fig. 10). Conversely, when nocodazole was removed again, an apparently normal microtubule network reappeared within about 15 min (data not shown), originating at the MTOC, and the phenotype of mitochondrial clustering, clearly visible already within ~1 h, was fully restored after ~4 h, indicating that the organelles were actively transported towards the MTOC (see Figs. 6 and 10). Taken together, these data suggest that a shift in the balance between plus- and minus-end–directed motor activities through tau-overexpression is responsible for the phenotypic effects. Thus, the ratio of mitochondria actively transported towards the cell periphery (that is plus-end directed) or towards the cell center (the MTOC, minus-end directed) in tau-stable versus control cells is expected to be altered by tau overexpression. To investigate this in further detail, cells were treated with nocodazole to achieve an even distribution of mitochondria in both cell lines. The microtubule array was then allowed to recover by washing out the drug and the direction of the movement of individual mitochondria was analyzed. Although transport in general is affected by tau overexpression (Fig. 7), irrespective of the kind of motor activity, plus-end–directed transport decreased ~10-fold, whereas minus-end–directed transport dropped only about twofold, resulting in a net inward transport of mitochondria in tau-stable cells compared with control cells.

These results demonstrate that microtubule-dependent processes are responsible for the clustering of mitochondria. It also means that mitochondria can become dispersed independently of microtubules, presumably by diffusion (in contrast to the ER, see below). The fact that reconstitution of the MT network leads to a relocalization of mitochondria near the cell center suggests that the overexpression of tau impairs predominantly the plus-end–directed transport of these organelles (see below). If this were true, one would expect that the normal distribution of mitochondria could be restored even in tau-overexpressing cells by abolishing the polar organization of microtubules. This can be achieved by treatment of cells with taxotere (at final concentrations of up to 10 μM), which leads to microtubule nucleation with random orientations throughout the cytoplasm. Fig. 8 shows that taxotere-
directed transport. In other words, a phenotype caused by impairment of plus-end–directed transport can manifest itself only in the presence of a polarized microtubule network where microtubule minus ends converge on the MTOC. Randomizing microtubule polarity by addition of taxotere allows transport of mitochondria to the cell periphery by minus-end–directed motors and thus leads to a redispersion of these organelles.

**Inhibition of Dynein by Overexpression of p50-Dynamitin Reverses the Effect of Mitochondrial Clustering by Tau**

The minus-end–directed transport of mitochondria is thought to depend on dynein (Hirokawa et al., 1990; Vallee et al., 1995), and therefore one would expect that inhibition of dynein would counteract the clustering of mitochondria induced by overexpression of tau. We inhibited dynein by transient transfection of the tau-overexpressing cells with dynamitin, a subunit of the dynactin complex that is crucial for the attachment between organelles and microtubules (Burkhardt et al., 1997). Overexpression of dynamitin in tau-stable cells significantly reduced the clustering of mitochondria, whereas EGFP transfection as a control had no effect on the mitochondrial distribution (Fig. 9). The area containing mitochondria within the cells after transfection increased from ~28% in EGFP-transfected tau-stable cells to 47.9% in dynamitin-overexpressing cells (P < 0.0001, two-tailed Student’s t test; Fig. 10). The fact

![Figure 8. Randomization of microtubule polarity by taxotere allows clustered mitochondria to disperse again. Tau-stable cells were treated with 10 μM taxotere for 6 h, which leads to an altered microtubule organization. Note that the MTOC in taxotere-treated cells is barely visible in the tubulin stain (b), in contrast to nontreated cells (compare Fig. 2). Microtubules are nucleated randomly throughout the cytoplasm, thus abolishing the role of the MTOC as an organizing element and therefore abolishing the polarity of microtubules. Minus-end–directed motors are now able to transport mitochondria back to peripheral regions in the tau-stable cells. Cells were fixed in methanol and mitochondria were stained with MitoTracker™ Green (a), tubulin was immunostained with DM1A (b). Bar, 10 μm.](#)

