

Published in final edited form as:

J Neurosci Res. 2009 January ; 87(1): 123–129. doi:10.1002/jnr.21831.

Mitochondrial Localization of DJ-1 Leads to Enhanced Neuroprotection

Eunsung Junn^a, Won Hee Jang^{a,b}, Xin Zhao^a, Byeong Seon Jeong^a, and M. Maral Mouradian^{a,*}

^aCenter for Neurodegenerative and Neuroimmunologic diseases, Department of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854

^bDepartment of Biochemistry, College of Medicine, Inje University, Busan Korea

Abstract

Mutations in *DJ-1* (*PARK7*) cause recessively inherited Parkinson's disease. DJ-1 is a multifunctional protein with anti-oxidant and transcription modulatory activity. Its localization in cytoplasm, mitochondria and nucleus is recognized, but the relevance of this sub-cellular compartmentalization to its cytoprotective activity is not fully understood. Here we report that at basal conditions DJ-1 is present mostly in the cytoplasm and to a lesser extent in mitochondria and nucleus of dopaminergic neuroblastoma SK-N-BE(2)C cells. Upon oxidant challenge, more DJ-1 translocates to mitochondria within 3 hours and subsequently to the nucleus by 12 hours. The predominant DJ-1 species in both mitochondria and nucleus is a dimer believed to be the functional form. Mutating cysteine 106, 53 or 46 had no impact on the translocation of DJ-1 to mitochondria. To study the relative neuroprotective activity of DJ-1 in mitochondria and nucleus, DJ-1 cDNA constructs fused to the appropriate localization signal were transfected into cells. Compared with 30% protection against oxidant induced cell death in wild-type DJ-1-transfected cells, mitochondrial targeting of DJ-1 provided a significantly stronger (55%) cytoprotection based on LDH release. Nuclear targeting of DJ-1 preserved cells equally as with the wild-type protein. These observations suggest that the time frame for the translocation of DJ-1 from the cytoplasm to mitochondria and to the nucleus following oxidative stress is quite different, and that dimerized DJ-1 in mitochondria is functional as anti-oxidant not related to cysteine modification. These findings further highlight the multi-faceted functions of DJ-1 as a cytoprotector in different cellular compartments.

Keywords

Parkinson's disease; Mitochondria; Neurodegenerative disease; Neuroprotection; Oxidative stress

Introduction

DJ-1 is one of the genes that cause inherited forms of Parkinson's disease (PD) when mutated (Bonifati et al. 2003). *DJ-1* (*PARK7*) maps to chromosome 1p36 (van Duijn et al. 2001) and encodes a 189 amino acid protein. Mutations in this gene were originally found to segregate with PD in two consanguineous families, one with a large genomic deletion leading to absence of gene product, and the other with a missense mutation substituting a highly conserved leucine with a proline at position 166 (L166P) (Bonifati et al. 2003). Both

*To whom correspondence should be addressed at: UMDNJ-RWJMS 683 Hoes Lane, Room 182 Piscataway, NJ 08854 Phone: 732-235-4772 Fax: 732-235-4773 Email: Mouradian@umdnj.edu..

