Parkin Is Associated with Actin Filaments in Neuronal and Nonneural Cells

Duong P. Huynh, PhD,* Daniel R. Scoles, PhD,* Trang H. Ho, BS,* Marc R. Del Bigio, MD, PhD,† and Stefan-M. Pulst, MD*‡

Inactivating mutations of the gene encoding parkin are responsible for autosomal recessive juvenile parkinsonism (AR-JP). However, little information is known about the function and distribution of parkin. We generated antibodies to two different peptides of parkin. By Western blot analysis and immunohistochemistry, we found that parkin is a 50-kd protein that is expressed in neuronal processes and cytoplasm of selected neurons in the basal ganglia, midbrain, cerebellum, and cerebral cortex. Unlike ubiquitin and α-synuclein, parkin labeling was not found in Lewy bodies of four sporadic Parkinson disease brains. Parkin was colocalized with actin filaments but not with microtubules in COS1 kidney cells and nerve growth factor–induced PC12 neurons. These results point to the importance of the cytoskeleton and associated proteins in neurodegeneration.

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Parkinson's disease (PD) is a progressive neurodegenerative disorder that predominantly leads to loss of pigmented neurons in the substantia nigra and other pigmented nuclei. Mutations in two genes result in mendelian forms of PD. Mutations in the α-*synuclein* gene cause autosomal dominant PD.[1,2] Two mutations in the α-*synuclein* gene (A53T and A30P) are responsible for an early-onset form of autosomal dominant PD and cause accelerated α-synuclein fibril formation.[3] α-Synuclein is a small protein associated with the 5- and 10-nm filaments in the nervous system,[4] and it is also found in the nucleus and presynaptic terminals of neurons,[5] perhaps contributing to early onset. A unique pathological feature of sporadic and autosomal dominant PD is the presence of Lewy bodies in neurons of the substantia nigra, locus ceruleus, nucleus basalis, hypothalamus, and cerebral cortex.[6,7] Lewy bodies are cytoplasmic inclusion structures that consist of aggregated proteins, including, among others, α-synuclein,[5] ubiquitin,[5] and ubiquitin C-terminal hydroxylase (UCH-L1).[8]

Autosomal recessive juvenile parkinsonism (AR-JP) is characterized by loss of dopaminergic neurons in the substantia nigra without Lewy body formation.[9–11] The mutated gene causing AR-JP was identified and its gene product designated parkin.[12] Missense and exon-deletion mutations in the parkin gene were also found in older PD patients.[13] The parkin gene is 1,496 nucleotides in length, has 12 exons, and is predicted to encode a 51.6-kd protein containing 465 amino acid residues. Parkin has a ubiquitin-like domain consisting of 76 amino acid residues at the N-terminus (Fig 1) and a RING-finger motif rich with cysteine residues between amino acids 417 and 450.[12] Parkin was strongly expressed in the brains of normal individuals and sporadic PD patients, but it was absent from AR-JP patient brains, suggesting that mutated parkin was unstable.[14]

In this study, we used confocal immunofluorescent labeling to show that parkin localizes to cytoskeletal structures. In contrast to previous findings,[14] parkin did not localize in the Golgi apparatus or to mitochondria.

Materials and Methods

Construction of pEGFPC1 Parkin (89–1,499 bp)

We obtained the full-length parkin cDNA by a two-step polymerase chain reaction (PCR) from an adult human brain cDNA library. We designed a 5′ fragment primer pair (parkFLA: CTACCCAGTGACCATGATAG, bp 89–97; parkB: CTCTCCCAGAATCCTGAAGTGA, complementary to bp 1,007–1,027) to amplify the 5′ cDNA fragment of bp 89–1,027, and a 3′ primer pair (parkC: CTGTCCCAACTCCTTGA TTAAA, bp 977–998; parkFLB: CTACACGTCGAACCAGTG, complementary to bp 1481–1499) to

From the *Rose Moss Laboratory for Parkinson's Disease and Neurodegenerative Disorders, CSMC Burns and Allen Research Institute, Cedars-Sinai Medical Center, and ‡Division of Neurology, Cedars-Sinai Medical Center, School of Medicine, University of California at Los Angeles, Los Angeles, CA; and †Department of Pathology, Health Sciences Centre and University of Manitoba, Manitoba Winnipeg, Canada.

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Address correspondence to Dr Pulst, Division of Neurology, Room 8909, Cedars-Sinai Medical Center, 8700 Beverly Boulevard, Los Angeles, CA 90048.