![Figure 9. Dynamitin overexpression restores the wild-type distribution of mitochondria. Tau-stable cells were transfected with dynamitin (p50), a subunit of the dynactin complex. 6 h after transfection, the mitochondria began to disperse again, visualized with MitoTracker™ Red after paraformaldehyde fixation (a). Note that the cell transfected with tau and dynamitin (arrowheads) shows dispersed mitochondria, whereas the neighboring tau-stable cells show the typical clustering near the MTOC. Dynamitin was stained with antidynamitin 50-1 antibody (b) and tubulin with YL1/2 antibody (c). Bar, 20 μm.](#)
stained living cells with DiOC6(3), a fluorescent marker (Hamm-Alvarez et al., 1993; Feiguin et al., 1994). To investigate an intact microtubule network (Vale and Hotani, 1988; Terasaki and Reese, 1994). Its distribution appears to depend both on kinesin-dependent transport and dynein. The distribution of mitochondria was quantified as described in Materials and Methods. A minimum of 30 cells were analyzed per experiment (n). In mock-transfected cells, mitochondria occupy up to 65% of the total cell area. The same is true in cells stably transfected with K23, a tau construct lacking the microtubule-binding repeats (CHO-K23). This value drops to ~30% in tau40-stable cells (CHO-tau). Treatment of the stably transfected cells with either nocodazole or taxotere restores the wild-type distribution of mitochondria (CHO-tau nocodazole or CHO-tau taxotere, respectively). The same effect can be seen after transfection with dynamitin (CHO-tau transfection dynamitin), but not after transfection of EGFP as a control protein (CHO-tau transfection EGFP; P < 0.0001, two-tailed Student’s t test). Removal of nocodazole again leads to clustering of mitochondria at the nucleus (CHO-tau noc. removal; 4 h).

that mitochondria were not completely redispersed (in contrast to the nocodazole-treated cells, where dispersal is complete) is probably due to fixation of cells 6 h after transfection. Since the overexpression of dynamitin affects the organization of the MTOC (Burkhardt et al., 1997), which in turn interferes with directed microtubule-dependent transport of mitochondria, we analyzed only those cells whose microtubule network was clearly unaffected. However, the experiment demonstrates that dynein motors are actively involved in the mitochondrial transport and argues against different types of mechanisms that operate in other situations (e.g., tethering to shrinking microtubules; Lombillo et al., 1995).

Shape and Extension of the Endoplasmic Reticulum Is Altered by the Overexpression of Tau

Thus far, we concentrated on the microtubule-dependent transport of mitochondria in transfected CHO cells. However, microtubules are responsible for transporting other vesicles and organelles as well, notably the endoplasmic reticulum (Terasaki and Reese, 1994). Its distribution appears to depend both on kinesin-dependent transport and on an intact microtubule network (Vale and Hotani, 1988; Hamm-Alvarez et al., 1993; Feiguin et al., 1994). To investigate if overexpression of tau would affect the ER, we stained living cells with DiOC6(3), a fluorescent marker for the ER. Overexpression of tau caused pronounced changes in shape and extension of the ER (Fig. 11). First, in tau-stable cells, the extension of the ER was strongly reduced so that it no longer reached the cell periphery. Secondly, the density and branching of the ER was significantly decreased. As in the case of mitochondria, this result is consistent with the assumption that plus-end-directed transport along microtubules is perturbed.

On the other hand, observations with the microtubule drugs taxotere and nocodazole showed that the behavior of the ER is more complex. Taxotere partially restored the wild-type distribution, although the ER did not fully reach the cell periphery (data not shown). In contrast, after disruption of microtubules with nocodazole, the ER did not expand again. These results confirm that organized microtubules are necessary for the general constitution of the ER, in addition to kinesin (Lippincott-Schwartz, 1998).

Recycling of Transferrin in Tau-overexpressing CHO Cells Is Slowed Down

Endo- and exocytotic processes depend on an intact microtubule cytoskeleton. We therefore asked whether the overexpression of tau influences these processes as well, using rhodamine-labeled transferrin as a marker. Transferrin is endocytosed via transferrin receptors and clathrin-coated vesicles and is actively transported along microtubules by motor proteins through early and sorting endosomes to recycling compartments in the pericentriolar region of the cell. It is finally released by exocytosis, again via microtubule-dependent transport processes (Martys et al., 1995). The efflux of transferrin was measured by removal of the supernatant at different time points and by quantitating the exocytosed transferrin by fluorescence (Fig. 12 a). At early time points (a few minutes after removal of excess transferrin) the tau-stable cell lines showed a significantly lower level of exocytosed transferrin than the control cells, but this difference disappeared later on (~20 min). Thus, the outward flow of exocytic vesicles was retarded but not interrupted.