mutations are homozygous and the disease is transmitted recessively. The clinical phenotype is characterized by an onset in the mid-thirties, good levodopa response and slow disease progression (Bonifati et al. 2003). DJ-1 is involved in diverse biological processes (Cookson 2003). First, several lines of evidence suggest that it plays a role in the oxidative stress response (Canet-Aviles et al. 2004; Mitumoto and Nakagawa 2001). DJ-1 is induced transcriptionally by insults that promote oxidative stress (Mitumoto and Nakagawa 2001; Mitumoto et al. 2001; Taira et al. 2004b). It quenches reactive oxygen species (ROS), and is converted into a variant with a more acidic isoelectric point (pI) in response to oxidants (Bandopadhyay et al. 2004; Kinumi et al. 2004; Mitumoto and Nakagawa 2001; Mitumoto et al. 2001; Taira et al. 2004b; Zhou and Freed 2005). Several reports have demonstrated that over-expression of wild-type DJ-1, but not the L166P mutant, protects cells against insults that promote ROS generation (Canet-Aviles et al. 2004; Junn et al. 2005; Taira et al. 2004b; Takahashi-Niki et al. 2004; Yokota et al. 2003; Zhou and Freed 2005). The protective effect of recombinant DJ-1 injected into the nigra has also been shown in the rat 6-OHDA model (Inden et al. 2006). Conversely, down-regulation of DJ-1 renders cells more susceptible to such insults (Junn et al. 2005; Martinat et al. 2004; Taira et al. 2004b; Takahashi-Niki et al. 2004; Yokota et al. 2003; Zhou and Freed 2005). Studies in *Drosophila* confirm that inactivation of the *DJ-1* homolog impairs the oxidative stress response in dopaminergic neurons (Menzies et al. 2005; Meulener et al. 2005; Park et al. 2005; Yang et al. 2005), and knock-down of *DJ-1* in *C.elegans* increases vulnerability to mitochondrial complex I inhibition (Ved et al. 2005). Similarly, *DJ-1* null mice are more vulnerable to MPTP than their wild-type littermates (Kim et al. 2005). Second, DJ-1 modulates transcription through interacting with DJ-1 binding protein (DJBP) (Niki et al. 2003) as well as with PIAS (protein inhibitor of activated STAT) (Takahashi et al. 2001), which modulates the activity of various transcription factors (Kotaja et al. 2002). In dopaminergic neuronal cells, DJ-1 interacts with p54nrb and PSF (pyrimidine tract-binding protein-associated splicing factor), two heterodimeric multifunctional regulators of transcription and RNA metabolism, and inhibits PSF-induced transcriptional silencing and apoptosis (Xu et al. 2005). Third, DJ-1 is a regulatory subunit (RS) of an RNA-binding protein (Hod et al. 1999). Fourth, DJ-1 may have chaperone activity, preventing heat-induced aggregation of substrate proteins (Lee et al. 2003) including α -synuclein (Shendelman et al. 2004; Zhou and Freed 2005) in a redox dependent manner (Shendelman et al. 2004; Zhou et al. 2006). Fifth, catalytic activities for DJ-1 have been suggested, including a cysteine protease function (Honbou et al. 2003; Olzmann et al. 2003), but not confirmed (Lee et al. 2003; Shendelman et al. 2004; Wilson et al. 2003). It is conceivable that many if not all of these functions relate to the pathogenetic role of DJ-1 in PD.

Subcellular localization studies have shown DJ-1 to be present in the cytosol, mitochondria and nucleus (Canet-Aviles et al. 2004; Taira et al. 2004a; Zhang et al. 2005). However, it is not clear if this compartmentalization of DJ-1 has a bearing on its cell protective activity, and if it does, which compartment is more involved in this function. Here we show that, in response to oxidative stress, some of the DJ-1 protein is translocated from its major cytosolic pool to mitochondria and nucleus. Furthermore, DJ-1 tagged with the mitochondrial localization signal and therefore fully localized to these organelles could protect cells against reactive oxygen species to a greater extent than wild-type DJ-1 or DJ-1 tagged with the nucleus translocation signal. These findings suggest that mitochondrial localization confers DJ-1 superior neuroprotective function against oxidative stress than cytosolic or nuclear localization.

Materials and Methods

Plasmids

Full-length cDNA vectors expressing Flag-tagged DJ-1 have been described previously (Junn et al. 2005). DJ-1 cysteine mutants, C46S, C53S and C106S were generated using the QuikChange site-directed mutagenesis kit (Stratagene), and DNA sequences were confirmed. To construct DJ-1 targeted to the outer-membrane of mitochondria, a peptide fragment derived from Bcl-X_L (aa 202-233), which has a mitochondrial targeting signal (Kaufmann et al. 2003), was fused to the C-terminus of myc-tagged DJ-1, yielding mitochondrial DJ-1 (DJ-1^{Mito}). To make DJ-1 targeted to the nucleus, DJ-1 cDNA was cloned in pCMV/myc/nuc (Invitrogen), a nuclear-targeting vector, yielding nuclear DJ-1 (DJ-1^{Nuc}).

Cell culture and transfection

Human neuroblastoma SK-N-BE(2)C cells were maintained in Ham's F-12:minimal essential media (MEM) (1:1) supplemented with 10% fetal bovine serum (FBS). Transfections were performed using FuGene 6 reagent (Roche Molecular Biochemicals) according to the supplier's instructions. To screen for cells that stably express DJ-1-myc, mitochondrial DJ-1 or nuclear DJ-1, transfected cells were diluted 10 fold in 100 mm culture dishes and treated with G-418 at a concentration of 0.5 mg/ml. After 3-4 weeks, isolated colonies were analyzed for transgene expression

Cell fractionation, chemical cross-linking and Western blot analysis

Mitochondrial, nuclear and cytosolic fractions were isolated using a commercial kit (Activemotif) according to the manufacturer's protocol. For cross-linking experiments, cells were rinsed with PBS, exposed to 100 μ M disuccinimidyl suberate (DSS) (Pierce) for 30 min at room temperature, and then rinsed with PBS containing 50 mM Tris-HCl (pH 7.6) to quench further DSS reaction. Subsequently, cells were processed for fractionation. Fractionated samples as well as total lysates were analyzed by Western blotting using mouse monoclonal anti-DJ-1 antibody (Stressgen) or peroxidase conjugated anti-Flag antibody (Sigma). Specificity of mitochondrial and nuclear fractions were assessed by SDS-PAGE followed by Western blot analysis with mouse monoclonal anti-bovine cytochrome oxidase subunit IV antibody (Molecular Probes) and rabbit polyclonal anti-Sp1 antibody (Santa Cruz Biotechnology), respectively. Band intensities were measured using graph digitizing software, UN-SCAN-ITTM (Silk Scientific Inc.).