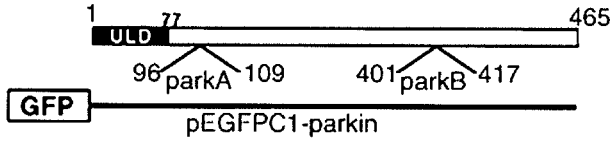

Fig 1. Map of parkin and peptides. The parkin map is based on the primary amino acid sequence published in Kitada and colleagues.[12] ULD represents the ubiquitin-like domain of the first 76 amino acids. ParkA and parkB are two peptides used to produce antibodies against parkin. pEGFPC1-parkin is a mammalian expression construct expressing the full-length parkin conjugated to the reporter protein, green fluorescent protein (GFP).

amplify the 3′ cDNA fragment of bp 977–1,499. Reverse transcriptase PCR was performed in a human brain cDNA library using the Expand High Fidelity PCR System following the vendor's protocol (Boehringer Mannheim, Indianapolis, IN). The full-length parkin cDNA (at bp 89–1,499 in Kitada and colleagues[12]) was obtained by ligating the two fragments at the *Sac*I site (at bp 1,009–1,012) and then ligated in-frame into pEGFPC1 vector (Clontech).

Antibody Production

Two peptides at amino acids (aa) 96–109 (parkA) and aa 440–415 (parkB)[12] were conjugated to keyhole limpet hemocyanin and injected into 2 rabbits. Antisera were purified by peptide column affinity purification.[15] These peptides did not have any sequence homology to actin. Antibodies were produced at Quality Controlled Biochemicals (Hopkinton, MA).

Protein Extraction and Western Blots

Frozen brain tissues from neurological normal brains or from a human neuron-like neuroblastoma cell line, HTB10, were used to isolate subcellular protein fractions using differential centrifugation as previously described.[15] Protein concentrations were determined using the Bradford Protein Assay Kit (BioRad, Hercules, CA).

Approximately 100 μg of total protein extract was loaded in each lane of a 15-well, precast 4 to 20% gradient SDS-polyacrylamide mini-gel (BioRad) and run at 100 V for 1 to 2 hours. Proteins were transferred to nitrocellulose filter (Amersham, Piscataway, NJ). The filter was rinsed briefly with Tris-buffered saline (TBS) (150 mM NaCl, 50 mM Tris-HCl, pH 8.0), blocked with 5% nonfat dried milk (BioRad), and incubated with desired dilutions of tested antibodies in TBST (TBS + 0.1% Tween20) for 1 hour at room temperature. Primary antibodies were detected using the ECL Western Blotting Detection System (Amersham).

Immunofluorescent Cytochemistry

For immunofluorescent studies, COS1 cells were washed with Dulbecco phosphate buffered saline (DPBS) three times at room temperature and incubated with 4% paraformaldehyde (Sigma, St Louis, MO) for 10 minutes. Fixed cells were pretreated with 0.1% Triton X-100, then incubated for 30 minutes with 3% goat serum in DPBS and colabeled for 1 hour at room temperature with 15 μg IgG/ml parkA or 120 μg/ml parkB antibody and mouse monoclonal antibody (MAb) to γ-adaptin (Sigma), Golgi58K (Calbiochem, San Diego, CA), clathrin (Chemicon, Temecula, CA), or β-tubulin (Sigma). Primary antibodies were detected with fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG and tetramethyl rhodamine isothiocyanate (TRITC)-conjugated anti-mouse IgG (Sigma). To visualize colocalization with actin filaments, labeled COS1 cells were incubated with TRITC-conjugated phalloidin A (Sigma) for 5 minutes. Images were acquired using a Zeiss LSM 310 confocal microscope at the Brain Imaging Center, UCLA School of Medicine, Los Angeles, CA.

Treatment of COS1 Cells with Cytoskeletal Dispersing Agents

To determine which types of filaments parkin binds, COS1 cells were treated with either cytochalasin D or nocodazole (Sigma). To disrupt the organization of actin filaments, COS1 cells were grown in four-well slides for 48 hours, treated with 20 μM cytochalasin D for 2 hours, and washed with culture medium. Cytochalasin D is a fungal toxin that causes actin filaments to depolymerize.[16] COS1 cells were also treated with 5 nM nocodazole for 30 minutes to destabilize microtubule organization. Treated and untreated cells were labeled with parkA antibody and β-tubulin MAb, or parkA antibody and TRITC-phalloidin A.

PC12 Culture and NGF Neuronal Induction

Twenty thousand rat PC12 cells were grown in four-well culture slides coated with 30 μg/ml of Engelbeth-Holm-Swarm manx sarcoma (EHS) cell matrix (Promega, Madison, WI) overnight. The next day, the culture medium was replaced with fresh medium containing 100 ng/ml 2.5S nerve growth factor (NGF) for 7 days to induce neuronal differentiation.