This observation was confirmed by quantitating the immunofluorescence of residual TRITC-labeled transferrin in methanol-fixed tau-stable cells and mock-transfected controls. Methanol-fixed tau-stable cells yielded a significantly higher amount of transferrin 7 min after removal of transferrin-containing media compared with mock-transfected cells (Fig. 12 a; P < 0.0001, two-tailed Student’s t test), mostly in the vicinity of the MTOC (the region of the recycling compartment; Martys et al., 1995). Whereas ~50% of the initial intracellular transferrin had been exocytosed in mock-transfected cells, only 10% had been recycled in tau-stable cells during the same time period. This correlates well with the experiment shown in Fig. 12 b, where, conversely, the exocytosed transferrin was quantitated. Again, after ~7 min, only 50% of transferrin was exocytosed in tau-stable cells compared with control cells.

Transport of Mitochondria and Endoplasmic Reticulum into the Neurites of Differentiated N2a-Neuroblastoma Cells Is Impaired by Overexpression of Tau

In the experiments described so far, we focussed on CHO cells to investigate tau’s effects on transport processes. The chief reasons were that CHO cells are well suited for studying transport due to their flattened cell form and their size, which facilitates observation and microinjection. Secondly, CHO cells do not normally express tau so that the discrimination between endogenous MAPs (e.g., MAP4) and stably introduced tau is much easier than in neuroblastoma cells. On the other hand, tau is expressed mainly in neuronal tissue, raising the question whether the effects of tau overexpression manifest themselves in neuronal cells as well. For this reason, we stably transfected
human tau40 into the mouse neuroblastoma cell line N2a. Although the cell body of N2a cells is very compact and small (≈10–20 μm, compared with ≈40 μm for CHO cells) so that it is difficult to detect differences in mitochondrial localization, the organelles appear to be clustered in the vicinity of the nucleus in tau-stable compared with control cells. Transport processes in the neurites of differentiated N2a cells on the other hand can be monitored much more easily because transport deficits in these structures accumulate to a more significant extent. As shown by several reports, tau protein is important for the generation of neurites, and transport of material into the neurites depends on kinesin (Baas et al., 1991; Feiguin et al., 1994).

Differentiated wild-type N2a cells contain abundant mitochondria in their neurites, extending up to the tip (Fig. 13 d). In striking contrast, the neurites of differentiated N2a cells transfected with tau showed very few mitochondria (Fig. 13 a and Table I). The remarkable depletion of mitochondria from neuritic extensions in these cells indicates that tau overexpression in neuronal cells leads to the same phenotype as seen in CHO cells, and again can be explained by an impairment of plus-end–directed microtubule-dependent transport of mitochondria. The absence of these organelles would severely limit the energy supply of neurites and could cause their degeneration. These observations have implications for the cellular causes of Alzheimer’s disease where elevated and mislocalized tau occurs concomitant with neuronal degeneration (as discussed later).

Since the distribution of mitochondria was impaired in differentiated N2a neuroblastoma cells overexpressing tau, we asked whether the ER was affected as well (judging by immunofluorescence with an ER-specific antibody; Louvard et al., 1982). Control cells showed ER elements throughout the cell body mostly surrounding the nucleus, but visible in the neuritic structures. By contrast, the majority of tau-overexpressing cells (≈82%, Table I) showed that the ER was collapsed towards the cell center, without extending around the nucleus (Fig. 14 a). The overall intensity of ER-staining remained the same in the two cell
In this study, we have investigated how an increased level of tau protein (generated by overexpression or microinjection) affects intracellular transport in CHO and neuroblastoma cells. The main results can be summarized as follows: (a) increased tau leads to a clustering of mitochondria and peroxisomes near the MTOC (Fig. 2), to a contraction of the intermediate filament system and the ER (Fig. 11), and to a reduced rate of exocytosis (e.g., of transferrin receptor, Fig. 12). The cells lose their elongated shape and become rounded (Fig. 2), and their growth rate slows down. Mitochondria and ER are decreased in neuritic processes (Figs. 13 and 14; Table I). (b) All of the above effects are observed at relatively low levels of tau overexpression where the microtubule network is not impaired (no bundling, no detachment from MTOC); in fact, they depend on the integrity of the microtubule network. The effects also depend on a strong binding of tau to microtubules ($K_a \sim 1 \mu M$, provided by the repeats and proline-rich flanking domain). (c) If the microtubule network is disrupted by drugs (i.e., disassembly by nocodazole, Fig. 5), or detachment of microtubules from MTOC and randomization by taxotere (Fig. 8), the tau-induced changes in the distribution of mitochondria are reversed so that the organelles become dispersed throughout the cell again (Fig. 10). (d) Inhibition of dynein (via overexpression of dynamin) reverses the effects of tau overexpression and allows the dispersion of mitochondria (Figs. 9 and 10).

