L166P Mutant DJ-1, Causative for Recessive Parkinson’s Disease, Is Degraded through the Ubiquitin-Proteasome System*

Received for publication, April 23, 2003, and in revised form, June 19, 2003
Published, JBC Papers in Press, July 8, 2003, DOI 10.1074/jbc.M304272200

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Mutations in a gene on chromosome 1, DJ-1, have been reported recently to be associated with early-onset Parkinson’s disease. While one mutation is a large deletion that is predicted to produce an effective knock-out of the gene, the second is a point mutation, L166P, whose precise effects on protein function are unclear. In the present study, we show that L166P destabilizes DJ-1 protein and promotes its degradation through the ubiquitin-proteasome system. A double mutant (K130R, L166P) was more stable than L166P, suggesting that this lysine residue contributes to stability of the protein. Subcellular localization was broadly similar for both wild type and L166P forms of the protein, indicating that the effect of the mutation is predominantly on protein stability. These observations are reminiscent of other recessive gene mutations that produce an effective loss of function. The L166P mutation has the simple effect of promoting DJ-1 degradation, thereby reducing net DJ-1 protein within the cell.

Several genes have been unambiguously shown to be causal in rare familial forms of Parkinson’s disease (PD) (1) (reviewed in Ref. 1). These include two dominant point mutations in α-synuclein (2, 3), a variety of recessive mutations in parkin (4), and two recessive mutations in DJ-1 (5). Clinical phenotypes vary somewhat between patients with mutations in these genes, but all produce symptoms that overlap between each other and with sporadic PD. This suggests that mutations in different genes would have common effects on those neurons that are susceptible to degeneration in PD. Although our knowledge of common cellular effects of these different proteins is incomplete, it has been shown, for example, that parkin can protect cells against the toxic effects of mutant α-synuclein both in vitro (6) and in vivo (7). A current hypothesis for the pathogenic pathway that leads to cell death in PD is that there is a deficiency in the ubiquitin-proteasome system. Identification of mutations in DJ-1 may allow us to test this hypothesis (8). The function of DJ-1 protein appears to be multifaceted (8). It was identified through an interaction with c-myc (9), as part of a multiprotein complex that stabilizes mRNA (10) and as a protein involved in infertility in the rat (11). DJ-1 may affect mRNA expression via protein-protein interactions with a complex including the androgen receptor and the sumoylation enzyme, PIASx (12, 13). DJ-1 has been found to shift to a more acidic isoform under oxidative conditions (14, 15). These studies provide evidence that DJ-1 might be involved in (i) control of gene regulation, (ii) post-translational modifications of proteins by SUMO, a ubiquitin-like modifier, and (iii) oxidative events. DJ-1 has moderate homology to the bacterial proteins Thjd and Pfp1, which are involved in thiamine synthesis and protease activity respectively. As the Thjd/Pfp1 superfamily (pfam01965) is large, there may be other unidentified functions of DJ-1. For example, other members of the same family have chaperone-like activity (16).

The DJ-1 gene has 7 exons and encodes a 189 amino acid (predicted 20 kDa) protein. One of the reported DJ-1 mutations is a large genomic deletion removing the first 5 exons, and thus, it is unlikely to produce any protein. The second is a point mutation (L166P) of a highly conserved amino acid in a region that is predicted to affect oligomerization, based on sequence similarity to the bacterial protein PH1704 (5). The crystal structure of human DJ-1 has been solved recently (17, 18) and leucine 166 has been localized to the penultimate C-terminal α-helix of the protein. DJ-1 is localized to both nuclei and cytoplasm in different cell types (9, 10). In their functional analysis of DJ-1, Bonifati et al. (5) suggested that the L166P mutant protein mislocalizes to mitochondria and that loss of cytoplasmic function may be sufficient to cause disease. Here we show that the L166P mutation makes DJ-1 dramatically less stable and promotes degradation through the ubiquitin-proteasome pathway, resulting in lower steady state protein levels and functionally mimicking the deletion mutation in resulting in low or absent levels of protein.