Immunohistochemistry

Human brain tissues obtained at necropsy within 24 hours of death were fixed with 10% formalin for at least 2 weeks. Selected brain regions were removed and embedded in paraffin. Six-micron-thick sections were obtained from 3 patients with sporadic PD who died at 59, 70, and 78 years of age, 2 patients with Alzheimer's disease who died at ages 75 and 85 years, and 2 neurologically normal individuals who died suddenly of cardiac causes at 60 and 70 years of age. Sections were rehydrated by rinsing twice with 5-minute interval in xylene, 100% ethanol, 95% ethanol, and 70% ethanol. Immunohistochemical labeling procedures were as described in Huynh and colleagues.[15]

Results

Biochemical Analysis of Parkin

We generated rabbit antibodies to peptides parkA and parkB, located at opposite ends of parkin (see Fig 1). Affinity purified parkA and parkB antibodies specifically detected a 70-kd green fluorescent protein (GFP)–parkin fusion protein expressed in COS1 cells that were transiently transfected with pEGFPC1-parkin vector, but they did not detect the 70-kd band in the

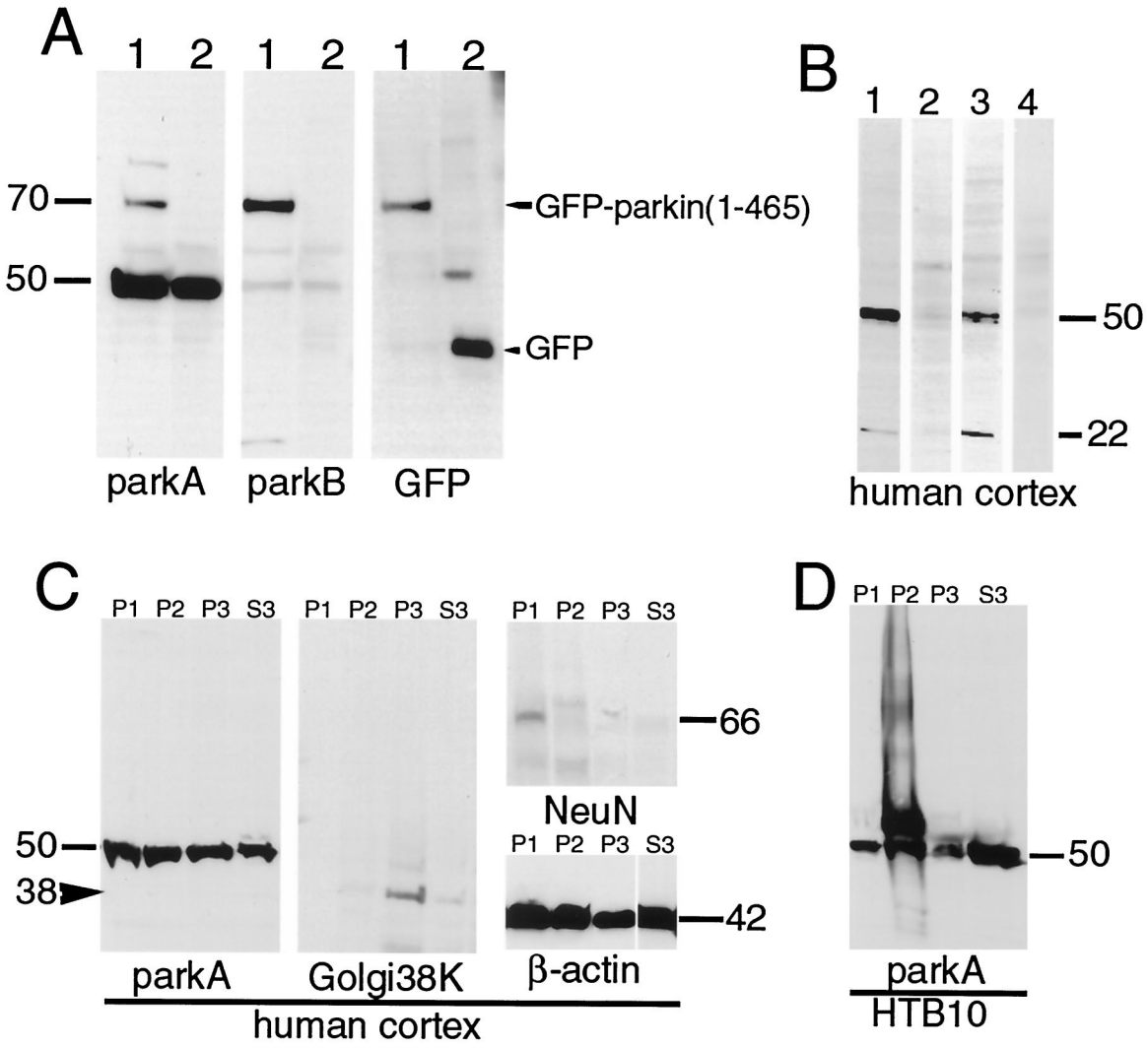

Fig 2. Biochemical analysis of parkin. (A) Immunoblots of protein extracts from pEGFPC1-parkin (lane 1) and pEGFPC1 (lane 2) transfected COS1 cells with antibodies to parkA, parkB, and green fluorescent protein (GFP). Each lane contained 100 μg of total protein extract. All antibodies detected the GFP-parkin fusion protein at 70 kd (lane 1). The native parkin at 50 kd was detected by antibodies to parkA and parkB but not by GFP antibody. The 27-kd GFP band was detected only by the GFP antibody. (B) Specificity of parkA and parkB antibodies. Immunoblot strips containing protein extract from human brain cortex were incubated with 1 μg IgG/ml of parkA antibody (lane 1), parkA antibody plus 100 μM parkA peptide (lane 2), parkB antibody (lane 3), or parkB antibody plus parkB peptide (lane 4). Both parkA and parkB antibodies detected the full-length parkin at 50 kd and a smaller band at 22 kd, and both protein bands were undetectable by peptide preabsorbed antibodies. In both COS1 and human brain protein extracts, the parkA antibody exhibited stronger immunoreactivity than the parkB