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Probing Modifications of the Neuronal Cytoskeleton

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Contents

Abstract

The prominent death of central neurons in Alzheimer's and Parkinson's is reflected by changes in cell shape and by the formation of characteristic cytoskeletal inclusions (neurofibrillary tangles, Lewy bodies). This review focuses on the biology of neurofilaments and microtubule-associated proteins and identifies changes that can occur to these elements from basic and clinical research perspectives. Attention is directed at certain advances in

neurobiology that have been especially integral to the identification of epitope domains, protein isoforms, and posttranslational (phosphorylation) events related to the composition, development, and structure of the common cytoskeletal modifications.

Recently, a number of experimental strategies have emerged to simulate the aberrant changes in neurodegenerative disorders and gain insight into possible molecular events that contribute to alterations of the cytoskeleton. Descriptions of specific systems used to induce modifications are presented. In particular, unique neural transplantation methods in animals have been used to probe possible molecular and cellular conditions concerned with abnormal cytoskeletal changes in neurons.

Index Entries: Alzheimer's; amyloid protein; cytoskeleton; Lewy body; microtubule-associated protein; neural transplant; neurofibrillary tangle; neurofilaments; Parkinson's; phosphorylation; transgenic animal.

Introduction

The neuronal cytoskeleton is firmly recognized as integral to the shape and function of neurons. Components of the cytoskeleton are capable of dramatic change (plasticity) in response to experimental injury and undergo significant modifications during the process of neuronal death. In cases of neuronal degeneration, pronounced disarrays of filaments become evident, often in the form of highly defined inclusions that alter neuronal morphology, likely interfere with axonal transport systems, and may contribute to the accelerated degeneration of specific neuronal populations.

One of the goals of modern neuroscience is to determine the molecular/cellular etiology of prevalent neurodegenerative disorders, including Alzheimer's and Parkinson's. Interference with cytoskeletal metabolism may be the primary cause of neuronal loss in these conditions. Although the formation of abnormal structures and subsequent cellular changes in neuronal morphology associated with these two disorders have been recognized since the turn of the century (Alzheimer, 1907; Simchowicz, 1911; Lewy, 1912), the underlying factor(s) responsible for the genesis of these structures are unknown. Application of new cellular and molecular techniques continue to help elucidate the composition of aberrant inclusions and provide insight into the determination of specific gene products involved with cytoskeletal reorganization. In this review, descriptions of the classical cytoskeletal inclusions within the various neuronal domains and the proteins that contribute to the inclusions are presented.

Throughout the review, attention has been directed at immunocytochemical concepts of the adult cytoskeleton when affected by degeneration. Events that may possibly upset normal cytoskeletal dynamics are given. An emphasis is placed on the most recent systems used to model and assess mechanisms of dysfunction in neurodegeneration. Included in the group of new experimental strategies now available are transgenic animals that overexpress amyloid isoforms and neural transplantation procedures that influence the cytoskeleton and induce neuronal death. Systems in the transplantation field are discussed and critically assessed.

Major Components of the Neuronal Cytoskeleton

The cytoskeleton provides a framework that defines, supports, and maintains the shape of neurons. A highly crosslinked network of microfilaments, neurofilaments, microtubules, and specific associated proteins significantly govern the internal architecture. Neurons display cytoskeletal polarity that is linked to their function, and this network defines a highly characteristic morphology in neurons within the central and peripheral nervous systems. Furthermore, the elements are crosslinked in an arrangement that provides a system for the segregated distribution of organelles and proteins within the neuron.

Neurofilaments

In mammals, neurofilaments (NF) are composed primarily of three subunits with different electrophoretic mobilities. They are designated NF-H, NF-M, and NF-L, with relative molecular masses of 200, 168, and 70 kDa, respectively (Hoffman and Lasek, 1975). Each NF subunit is divided into three domains—a highly conserved central α-helical rod domain, an amino-terminal head domain, and a carboxy-terminal tail domain (Geisler et al., 1983, 1985). However, the concept of the "NF triplet" in the peripheral and central nervous system is changing because of the characterization of novel intermediate filament proteins, including peripherin (Leonard et al., 1988) and α-internexin (Chiu et al., 1989; Kaplan et al., 1990).

Neurofilaments are among the most highly phosphorylated neuronal proteins (Julien and Mushynski, 1982). The NF-M and NF-H contain large numbers of phosphate groups on their carboxy-terminal tail regions (Julien and Mushynski, 1983; Carden et al., 1985). Recent studies show that NF-protein subunits are phosphorylated at both the amino-terminal head domain and the carboxy-terminal tails by different kinases (Nixon and Sihag, 1991). Major phosphorylation sites of the NF-H are shown to be located in a repetitive sequence motif of lysine–serine–proline (Geisler et al., 1987). The site specific phosphorylation or dephosphorylation within the domains of each NF subunit is thought to regulate neurofilament assembly, axonal transport, and interactions with other cytoskeletal proteins (Nixon, 1993). The extensive phosphorylation of these domains, thought to project outward from the filament core, are considered as a mechanism by which NFs crosslink with each other and with cytoplasmic organelles and thereby help to stabilize the axonal cytoskeleton (Julien and Mushynski, 1983; Carden et al., 1985; Leterrier et al., 1982; Hirokawa et al., 1984). Immunocytochemical studies have revealed that several antibodies directed against the carboxy-terminal tail domains of NF-H and NF-M bind to the peripheral regions of NF (Willard and Simon, 1981; Liem et al., 1985) and to the crossbridges (Hirokawa et al., 1984).

Immunocytochemical and in vitro reassembly experiments suggest that the long-tail domains of the NF-M and NF-H subunits extend from the core of the filament and seem likely to be involved in establishing proper filament spacing to other axonal structures (Hirokawa et al., 1984; Hisanaga and Hirokawa, 1988). Phosphorylated NFs are more abundantly and closely packed in the axon vs the dendrite. Neurofilaments appear to be the intrinsic determinants of axonal diameter and are selectively phosphorylated in the axonal compartment.

In 1983, Sternberger and Sternberger used monoclonal antibodies directed against phosphorylated (P) and nonphosphorylated (NP) neurofilament epitopes to unequivocally demonstrate that NFs in the axon are highly phosphorylated, whereas cell bodies and dendrites react minimally or not at all with phosphorylated antibodies. A number of other laboratories have subsequently confirmed the differential phosphate distribution with similar antibodies (Peng et al., 1986; Hart et al., 1987; Lee et al., 1987). The eloquent quick-freeze, deep-etch technique used by Hirokawa and associates indicates that NFs in the axon run parallel and are extremely crossbridged. In the cell body, NFs are randomly oriented and the number of crossbridges are reduced to about two-fifths of the axon. In the dendrites, NFs run parallel but are dispersed or exist as small fasicles and the average frequency of crossbridges is about three-fifths of the axon (Hirokawa, 1982). Interestingly, dephosphorylation of the carboxy-terminal tail domain of NF-M and NF-H does not change the structure of NF in vitro when examined by quick-freeze, deep-etched electron microscopy (Hisanaga and Hirokawa, 1989).