EXPERIMENTAL PROCEDURES

DJ-1 Constructs and Transfections—Full-length cDNA for DJ-1 (GenBank™ accession number AF021819) cloned into pCDNA3.1/GS was purchased from Invitrogen. This construct has an in-frame C-terminal V5 epitope-His6 fusion tag. The L166P and K130R mutants were generated using the QuickChange mutagenesis kit (Stratagene, La Jolla, CA). Primer sequences are available on request from the authors. We also made an N-terminal myc-tagged DJ-1 construct by subcloning

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* The abbreviations used are: PD, Parkinson’s disease; FPLC, fast performance liquid chromatography; PBS, phosphate-buffered saline; DPBS, Dulbecco’s PBS; PIAS, protein inhibitor of activated STAT (signal transducers and activators of transcription); SUMO, small ubiquitin-like modifier; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; GFP, green fluorescent protein; wt, wild type.

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the full coding sequence into the EcoRI site of pGWImyc2b, with mutations introduced as above. The inserts of all constructs were fully sequenced using BigDye terminator kit on an ABI 3100 sequencer (Applied Biosystems Inc., Foster City, CA) prior to use. Human HEK293, M17 neuroblastoma, or monkey COS-7 cells were grown in Opti-MEM supplemented with 10% (v/v) fetal bovine serum (both from Invitrogen) and were transiently transfected using FuGENE (Roche Applied Science). To generate pools of stable transfectants, cells were selected with 50 μg/ml Zeocin(24) (Invitrogen).

Yeast Two-hybrid Screening—Yeast two-hybrid tests were performed as described previously (19) using the L40 yeast strain harboring the reporter genes HIS3 and β-galactosidase under the control of upstream LexA binding sites. The DJ-1 bait consisted of the entire coding sequence of PIASx—Turbo (Stratagene), incorporating EcoRI sites. The DJ-1 bait was amplified by PCR using Pfu-Turbo (Stratagene), incorporating EcoRI ends for subcloning into pBHA or pGAD10. Site-directed mutagenesis was performed as above. An EcoRI fragment containing residues 433–572 of PIASx was produced using PCR amplification and subcloned into pGAD10. Yeast two-hybrid interactions were semi-quantified as described previously (19) based on growth on His/Leu/Trp (—HLT) plates (supplemented with 3 mM 3-amino-1,2,4-triazole) and X-gal filter lysates at room temperature, with similar results obtained in at least three experiments.

Western Blotting and Co-immunoprecipitation—For Western blotting, cells were lysed as described previously (6), and 10 μg of total protein were separated on 10–20% SDS-PAGE gels and transferred to Immobilon polyvinylidene fluoride membranes (Millipore, Bilheric, MA). Primary antibodies used were monoclonal anti-DJ-1 (clone 3E8, Stressgen, San Diego, CA), monoclonal anti-V5 (Invitrogen), monoclonal anti-myc (clone 9E10, Roche Applied Science), and monoclonal anti-β-actin (clone AC-15, Sigma). Blots were developed with peroxidase-labeled secondary antibodies (Jackson Immunochemicals, West Grove, PA) using ECL-plus (Amersham Biosciences). Quantitation of protein expression was performed by capturing ECL-plus with a Storm phosphorimager using the blue fluorescence mode.

For immunoprecipitation experiments, cells were scraped in cold phosphate-buffered saline (PBS, pH 7.4) collected by centrifugation and then resuspended by briefly sonicating in buffer containing 150 mM NaCl, 5 mM EDTA, 50 mM Tris-HCl, pH 7.5, 1 mM dithiothreitol plus protease inhibitors and lysed using a hand-held homogenizer. We centrifuged the lysate sequentially at 1000 g, 30 min. The extract was applied to a FPLC Superdex 200 HR 10/30 column (Amersham Biosciences), at a flow rate of 0.25 ml/min in PBS, 0.1% Triton X-100. Fractions (0.25 ml) were collected and proteins resolved by SDS-PAGE then immunoblotted using anti-myc antibodies to detect DJ-1. Protein standards (Amersham Biosciences and Sigma) were applied onto the same column under the same conditions to prepare a standard curve, from which the native molecular weight of DJ-1 was calculated.