antibody. (C) Parkin is present in all subcellular protein fractions from human brain cortical protein extracts. Fifty μg of subcellular proteins from nuclear (P1), mitochondrial (P2), microsomal/Golgi (P3), and cytosol (S3) fractions were loaded in each lane and immunoblotted with antibodies to parkA, Golgi38K, NeuN, and β-actin. Both antibodies to parkin and β-actin detected their respective antigens in all subcellular fractions. The subcellular proteins were further confirmed by the enhanced level of the Golgi38K in the P3, and the 66-kd neuronal nuclear protein, NeuN, in the P1 fractions. (D) Parkin was also found in subcellular fractions P1, P2, P3, and S3 from a neuroblastoma cell line, HTB10. Interestingly, in this neuronal-like cell line, an unknown additional band was detected in the mitochondrial P2 fraction.

pEGFPC1 control vector–transfected COS1 cells (Fig 2A). An antibody GFP also detected the 70-kd GFP-parkin fusion protein, and a 27-kd band representing GFP in COS1 cells transiently transfected with the pEGFPC1 vector. The parkA antibody also strongly recognized a 50-kd protein representing endogenous parkin in COS1 cells. The 50-kd protein was faintly detected by the parkB antibody.

In extracts of human cortex, both parkA and parkB antibodies detected an intense band at 50 kd and a

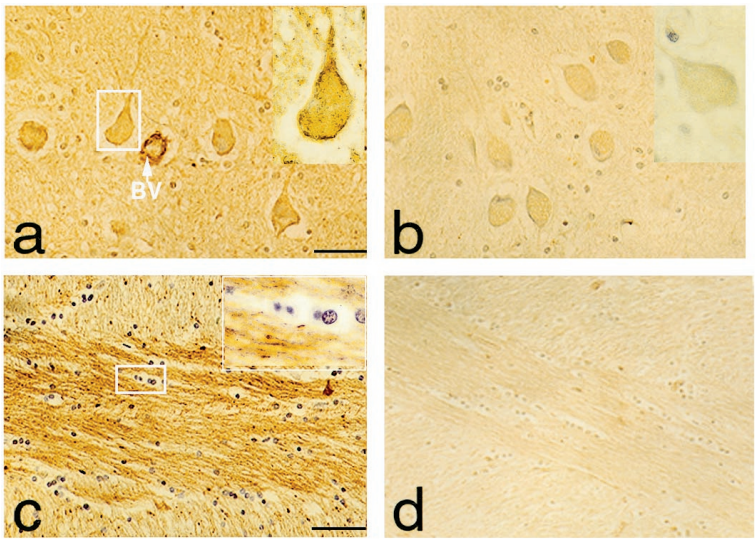

Fig 3. Immunohistochemical analysis of the antibody to parkA peptide in human basal ganglia. To determine the specificity of parkA antibody immunoreactivities, human basal ganglia sections (putamen and globus pallidus) were stained with 10 μg IgG/ml of affinity purified parkA antibody (a, c) or preabsorbed parkA antibody (b, d). ParkA antibody labeled neuronal cell bodies and fibers in the putamen with moderate intensity (a, c). Blood vessel (bv in a) wall was labeled most intensely. Bar = 50 μm. (Insets) *Higher-magnification views of the labeling of putamen neurons and fibers.*

weaker 22-kd protein (see Fig 2B). Neither the p22 nor p50 proteins was detectable with either parkA or parkB antibodies preabsorbed with 100 μM of the respective peptide. Preincubating parkA antibody with parkB peptide or parkB antibody with parkA peptide resulted in no absorption (data not shown).