Microtubules and Microtubule-Associated Proteins (MAPs)

The monomeric unit of microtubules is tubulin, a heterodimer of two closely related globular proteins termed α- and β-tubulin (each with M_r

of 50 kDa). Dimers of α- and β-tubulin assemble to form the linear polymers in the shape of a hollow tube. Microtubules, in association with microfilaments and NF, have been implicated in many neuronal functions including cell division, motility, growth of axons and dendrites, maintenance of neuronal morphology, and the transport of cytoplasmic components (Burgoyne, 1991).

Tubulin can be purified and induced to cycle between polymerization and depolymerization in vitro. The tubules are also polar structures, since one end (plus) grows faster than the other (minus) end. Microtubules in the axon are all orientated with the plus end toward the axon tip (Heidemann et al., 1981). In contrast, microtubules in dendrites are mixed in polarity orientation (Baas et al., 1988). The difference in this orientation may provide for selective transport into the axon or dendrites. Neurons contain a population of acetylated and detyrosinated microtubules that have distinct properties related to stability (Black et al., 1989; Baas and Black, 1990). The spatial organization of posttranslationally modified microtubules can be analyzed by antibodies that recognize detyrosinated (stable), tyrosinated (unstable) (Gunderson et al., 1984, 1987; Kreis, 1987), or acetylated α-tubulin (Piperno and Fuller, 1985).

Microtubules associate with a heterogenous collection of proteins known as the MAPs. MAPs are involved with the assembly and stabilization of microtubules and in the interaction of microtubules with other cytoskeletal elements and organelles (Matus, 1988; Goedert et al., 1991). Since they influence microtubule stability, they are integral to the dynamics of cellular morphology and are linked to the determinants of neuronal form during growth, differentiation, and aging. A series of major MAPs and subsets have been identified and classified according to their molecular mass as MAP1 through MAP5. The MAPs can be split into two groups consisting of very high molecular mass polypeptides (MAP1 and MAP2 are highly represented in the adult CNS) and a group of lower mol-mass MAPs called tau.

Biochemical and immunological approaches have identified MAP1, MAP2, and tau as the main MAPs in adult mammalian nerve cells (Shiomura and Hirokawa, 1987). MAP1 is found in axons and dendrites (Bloom et al., 1984; Huber and Matus, 1984; Peng et al., 1986). MAP2 is located mainly in the perikarya and dendrites (Bernhard and Matus, 1984; Peng et al., 1986), and tau is almost exclusively an axonal marker (Binder et al., 1985). The fine filamentous crossbridges that interconnect microtubules and NF (Hirokawa, 1982) can be labeled with MAP antibodies (Hirokawa et al., 1988a; Sato-Yoshitake et al., 1989). The spacing between adjacent microtubules is approx 20 nm in the presence of tau and approx 100 nm in the presence of high-molmass MAP2 (Hirokawa et al., 1988b; Goedert et al., 1991). Expression of MAP2 or tau in the nonneuronal insect (Sf9) cell line has indicated that the spacing between microtubules depends on the MAP expressed. In cells that synthesize MAP2, the distance between microtubules is similar to that found in dendrites, whereas the spacing between microtubules in Sf9 cells expressing tau approximates the spacing found in axons (Chen et al., 1992). LeClerc et al. (1993) have indicated that MAP2C and tau expression in Sf9 cells have differential effects on microtubule assembly and/or transport events. The detailed organization of microtubules induced by each of the two constructs is distinctive, and these differences may be relevant to axonal and dendritic differentiation.

Many of the MAPs have been sequenced and cDNAs have been produced, including MAP1 (Lewis et al., 1986a; Safaei and Fischer, 1989), MAP2 (Garner and Matus, 1988; Kosik et al., 1988; Lewis et al., 1986b), and MAP5 (Garner et al., 1988b). These probes have been used to indicate that the heterogeneity of the high-mol-wt MAPs is not caused by the presence of multiple genes, but more likely by posttranslational modification or the presence of separate transcripts via alternative slicing from the same gene.

MAP1

MAP1 is a component of the filamentous structures between microtubules in axons and dendrites (Hirokawa et al., 1985; Shiomura and Hirokawa, 1987). MAP1 is comprised of two forms designated as MAP1A (350 kDa) and MAP1B (320 kDa). MAP1B has been named MAP1.2 (Aletta et al., 1988), MAP1(x) (Calvert and Anderton, 1985), and MAP5 (Riederer et al., 1986). Immunoelectron microscopy has been used to show that MAP1A and MAP2 colocalize on microtubules and may have an interrelationship in vivo that is responsible for forming the network of bridges between microtubules (Shiomura and Hirokawa, 1987). MAP1B/MAP5 appears very early during differentiation and is coupled to neuronal morphogenesis (Matus, 1991).

Phosphorylation events also appear to be important for MAP1B function (Aletta et al., 1988; Riederer et al., 1991). Protein kinase activities have been identified that are capable of phosphorylating MAP5 (Diaz-Nido et al., 1988; Hoshi et al., 1990; Tsao et al., 1990). MAP1A and 1B have been sequenced (Noble et al., 1989; Garner et al., 1990) and the tubulin-binding domain of MAP1B has been determined as a repeat motif sequence of four amino acids that is distinct from the microtubule-binding domains of MAP2 or tau (Noble et al., 1989).

MAP2

MAP2 is one of the most highly phosphorylated proteins in the brain (Theurkauf and Vallee, 1983). It consists of at least three isoforms produced by a single gene—high-mol-mass MAP2a and 2b, and low-mol-mass MAP2c (Goedert et al., 1991). MAP2a and 2b have apparent molecular masses of 288 and 280 kDa, whereas MAP2c has a mol mass of 70 kDa (Matus, 1988; Nunez, 1988). MAP2b and MAP2c are transcribed from the same gene and are produced by alternative splicing of a primary transcript (Garner and Matus, 1988). It is not known whether MAP2a is produced by alternative splicing or whether it is the product of MAP2b posttranslational modification (Kindler et al., 1990; Goedert et al., 1991). The complete amino acid sequences of rodent high-mol-mass MAP2 and MAP2c have been determined by molecular cloning (Lewis et al., 1988; Kindler et al., 1990). The subsets of MAP2 are differentially expressed during development. In general, MAP2c is expressed mainly during the embryonic/early postnatal period, remains low in the adult brain (Riederer and Matus, 1985), and is confined to axons; MAP2a appears to replace MAP2c during development, whereas MAP2b is present from the embryonic period and continues to be expressed throughout life (Binder et al., 1984; Burgoyne and Cummings, 1984).