Gel-exclusion Chromatography—Cells expressing myc-DJ-1 were lysed in PBS, 0.1% Triton X-100 and centrifuged by straining (130,000 × g, 30 min). The extract was applied to a FPLC Superdex 200 HR 10/30 column (Amersham Biosciences), at a flow rate of 0.25 ml/min in PBS, 0.1% Triton X-100. Fractions (0.25 ml) were collected and proteins resolved by SDS-PAGE then immunoblotted using anti-myc antibodies to detect DJ-1. Protein standards (Amersham Biosciences and Sigma) were applied onto the same column under the same conditions to prepare a standard curve, from which the native molecular weight of DJ-1 was calculated.

Subcellular Fractionation—Subcellular fractionation was performed using procedures similar to those described in Ref. 21, with modifications. Briefly, cells transfected with various constructs were resuspended in 0.25 M sucrose, 10 mM Hepes/NaOH, pH 7.5, 1 mM dithiothreitol plus protease inhibitors and lysed using a hand-held homogenizer. We centrifuged the lysate sequentially at 1000 × g, 10,000 × g, and 100,000 × g, retaining pellets for each step and using the supernatant for each subsequent step. The pellets at 1000 × g (P1) and 10,000 × g (P10) were used as crude nuclear/whole cell and mitochondrial fractions, while the final 100,000 × g supernatant (S100) was taken as soluble cytosolic proteins. Similar total amounts (5 μg) of protein were loaded from each lane, and Western blotting was performed as described above. We used the following additional markers for specific fractions: anti-Tim23 for mitochondria (BD Transduction Laboratories, 1:2500) and anti-nucleoporin p62 for nuclei (BD Transduction Laboratories, 1:5000).

Quantitative Reverse Transcription-PCR—Total RNA was extracted using TRIzol, and cDNA was synthesized by priming 1 μg of total RNA with oligo(dT), using Superscript II RNA polymerase according to manufacturer’s instructions (Invitrogen). cDNA templates were diluted 5-fold before use in reverse transcription-PCR using primers for DJ-1 (forward, 5′-GTGAGTTAAGCGAGATCGCCAGCAG-3′; reverse, 5′-TCGAGTTAATACTTTGTTCCGCCC-3′, 5′-TCGAGTTAATACTTTGTTCCGCCC-3′). Real-time quantitative PCR was performed and analyzed using the ABI 7900 system (Applied Biosystems Inc.) as described previously (22).

Immunofluorescence Staining—Cells were grown on poly-d-lysine-coated coverslips, transfected with V5-tagged DJ-1 variants (or GFP as a control) and fixed in 4% paraformaldehyde in Dulbecco’s PBS (pH 7.4) at room temperature, permeabilized with 0.1% Triton X-100, and quenched with 0.1% glycine. Coverslips were then washed in DPBS, non-specific immunoreactivity blocked with DPBS containing 10% fetal bovine serum, 0.1% Triton X-100, and incubated with monoclonal anti-V5 (diluted 1:200) overnight at 4 °C. Cells were incubated with AlexaFluor 488-conjugated goat anti-mouse IgG conjugated prior to mounting under ProLong Antifade medium (Molecular Probes, Eugene, OR). Cells were either preincubated with 500 nm Mitotracker CMTMXos (Molecular Probes) for 30 min at 37 °C prior to staining or counterstained with SYTO-64 to label nuclei after staining (both from Molecular probes). Slides were examined using a Zeiss LSM510 confocal microscope using independent excitation for both channels. Omission of primary antibody was used to evaluate non-specific fluorescence and in all cases gave no signal, whereas staining for V5 alone was similar to that seen in dual labeling experiments. Because the signal strength for the L166P variant was substantially lower that that for wild type (see “Results”), we scanned antibody stained cells using a higher sensitivity, by increasing the photomultiplier tube voltage on this channel, hence the collected signals are similar in appearance. In this fashion we maintained separate signals for both channels with minimal bleed-through.