These effects can be explained by the assumption that tau interferes preferentially with the plus-end–directed transport along microtubules involving kinesin, while the minus-end transport involving dynein is less affected. The directionality of organelle transport observed with the tau-transfected cells is consistent with a number of reports showing that plus-end–directed transport is achieved mainly by kinesin-like motors, and the reverse transport by dynein (Vallee and Sheetz, 1996; Rickard and Kreis, 1996; Hirokawa, 1998; Lippincott-Schwartz, 1998). For example, mitochondria are transported by the kinesin motors KIF1B (Nangaku et al., 1994) and KIF5B (Tanaka et al., 1998), or, in the opposite direction by dynein (Hirokawa et al., 1990), and, therefore, inhibition of kinesin leads to a net retrograde transport of mitochondria. Rodionov et al. (1993) have shown that microinjection of an inhibitory kinesin antibody, which cross-reacts with several members of the kinesin superfamily, leads to a strikingly similar phenotype of mitochondrial clustering. Furthermore, the targeted disruption of mouse conventional kinesin heavy chain KIF5B results in the perinuclear clustering of mitochondria (Tanaka et al., 1998). Similarly, the extension of the ER and the intermediate filament system or the trafficking of vesicles is determined by a balance between kinesin- and dynein-dependent movements so that the ER and the vimentin-network collapses when kinesin is suppressed (Feiguin et al., 1994; Gyoeva and Gelfand, 1991). The term “anterograde” is used for the plus-end–directed axonal transport along microtubules, but also for the minus-end–directed transport from ER to Golgi, so that we prefer the term “plus-end–directed” for the present discussion.

The function of tau or MAPs is usually discussed in terms of their role in microtubule stabilization (Matus, 1994), the generation of cell processes (Kosik and McConlogue, 1994), in their spacer function between microtubules (Chen et al., 1992), or as anchoring sites for other proteins (Obar et al., 1989; Ookata et al., 1995). The data presented here point to a new function of tau; that is, regulation of transport within cells by the variation of the intracellular concentration of tau on the surface of microtubules. Since related effects have been observed with MAP4 (Bulinski et al., 1997), this may be a general function of MAPs. In this context, it is of interest to note that, although MAP4 efficiently stabilizes microtubules in vitro and in vivo, the mitochondrial distribution in MAP4-overexpressing cells has been reported to not be altered (Nguyen et al., 1997). This argues against the possibility that a change in lifetime of microtubules accounts for the
effect of mitochondrial clustering, because the lifetime would be increased both by MAP4 and tau. More importantly, the comparison perhaps points to different functions of tau and MAP4 in vivo.

Inhibition of Motor Activity by MAPs

The question of whether and how MAPs and motors could interfere with each other on a microtubule has been the subject of extended discussions, mainly because experimental evidence obtained in vitro remained ambiguous (e.g., Paschal et al., 1989; Von Massow et al., 1989; Rodinonov et al., 1990; Lopez and Sheetz, 1993; Hagiwara et al., 1994). The results depended not only on the type of MAPs and motors used, their effective concentrations (in solution or bound to a surface), but also on the experimental design (microtubule gliding or bead movement assay). The majority of the in vitro results suggested that tight binding of MAPs to microtubules via the microtubule-binding domain and a large projection domain was a prerequisite for observing any inhibition in motility. Thus, MAP2 could retard kinesin or dynein, while smaller MAPs (e.g., tau, MAP2c) had a much smaller effect or none at all (Von Massow et al., 1989; Lopez and Sheetz, 1993).