Immunofluorescence

SK-N-BE(2)C cells were transiently transfected as described in *Results*, and cultured in glass chamber slides (Becton Dickinson) for 24 h. Cells were fixed in 4% paraformaldehyde in PBS for 20 min, washed with PBS three times, and permeabilized with 0.5% Triton X-100 in PBS for 10 min. After washing with PBS again and blocking with 5% BSA for 20 min, cells were incubated with the indicated antibodies diluted in 1% BSA at room temperature for 1 h, and washed five times with PBS. For nuclear staining, cells were incubated with 10 μ M 4',6-diamidino-2-phenylindole (DAPI) for 1 min, washed five times with PBS, mounted with ProLong[®] antifade mounting material (Molecular Probes) under a coverglass, and analyzed under a fluorescence microscope (Axiovert 200, Zeiss).

Cell death assay

SK-N-BE(2)C cells stably expressing DJ-1-myc, mitochondrial (DJ-1^{Mito}) or nuclear DJ-1 (DJ-1^{Nuc}) were exposed to hydrogen peroxide, and cell death was assessed using lactate dehydrogenase (LDH) Cytotoxicity Detection Kit (Roche Molecular Biochemicals). This

method quantifies cell death in culture based on the measurement of LDH released into the growth medium when cell membrane integrity is lost. Apoptotic cell death was measured using the terminal dUTP transferase nick-end labeling method (TUNEL, In Situ Cell Death Detection Kit TMR-Red, Roche Molecular Biochemicals) as described by the manufacturer. For quantification of TUNEL-positive cells, eight microscopic fields were randomly selected and the percentage of TUNEL-positive cells was counted.

Statistics

For cell death assays, statistical analysis was performed by analysis of variance (ANOVA) followed by Newman-Keuls' *post hoc* test. Data are expressed as means \pm S.E.M. A *p* value < 0.05 was accepted as statistically significant.

Results

Localization of DJ-1 to mitochondria and nucleus in response to oxidative stress

Sub-cellular fractionation studies were carried out to identify the localization of endogenous DJ-1 in response to hydrogen peroxide challenge. Human dopaminergic neuroblastoma cells SK-N-BE(2)C were treated with 100 μ M H₂O₂ for the indicated times (Fig. 1). At basal conditions, the bulk of DJ-1 was found in the cytosol, with little expression in mitochondria and nucleus. Following H₂O₂ treatment, more DJ-1 was detected in the mitochondria as early as 1 hour, increasing further at 3 hours. However, no change in nuclear localization of DJ-1 was seen within this time frame. The integrity of our fractionation procedures was confirmed by the presence of the mitochondrial marker cytochrome oxidase subunit IV exclusively in the mitochondrial fraction, and the presence of the transcription factor Sp1 exclusively in the nuclear fraction (Fig. 1A). We also verified the sub-mitochondrial localization of DJ-1 to the cytoplasmic side of the outer mitochondrial membrane by limited trypsin digestion studies (data not shown), as reported previously (Canet-Aviles et al. 2004). Longer exposure of cells to H₂O₂ to 12 and 24 hours resulted in the translocation of DJ-1 into the nucleus, at which time mitochondrial DJ-1 was no different than basal levels (Fig. 1B). These data demonstrate that endogenous DJ-1 moves from the cytoplasm to mitochondria and nucleus at different time frames following H₂O₂ challenge. The importance and function of these localizations in response to oxidative stress were studied next.