There are many sites on the MAP2 molecule that can be phosphorylated and a variety of phosphorylation states exist in vivo (Tsuyama et al., 1987). In vitro, it is site-specific, rather than the total phosphorylation of MAP2 that governs intracellular interactions (Brugg and Matus, 1991). It remains to be determined if different phosphorylation sites actually regulate different functions of MAP2. Phosphorylation of MAP2 plays a role in the modulation of its function indicated by the observation that activation of glutamate receptors leads to the rapid and specific dephosphorylation of MAP2 (Halpain and Greengard, 1990). Protein kinases may preferentially target a distinct subset of the phosphorylation sites on MAP2 and the increase or decrease in phosphorylation may be important in regulating MAP2 function in response to extracellular signals (Halpain and Greengard, 1992).

MAP2 in the adult is a somatodendritic marker (Matus et al., 1981; Miller et al., 1982; DeCamilli et al., 1984) and the mRNAs for MAP2 are correspondingly expressed in neuronal cell bodies and dendrites (Garner et al., 1988b; Goedert et al., 1991). The occurrence of high-mol-mass MAP2 mRNA in the dendritic domain represented the first demonstration of the spatial segregation of a mRNA in neurons (Garner et al., 1988a). In the carboxy-terminal of MAP2 there is a series of amino acid repeats that is involved in binding tubulin (Joly et al., 1989; Lewis et al., 1988). This

repeat region of MAP2 can bind to microtubules when expressed in cell lines, indicating that the sequences flanking the repeats are important for the binding of MAP2 to microtubules (Lewis et al., 1989; Lewis and Cowan, 1990). MAP2 also has the tendency to self-associate and form antiparallel dimers in vitro (Wille et al., 1992b).

Tau

Tau represents a group of phosphoproteins that copurify with tubulin during assembly and disassembly cycles (Cleveland et al., 1977). Tau was the first MAP shown to promote microtubule assembly in vitro (Weingarten et al., 1975). This MAP consists of a group of isoforms with true mol masses from adult human brain of 37–45 kDa (Goedert and Jakes, 1990). In the human brain, six isoforms of tau have been identified that arise from alternatively spliced transcripts originating from a single gene (Neve et al., 1986; Lee et al., 1988b; Goedert and Jakes, 1990; Kosik et al., 1989b, Himmler et al., 1989). In addition to the group of low-mol-wt tau proteins, a high-mol-wt tau isoform has been described (Georgieff et al., 1991; Oblinger et al., 1991). The high-mol-wt isoform appears to be only expressed in the PNS (Georgieff et al., 1991; Oblinger et al., 1991) and is phosphorylated to a lesser degree compared to the low-mol-wt form (Taleghany and Oblinger, 1992). Tau is a rodlike structure that associates with microtubules via armlike projections (Hirokawa et al., 1988b). Three imperfectly repeated sequences near the carboxy-terminus of tau can bind to the exterior of microtubules (Lee et al., 1989; Himmler et al., 1989).

Tau is abundant in the axonal domain (Binder et al., 1985; Peng et al., 1986; Kowall and Kosik, 1987; Brion et al., 1988; Trojanowski et al., 1989) and binds selectively to axonal microtubules (Ferreira et al., 1989). Experiments by Caceres and Kosik (1990) indicate that the selective stabilization of microtubules by tau is important to axonal determinants. The formation of microtubule bundles by tau was initially shown in a fibroblast cell line transfected with a tau cDNA (Kanai et al., 1989). Deletion mutants indicate that the N-terminal neutral region or the C-terminal tail region of tau are important to the microtubule-binding function (Kanai et al., 1992). Furthermore, hyperexpression of tau will induce the formation of microtubule bundles with uniform polarity in the cell body and in the processes of transfected Sf9 cells (Baas et al., 1991). The expression of tau protein in a foreign host results in the elaboration of a long, relatively unbranched processes that resembles the morphology of axons (Knops et al., 1991). These experiments indicate that tau can alter cell morphology by transducing microtubule elongation into a specific cellular shape, i.e., axon-like processes.

There are reports that tau may not be exclusively confined to the axon. Localization of tau immunoreactivity to the somatodendritic compartment (Migheli et al., 1988; Papasozomenas and Binder, 1987) and by *in situ* hybridization to somata and dendrites (Kosik et al., 1989a) have been reported in the literature. Reasons for the discrepancies in the immunocytochemical location of tau epitopes within neurons have not been resolved.

Cytoskeletal Changes Associated with Alzheimer's and Parkinson's

In Alzheimer's and Parkinson's, specific regions of the brain typically contain significant numbers of neurons in various stages of degeneration. A distinctive and especially prominent change in the somatic, axonal, and dendritic domains is the formation of paired helical filaments (PHF). Aggregates of PHF are found in the cell body as constituents of neurofibrillary tangles (NFT), in dendrites or axons (neuropil threads or curly fibers), or are described as dystrophic neurites when associated with amyloid formation.

Although abnormal filamentous structures, such as PHF and NFT, signify dysfunctional neurons in the CNS, it is not known if defects in cytoskeletal protein metabolism represent the primary or secondary changes in the process of degeneration. Moreover, it remains to be deter-

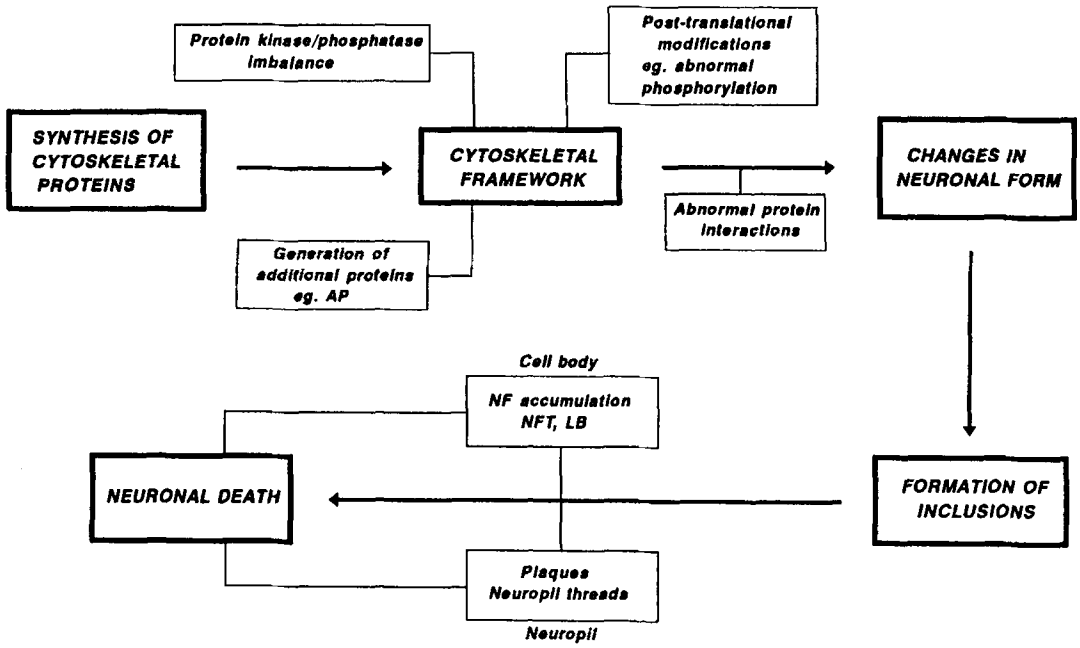

Fig. 1. Potential determinants of cytoskeletal modifications. Schematic diagram illustrates possible factors in cytoskeletal dynamics that may contribute to cytoskeletal change and neuronal degeneration in Alzheimer's and Parkinson's. Neuropil changes (senile plaques and neuropil threads) and intraneuronal inclusions (Lewy bodies and neurofibrillary tangles) occur as overlapping spectrums of degenerative conditions in both disorders. Abbreviations: AP, amyloid protein; NF, neurofilaments; NFT, neurofibrillary tangles; LB, Lewy bodies.