Conceptually, one can analyze the interference between MAPs and motors in terms of competition for common binding sites on a microtubule and/or in terms of steric hindrance (Lopez and Sheetz, 1993). A purely competitive mechanism would require only the microtubule-binding domain of MAPs (repeats plus flanking regions), while steric hindrance would depend on both microtubule binding plus the NH2-terminal projection domain. Since several of the earlier studies had emphasized that large projection domains of MAPs were necessary for motor inhibition, we were surprised to find that a small MAP such as tau, overexpressed only moderately in cells, could be inhibitory for plus-end-directed transport. This could be achieved even with constructs having no projection do-
Table I. Quantitation of the Phenotypic Effects of Tau Overexpression in N2a-Neuroblastoma Cells

| Phenotype                      | N2a tau40 | N2a mock |
|--------------------------------|-----------|----------|
| Neurites (length >30 μm)       | 5.4%      | 15%      |
| Mitochondria in neurites (>6 per neurite) | 17%      | 94%      |
| ER distribution (clustered)   | 82%       | 23%      |

A minimum of 150 cells were analyzed in four (neurite length and mitochondria) or three (ER distribution) independent experiments. In differentiated, mock-transfected cells, 15% of the cells developed neurites longer than 30 μm, whereas, in tau-stable cells, this value drops to only 5.4%. The effect of tau-overexpression becomes even more impressive when analyzing the mitochondria present in the neuritic structures: only 17% of tau-stable cells transported more than six mitochondria into their neurites, compared with 94% of control cells. The average number of mitochondria in the control cells was ~10–20 per neurite (data not shown). The ER was regarded as clustered when it did not surround the nucleus of the cells. In tau-stable cells, up to 82% displayed this phenotype. In contrast, in mock-transfected cells, only 23% of cells had a similar appearance.

Having determined the effects of tau overexpression in CHO cells, an obvious question was whether analogous effects would be observable in cells of neuronal origin. In this case, the experimental approaches are more limited because of the smaller size of the cells. For example, a redistribution of organelles in the cell body is difficult to observe and the level of endogenous tau is too low for many biochemical assays. On the other hand, vesicles and organelles are easily detectable in the long neuritic processes of differentiated cells. Thus, the neurites of control N2a cells show staining with the markers of mitochondria and the ER, but N2a cells overexpressing tau only at a significantly lower level. In agreement with the previous interpretation, this means that plus-end–directed transport, at least in the case of mitochondria, is impaired, leading to an accumulation of these elements in the cell body.

The effect of tau on vesicle trafficking described here represents a new function that has attracted little attention thus far. This was due in part to the fact that the influence of MAPs on the activity of motor proteins were mainly investigated in vitro, showed only weak effects, visible only at high MAP concentrations, and were apparently restricted to high molecular weight MAPs (Paschal et al., 1989; Von Massow et al., 1989; Lopez and Sheetz, 1993; Hagiwara et al., 1994). Nevertheless, in the context of...
cells, the subtle effects become magnified and visible as redistribution of cellular components. The most important prerequisite for tau's influence on intracellular traffic is the tight binding to microtubules due to the repeats and the flanking domains (∼1–2 μM) while the projection domain has only a minor effect. This implies that the phosphorylation of tau should play an important role since this regulates tau's affinity to microtubules. One can broadly distinguish two types of phosphorylation of tau, proline directed (affecting Ser-Pro or Thr-Pro motifs and induced by proline-directed kinases such as MAP kinase, GSK3, cdk5) and non–proline directed (induced by second-messenger–dependent kinases such as PKA, PKC, CaMK, MARK; for review see Mandelkow et al., 1995). Proline-directed phosphorylation has only a moderate influence on tau-microtubule interactions (Biernat et al., 1993), whereas two other sites potently disrupt the interaction, Ser262 (phosphorylated by MARK; Drewes et al., 1997) and Ser214 (phosphorylated by PKA; Zheng-Fischhöfer et al., 1998; both these sites are enhanced in Alzheimer's tau). Thus far, such sites were mainly seen as modulators of microtubule stability. However, in the light of our present results, one should also view the MAP-microtubule affinity-regulating phosphorylation reactions as modulators of intracellular traffic. In this scenario, tau bound to microtubules would present resistance to plus-end–directed movement, which would be relieved by tau's phosphorylation and detachment from microtubules. Since the outward flow of material is a prerequisite for cell polarity and differentiation (Feiguin et al., 1994; Bradke and Dotti, 1997), the activity of tau kinases would become important at this point. It is interesting to note that vesicle transport can be inhibited by MAP2, but relieved by a kinase that is probably related to MARK (Drewes et al., 1997; Lopez and Sheetz, 1995), a kinase that phosphorylates MAP2c and MAP4 in vitro (Illenberger et al., 1996), and therefore can

