Microtubule: A Common Target for Parkin and Parkinson's Disease Toxins

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Parkinson's disease (PD) is characterized by the selective loss of nigral dopaminergic (DA) neurons, which have long axons enriched with microtubules. Depolymerization of microtubules by PD toxins such as rotenone disrupts vesicular transport. The ensuing accumulation of vesicles in the cell body leads to increased cytosolic concentration of dopamine due to leakage of the vesicles. Elevated oxidative stress induced by dopamine oxidation may thus trigger the selective demise of DA neurons. Many strategies have been developed to protect DA neurons by stabilizing microtubules either directly or through intracellular signaling cascades. On the other hand, parkin, one of the most frequently mutated genes in PD, encodes for a protein-ubiquitin E3 ligase that strongly binds to microtubules. Parkin stabilizes microtubules through three domains that provide strong and independent interactions with tubulin and microtubules. These interactions anchor parkin on microtubules and may facilitate its E3 ligase activity on misfolded proteins transported along microtubules. Thus, parkin and rotenone, two prominent genetic and environmental factors linked to PD, act in an opposing manner on the same molecular target in the cell, microtubules, whose destruction underlies the selective vulnerability of dopaminergic neurons. NEUROSCIENTIST 12(6):469–476, 2006. DOI: 10.1177/1073858406293853

KEY WORDS *Microtubules*, *Parkinson's disease*, *Rotenone*, *Parkin*, *Tubulin*, *Dopamine*

Parkinson's disease (PD) is characterized by the relatively selective degeneration of dopaminergic neurons in substantia nigra pars compacta. Loss of these neurons leads to reduced dopamine input in the striatum, a brain region controlling voluntary locomotor activities. Most PD occurs sporadically, with no obvious inheritance pattern. A large number of epidemiological studies (Langston 2002), especially those performed on twins (Tanner and others 1999), reveal strong environmental connections to the disease. The concordance rates are virtually identical in monozygotic and dizygotic twins with age at onset older than 50. However, in PD twin pairs with age at onset younger than 50, the concordance rate in monozygotic twins (100%) is significantly higher than that in dizygotic twins (17%) (Tanner and others 1999). This study provides very clear evidence that the common, sporadic forms of late-onset PD are highly influenced by environmental factors, whereas the early-onset forms of PD have a strong genetic basis. Among the environmental factors examined, the use of pesticides and herbicides has consistently been found to be a significant risk factor (Di Monte 2003). Recent studies in animal models, using pesticides such as rotenone (Betarbet and others 2000), have demonstrated that PD-like symptoms and degeneration of dopaminergic (DA) neurons do occur after long-term exposure to these toxins.

On the other hand, mutations of five genes have been definitively linked to familial forms of PD (Moore and others 2005). Gain-of-function mutations of a synuclein or triplication of the wild-type allele are linked to a rare, early-onset form of PD. Dominant mutations of the LRRK2 gene appear to be a frequent cause of familial PD. In contrast, mutations of parkin, DJ-1, and PINK1 cause PD largely in a recessive manner. Among recessively inherited PD cases, mutations of parkin seem to be most prevalent (Moore and others 2005). In addition to these genes, mutations of UCH-L1, NR4A2 (Nurr1), and synphilin-1 have been implicated in rare cases of PD. Furthermore, several loci have been linked to familial forms of PD, although the responsible genes have not been identified (Moore and others 2005). Many lines of evidence have increasingly suggested that environmental and genetic factors associated with PD may impinge on common targets that are critical to the survival of dopaminergic neurons.

Parkin Strongly Binds to Microtubules and Ubiquitinates Tubulin

Microtubules are dynamic polymers of tubulin α/β heterodimers. A typical microtubule consists of 13 protofilaments wrapped laterally into a stiff, hollow tube. Each

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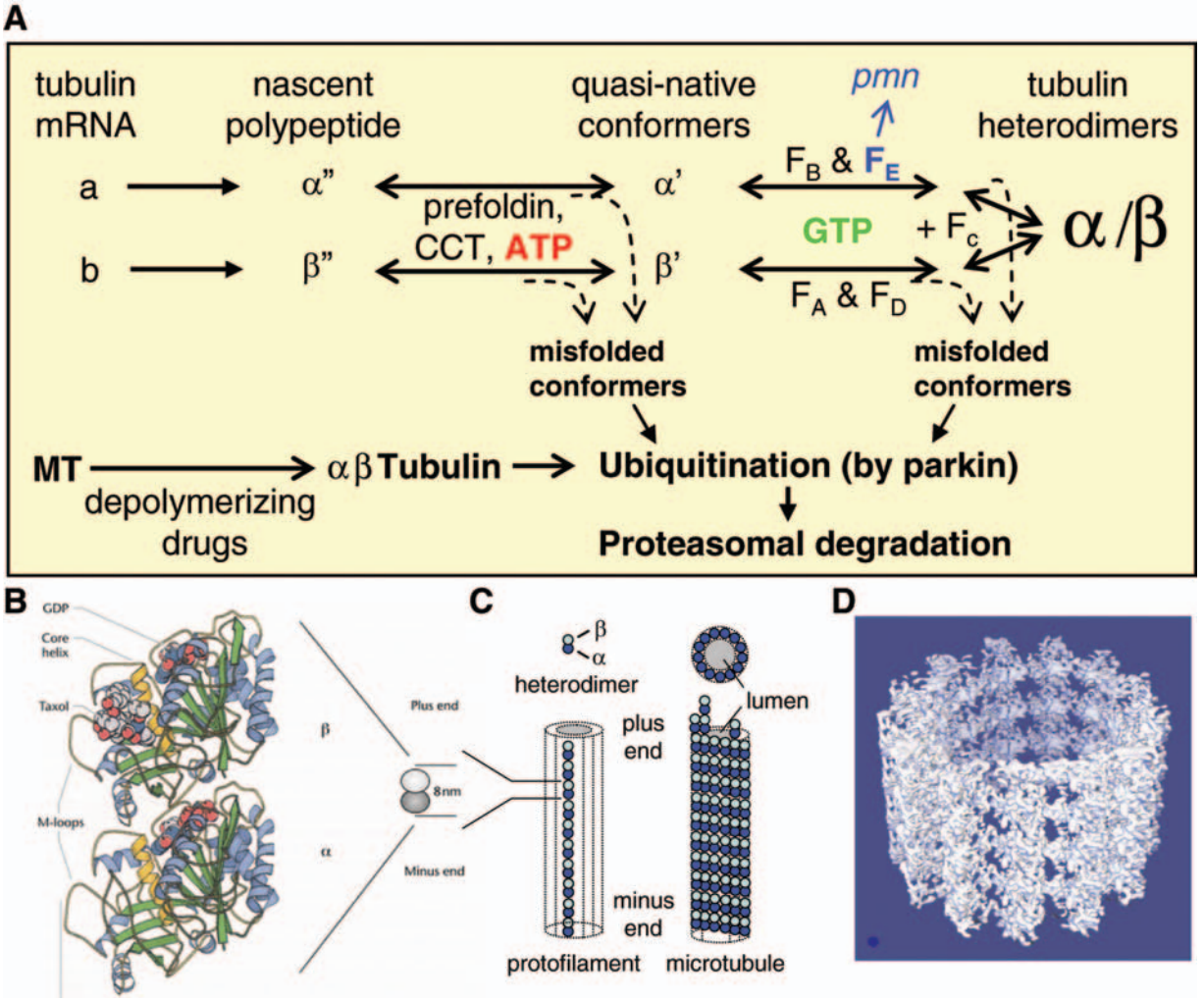