mined whether the various changes in cytoskeletal morphology are indeed primary and specific representations of neural degeneration or if they are simply nonspecific filamentous insults that reflect basic cellular dysfunctions (Fig. 1). It is recognized that cytoskeletal changes may represent a response owing to metabolic disturbances in the cytosol, reduction in neuronal (synaptic) interactions, or a change in trophic factor availability. Basic research has related these conditions to neuronal loss in various animal models of experimental neurodegeneration.

Modified Tau Proteins and Paired Helical Filaments

Peptide sequence analysis and immunohistochemical techniques have indicated that PHF share epitopes with a variety of tau protein isoforms (Goedert et al., 1989; Wischik et al., 1988a,b; Kondo et al., 1988). It is now apparent that modified tau (abnormally phosphorylated forms) is the major, if not the exclusive component of PHF in Alzheimer's (Lee et al., 1991; Greenberg and Davies, 1990). The microtubule-binding domains of tau are required for PHF stability (Ksiezak-Reding and Yen, 1991). Wille et al. (1992a) showed that the repeat region of recombinant human tau expressed in bacteria can dimerize in an antiparallel fashion that resembles the PHF from Alzheimer's. However, the self-assembly process in vitro apparently does not require phosphorylation of the protein.

Many studies have indicated that tau is posttranslationally modified in Alzheimer's via abnormal phosphorylation (Wood et al., 1986; Wolozin et al., 1986; Grundke-Iqbal et al., 1986; Flament et al., 1990a; Ksiezak-Reding et al., 1990a). The switch of tau protein to the modified state in Alzheimer's includes the phosphorylation of serine^{199}, serine^{202}, and/or serine^{396} motifs upstream of the microtubule-binding region (Lee et al., 1991; Biernat et al., 1992; Liu et al., 1993). The kinase activity at the former two serine sites

has the characteristics of a proline-directed kinase (Biernat et al., 1992). It is not known whether abnormal phosphorylation represents a casual event in PHF formation or whether it is a consequence of a proportion of tau being immobilized in cell bodies and dendrites rather than moving into the axon.

An initial reference to modified tau proteins (also named Alzheimer disease proteins) was by the term A68 (Wolozin et al., 1986). These proteins have been definitively identified as tau by antibody crossreactivity (Greenberg and Davies, 1990; Ksiezak-Reding et al., 1990b) and by direct protein sequencing (Lee et al., 1991). The distinct features of modified tau are now labeled as PHF-tau. PHF-tau is extremely insoluble in contrast to normal tau (Kosik, 1992). Enzymatic dephosphorylation of highly purified A68 results in reduction of the apparent mol wt and a shift in the electrophoretic mobility in a manner resembling normal tau (Goedert et al., 1992; Brion et al., 1991; Greenberg et al., 1992). All six brain tau isoforms are abnormally phosphorylated and contribute to the formation of A68 (Goedert et al., 1992). Recent amino acid composition analysis suggests that A68 contains more glycine and less lysine residues than normal adult tau (Liu et al., 1991). Hence, abnormal alternative gene splicing of tau or posttranslational modifications may be alternative processes to generate the A68 protein. A68 injections into the adult rat brain resist proteolysis in contrast to dephosphorylated forms of the A68 protein (Shin et al., 1993).

When tau is hyperphosphorylated, it loses the capacity to bind to microtubules (Rosenblum et al., 1990) and modified tau from Alzheimer brain tissue stimulates microtubule assembly with slower kinetics vs tau from age-matched controls (Lu and Wood, 1993). Bramblett et al. (1992) have shown a high correlation of brain areas affected with neurofibrillary abnormalities with reduced levels of microtubule binding competent tau. The abnormal phosphorylation of serine^{396} specifically reduces microtubule binding, and this may be mediated by the inappropriate activation of fetal kinases or by the reduced activity of tau protein phosphatases (Bramblett et al., 1993). They hypothesize that depletion of microtubule binding competent tau may adversely affect microtubule function and contribute to the degeneration of neurons in Alzheimer's.

Monoclonal antibodies that recognize PHF-tau epitopes include Alz-50 (Ksiezak-Reding et al., 1990a; Goedert et al., 1991), Tau-1 after dephosphorylation of PHF-tau (Grundke-Iqbal et al., 1986), and AT8 (Mercken et al., 1992). Alz-50, a monoclonal antibody raised against a basal forebrain homogenate of Alzheimer brain tissue, received considerable attention with the initial report that the antibody only recognized epitopes in the brains of Alzheimer patients (Wolozin et al., 1986). It is now apparent that this antibody is an isoform of tau, recognizes a phosphorylated epitope in the carboxy-terminus of tau (Uéda et al., 1990), and also identifies a limited population of normal neurons (Davies, 1992). In regard to Tau-1, Szendrei et al. (1993) identified a sequence of 13 amino acids that includes four serine residues in normal tau that bind to the Tau-1 monoclonal. Also, the selectivity of Tau-1 depends on the presence of a phosphate group and not on its location or change in secondary structure after phosphorylation (Szendrei et al., 1993). The AT8 antibody is currently considered to be most specific for the Alzheimer state since it reports on the state of tau phosphorylation (Biernat et al., 1992). This antibody is selective for PHF-tau without crossreactivity with normal tau. In addition, AT8 shares complete specificity for NFT, neuropil threads, and dystrophic neurites of senile plaques by immunocytochemistry in sections of the hippocampus taken from Alzheimer patients (Mercken et al., 1992). Another series of antibodies directed against altered phosphorylation states of tau proteins, named tau 55, 64, and 69, are also thought to be early markers of degeneration in Alzheimer's (Bancher et al., 1989a; Flament et al., 1990b). These antibodies have been mapped to cortical (Brodmann) areas of Alzheimer brain to show very heterogeneous patterns of neurofibrillary degeneration (Vermersch et al., 1992).