To determine the subcellular localization of parkin, protein extracts from a control human brain cortex and HTB10 cells (see Fig 2C and D) were separated by differential centrifugation into subcellular fractions. These fractions include the nuclear P1, mitochondrial P2, microsomal/Golgi-ER P3, and cytosol S3 fractions. The 50-kd protein was detected in all four fractions in human cerebral cortex and in HTB10 neuroblastoma cells. Interestingly, a band of approximately 60 kd in the P2 fraction from the HTB10 cells was detected by the parkA antibody, suggesting that a larger parkin isoform may reside specifically in this fraction. The purity of subcellular fractions was determined by detection of marker proteins. A 38-kd Golgi-resident protein was detected only in the P3 fraction by a Golgi38K antibody, and the nuclear 66-kd NeuN protein was detected only in the P1 fraction by a NeuN antibody.

Since the distribution of parkin in all four fractions could not be explained by improper fractionation, we hypothesized that it might be due to attachment to cytoskeletal proteins (see later). Indeed, when the fractionated protein extracts were labeled with a mouse MAb to β-actin, a 42-kd band was detected in all protein fractions (see Fig 2C).

Parkin Is Associated with Actin Filament Fibers

In a previous study, it was suggested that parkin was localized in the Golgi apparatus.[14] To repeat these observations, we first determined the specificity of the parkA antibody for cytochemistry. Two adjacent sections of basal ganglia from a neurological normal adult brain were labeled with 10 μg IgG/ml parkA antibody or with parkA antibody preabsorbed with 100 μM parkA peptide (Fig 3). The parkA antibody labeled neuronal processes and cell bodies of neurons in the putamen. The immunoreactivity was abolished with preabsorbed parkA antibody. To determine the subcellular distribution of parkin, we colabeled COS1 cells with the parkA antibody and a trans-Golgi58K MAb (Fig 4A). The trans-Golgi58K protein is located in the microtubule-binding peripheral Golgi membrane. Confocal fluorescent microscopy imaging of these cells showed that the parkA antibody exhibited strong labeling of cytoskeletal fibers and membrane ruffles. However, colocalization with the Golgi58K protein was absent. Antibodies to other resident Golgi proteins (γ-adaptin and clathrin) and mitochondria (mitofilin) also failed to show any colocalization with the parkA antibody (not shown).

Because the pattern of parkin immunoreactivity resembled actin filament or microtubule staining, we colabeled COS1 cells with the parkA Ab (see Fig 4B, a and e) and TRITC-conjugated phalloidin (see Fig 4B, b and f). The parkA antibody labeling colocalized with phalloidin staining (see Fig 4B, c). At high magnification, both the parkA antibody and phalloidin labeled the same actin fibers (see Fig 4B, e–g). An identical labeling pattern was observed in HTB10 cells (not shown). Although the parkB antibody binds less avidly to endogenous parkin in COS1 cells (see Fig 2A), it showed a similar labeling pattern as the parkA antibody, including colabeling with phalloidin at higher concentrations (see Fig 4B, m–o).

To further differentiate whether parkin was localized to actin filaments or microtubules, COS1 cells were treated with either 20 μM cytochalasin D (see Fig 4B, h–j) for 24 hours or 5 nM nocodazole (Fig 5e–k) for