Statistical Analyses—Quantitative Western blotting data were analyzed by one-way analysis of variance with Neumann-Kelau’s post-hoc tests to determine differences between groups.

RESULTS

DJ1 Forms Homo-oligomers in Yeast and Mammalian Cells—As other proteins in the DJ-1 superfamily are oligomeric (5, 16), we tested for a self-interaction of human DJ-1 using yeast two-hybrid assays. Using full-length DJ-1 in both bait and prey fusion proteins, we found evidence for a strong self-interaction of DJ-1 (Fig. 1, A and B), which was markedly attenuated or abolished by deletion of relatively small sequences at either N or C termini. These results imply that full-length DJ-1 protein is required for self-interaction. Similar results were seen using deletion analysis of either bait or prey molecules. Substitution of L166P for wt protein in either bait or prey constructs produced a substantial decrease in the signal from yeast two-hybrid assays, which was undetectable if both constructs had the L166P mutation (Fig. 1C). We also tested a K130R mutant that has been reported to be deficient in sumoylation (13), which did not affect the yeast two-hybrid interaction. We were able to confirm the previously reported interaction of DJ-1 with PIASx (13). The interaction with PIASx was much less robust than the self-interaction of DJ-1 and was not detectable in the presence of the L166P DJ-1 mutation.

We were able to demonstrate a self-interaction of DJ-1 in mammalian cells by transfecting in V5His-tagged DJ-1 and co-immunoprecipitating endogenous, untagged DJ-1 from HEK293 cells (Fig. 1D). The exogenous DJ-1 interacted with endogenous protein, indicating that DJ-1 forms at least a dimer and that the V5-His-tagged protein retains this property. α-Synuclein was not co-immunoprecipitated under the same conditions. We also extracted DJ-1 from transfected cells and analyzed the native protein size by FPLC gel-exclusion chromatography. A single major peak was seen at about 55 kDa, consistent with oligomerization and slightly larger than the predicted size of a DJ-1 homodimer (Fig. 1E).
Low Steady State Protein Levels of L166P DJ-1 Are Due to Proteasomal Degradation—We next examined the level of expression of wild type and L166P DJ-1 in mammalian cells. All of the cell types examined (HEK293, M17, and COS-7) show robust basal expression of the DJ-1 protein. To distinguish the transfected DJ-1 from endogenous protein, we took advantage of the C-terminal V5-His tag in transfected DJ-1 to provide an additional epitope and a small increase in molecular mass from approximately 21 to 25 kDa (Fig. 2A). In transient transfection experiments, expression was similar to that of the endogenous protein in HEK293 cells and in human brain extracts (Fig. 2A). However, when we transfected L166P DJ-1 into the same cells, we found very low levels of V5-tagged protein (Fig. 2B). We considered that this might be a result of a defective plasmid, such as a deletion outside of the open reading frame that was not sequenced, but three independent clones of the same V5-tagged L166P construct gave similar results. Furthermore, an N-terminally myc-tagged construct also showed much lower levels of protein when mutated to L166P (see below). The effect was not cell type-dependent as this was seen in both HEK293 and M17 cells (Fig. 2B) and in COS-7 cells (data not shown) and was reproducible in at least triplicate independent experiments in each cell type.

We compared protein and mRNA expression of wt and L166P DJ-1 in pools of stably transfected M17 human dopaminergic neuroblastoma cells (Fig. 2, C and D). As in transiently transfected cells, mutant protein was difficult to detect using either V5 or DJ-1 antibodies, requiring loading of increased protein. This was confirmed in measurements made in three lines, with eight replicate measurements per cell line (Fig. 2D), where protein expression was similarly low (data not shown).