Fig. 1. Tubulin folding pathway and the assembly of tubulin into microtubules. *A*, Folding of nascent α and β tubulin polypeptides into polymerization-competent α/β heterodimers requires prefoldin, cytosolic chaperonin (CCT) and five tubulin-specific folding cofactors (F_A-F_E). The folding reactions are driven by ATP and GTP hydrolysis. Misfolded conformers may be ubiquitinated by parkin and are degraded by the proteasome. Microtubule (MT) depolymerization also triggers rapid degradation of tubulin, which may be mediated by ubiquitination and proteasome. A spontaneous mutation of tubulin folding cofactor E (F_E) causes progressive motorneuronopathy (pmn) in mice, illustrating the role of misfolded tubulin in selective neurodegeneration. *B*, Structure of tubulin α/β heterodimer with bound taxol. (Modified from *Encyclopedia of Life Sciences*, with permission from John Wiley & Sons Ltd.; Amos 2005). *C*, Assembly of tubulin heterodimers into microtubules, which typically consist of 13 protofilaments of tubulin arranged in head-to-tail fashion. *D*, Cryo-EM structure of microtubule. (Modified from Li and others 2002, with permission from Elsevier). GDP = guanosine diphosphate.

protofilament contains tubulin heterodimers arranged in a head-to-tail fashion (Fig. 1*B–D*). The noncovalent association between α and β tubulin is so tight that the tubulin heterodimer can be viewed as a single entity and is often referred to as tubulin for brevity. Our previous study has shown that parkin, a protein-ubiquitin E3 ligase linked to PD, ubiquitinates α and β tubulin and accelerates their degradation by the 26S proteasome; these effects are abolished by PD-linked mutations of parkin (Ren and others 2003). The synthesis of α- and β-tubulin polypeptides is very tightly regulated at the transcriptional and translational levels to ensure the equimolar production of both tubulins (Cleveland 1983), as overexpression of either tubulin gene is toxic to yeast cells at least (Burke and others 1989). The formation of polymerization-competent α/β heterodimers requires a series of folding reactions that are perhaps the most complicated among all known proteins (Lewis and others 1997). First, α or β polypeptide is folded into quasi-native conformers with the help of cytosolic chaperonins, which are ribosome-sized multisubunit complexes that facilitate protein folding in an ATP-dependent manner. Second, α and β monomers are captured by tubulin-specific folding cofactors. In a sequential, coordinated, and reversible folding process catalyzed by cofactors A through E, α/β heterodimers are formed with the hydrolysis of GTP. Misfolded tubulins

produced during this complicated process are quickly degraded by the proteasome (Wang and others 2006). As an E3 ligase for tubulins, parkin may ubiquitinate misfolded tubulins to facilitate their degradation (Fig. 1*A*).

The crucial role of misfolded tubulin in selective neurodegeneration is clearly illustrated in pmn mice, which exhibit progressive motor neuronopathy caused by a spontaneous mutation in tubulin-specific folding cofactor E (Martin and others 2002). Although this mutation should influence all types of cells owing to the critical role of cofactor E in the formation of native tubulin α/β heterodimers (Lewis and others 1997), only motor neurons are significantly affected in pmn mice. It is perhaps related to the length of axons in motor neurons, because such neurons from pmn mice have significantly shorter and swelling axons, as well as irregularly structured microtubules (Martin and others 2002).