A number of reports indicated that P-NF antibodies react with NFT determinants in Alzheimer's (Anderton et al., 1982; Sternberger et al., 1985; Cork et al., 1986). It is presently known

that some of the antibodies thought to exclusively identify NF also crossreact with other phosphoproteins. For example, certain antisera to P-NF (medium and heavy NF forms) that identify protein domains on PHF and NFT also crossreact with the tau protein (Nukina and Ihara, 1986; Kosik et al., 1986; Miller et al., 1986; Ksiezak-Reding and Yen, 1987; Yen et al., 1987). However, the multiphosphorylation repeat domains specific for the carboxy-termini of NF-H and NF-M have been detected in NFT (Lee et al., 1988a; Zang et al., 1989).

Antibodies to multiple epitopes on the tau molecule indicate that there are at least two antigenically defined populations that associate with intracellular and extracellular NFT (also called ghost tangles) (Bondareff et al., 1990; Dickson et al., 1992). The intracellular NFT reacted with the carboxy- and amino-terminal regions of tau, but not with the microtubule-binding domain. Conversely, the extracellular tangles reacted preferentially with antibodies to the microtubule-binding region. Results by Endoh et al. (1993) also indicate that the extracellular NFT represents the final form of the tangle that has undergone extensive processing.

Neuropil Threads

In Alzheimer's (Kowall and Kosik, 1987) and Parkinson's (Ohtsubo et al., 1990), tau antibodies identify neuronal processes with normal morphology and fibers that are thickened, curly, or kinked (Kowall and Kosik, 1987; Braak and Braak, 1988). These abnormal appearing processes were originally termed neuropil threads and described as argyrophilic neuropil processes presenting as PHF by electron microscopy (Braak et al., 1986). The majority of neuropil threads contain straight filaments or PHF, but no NF (Perry et al., 1991).

Extensive meshworks of abnormal processes throughout Alzheimer's cortex in addition to NFT and senile plaques are revealed by tau immunoreactivity (Braak et al., 1986; Kowall and Kosik, 1987; Ihara, 1988). The meshworks consist of curly fibers, 5–30 μm long, that are densest in the pyramidal cell layer of CA1 of the hippocampus, layers II and IV of the entorhinal cortex, and layers III and V of the association cortex (Ihara, 1988). Curly fibers examined by the freeze-etched/replica method consist of PHF in both pre- and postsynaptic elements (Ohtsubo et al., 1990). It has been suggested that curly fibers may represent new regrowth of neuronal processes (Ihara, 1988). The role of the abnormal phosphorylated tau isoforms in relation to microtubule instability may be a factor in the generation of neuropil threads as they acquire aberrant morphology.

Lewy Bodies

Brainstem Lewy bodies (LB) are the distinctive inclusions of idiopathic Parkinson's. Subcortical LB have a dense central core, consisting of circular profiles and a rim of radiating peripheral filaments. In the cortex, the radial arrangement of filaments is not apparent. LB were originally shown to contain NF antigens by Goldman et al. (1983). The core and rim of LB can be immunolabeled with antibodies to NP-NF, P-NF, PHF, MAP1, MAP2, and β-APP (Forno et al., 1986; Goldman and Yen, 1986; Galloway et al., 1988, 1992; Bancher et al., 1989b; Arai et al., 1992a,b). LB react with antibodies that recognize all phosphoisoforms of NF-H and NF-M, including the least and most heavily phosphorylated variants (Hill et al., 1991). The entire extent of each NF subunit is found in LB but the NF subunits may be altered during the processing of these filaments into LB. Hill et al. (1991) have proposed that the NF polypeptide fragments generated as a result of LB formation may act as a signal to reduce NF protein synthesis. Reduced NF protein production would result in reduced movement of assembled NF into axons. The continuation of this cycle may then lead to a reduction in axonal diameter and synaptic dysfunction, eventually resulting in permanent loss of the nigrostriatal neurons.

In addition, LB, NFT, neuropil threads, and plaque-related dystrophic neurites can be visualized with antibodies to ubiquitin. This protein activates adenosine-triphosphate dependent proteins (Leigh et al., 1989) and is part of a carefully controlled multienzyme pathway for destroying abnormal and damaged proteins (Lowe et al.,

1993). Cortical LB react strongly with antibodies to ubiquitin (Love and Nicoll, 1992; Kuzuhara et al., 1988; Bancher et al., 1989b). Tau antibodies do not react with LB (Bancher et al., 1989b; Hill et al., 1991). It has also been determined that the ubiquitin targeted protein in PHF is tau and at least four sites in the microtubule-binding region of tau serve as ubiquitin acceptors (Morishima-Kawashima, 1993).

It is now apparent that LB are not specific to the pigmented nuclei in Parkinson's. With careful analyses, occasional LB can be found in the cerebral cortex in every case of Parkinson's (Quinn and Steiger, 1991). Neocortical LB are observed in the brains of patients affected with late life dementia that is clinically similar to Alzheimer's (Hansen et al., 1990). A variety of new terms have thus recently emerged to describe LB in combination with dementia and Alzheimer's (Quinn and Steiger, 1991). For example, LB can define a late life dementia known as diffuse LB disease, and a variation of Alzheimer's called the LB variant of Alzheimer's (Dickson et al., 1987, 1989; Perry et al., 1990).

The Integral Role of Phosphorylation and Dephosphorylation to Cytoskeletal Change

Considerable evidence exists to indicate that protein phosphorylation is important for a number of neuronal functions (Greengard, 1987). The addition of one or more phosphate groups to a protein alters the charge, thereby potentially changing its conformation and functional state. In relation to the cytoskeleton, proteins such as tau (Lindwall and Cole, 1984), MAP2 (Garner et al., 1988a; Tsuyama et al., 1986; Tucker et al., 1988), and the NP triplet (Julien and Mushynski, 1982) have been identified as substrates for specific protein kinases. Inappropriate phosphorylation events may modify the dynamics of microtubule and NF interactions and create highly specific changes in the cytoskeletal proteins that set up an irreversible cascade of filamentous modifications.

Proper control of phosphorylation is paramount to the maintenance of cytoskeletal function and intracellular protein interactions. At least two categories of modifications can modulate the binding of tau to microtubules (Kosik and Caceres, 1991). These include a splicing event in the microtubule-binding domain of tau and the phosphorylation state of tau. Phosphorylation is the only known posttranslational modification of tau (Kosik, 1993). Many of the identified and the putative phosphorylation sites of tau contain the dipeptide ser–pro or thr–pro. Phosphorylation at ser–pro motifs is capable of decreasing the microtubule binding of tau (Gustke et al., 1992) and alkaline phosphatase treatment of tau results in a rapid, extensive polymerization of microtubules (Lindwall and Cole, 1984).