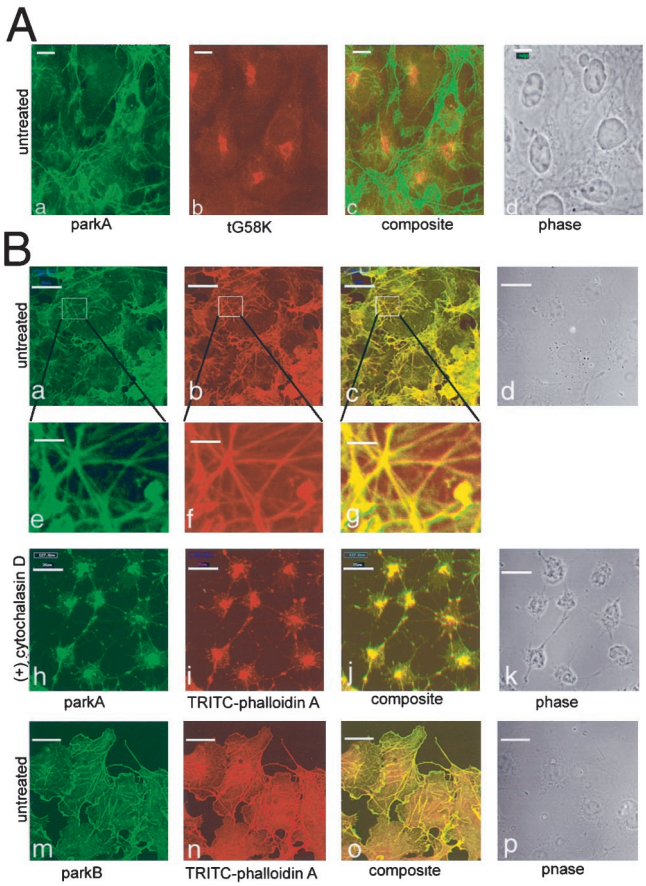

Fig 4. Parkin is localized to actin filaments but not to microtubules or the Golgi apparatus in COS1 cells. Immunofluorescent cells were viewed with confocal microscopy, and images were digitally recorded. (A) Parkin did not localize to the Golgi apparatus. Cells were stained with 10 μg IgG/ml parkA antibody (a) and 1/100 dilution of Golgi58K mouse monoclonal antibody (b). Panel c represents the overlay images of parkin and Golgi58K labeling. Bar = 10 μm. (B) Parkin colocalizes with actin filaments. Panels a–c are images of untreated cells stained with parkA antibody and phalloidin, a strong actin filament binder. Higher magnification views (e–g) show parkA antibody and phalloidin labeled the same actin filaments. When COS1 cells were treated with cytochalasin D, a fungal compound that destabilizes actin filaments, both parkA antibody– and phalloidin-labeled fibers regressed juxtanuclearly. In untreated cells, parkB antibody also co-labeled with phalloidin (m–o). Bars = 25 μm in a–d, h–k, and m–o, and 5 μm in e–g.

30 minutes. Treated cells were labeled either with parkA antibody and TRITC-phalloidin (see Fig 4B, h–j, and Fig 5i–k) or with parkA and β-tubulin (see Fig 5e–g) antibodies. Cytochalasin D is a fungal product that blocks the polymerization of actin filaments, whereas nocodazole inhibits the polymerization of tubulin to form microtubules.

Treatment with cytochalasin D resulted in the contraction of actin filaments to a juxtanuclear position in COS1 cells. Both the parkA antibody and phalloidin labeled the same cytostructural elements in cytochalasin D–treated cells (see Fig 4B, h–j). As expected, nocodazole did not have any effects on actin filaments, and the patterns of parkA and phalloidin labeling were similar to untreated cells (see Fig 4B, a–c, and Fig 5i–k). Nocodazole treatment, on the other hand, disrupted microtubules as evidenced by the diffuse cytoplasmic staining with a β-tubulin MAb (see Fig 5f and g). We also labeled untreated COS1 cells with the parkA antibody and the β-tubulin antibody (see Fig 5a–c). ParkA labeling did not colocalize with β-tubulin (see Fig 5c).

In NGF-differentiated rat PC12 neurons, the parkA antibody exhibited a punctate labeling in cell bodies and neurites (see Fig 5m). When the images of parkin and phalloidin labeling (see Fig 5n) were superimposed, both were clearly colocalized (see Fig 5o).

Parkin Expression in the Human Nervous System

To determine the distribution of parkin in the human central nervous system, we labeled paraffin-embedded sections of the midbrain, basal ganglia, cerebral cortex, and cerebellum from three neurologically normal individuals using the parkA antibody. We also labeled tissues from 4 individuals who had a clinical diagnosis of sporadic PD confirmed by the presence of Lewy bodies in the substantia nigra. In all brain regions, immunoreactivity was particularly strong in neuronal processes and cell bodies of selected neurons. No labeling was observed in glial cells (Fig 6).

In the basal ganglia, parkin immunoreactivity was more intense in neuronal processes than in other regions of the brain (Fig 3a and c). Parkin was found in the cytoplasm and neuronal processes of neurons in the putamen and globus pallidus (see Figs 3 and 6). Similar observations were made in the substantia nigra (see Fig 6c and d). Lewy bodies were not labeled by the parkin antibody (see Fig 6a). The overall intensity of labeling appeared to be stronger in PD brain than in controls (see Fig 6d).