Because the tubulin folding process is dependent on ATP and GTP hydrolysis (Lewis and others 1997), complex I-inhibiting toxins that reduce ATP production (e.g., rotenone and MPP^+ [N-methyl-4-phenylpyridinium]) may adversely affect the folding reaction and lead to increased production of misfolded tubulin. In addition, the ability of these PD toxins to depolymerize microtubules (Brinkley and others 1974; Marshall and Himes 1978; Cappelletti and others 1999; Cappelletti and others 2001) would further increase the amount of tubulin that needs to be degraded. Microtubule depolymerization leads to rapid degradation of tubulin, a protein that normally has a very long half-life (Cleveland 1989). Without functional parkin to ubiquitinate and degrade tubulin, cells may suffer from the same kind of toxicity caused by overexpression of tubulin (Burke and others 1989). As nigral DA neurons have very long axons projecting to the striatum, a very high percentage (>95%) of total cell volume is estimated to be in the axon, which contains large quantities of microtubules. Depolymerization of microtubules would cause more harm to neuronal processes than to the soma, because tubulin synthesis can rapidly replenish lost microtubules in the cell body, but not those in distal processes. This is because in neurons, microtubules in the processes are not assembled locally but are transported along existing microtubules from the cell body, where they are nucleated, assembled, and released from the centrosome (Baas 2002). Thus, microtubule depolymerization in the processes could have an avalanche effect owing to the inability to restore lost microtubules. Consequently, exposure of PD toxins such as rotenone or MPP^+ may result in much higher demand to ubiquitinate and degrade tubulin in nigral DA neurons than that in other types of cells with much smaller volume and shorter processes. Mutations that abrogate the E3 ligase activity of parkin toward tubulin may leave nigral DA neurons unprotected from excessive amount of tubulin, which may be toxic to the cell (Burke and others 1989).

In addition to its E3 ligase activity on tubulin, parkin binds strongly to microtubules through direct interactions with tubulin (Ren and others 2003). In taxol-mediated microtubule co-assembly assays, almost all parkin exists as a complex with microtubules in the pellet fraction. The binding between parkin and microtubules is so strong that they cannot be separated even with 3.8 M of NaCl. Parkin co-purifies with tubulin and is found in >99% pure tubulin preparation (Yang and others 2005). This tight binding of parkin with tubulin and microtubules is mediated by three separate domains of parkin that independently bind to tubulin and microtubules with high affinity (Fig. 2*A*). Consequently, the association of parkin with tubulin and microtubules is not significantly affected by PD-linked mutations that abrogate the E3 ligase activity of parkin, because each of these mutations would at best affect only one domain of parkin that binds to tubulin and microtubules (Yang and others 2005).

Consistent with the tight binding, parkin exhibits punctate subcellular localization along microtubules (Fig. 2*B*) (Ren and others 2003). This strategic location would greatly facilitate the E3 ligase activity of parkin toward many of its substrates, which are transmembrane proteins or membrane-associated proteins, such as Pael-R (Imai and others 2001), DAT (Jiang and others 2004), synaptotagmin XI (Huynh and others 2003), and CDCrel-1 (Zhang and others 2000). At least some of these proteins are prone to misfold in the endoplasmic reticulum (ER) (Imai and others 2001; Jiang and others 2004), which causes unfolded protein stress if left unchecked (Hampton 2002). Previous studies have demonstrated that misfolded membrane proteins are reversely translocated from the ER to the cytosol, where they must be immediately ubiquitinated to avoid aggregation due to the abundance of hydrophobic residues left exposed by the disordered polypeptide chain (Tsai and others 2002). Under normal situations, the ER is attached to microtubules to maintain its morphology and stability (Cole and Lippincott-Schwartz 1995). The proximity of the ER to parkin, which is anchored on microtubules, gives parkin ideal access to misfolded substrates as they are retrotranslocated from the ER (Fig. 2*C*).

Selective Vulnerability of DA Neurons to Microtubule Depolymerization

Systemic, chronic administration of rotenone, a widely used agricultural pesticide, produces selective degeneration of nigral DA neurons and PD-like locomotor symptoms in animal models (Betarbet and others 2000). Rotenone acts directly on two targets in the cell; it inhibits complex I of the mitochondrial respiratory chain (Chance and others 1963) and depolymerizes microtubules (Marshall and Himes 1978). The former activity impacts complex I in all cells of the brain and does not readily explain the selectivity of rotenone toxicity on DA neurons (Betarbet and others 2000). Our recent study has shown that the microtubule-depolymerizing activity of rotenone is critical in determining its selective toxicity (Ren and others 2005).

Rotenone depolymerizes purified microtubules in vitro, as well as microtubules in the cell (Brinkley and others 1974; Marshall and Himes 1978; Ren and others 2005), by binding to the colchicine site on tubulin heterodimers (Marshall and Himes 1978; Ren and others