The similar levels of DJ-1 mRNA in the transfected cells suggests that the low levels of L166P protein are not due to a lack of transcription. We, therefore, considered whether this post-transcriptional effect was due to increased degradation of the protein. Two major routes for protein degradation in the...
Protein Instability of L166P Mutant DJ-1

Fig. 2. Lower steady state protein levels of L166P protein compared with wild type DJ-1. A, endogenous and transfected DJ-1 protein. HEK293 cells were transiently transfected with V5-tagged DJ-1 (lane 2) or GFP as a control (lane 1), and lysates were blotted for V5, which revealed strong expression of 25-kDa V5-tagged DJ-1 (arrow, top panel). Immunoblot for DJ-1 (second panel) confirmed the expression of V5-tagged DJ-1 and also revealed significant levels of endogenous DJ-1 (20 kDa, arrowhead) in HEK293 cells and in human brain (lane 3). This blot was reprobed for GFP as a transfection control and for β-actin (open arrowhead) to confirm equal loading (10 μg of protein per lane). Marker sizes on the right of all blots are in kilodaltons (also shown in B and C). B, L166P is less stable than wild type DJ-1. HEK293 (top panel) or M17 cells (middle panel) were transiently transfected with various V5-tagged DJ-1 constructs and blotted using V5 antibodies. Wild type DJ-1 (lane 1) is robustly expressed in both cell types, while the L166P mutant (lanes 2–4 indicate different expression constructs) is weakly expressed, suggesting that this form of DJ-1 is less stable. A lower band (*) was seen in HEK293 cells, which indicates C-terminal breakdown product of L166P. GFP-transfected HEK293 cells (lane 6) lacked V5-immunoreactive protein. The same blot was reprobed with β-actin to demonstrate equal loading (10 μg of protein per lane). C, M17 human neuroblastoma cells were stably transfected with V5-tagged wt (lane 2) or L166P DJ1 (lane 3) or control vector (lane 1). V5-immunoblot revealed strong expression of V5-tagged wild type, but weak expression of L166P DJ-1 (top panel, arrow), which was confirmed using an antibody to DJ1 antibody (middle panel, arrow indicates transfected DJ-1, arrowhead indicates endogenous DJ-1). The same blot was reprobed with β-actin to demonstrate equal loading (40 μg of protein per lane). D, mRNA expression of DJ-1. Quantitative reverse transcription-PCR was used to measure the abundance of DJ-1 mRNA in M17 cells stably transfected with either wild type or L166P forms of DJ-1 as indicated. Note that each of these stable cell lines expresses similar levels of DJ-1 transcript relative to untransfected M17 cells, corrected for expression of β-actin mRNA. Error bars indicate S.E., n = 3 cell lines for each construct, with eight measurements of mRNA for each cell line.

In some experiments we used a construct myc-tagged at the N terminus of DJ-1. As for the constructs that were tagged with V5 at the C terminus, myc-tagged L166P DJ-1 was unstable, and steady state levels of protein were increased in the presence of MG132 (Fig. 4, lanes 3 and 4). Using these high levels of expression, we saw an additional DJ-1 band of ~29 kDa, which was more prominent after treatment with MG132. We have not been able to unambiguously identify this protein, but this is likely to be a covalent modification of DJ-1 as it is stable to SDS. We reasoned that as the L166P mutant was responsive to proteasome inhibition it is likely to be ubiquitylated and that modifying lysine acceptor sites for ubiquitin might stabilize the protein. To test this hypothesis, we generated an artificial K130R,L166P double mutant. K130R is reported to be sumoylation-deficient (13), and as SUMO and ubiquitin can target the same lysine residue (e.g. 24), we surmised that K130R might also be deficient in ubiquitylation. K130R has recently shown to be appropriately folded (18). The double mutant K130R, L166P DJ-1 was more stable than L166P alone but less stable.
than wild type protein, and the additional 29-kDa band was not present. MG132 increased steady state protein levels of the double mutant, indicating that the rescue of the L166P-induced protein instability was incomplete. Therefore, additional factors may play a role in the instability of the L166P protein (see “Discussion”). We have also noted distinct degradation products of K130R mutants that are not readily detected with the wild type protein. As proteasomal processing of ubiquitylated proteins usually results in small peptides and amino acids, these lower molecular weight species may represent non-proteasomal degradation of the double mutant.