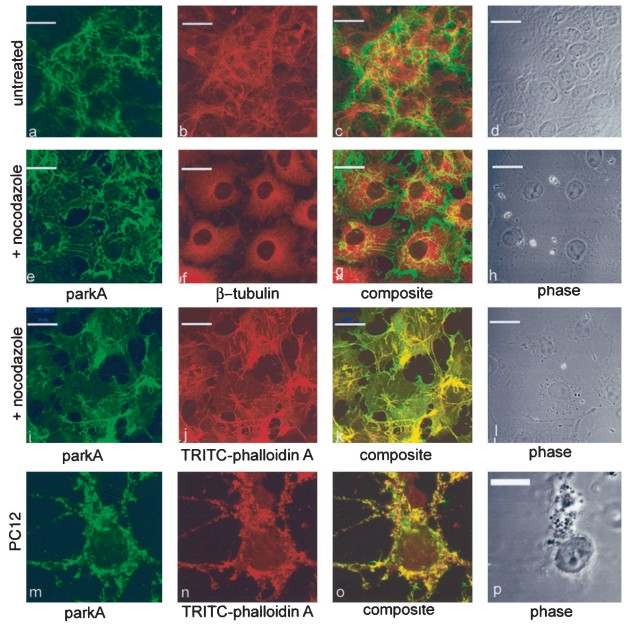

Fig 5. Parkin did not colocalize with microtubules. To determine whether parkin localizes with microtubules, COS1 cells were stained with parkA antibody (a) and β-tubulin mouse monoclonal antibody (b). A composite of these two images (c) shows no colocalization between parkin and β-tubulin. To further confirm this observation, cells were treated with nocodazole (e–l), a microtubule depolymerizer, and stained with parkA antibody (e) and β-tubulin (f) or parkA antibody (i) and phalloidin (j). Panels g and k are composite of panels e–f and i–j, respectively. Nocodazole caused a redistribution of β-tubulin labeling (f) but had no effect on the distribution of parkin (e, i, g, k) and phalloidin (j, k). Panels m–o show that parkin is localized in the punctate structures along the cell membrane and neuronal processes in nerve growth factor–induced differentiated PC12 cells. PC12 cells were induced with 100 ng/ml nerve growth factor for 7 days and then stained with 10 μg/ml parkA antibody (m) and with 1 ng/ml TRITC-conjugated phalloidin to stain actin filaments (n). An overlay image shows that parkA is colocalized with phalloidin (o). Bars = 25 μm in a–i, and 10 μm in m–p.

In normal cerebral cortex, the overall immunoreactivity intensity was weaker than that observed in the midbrain and basal ganglia (data not shown). The molecular layer was weakly labeled with the parkA antibody. Parkin immunoreactivity was undetectable in granule neurons, while the cytoplasm and neuronal processes of large pyramidal neurons in layers III, IV, and V were labeled weakly. There was no detectable IR in fusiform cells of layer VI. Nerve fibers in the white matter were labeled weakly, but there was no immunoreactivity in the glial cells in this region (data not shown).

In the cerebellum, parkA immunoreactivity was strong in the molecular layer of the cerebellum (see Fig 6e and f). High-magnification views of the molecular layer showed that Purkinje cell nerve fibers were strongly labeled in a punctate pattern, whereas labeling of cell bodies was weak. Neurons in the granular layer were not labeled, but cerebellar glomeruli, which consist of mossy fiber terminals, Golgi cell axons, and granule cell dendrites, were strongly labeled (Fig 6, panel e arrow).

Discussion

PD is a major neurodegenerative disease characterized by muscle rigidity, tremor, and bradykinesia (see review by Dunnett and Bjorklund[17]). Although most idiopathic PD cases are sporadic and probably are influenced by environmental factors, at least five loci have been identified to be involved in familial PD, and three genes have been identified to be involved in familial PD.[2,8,12,17,18] Mutations in the parkin gene were identified in patients with juvenile-onset, L-dopa–responsive PD without Lewy bodies.[12] Recent genetic analyses have expanded the spectrum of parkin mutations to older PD patients.[13] To elucidate the function of parkin, we generated two antibodies to different peptides at opposite ends of parkin and investigated the distribution of parkin in cell lines and in human basal ganglia, midbrain, cerebral cortex, and cerebellum. Although some of our results confirm a previous study of parkin distribution,[14] important differences emerge with regard to subcellular distribution of parkin and its localization in human and rat cell lines.

Biochemical Analysis of Parkin in Human Brain Tissue and COS1 Cells

In Western blots of protein extracts from human cerebral cortex, HTB10 cells, and COS1 cells, parkA and B antibodies detected a 50-kd protein close to the calculated molecular weight of parkin[12] (see Fig 2). It is likely the normal full-length parkin based on the following observations: (1) antibodies to different peptides of parkin recognized the same p50 band and it is identical to the larger form of the rat parkin[19]; (2) antibodies preabsorbed with their respective peptide failed to detect it (see Fig 3); and (3) both parkA and parkB antibodies detected the GFP-parkin full-length fusion protein.