Evidence can be provided to illustrate different modes of cytoskeletal arrangement based either on a sequence of phosphorylation events or perhaps even a single phosphate modification. In the case of NF, a variety of protein kinases and phosphatases related to the assembly, transport, and disposition of NF provides many possibilities for metabolic abnormalities that could have direct or indirect abnormal consequences on the spatial arrangements and aggregations of NF. For example, the differential timing of phosphate additions and turnover at specific sites on the NF subunits as they move out from the perikaryon to the axon may explain why inclusions tend to occur in specific sites (cell body, proximal, or distal axonal segments) in certain diseases (Sayre et al., 1985; Goldman and Yen, 1986; Watson et al., 1989). Implications of the complex regulation of NF function have been outlined by Nixon and Sihag (1991). Decreased carboxy-terminal phosphorylation may cause NF to move at faster than normal rates and hyperphosphorylation at the carboxy-terminal sites may promote abnormal accumulation of NF in axons. On the other hand, interference with phosphorylation at head domain sites, or premature phosphorylation of carboxy-terminal tails in the cell body, could be expected to promote inappropriate interaction of

NF with the cytoskeletal elements, in turn preventing NF entry into axons and thereby initiating the formation of neurofibrillary lesions within perikarya (Nixon and Sihag, 1991).

In contrast to the complex regulation of NF dynamics, can a single abnormal molecular event (e.g., phosphorylation of a single motif) be enough to drive the expression of a very prominent cytoskeletal change? On the basis of current data, the abnormal phosphorylation of tau appears conducive to both PHF formation and dissociation from the microtubule system. The possibility exists that normal tau has regulatory sequences outside the repeats that would normally prevent aggregation into PHF. Should these regions become phosphorylated, then bundles of PHF would form and potentially trigger the development of NFT.

Amyloid Protein and the Senile Plaque

Senile plaques represent foci in the neuropil that consist of amyloid, normal, and abnormal (referred to as dystrophic) neuronal processes. The major protein in the senile plaque is a 39–42 amino acid polypeptide referred to as the β-amyloid protein (β-AP, β/A4, or the A4 protein) (Masters et al., 1985; Selkoe et al., 1986) and it is derived from a large protein termed the β-amyloid protein precursor (β-APP). β-APP is a phosphoprotein that spans the transmembrane region of cells and is composed of at least four major isoforms, designated $\beta\text{-APP}_{695}$, $\beta\text{-APP}_{714}$, $\beta\text{-APP}_{751}$, and $\beta\text{-APP}_{770}$, which arise from alternate splicing of a single gene (Kang et al., 1987; Ponte et al., 1988; Tanzi et al., 1988; Golde et al., 1990). The β-AP portion of the precursor protein is located partially in the extracellular space and partially within the plasma membrane. A sequence in the transmembrane and cytoplasmic domains serves as a substrate for protein kinase C (Gandy et al., 1988; Oltersdorf et al., 1989). Phosphorylation of β-APP may regulate its internalization and catabolism (Buxbaum et al., 1990) and abnormal phosphorylation events may alter the metabolism of β-APP and lead to the deposition of β-AP (Masters et al., 1985; Gandy et al., 1988). Two routes of β-APP processing have been identified. Cleavage can occur within the β-AP domain of a secretory pathway to generate secreted forms of β-APP and non-amyloidogenic peptide fragments (Esch et al., 1990; Sisodia et al., 1990). A secreted form of β-APP produced by cleavage at the amino-terminus has been detected in human brain cell cultures and in cerebrospinal fluid (Seubert et al., 1993). In addition, cleavage may also occur within the endosomal–lysosomal pathway (Haass et al., 1991; Golde et al., 1992) to produce intact β-AP (Shoji et al., 1992; Seubert et al., 1992; Haass et al., 1992).

Ultrastructural methods indicate that β-AP epitopes are present in the intracellular (Yamaguchi et al., 1990, 1992) and the extracellular form of NFT (Tabaton et al., 1991). The major part of β-APP in the neurites of senile plaques is a fragment representing the N-terminal part of the molecule (Cole et al., 1991). Cras et al. (1991) observed that in the Alzheimer condition, immunoreactive dystrophic neurites within plaques are also positive to tau, ubiquitin, and P-NF. The abnormal distribution of tau and the NF epitopes in the dystrophic β-APP neurites also correlates with the extent of aberrant neurofibrillary staining in the surrounding brain tissue. Arai et al. (1990) suggest that A4 plaque protein is deposited as a result of local β-APP processing in association with tau and NF protein fragments.

Full-length β-APP undergoes fast anterograde axonal transport to nerve terminals (Koo et al., 1990; Schubert et al., 1991). Within sensory neurons of the dorsal root ganglia, evidence has been provided for $\beta\text{-APP}_{695}$ as the predominantly synthesized form to be anterogradely transported (Sisodia et al., 1993). It is likely that this isoform is also transported to nerve terminals in central neurons (Sisodia et al., 1993). The possibility exists that abnormal processing of β-APP in relation to axonal transport and interaction with other cytoskeletal components may significantly influence transport dynamics. Hence, abnormal processing of this protein may contribute to altered

cytoskeletal protein turnover and generate fragments that associate with PHF in neuronal cell bodies and the dystrophic processes in senile plaques.

Experimental Strategies to Model Cytoskeletal Change and Neurodegeneration

One of the most characteristic hallmarks of normal aging and of neurodegeneration is the shift in P-NF immunoreactivity from the axon to the somata. A number of animal models have been used to initiate defined changes in the cytoskeleton, including the expression of P-NF in the perikarya and proximal dendrites. Exposure to certain chemicals, aluminum salts, and physical injury to axons are the primary experimental methods that have been used to investigate mechanisms of cytoskeletal (primarily NF) dysfunction. Representative aspects of these systems are presented here.

Neurotoxins

The neurotoxins acrylamide, 2,5-hexanedione (2,5-HD), β,β'-iminodipropronitrile (IDPN), and acrylamide are chemicals used to create specific NF accumulations (focal swellings) in central and peripheral axons. Exposure to 2,5-HD produces NF aggregates within the preterminal segments of peripheral and central axons (Sayre et al., 1985). The appearance of axonal enlargements by 2,5-HD is thought to be directly related to accelerated NF transport (Monaco et al., 1990). When 2,5-HD is infused into the fimbria of adult rats, abnormalities in the fibers of basal forebrain cholinergic neurons are detected with an antibody against the low-mol-wt NF subunit (DiPatri and Butcher, 1991). In the case of IDPN administration, large axonal swellings are produced in the first proximal internodes of large myelinated sensory and motor nerve fibers without axonal degeneration or neuronal loss (Griffin and Price, 1980). IDPN selectively impairs the slow transport of NF proteins, likely by dissociating NF from microtubules (Griffin et al., 1978,1983) and the impaired anterograde transport causes the axonal enlargements. Aberrant NF phosphorylation of dorsal root ganglia neuronal cell bodies and an increase in the appearance of P-NF epitopes distal to IDPN-induced axonal swellings is also observed (Gold and Austin, 1991).