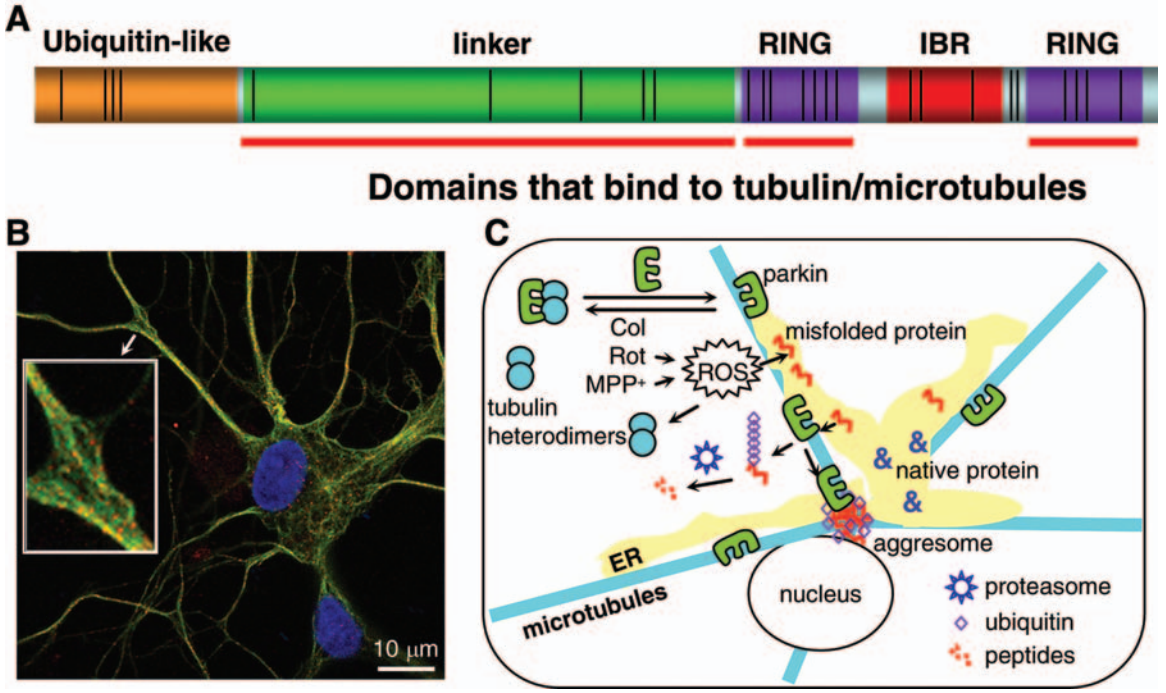

Fig. 2. Cellular functions of parkin in the context of microtubules. *A*, Functional domains of parkin. Three of the five domains of parkin provide strong, independent, and redundant binding to tubulin and microtubules. Parkinson's disease (PD)-linked point mutations in parkin (indicated by vertical bars) are generally clustered in domains critical for its protein-ubiquitin E3 ligase activity. *B*, Parkin exhibits punctate localization along microtubules. Cultured cortical neurons were co-stained for α-tubulin (green), parkin (red), and DNA (blue). Inset, an enlarged portion of the main image. (Modified from Ren and others 2003, with permission from the Society for Neuroscience). *C*, Parkin exerts its E3 ligase functions in the context of its interaction with tubulin and microtubules. The binding between parkin and microtubules stabilizes the microtubule network against depolymerizing agents including PD toxins rotenone and MPP^+ [N-methyl-4-phenylpyridinium]. The location of parkin on microtubules and the attachment of ER to microtubules suggest that parkin may efficiently ubiquitinate misfolded transmembrane protein substrates, which have to be retrotranslocated to the cytosol for ubiquitination and degradation. Parkin also ubiquitinates misfolded proteins transported along microtubules to the aggresome, which is located near the centrosome, an organelle anchoring microtubules (Modified from Yang and others 2005, with permission from ASBMB). IBR = in between ring finger; col = colchicine; rot = rotenone; ER = endoplasmic reticulum.

2005). Microtubule depolymerization disrupts vesicular transport, which leads to accumulation of vesicles in the soma. Because vesicles containing neurotransmitters are inherently leaky (Floor and others 1995; Liu and Edwards 1997), the accumulation of vesicles in the cell body increases cytosolic concentration of the neurotransmitter. In DA neurons, leakage of dopamine from the vesicles to the cytosol greatly elevates oxidative stress induced by DA oxidation, which triggers cell death (Fig. 3*B*) (Ren and others 2005). Neurons that do not contain an oxidizable neurotransmitter (e.g., GABAergic or glutamatergic neurons) are spared even though their microtubules are depolymerized to a similar extent (Ren and others 2005). In fact, the K_m values of vesicular monoamine transporter 2 (VMAT2) for monoamines are around 0.2 μM, whereas the K_ms for vesicular uptake of glutamate or GABA range from 0.3 to ~3 μM (Liu and Edwards 1997). This >1000-fold difference in the affinity of vesicular transporters toward various neurotransmitters ensures that the cytosolic concentrations of monoamines are kept at much lower levels than those of other neurotransmitters. The high efficiency of VMAT2 in sequestering monoamines greatly minimizes the oxidation of cytosolic monoamines including dopamine.

The selective toxicity of rotenone on DA neurons is mimicked by other microtubule-depolymerizing drugs such as colchicine or nocodazole and attenuated by the microtubule-stabilizing agent taxol (Fig. 3*C–E*) (Ren and others 2005). The model illustrated in Figure 3*B* is further substantiated by the results that the selective toxicity of microtubule-depolymerizing drugs (rotenone or colchicine) is greatly reduced by blocking dopamine synthesis or degradation. Thus, the selective vulnerability of midbrain DA neurons stems from the unique combination of their cell morphology and neurochemistry. These neurons have to deal with a toxic neurotransmitter that must be highly sequestered in vesicles, which are transported in axons along microtubules for a long distance to the target area (striatum).