Figure 14. The expansion of the endoplasmic reticulum in differentiated neuroblastoma cells is significantly reduced by overexpression of tau. Tau-stable N2a cells (a–c) or mock-transfected cells (d–f) were induced to differentiate, and then fixed in paraformaldehyde and triple-labeled with antibodies against the ER (a and d), tubulin (b and e), and tau (c and f). In contrast to control cells, ∼82% of tau-stable neuroblastoma cells show the ER clustered at one side of the nucleus (a), whereas in mock-transfected cells the ER surrounds the entire nucleus (d; compare Table 1). Furthermore, the expansion of the ER into the neurites in control cells (d) can clearly be seen. In tau-stable cells, only a faint signal of ER-immunoreactivity is visible in neuritic processes. The total intensity of the ER stain is the same in both cells. Arrowheads in a and d are indicating the position of the neurite relative to the cell body. Bar, 10 μm.
be expected to regulate the affinity of MAPs to microtubules in general. This points to another interesting observation: it has been shown that MAP1A can compensate for the lack of tau in knock-out mice (Harada et al., 1994), suggesting that the function of MAPs is partially redundant. Whereas axonal elongation was not affected in these animals, microtubule stability was decreased in some axons. It is conceivable that different MAPs regulate the transport of different organelles and in different cell compartments. Thus, it would be interesting to correlate the distribution of organelles and MAPs in mouse models lacking or overexpressing MAPs to get further clues for the functions of MAPs in intracellular transport.

**Implications for Neuronal Degeneration in Alzheimer’s Disease**

The effects of tau overexpression on mitochondrial distribution, vesicle-trafficking, and the ER have important implications for Alzheimer’s disease. In AD, tau is abnormally phosphorylated, aggregated into paired helical filaments, and mislocalized from the axon to the somatodendritic compartment (Binder et al., 1985; Wischik et al., 1988; Braak et al., 1994). In addition, it has been reported that intracellular tau-protein concentration in AD brain is elevated (Khatooon et al., 1992). In neurons, this mislocalization and elevated intracellular concentration of tau would affect the transport of mitochondria or ER to the most peripheral regions of the axons and would lead to a decrease in glucose metabolism and ATP synthesis in these compartments, causing energy deficits, loss of Ca2+ homeostasis, or lipid synthesis (Futerman and Banker, 1996; Mattson and Guo, 1997). Finally, this may lead to degeneration of the neuron, presumably beginning from the most distal regions, leading to the so called “dying back” of axons (Braak et al., 1994; Trojanowski and Lee, 1995). In this context, it is of special interest that compromised mitochondrial function has indeed been shown to associate with late onset Alzheimer’s disease (Davis et al., 1997). In addition, a slowing down of transport processes could affect the proper distribution of proteins, particularly of amyloid precursor protein (APP), another key player in the progression of Alzheimer’s disease that is rerouted by transcytosis (Simons et al., 1995), and whose overproduction of amyloidogenic peptide Aβ1-42 takes place in the endoplasmic reticulum (Cook et al., 1997; Hartmann et al., 1997). Thus, a transport inhibition due to the overexpression and mislocalization of tau in neurons might increase production of Aβ1-42. Conversely, overproduction of amyloidogenic variants of APP correlates with an increased level of tau protein and tau mRNA (Lee et al., 1996), and, in the Alzheimer’s-related Down syndrome, APP and tau are both upregulated (Oyama et al., 1994). Finally, the mutant presenilins PS1 and PS2 that are responsible for familial Alzheimer’s disease have been shown to interact with APP in the endoplasmic reticulum, thereby enhancing the production of Aβ (Xia et al., 1997). Thus, future work might give new clues to an understanding of Alzheimer’s disease since it might experimentally link the function of AD-specific gene products, such as APP and the presenilins, with mitochondrial energy supply and tau protein.

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