Animals chronically intoxicated with acrylamide show abnormalities of NF transport (Gold et al., 1985). Subsequent to daily ip injections of acrylamide, antibodies to phosphorylated epitopes identify a population of neuronal cell bodies in the dorsal root ganglia of rodents (Gold et al., 1988). A single high dose of acrylamide does not produce the abnormal immunoreactivity, suggesting that the changes in the spatial distribution of this NF reflect a secondary response of neurons to axonal injury and are not a result of a direct toxic effect (Gold et al., 1988). Aspects of MAP1 and MAP2 distribution in the rat brain after treatment with acrylamide are now being explored. This neurotoxicant differentially depletes the immunoreactivity of MAP1 and MAP2 in the extrapyramidal system (Chauhan et al., 1993).

Subpopulations of embryonic spinal cord and cortical neurons when treated with glutamate receptor agonists (including quisqualate and kainic acid) in culture induce the stabilization of the microtubular network by increasing MAP2 binding either directly or indirectly to microtubules (Bigot and Hunt, 1991). However, the excitatory amino acid-induced cytoskeletal rearrangement of MAP2 is reversible and is not linked to neuronal degeneration (Bigot and Hunt, 1991). These experiments indirectly indicate that modulation of glutamate receptors can influence the state of MAP2. Intrathecal injections of the excitatory amino acid kainate to the rat spinal cord produce abnormal immunoreactivity to phosphorylated NF in the perikarya and proximal dendrites of ventral horn neurons (Hugon and Vallat, 1990).

Aluminum Salts

Administration of aluminum (Al) salts to laboratory animals and to cells in vitro has been used

for almost three decades to investigate cytoskeletal changes. Most of the attention has centered on the spatial distribution of NF subtypes subsequent to application of Al derivatives.

The intrathecal exposure of Al salts into young rabbits causes prominent accumulations of neurofilaments (Selkoe et al., 1979; Troncosco et al., 1982; Bizzi and Gambetti, 1986; Muma et al., 1988) and induces abnormal phosphorylation states (Bizzi and Gambetti, 1986; Troncosco et al., 1986). Intrathecal administration of Al lactate to rabbits causes the expression of phosphorylated NF in the perikarya and proximal axons of spinal cord motor neurons (Troncosco et al., 1986) and subacute or chronic administration of Al maltol produces expression of perikaryal NF in cortical pyramidal neurons, projection neurons of the diencephalon, and spinal cord neurons (Katsetos et al., 1990). The accumulation of P-NF epitopes may result as an impairment of the slow axonal transport system (Bizzi et al., 1984; Troncoso et al., 1985).

Multivalent cations such as Al cause aggregations of purified NF in vitro (Troncosco et al., 1990). Explant cultures of rat dorsal root ganglia (Gilbert et al., 1992) and dissociated cultures of embryonic cortical neurons (Langui et al., 1990) have been used to study Al induced NF changes (Gilbert et al., 1992). The model of Gilbert et al. (1992) produces changes that are similar to the accumulations of NF and to the modifications of NF gene expression observed *in situ*. In addition to the effect on NF aggregation, Al inhibits calpain-mediated proteolysis of NF (Nixon et al., 1990), indicating that Al affects the turnover rate of NF.

Recently, dendrite arbor degeneration visualized by MAP2 immunostaining as a response to Al phosphate intoxication has been reported (Takeda et al., 1991; Wakayama et al., 1993). The content and phosphorylation of MAP2 in the brain are affected by exposure to Al. Elevated dietary aluminum levels significantly decrease the MAP2 levels in developing and adult rat hippocampus as determined by immunoblot and immunocytochemical analyses (Johnson et al., 1992). The mechanism concerned with reduction of MAP2 in dendrites has not been identified.

Axonal Lesions

Transection of axonal processes is used to analyze a wide variety of degenerative responses in neurons. In regard to NF distribution, axon specific antibodies can be detected in neuronal cell bodies and proximal dendrites subsequent to axonal injury. Different types of neurons in the CNS, including retinal ganglion cells (Dräger and Hofbauer, 1984), spinal cord neurons (Goldstein et al., 1987), dorsal root ganglia sensory neurons (Rosenfeld et al., 1987), septal cholinergic neurons (Koliatsos et al., 1989), and dopaminergic nigral neurons (Klosen et al., 1990) undergo a shift in P-NF immunoreactivity from the axon to the cell body after sectioning the axons. The change in P-NF distribution from the axonal to the somatic domain has been suggested to result from alterations in NF phosphorylation rather than from changes in the transport rate of P-NF (Goldstein et al., 1987).

Transgenic Animals

A number of lines of transgenic mice carrying various sequences for the β-APP under different promoters have been used to explore the relationship of amyloid protein over production with the neuronal abnormalities in Alzheimer's. Wirak et al. (1991) introduced a construct that encodes the 42 amino acid β-AP under control of the promoter for the human β-APP into two lines of transgenic mice. Descriptions of human β-AP accumulation in the hippocampi of the transgenic mice were presented. Quon et al. (1991) prepared transgenic mice that overexpressed the β-APP_{751} isoform. The cortical and hippocampal regions displayed β-amyloid immunoreactivity in neuronal processes and in extracellular regions as compact deposits (10–50 μm in diameter). No cytoskeletal changes were given. Another transgenic line carrying the sequence for the carboxy-terminal 104 amino acids of β-APP under control of the dystrophin promoter has also been described. In this line of mice, β/A4 immunoreactivity develops in neuronal cell bodies of the hippocampus and in processes described as curly, abnormal-appearing fibers (Kammesheidt et al., 1992). However, Jucker et al. (1992) have noted β-AP

deposits in nontransgenic mice. Hence, the conclusion that the β-AP transgene causes the formation of amyloid-like deposits cannot be considered valid until transgene-dependent phenotypes can be generated that are not predisposed to β-AP accumulation.

Transgenic lines of mice have been created by the microinjection of DNA with a leucine substitution in the codon homologous to the human prion protein (codon linked to the Gerstmann-Sträussler-Scheinker syndrome) (Hsiao et al., 1990). The mice carrying the transgene were described to develop clinical and pathological features of the GSS syndrome. Although these transgenic mice were described as a model of spontaneous neurodegeneration, no specific descriptions of cytoskeletal changes nor evidence of neuronal death (qualitative or quantitative) were reported. Only vacuoles (5–15 μm) containing an amorphous substance were shown to occur in a generalized manner in the white and gray matter. Overexpression of the *Mos* protooncogene in mice can also produce vacuolation in the neuropil and profiles of swollen degenerating neuronal processes (Propst et al., 1990). In addition, this study reported P-NF epitope expression in neuronal perikarya but showed no qualitative data (Propst et al., 1990).