**Localization of L166P Mutant DJ-1**—Previous studies have shown that DJ-1 is found in cytoplasm and nuclei and L166P has been suggested to mislocalize to mitochondria in COS-7 (5). We stained transiently transfected cells (COS-7 or M17) for transfected DJ-1 and counterstained with either nuclear or mitochondrial markers (Fig. 5). Consistent with previous reports, wt DJ-1 was found in both cytoplasm and nuclei, as evidenced by overlap with nuclear dyes SYTO-64 (Fig. 5, L and Q). In most cells, there was overlap with Mitotracker, but no specific localization to mitochondria. However, in a proportion of cells, we saw decreased cytoplasmic staining and specific
localization to mitochondria, although nuclear labeling was maintained (Fig. 5, C and E). This was true for both cell types examined and represented ~10% of the total transfected cells. The signal with L166P DJ-1 was lower than that for wild type, but we were able to distinguish sufficient specific signal in transfected cells compared with untransfected cells in the same cultures by increasing sensitivity of collection on this channel (see “Experimental Procedures”). We found that the overall distribution of L166P was similar to wild type protein and was localized to mitochondria in some cells (Fig. 5, J and O), but not all (Fig. 5, D and N).

We additionally assessed the distribution of wt and L166P DJ-1 using subcellular fractionation by separating into crude nuclear/whole cell, mitochondrial, and cytosolic fractions (Fig. 6). Consistent with immunofluorescence data, wt DJ-1 was found in cytosolic and mitochondrial fractions, as well as a strong signal in the whole cell lysate that includes the nuclear fraction. We saw only a minor amount of DJ-1 in the microsomal (endoplasmic reticulum and Golgi) fraction. L166P DJ-1 was seen in the same fractions at lower levels, consistent with the immunofluorescence results. Therefore, the major effect of the L166P mutation is on steady state protein levels rather than distribution of the protein within the cell.

**DISCUSSION**

There are two critical elements of knowledge about genes that cause human diseases, namely the functions of the gene product under normal circumstances and how mutations in the gene alter that function. In the present study we have assessed how the L166P point mutation causes a loss of function in DJ-1. We have shown that DJ-1 self-associates and that the L166P mutation significantly reduces the signal in yeast two-hybrid experiments. This lack of signal could be due to a reduced capacity for self-interaction or due to lower steady state levels of the protein (see below). Gel-exclusion chromatography results indicated that native DJ-1 is oligomeric and likely to be either a dimer or trimer. These data are consistent with recent x-ray diffraction data (17, 18), which also demonstrate dimer formation. Different members of the DJ-1 superfamily of proteins form many different types of oligomeric species, and this data suggest that DJ-1 is more structurally similar to the bacterial Hsp31, which forms dimers (16) and less like PH1704, which forms hexamers (25). Full-length DJ-1 is necessary for its self-interaction, suggesting a contribution of both N and C termini to the proper folding or self-interactions of the protein. Leu166 is located in a C-terminal α-helix (17, 18) and may act as a helix breaker (5), but also plays a role in the determination of protein stability.

We were able to express wild type DJ-1 protein both transiently and stably in mammalian cells. We also demonstrated that DJ-1 is endogenously expressed at high levels by various
transfection, and the experiment was repeated in triplicate with similar results. Each duplicate lane is an independent experiment. 

**A** L166P V5-tagged DJ-1 as indicated. Whole cell lysates, microsomal (P100), mitochondrial (P10), and cytosolic (S100) fractions were separated (see **Experimental Procedures**). Blots were reprobed with monoclonal antibodies to the mitochondrial protein Tim23 (45, 23 kDa), nucleoporin p62 (C, 62 kDa). Each duplicate lane is an independent transfection, and the experiment was repeated in triplicate with similar results.