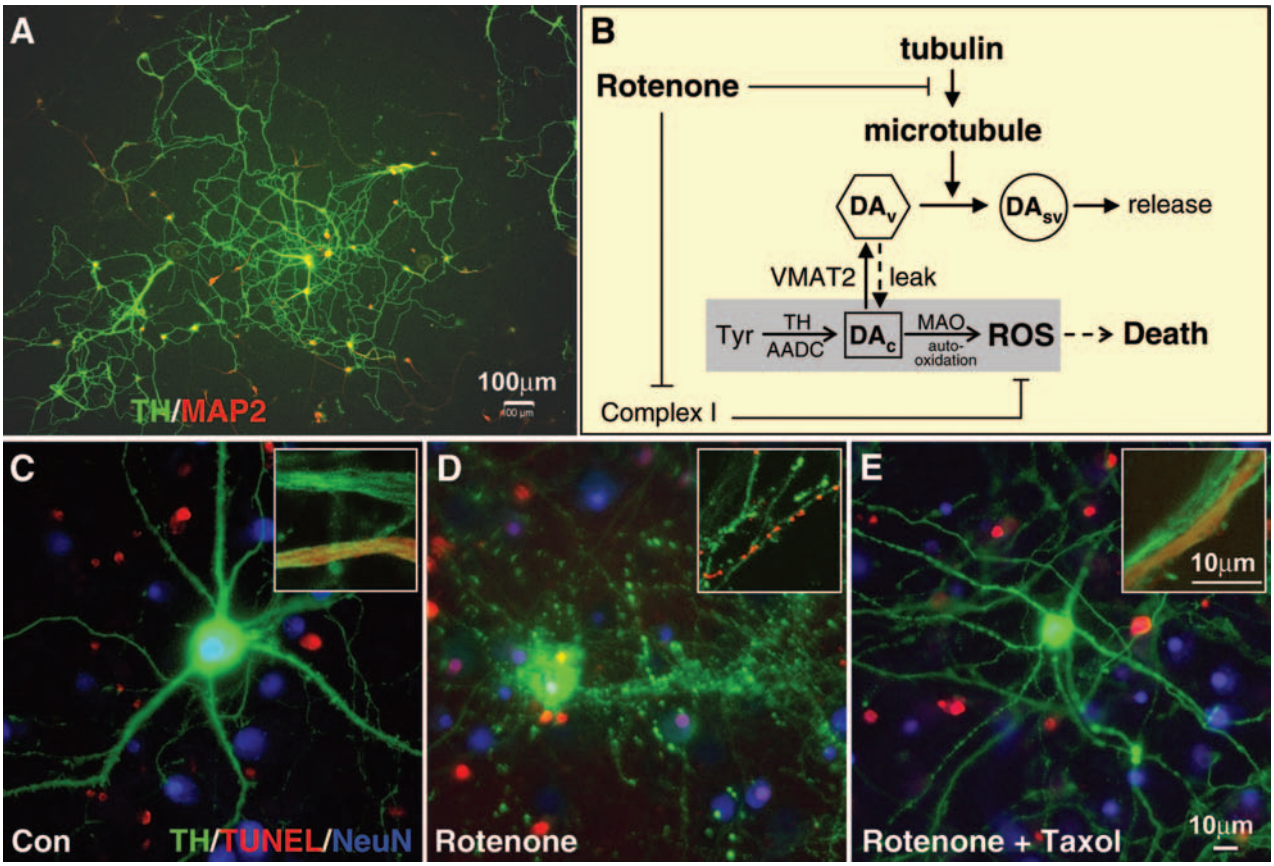

Fig. 3. Selective vulnerability of midbrain dopaminergic neurons to microtubule depolymerization. *A*, Cultured rat embryonic midbrain TH$^+$ neurons maintain the property of extending very long processes. Fixed cultures were co-stained for TH (green) and MAP2 (red, marking neurons). *B*, A working model on the selective toxicity of rotenone against midbrain dopaminergic (DA) neurons. The microtubule-depolymerizing activity of rotenone disrupts vesicle transport, which leads to vesicle accumulation in the soma and increased cytosolic concentration of dopamine due to vesicle leakage. Reactive oxygen species (ROS) produced by increased DA oxidation, in combination with those generated by complex I inhibition, render DA neurons much more vulnerable. DA metabolism (shaded gray) underlies the selectivity of rotenone toxicity. Arrows, positive effects; blunted bars, negative effects; DA $_{v, sv, c}$ = vesicular, synaptic vesicle, or cytosolic dopamine; Tyr = tyrosine; TH = tyrosine hydroxylase; AADC = L-aromatic amino acid decarboxylase; MAO = monoamine oxidase; VMAT2 = vesicular monoamine transporter 2; MAP = microtubule-associated protein. *C-E*, The selective toxicity of rotenone on midbrain TH$^+$ neurons and rotenone-induced microtubule depolymerization were significantly attenuated by the microtubule-stabilizing drug taxol. Midbrain neuronal cultures were treated without (*C*) or with (*D*) rotenone or rotenone plus taxol (*E*). Fixed cultures were stained for TH (green), TUNEL (red, marking cell death), and NeuN (blue, marking neurons). Inset, a portion of the process co-stained for TH (red) and α-tubulin (green) to illustrate the polymerization state of microtubules. Con = control; TUNEL = terminal deoxynucleotidyl transferase dUTP nick end labeling; NeuN = neuronal nuclear antigen. (*B-E* modified from Ren and others 2005, with permission from ASBMB.)

Superimposed on the microtubule-depolymerizing activity, the complex I–inhibiting activity of rotenone blocks the orderly transfer of high-energy electrons in the mitochondrial respiratory chain and produces reactive oxygen species in all cells (Takeshige and Minakami 1979), which apparently renders rotenone toxicity less selective than pure microtubule-depolymerizing agents such as colchicine or nocodazole (Ren and others 2005). Nevertheless, the combination of these two independent activities makes a potent PD toxin (Fig. 3*B*). It is perhaps not a coincidence that MPP$^+$, the active metabolite of the PD toxin MPTP, also inhibits complex I (Higgins and Greenamyre 1996) and depolymerizes microtubules in vivo and in vitro (Cappelletti and others 1999; Cappelletti and others 2001). Owing to its structural similarity to dopamine, MPP$^+$ is selectively taken up by dopaminergic neurons through the dopamine transport and thus causes the selective death of DA neurons.

A variety of natural and manmade chemicals in the environment have microtubule-destabilizing activities. For example, many plants synthesize microtubule-depolymerizing compounds such as cryptophycins, halichondrins, estramustine, colchicine, and so on (Jordan and Wilson 2004). On the other hand, many synthetic