Neural Transplants

Different transplantation paradigms have recently been developed to initiate neural degeneration and study the biological mechanisms that are possibly responsible for the cellular degeneration (Table 1). In 1987, the observation of neuronal death and aberrant cytoskeletal changes within CNS tissue transplanted to the PNS was made (Doering and Aguayo, 1987). Embryonic telencephalon was grafted to adult rat sciatic nerves in our initial experiments. Precursor cells differentiated into a variety of neuronal and glia cell types with normal morphological features and spatial distributions of cytoskeletal proteins. After 6 mo, a number of cytoskeletal changes were evident at the light and electron microscope levels. Specific alterations included axonal swellings, Hirano body formation, and the appearance of P-NF in the neuronal perikarya. Although a number of factors may trigger neuronal degeneration in these CNS transplants, it is now known that interactions with a neural target are critical to the long-term viability of the CNS neurons under circumstances of isolation in the PNS.

Support for the necessity of correct source–target neural interactions in the prevention of abnormal cytoskeletal changes has been obtained from three different transplant paradigms in rodents, namely the intracerebral, intraocular, and intrasciatic nerve systems. If intracerebral septal grafts do not make axonal connections with the host hippocampus, neurons express strong perikaryal immunoreactivity to the heavy P-NF subunit (Doering et al., 1991a). Grafts of fetal septum made to the anterior chamber of the eye also undergo a number of cytoskeletal changes that include axonal swellings, kinked axons, and abnormal fiber aggregates if not cografted with the hippocampal formation (Doering et al., 1991b). Most recently, evidence that an appropriate target is required to offset the cytoskeletal changes that parallel neuronal death has been obtained from the implantation of embryonic septal and hippocampal regions into adult sciatic nerves (Doering, 1992). When the septum is transplanted alone or with an inappropriate target area, septal neurons are not protected from the degenerative sequences.

Certain filamentous alterations that develop in these isolated CNS grafts within peripheral nerves are comparable to some of the changes that characterize the common neurodegenerative disorders. The particularly evident changes visualized with immunocytochemical probes include axonal swellings, curly, or kinked fibers, perikaryal expression of phosphorylated NF, often in tangled arrays, and the regression of dendrite arbors as monitored by MAP2 immunoreactivity. It is important to note that these changes occur *de novo* within the transplants. Unlike other methods to induce aberrations in the cytoskeleton, no physical or chemical intervention is required to generate these changes subsequent to the initial implantation of the fetal tissues into the peripheral nerves.

PC12 cells transfected with retroviral recombinants that express the carboxyl-terminal of β-

Table 1
Animal Transplant Paradigms to Study Neurodegeneration

Donor tissue	Host location	Key observations	Reference
Trisomy (Ts16) embryonic basal forebrain	Normal hippocampus	Cholinergic neuronal atrophy in transplant	Holtzman et al., 1992
Trisomy (Ts16) embryonic hippocampus	Normal retrosplenial cavity	β-APP, β/A4, PHF immunoreactivity in transplant	Richards et al., 1991
PC12 cells (transfected with carboxy terminal β-APP)	Normal cortex and hippocampus	Transplant degeneration Host cortical tissue atrophy Host Alz-50 and β-APP immunoreactivity adjacent to graft degeneration	Neve et al., 1992
Embryonic basal forebrain	Intracerebral aspiration cavity	P-NF in cholinergic neurons without long axonal connections	Doering et al., 1991a
Fetal septum	Anterior chamber of eye	Neuronal loss Decrease in NGF-R immunoreactivity P-NF staining in soma Swollen axons and abnormal fiber aggregates	Doering et al., 1991b
Fetal septum	Peripheral nerve	Neuronal loss Decrease in cholinergic and NGF-R staining P-NF in soma Tau positive curly fibers Dendrite (MAP2) regression	Doering, 1992

Abbreviations: β-APP, β-amyloid precursor protein; P-NF, phosphorylated neurofilaments; NGF-R, nerve growth factor receptor (p75); MAP2, microtubule-associated protein 2.

APP have been transplanted into the brains of newborn mice (Neve et al., 1992). Based on the massive cortical atrophy after 4 mo posttransplantation and the appearance of Alz-50 immunoreactivity, not within the degeneration but adjacent to the degenerative cortical zone, the authors reported specific neuropathology. However, no specific cytoskeletal changes or specific neuronal populations were identified other than a generalized cortical atrophy. Clearly, the effects reported are not directly related to the foreign cells since no abnormal features are seen until the transplants apparently die. Moreover, normal neurons can be labeled with Alz-50, and the appropriate controls in response to massive atrophy and blood-brain barrier insult were not reported.

Solid embryonic hippocampal grafts (Richards et al., 1991) and suspensions of fetal basal forebrain (Holtzman et al., 1992) or hippocampus (Fine et al., 1991) from trisomy 16 mice have been transplanted into normal young adult mice to

study the long-term survival and morphology of this tissue. In the study by Richards et al. (1991) antibodies to APP, β/A4, an abnormal form of tau, and a PHF preparation were employed to identify cells within the trisomy tissue after 4 mo posttransplantation. The grafted cells illustrated showed granular or diffuse immunoreactivity for these antibodies. No filamentous structures were reported in the cells and no cells were critically identified as neurons with components of the normal neuronal cytoskeleton. Although Richards et al. (1991) described the involvement of a slowly developing neuropathological process, no quantitation was provided in relation to age of the transplants. Atrophy of the transplanted cholinergic neurons in the hippocampus was observed by Holtzman et al. (1992). In the same model, denervation of the hippocampus produced a significant increase in the size of the grafted trisomy cholinergic neurons. No aspects of specific cytoskeletal morphology or abnormalities were reported in this study. In contrast to Richards et al. (1991), no β/A4 immunoreactivity was detected by either Holtzman et al. (1992) or by Fine et al. (1991) in the trisomy transplants.

Concluding Remarks

The factor(s) that trigger the sequence of degenerative events in Alzheimer's and Parkinson's are unknown. Whether specific modifications (e.g., hyperphosphorylation) of select cytoskeletal proteins set up an irreversible pattern of cytoskeletal reorganization or the aberrant alterations reflect a consequence concerned with other aspects of neuronal dysfunction (e.g., abnormal APP processing) remains to be determined.

Recent advances in biochemistry and molecular neurobiology have provided important insights into the cytoskeletal proteins that contribute to the common neuronal inclusions. Possible sequences of cytoskeletal modifications can now be reconstructed and evaluated. In particular, the abnormal phosphorylated forms of tau and the evidence for the self-assembly of tau protein into PHF-like structures represents a significant step in our understanding of potential neurofibrillary tangle formation. Knowledge of the types of proteins that comprise specific cytoskeletal modifications may eventually indicate the molecular events that precede the genesis of inclusions and provide clues to the etiology of the neuronal death disorders.

Finally, it is clear that continued refinement of the animal models of neurodegeneration in combination with the manipulation of the genome to govern the expression of selected proteins will provide the opportunity to explore new avenues of cytoskeletal rearrangement that parallel neuronal death.

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