mammalian cell lines in culture and in human brain. Despite the presence of similar amounts of mRNA, the L166P mutant protein was not expressed at robust levels, suggesting a post-translational regulation of protein abundance. We subsequently found that L166P DJ-1 is degraded by the proteasome. The structural basis for this is not clear. In other proteins, e.g. lysozyme, substitution of Pro for Leu destabilizes α-helical regions by altering hydrogen bonding and results in a protein that is dramatically less stable (26). Our data show that, in the case of DJ-1, a Leu to Pro substitution in a predicted helical region confers instability on this protein and that mammalian cells recognize the mutant and remove it via the proteasome. This is likely to be mediated by an as yet unknown E3 protein-ubiquitin ligase. There are multiple ways in which the E3 ubiquitylation enzymes recognize their cognate target proteins (reviewed in Ref. 27), but it has been hypothesized that mutant proteins may be targeted because of exposure of hydrophobic domains that are normally hidden by protein-protein interactions or within the core of the protein. A hypothesis for the effect of the L166P mutation would be that by changing the conformation in the C-terminal helix of DJ-1, the mutation may increase availability of such cryptic sites for recognition by its cognate E3 ligase. 

Lys^{130} is one possible site of ubiquitylation as the double K130R,L166P mutant was somewhat more stable in mammalian cells. This lysine side chain is solvent exposed and on the outer surface of the dimerized protein (18), thus making it available for modification. However, this double mutant was still less stable than the wild type protein, indicating that there are additional determinants of protein instability. There are 16 lysine residues in human DJ-1, and others may be ubiquitin acceptors when Lys^{130} is mutagenized. A systematic analysis of lysine residues that contribute to instability in L166P DJ-1 is under way in our laboratory. Although we have seen additional higher molecular weight species with L166P DJ-1 that were not present in the K130R,L166P double mutant, we have not been able to identify them with antibodies to either ubiquitin or SUMO-1. Therefore, additional covalent modifications of DJ-1 are possible. The role of sumoylation of DJ-1, and specifically the site of lysine acceptor for this modification, are not yet clear. Lys^{130} is not present in a canonical SUMO-1 addition site (ϕKKXE/D), and hence, additional work is required to characterize the mode of addition of ubiquitin-like molecules to DJ-1.

These data indicate that L166P would produce insufficient protein as a result of proteasomal degradation. This situation is similar to that seen with mutant forms of other recessive gene products such as the cystic fibrosis transmembrane receptor (28) or the neurofibromatosis type 2 gene product, schwannomin (29). The ubiquitin-proteasome system also degrades some dominant negative mutations such as cavedin-3 mutations associated with limb-girdle muscular dystrophy type 1C (30). However, not all mutant proteins are degraded by the proteasome, and some are degraded by lysosomes, including certain GTP cyclohydrolase mutations associated with DOPA (3,4-dihydroxyphenylalanine)-responsive dystonia (31).

Identification of multiple genetic mutations that are each causative for PD and knowledge of how the mutations alter function of the protein products will allow us to construct pathways that lead to pathogenesis in this disease. The relationship that DJ-1 has with other PD-related proteins is still an open question. For dominant gene mutations, such as the α-synuclein point mutations, there may be gain of function effects that promote damage to cells, such as the enhancement of oligomer formation (32, 33). However, not all recessive genes are associated with loss of normal function, such as the parkin mutations that lack ubiquitylation activity (34, 35). As DJ-1 appears to have multiple functions, of which several are potentially relevant to the pathogenesis of PD (8), it is important to assess how mutations affects protein function. It is likely that the L166P mutation has a simple effect, namely to reduce the cellular protein level to that which increases the effect of damage processes that are also triggered by loss of parkin function or dominant α-synuclein mutations. By promoting degradation, the L166P mutation functionally mimics the larger Dutch mutation and reduces net protein within the cell. The mechanism by which loss of DJ-1 protein is toxic to dopaminergic neurons remains to be elucidated.

Acknowledgments—We thank John Hardy and Andrew Singleton for critical comments on this manuscript and Joan Davis for help in preparation. We are also grateful to M. Wilson, J. Collins, Y. Hod, D. Ringe, and G. A. Petsko for sharing their unpublished data on the crystal structure of DJ-1 prior to publication.
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L166P Mutant DJ-1, Causative for Recessive Parkinson's Disease, Is Degraded through the Ubiquitin-Proteasome System

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J. Biol. Chem. 2003, 278:36588-36595.
doi: 10.1074/jbc.M304272200 originally published online July 8, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M304272200

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