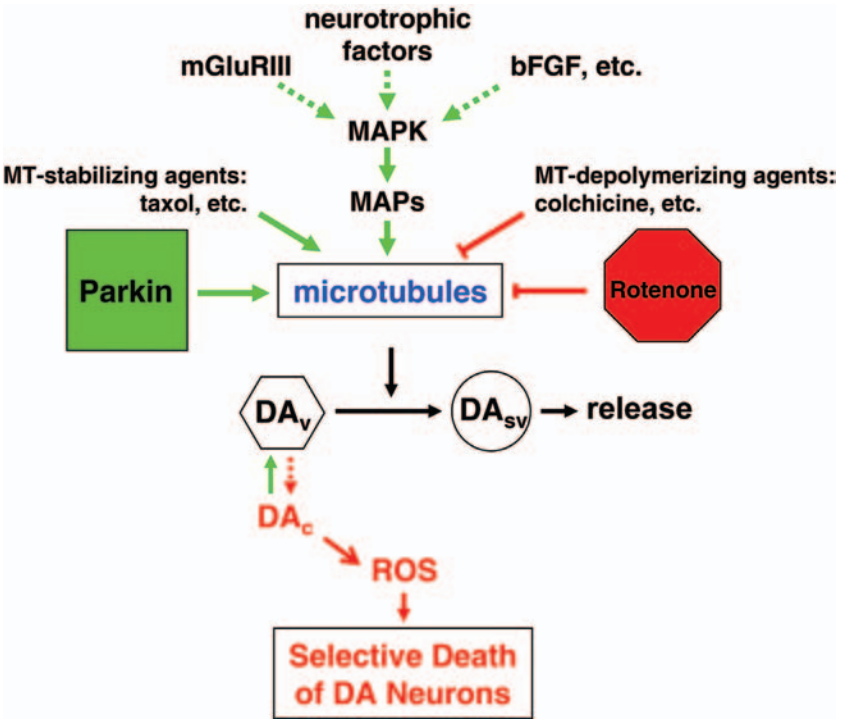

Fig. 4. Microtubule (MT) as a mechanistic and therapeutic target for Parkinson's disease. Parkin and rotenone, two prominent genetic and environmental factors for Parkinson's disease, converge their actions on microtubules, which are critical for the survival of dopaminergic (DA) neurons through obligatory functions in vesicle transport. Similar to rotenone, a variety of microtubule-depolymerizing agents in the environment may impact DA neurons through their actions on microtubules. Neuroprotective strategies can be developed by stabilizing microtubules either directly (e.g., using taxol) or indirectly through activation of intracellular signaling pathways that converge on the MAP kinase. mGluRIII = group III metabotropic glutamate receptors; bFGF = basic fibroblast growth factor; MAP = microtubule-associated protein; MAPK = MAP kinase; $DA_{v, sv, c}$ = vesicular, synaptic vesicle, or cytosolic dopamine; ROS = reactive oxygen species. Green arrow, positive action; blunted red bar, negative action; both regarding the survival of DA neurons.

herbicides, such as 2,4-Dichlorophenoxyacetic acid (Rosso and others 2000), dinitroaniline (Zeng and Baird 1999), and chlorpropham (Holy 1998) kill plants by disrupting microtubules. It seems that microtubule depolymerization is a general mechanism of action for many herbicides, despite their differences in chemical structures and efficacies in depolymerizing microtubules (Hoffman and Vaughn 1994). Furthermore, many antifungal or antiparasitic agents such as nocodazole, thiabendazole (Kiso and others 2004), and benzimidazole (Lacey and Gill 1994) also exert their actions through microtubule-depolymerization. The diversity and abundance of microtubule-depolymerizing agents in the environment make it worthwhile to examine their potential involvement in PD.

Protecting DA Neurons through Microtubule Stabilization

Our previous study has shown that application of the microtubule-stabilizing drug taxol greatly attenuates the selective toxicity of rotenone on cultured midbrain DA neurons (Ren and others 2005). In addition to using small-molecule compounds to directly stabilize microtubules, we have found novel methods to stabilize microtubules through activation of intracellular signaling pathways by group III metabotropic glutamate receptors (mGluRIII) (Jiang and others 2006b). Application of mGluRIII agonists such as L-AP4 attenuates the selective toxicity of rotenone on DA neurons by activating the microtubule-associated protein (MAP) kinase pathway to stabilize microtubules (Fig. 4) (Jiang and others 2006b). Recent studies increasingly suggest that manipulation of the metabotropic glutamatergic system in basal ganglia may be an effective therapeutic strategy for PD, which is characterized by a relatively selective loss of nigral DA neurons. Because the rest of the basal ganglia nuclei are largely intact, rebalancing the activities of the basal ganglia network by selective activation of certain metabotropic glutamate receptors appears to significantly alleviate PD-like symptoms in animal models (Conn and others 2005). For example, intracerebroventricular injection of L-AP4 markedly reduces locomotor deficiencies in various animal models of PD (Valenti and others 2003; Macinnes and others 2004). The neuroprotective effect of L-AP4 on DA neurons (Jiang and others 2006b) may work synergistically with its ability to modulate synaptic transmission in non-DA neurons in the basal ganglia (Conn and others 2005), especially at the early stage of PD before the heavy loss of nigral DA neurons.

Our recent study has shown that neurotrophic factors such as NGF (nerve growth factor), BDNF (brain-derived neurotrophic factor), and GDNF (glia cell line-derived neurotrophic factor) protect against the selective toxicity of rotenone on midbrain DA neurons through activation of the MAP kinase pathway and ensuing microtubule stabilization (Jiang and others 2006a). These neurotrophic factors significantly reduce the selective toxicity of rotenone or colchicine; they also stabilize microtubules against rotenone- or colchicine-induced depolymerization. Both effects are abolished when activation of the MAP kinase pathway is blocked by MAP kinase (MEK) inhibitors or a dominant-negative MEK1 construct. In addition to the MAP kinase pathway, the PI-3 kinase pathway is also involved in the protective effect of NGF against rotenone, but not colchicine. Activation of the PI-3 kinase pathway protects against complex I inhibition by rotenone or amytal, whereas activation of the MAP kinase pathway stabilizes microtubules (Jiang and others 2006a). Consistent with our findings, it has been shown recently that bFGF (basic fibroblast growth factor) attenuates rotenone toxicity through activation of MAP kinase and PI-3 kinase pathways (Hsuan and others 2006).