Parkin Is Associated with Actin Filaments

A recent study suggested that parkin was located in the Golgi apparatus.[14] When we performed subcellular fractionation, parkin was found in every fraction similar to β-actin, suggesting that parkin was a cytoskeletal-associated protein (see Fig 2). These results were consistent with our anatomical observations that parkin was not colocalized with Golgi proteins but instead as-

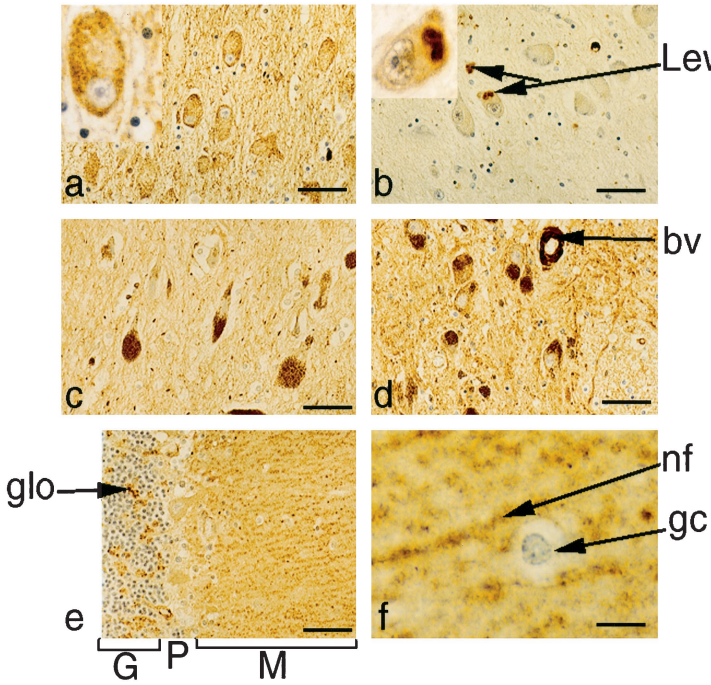

Fig 6. Parkin is widely expressed in sporadic Parkinson's disease (PD) and normal human brain tissues. To investigate the relative expression levels of parkin in human brain, 5-μm sections from different areas of a neurological normal and sporadic PD brain were stained with 10 μg IgG/ml parkA antibody (a, c–f). (a) The cytoplasm of neurons and axons in the putamen from a sporadic PD patient were labeled strongly with the parkA antibody. Lewy bodies were not stained with parkA antibody, and there was no nuclear staining. (b) An adjacent section of the putamen was stained with an antibody to ubiquitin. Note the spherical shaped ubiquitin-labeled Lewy bodies. (c) Midbrain from a neurologically normal individual with nigral neurons and nerve fibers that are moderately stained with parkA antibody. (d) Midbrain from an individual with sporadic PD. Nigral neurons and fibers are stained with parkA antibody. Note the apparent increase in parkin immunoreactivity in this section. (e) A cerebellar section from a normal individual. Note the intense punctate parkin immunoreactivity in the molecular layer and the glomerulus (glo), but weak parkin immunoreactivity in the Purkinje cell bodies. (f) High-magnification view of the parkin-labeled cerebellar molecular layer. Nerve fibers (nf) were strongly labeled; while glial cells were not. Bars = 50 μm in a–e, and 10 μm in f. bv = blood vessel; gc = glial cell; G = granule layer; P = Purkinje neuron layer; M = molecular layer.

sociated with actin filaments (see Fig 4). The parkA antibody colabeled with phalloidin, an actin filament binder. The localization to actin filaments was further demonstrated by treatment of cells with cytochalasin D, an actin filament destabilizer, which altered both parkA and phalloidin labeling. On the other hand, parkin labeling was not changed when cells were treated with nocodazole, a fungal toxin that disorganizes microtubules (see Fig 4). These results were also confirmed by staining with the parkB antibody. The actin association of parkin and a potential role in vesicular transport requires further study.

Parkin and α-Synuclein Have Different Localization Patterns in Neurons

In neurons, α-synuclein was found in the processes and nuclei.[3,4] It binds to presynaptic vesicles and to both the 5- and 10-nm filaments.[3,20,21] This contrasts with parkin localization. Parkin is found in neuronal processes (see Figs 3, 5, and 6), and it is associated with actin filaments (see Figs 4 and 5). α-Synuclein accumulates in Lewy bodies and Lewy fibrils in nigral neurons in autosomal dominant and sporadic PD.[3] In contrast, parkin does not accumulate in Lewy bodies in brains of sporadic PD patients (see Fig 6). Differences in the subcellular localization and pathological distribution between α-synuclein and parkin suggest that the pathogenetic mechanisms caused by mutations in these genes are at least in part distinct.

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