The convergence of protective effects from three diverse groups of agents—group III metabotropic glutamate receptor agonists, neurotrophic factors, and bFGF—on the MAP kinase pathway suggests that pharmacological manipulation of this pathway is an effective neuroprotective strategy for PD. Activated MAP kinase may phosphorylate microtubule-associated proteins to stabilize microtubules (Cassimeris and Spittle 2001). It may be possible to design small-molecule drugs to activate the MAP kinase pathway to stabilize microtubules against environmental PD toxins such as rotenone. Such a strategy may obviate the problem of delivering macromolecules such as neurotrophic factors, which have mixed results in clinical trials (Kirik and others 2004; Barker 2006). Obviously, bioavailability is a critical issue for this strategy to succeed. The poor penetration of taxol and L-AP4 across the blood-brain barrier significantly limits their potential values in treating PD.

Conclusions

Although the views on PD can be clouded by its diverse clinical manifestations and complex etiological factors, recent studies point to a general trend that would lead to defining the disease by its molecular and cellular mechanisms. This reductionist approach has been successfully used for many other complex diseases when a core set of clinical features are linked to a basic cellular mechanism. A prominent example is cancer, which is now viewed largely as a disease caused by genetic mutations that lead to uncontrolled cell proliferation (Vogelstein and Kinzler 2004). A parallel might be found for PD, where a significant body of work has overwhelmingly demonstrated the link between the degeneration of nigral DA neurons and the motor symptoms typically found in PD. By focusing on the selective demise of nigral DA neurons, it is possible to reduce the complexity of PD to a level malleable to mechanistic studies that address the root cause of the disease.

Within this conceptual framework, microtubules appear to be a critical piece for the survival and death of nigral DA neurons (Fig. 4). Unlike other cells or even many types of neurons, dopaminergic neurons in the substantia nigra need to transport a toxic substance sequestered in vesicles over a long distance on microtubules. Disruption of microtubules by PD toxins such as rotenone is particularly damaging to DA neurons owing to the constant spillage of neurotransmitters from vesicles and the propensity for dopamine to be oxidized. Based on such knowledge, a variety of strategies can be developed to protect nigral DA neurons from PD toxins like rotenone. These strategies either directly stabilize microtubules by compounds such as taxol or enhance microtubule stability through activation of intracellular signaling pathways that converge on MAP kinase. Another consequence of microtubule depolymerization is the increased demand to degrade tubulin freed up from microtubules. Parkin, a protein-ubiquitin E3 ligase linked to PD, catalyzes the ubiquitination and degradation of tubulin (Ren and others 2003) and stabilizes microtubules against depolymerizing agents (Yang and others 2005). The extremely tight binding between parkin and microtubules anchors this E3 ligase on microtubules so that it may act as a sentinel to efficiently ubiquitinate misfolded proteins being transported along microtubules or retrotranslocated from the ER. Thus, genetic factors such as parkin and environmental toxins such as rotenone act on the same molecular target—microtubules—to influence the survival and death of midbrain dopaminergic neurons.

References

Amos LA. 2005. Tubulin and microtubules. In: Encyclopedia of life sciences. Chichester (UK): Wiley.

Baas PW. 2002. Microtubule transport in the axon. Int Rev Cytol 212:41–62.

Barker RA. 2006. Continuing trials of GDNF in Parkinson's disease. Lancet Neurol 5:285–6.

Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT. 2000. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat Neurosci 3:1301–6.

Brinkley BR, Barham SS, Barranco SC, Fuller GM. 1974. Rotenone inhibition of spindle microtubule assembly in mammalian cells. Exp Cell Res 85:41–6.

Burke D, Gasdaska P, Hartwell L. 1989. Dominant effects of tubulin overexpression in saccharomyces-cerevisiae. Mol Cell Biol 9: 1049–59.

Cappelletti G, Maggioni MG, Maci R. 1999. Influence of MPP+ on the state of tubulin polymerisation in NGF-differentiated PC12 cells. J Neurosci Res 56:28–35.

Cappelletti G, Pedrotti B, Maggioni MG, Maci R. 2001. Microtubule assembly is directly affected by MPP(+) in vitro. Cell Biol Int 25:981–4.

Cassimeris L, Spittle C. 2001. Regulation of microtubule-associated proteins. Int Rev Cytol 210:163–226.

Chance B, Williams GR, Hollunger G. 1963. Inhibition of electron and energy transfer in mitochondria. I. Effects of amytal, thiopental, rotenone, progesterone, and methylene glycol. J Biol Chem 238: 418–31.

Cleveland DW. 1983. The tubulins: from DNA to RNA to protein and back again. Cell 34:330–2.

Cleveland DW. 1989. Autoregulated control of tubulin synthesis in animal cells. Curr Opin Cell Biol 1:10–14.
Cole NB, Lippincott-Schwartz J. 1995. Organization of organelles and membrane traffic by microtubules. Curr Opin Cell Biol 7:55–64.
Conn PJ, Battaglia G, Marino MJ, Nicoletti F. 2005. Metabotropic glutamate receptors in the basal ganglia motor circuit. Nat Rev Neurosci 6:787–98.
Di Monte DA. 2003. The environment and Parkinson's disease: is the nigrostriatal system preferentially targeted by neurotoxins? Lancet Neurol 2:531–8.
Floor E, Leventhal PS, Wang Y, Meng L, Chen W. 1995. Dynamic storage of dopamine in rat brain synaptic vesicles in vitro. J Neurochem 64:689–99.
Hampton RY. 2002. ER-associated degradation in protein quality control and cellular regulation. Curr Opin Cell Biol 14:476–82.
Higgins DS, Greenamyre JT. 1996. [H-3]dihydrorotenone binding to NADH: ubiquinone reductase (Complex I) of the electron transport chain: an autoradiographic study. J Neurosci 16:3807–16.
Hoffman JC, Vaughn KC. 1994. Mitotic disrupter herbicides act by a single mechanism but vary in efficacy. Protoplasma 179:16–25.
Holy J. 1998. Chlorpropham [isopropyl N-(3-chlorophenyl) carbamate] disrupts microtubule organization, cell division, and early development of sea urchin embryos. J Toxicol Environ Health A 54:319–33.
Hsuan SL, Klintworth HM, Xia Z. 2006. Basic fibroblast growth factor protects against rotenone-induced dopaminergic cell death through activation of extracellular signal-regulated kinases 1/2 and phosphatidylinositol-3 kinase pathways. J Neurosci 26:4481–91.
Huynh DP, Scoles DR, Nguyen D, Pulst SM. 2003. The autosomal recessive juvenile Parkinson disease gene product, parkin, interacts with and ubiquitinates synaptotagmin XI. Hum Mol Genet 12: 2587–97.
Imai Y, Soda M, Inoue H, Hattori N, Mizuno Y, Takahashi R. 2001. An unfolded putative transmembrane polypeptide, which can lead to endoplasmic reticulum stress, is a substrate of Parkin Cell 105:891–902.
Jiang H, Jiang Q, Feng J. 2004. Parkin increases dopamine uptake by enhancing the cell surface expression of dopamine transporter. J Biol Chem 279:54380–6.
Jiang Q, Yan Z, Feng J. 2006a. Neurotrophic factors stabilize microtubules and protect against rotenone toxicity on dopaminergic neurons. J Biol Chem 281:29391-400.
Jiang Q, Yan Z, Feng J. 2006b. Activation of group III metabotropic glutamate receptors attenuates rotenone toxicity on dopaminergic neurons through a microtubule-dependent mechanism. J Neurosci 26:4318–28.
Jordan MA, Wilson L. 2004. Microtubules as a target for anticancer drugs. Nat Rev Cancer 4:253–65.
Kirik D, Georgievska B, Bjorklund A. 2004. Localized striatal delivery of GDNF as a treatment for Parkinson disease. Nat Neurosci 7: 105–10.
Kiso T, Fujita K, Ping X, Tanaka T, Taniguchi M. 2004. Screening for microtubule-disrupting antifungal agents by using a mitotic-arrest mutant of Aspergillus nidulans and novel action of phenylalanine derivatives accompanying tubulin loss. Antimicrob Agents Chemother 48:1739–48.
Lacey E, Gill JH. 1994. Biochemistry of benzimidazole resistance. Acta Trop 56:245–62.
Langston JW. 2002. Parkinson's disease: current and future challenges. Neurotoxicology 23:443–50.
Lewis SA, Tian GL, Cowan NJ. 1997. The alpha- and beta-tubulin folding pathways. Trends Cell Biol 7:479–84.
Li H, DeRosier DJ, Nicholson WV, Nogales E, Downing KH. 2002. Microtubule structure at 8 A resolution. Structure 10:1317–28.
Liu Y, Edwards RH. 1997. The role of vesicular transport proteins in synaptic transmission and neural degeneration. Annu Rev Neurosci 20:125–56.
Macinnes N, Messenger MJ, Duty S. 2004. Activation of group III metabotropic glutamate receptors in selected regions of the basal ganglia alleviates akinesia in the reserpine-treated rat. Br J Pharmacol 141:15–22.
Marshall LE, Himes RH. 1978. Rotenone inhibition of tubulin self-assembly. Biochim Biophys Acta 543:590–4.
Martin N, Jaubert J, Gounon P, Salido E, Haase G, Szatanik M, and others. 2002. A missense mutation in Tbce causes progressive motor neuronopathy in mice. Nat Genet 32:443–7.
Moore DJ, West AB, Dawson VL, Dawson TM. 2005. Molecular pathophysiology of Parkinson's disease. Annu Rev Neurosci 28:57–87.
Ren Y, Liu W, Jiang H, Jiang Q, Feng J. 2005. Selective vulnerability of dopaminergic neurons to microtubule depolymerization. J Biol Chem 280:34105–12.
Ren Y, Zhao JH, Feng J. 2003. Parkin binds to alpha/beta tubulin and increases their ubiquitination and degradation. J Neurosci 23: 3316–24.
Rosso SB, Caceres AO, de Duffard AM, Duffard RO, Quiroga S. 2000. 2,4-Dichlorophenoxyacetic acid disrupts the cytoskeleton and disorganizes the Golgi apparatus of cultured neurons. Toxicol Sci 56:133–40.
Takeshige K, Minakami S. 1979. NADH- and NADPH-dependent formation of superoxide anions by bovine heart submitochondrial particles and NADH-ubiquinone reductase preparation. Biochem J 180:129–35.
Tanner CM, Ottman R, Goldman SM, Ellenberg J, Chan P, Mayeux R, Langston JW. 1999. Parkinson disease in twins: an etiologic study. JAMA 281:341–6.
Tsai B, Ye Y, Rapoport TA. 2002. Retro-translocation of proteins from the endoplasmic reticulum into the cytosol. Nat Rev Mol Cell Biol 3:246–55.
Valenti O, Marino MJ, Wittmann M, Lis E, DiLella AG, Kinney GG, and others. 2003. Group III metabotropic glutamate receptor-mediated modulation of the striatopallidal synapse. J Neurosci 23:7218–26.
Vogelstein B, Kinzler KW. 2004. Cancer genes and the pathways they control. Nat Med 10:789–99.
Wang Y, Tian G, Cowan NJ, Cabral F. 2006. Mutations affecting beta-tubulin folding and degradation. J Biol Chem 281:13628–35.
Yang F, Jiang Q, Zhao J, Ren Y, Sutton MD, Feng J. 2005. Parkin stabilizes microtubules through strong binding mediated by three independent domains. J Biol Chem 280:17154–62.
Zeng L, Baird WV. 1999. Inheritance of resistance to anti-microtubule dinitroaniline herbicides in an "intermediate" resistant biotype of Eleusine indica (Poaceae). Am J Bot 86:940.
Zhang Y, Gao J, Chung KK, Huang H, Dawson VL, Dawson TM. 2000. Parkin functions as an E2-dependent ubiquitin-protein ligase and promotes the degradation of the synaptic vesicle-associated protein, CDCrel-1. Proc Natl Acad Sci U S A 97:13354–59.