Dimerization of DJ-1 in mitochondria and nucleus

Crystallographic studies of DJ-1 have shown that the wild-type protein is a helix-strand-helix sandwich and forms a homodimer (Honbou et al. 2003; Huai et al. 2003; Lee et al. 2003; Tao and Tong 2003; Wilson et al. 2003). And studies using co-immunoprecipitation of different tagged forms of DJ-1 and yeast two hybrid screening support the ability of this protein to self-interact (Ito et al. 2006; Junn et al. 2005). The L166P mutation, which places the strong helix breaker proline in the C terminal helix located in the hydrophobic core of the molecule, interferes with dimerization (Macedo et al. 2003; Miller et al. 2003; Moore et al. 2003; Tao and Tong 2003). Therefore, loss of function of this mutant is presumably due to destabilization of the dimer interface (Taira et al. 2004b; Wilson et al. 2003), suggesting that dimerization is critical for the physiologic function(s) of DJ-1. We investigated whether DJ-1 localized in the mitochondria or nucleus is dimerized. To stabilize DJ-1 dimers, we employed disuccinimidyl suberate (DSS), a non-cleavable chemical cross-linker that forms covalent bonds between primary amines. SK-N-BE(2)C cells were incubated with 100 μ M DSS followed by sub-cellular fractionation into cytosolic, mitochondrial and nuclear fractions. Western blot analysis demonstrated that DJ-1 dimers are present in all these fractions (Fig. 2A). Considering that dimerization of DJ-1 is critical for its neuroprotective function, this finding suggests that DJ-1 present in mitochondria and nucleus is

physiologically active. On the other hand, dimer formation by L166P mutant DJ-1 in the presence of DSS was negligible (Fig. 2B), as reported previously (Baulac et al. 2004).

Cysteine mutants of DJ-1 can move to mitochondria and nucleus in response to H₂O₂

The highly conserved cysteine residue at position 106 of DJ-1 has previously been shown to be extremely sensitive to radiation (Wilson et al. 2003) and oxidative damage resulting in sulfinic acid modification (Canet-Aviles et al. 2004; Lee et al. 2003). Additionally, the C106A substitution reportedly blocks the mitochondrial localization of DJ-1 in response to paraquat (Canet-Aviles et al. 2004). Other cysteine residues, such as Cys 53 (located in the dimer interface) and Cys 46 may be oxidized to a certain extent as well (Kinumi et al. 2004), and the C53A mutant reportedly loses the ability to change pI in response to H₂O₂ or protect cells against this ROS generator (Shendelman et al. 2004; Taira et al. 2004b). To assess the importance of these three cysteine residues in mitochondrial and nuclear localization in response to oxidative stress, Flag-tagged DJ-1 with cysteine to serine substitutions at residues 46, 53 or 106 was each transfected into SK-N-BE(2)C cells, treated with H₂O₂ for 3 h (cytosol and mitochondrial samples) or 24 h (nuclear sample), followed by sub-cellular fractionation studies (Fig. 3). This experiment showed that all cysteine mutants could move to the mitochondria (8-10% of total DJ-1 protein) and nucleus (1-2%) similar to wild-type DJ-1 in response to H₂O₂. This result suggests that the localization of DJ-1 to the mitochondria or nucleus in response to H₂O₂ does not necessarily involve oxidation of its cysteine residues.

Mitochondria-localized DJ-1 exhibits enhanced neuroprotective activity

Next, we investigated the functional relevance of mitochondrial or nuclear localization of DJ-1 in response to oxidative stress, and addressed the question of which pool contributes to protection against oxidative stress. To achieve this, mitochondria-targeted DJ-1 (DJ-1^{Mito}) and nucleus-targeted DJ-1 (DJ-1^{Nuc}) were constructed as described in Materials and Methods section. These two constructs as well as wild-type DJ-1 were transfected into SK-N-BE(2)C cells and their sub-cellular localizations were confirmed by immunocytochemistry (Fig. 4A and 4B). DJ-1^{Mito} co-localized with MitoTracker as dotted and filamentous structures scattered in the cytoplasm, which is a typical mitochondrial pattern of staining (Fig. 4B). In addition, we confirmed that DJ-1^{Mito} protein partitions into the mitochondria by sub-cellular fractionation (data not shown). DJ-1^{Nuc}, on the other hand, was detected exclusively in the nucleus, perfectly co-localizing with DAPI staining (Fig. 4A).

With these confirmations, SK-N-BE(2)C cells were engineered to stably over-express wild-type, DJ-1^{Mito} and DJ-1^{Nuc}, respectively (Fig. 4C). Clones expressing DJ-1 isoforms at equivalent amounts were chosen for cell death experiments to minimize the effect of varied expression levels. These engineered cells were challenged with 200 μ M H₂O₂ for 24 h and cell death was measured using LDH assay in the supernatant (Fig. 4D). As expected, cell death was inhibited significantly by the over-expression of wild-type DJ-1. Notably, cells expressing mitochondrial localized DJ-1 (DJ-1^{Mito}) fared significantly better than those expressing wild-type DJ-1 or nuclear (DJ-1^{Nuc}). In addition, TUNEL assay showed that apoptotic cell death was inhibited to a greater degree by DJ-1^{Mito} than by wild-type DJ-1 (Fig. 4E). These results suggest that mitochondrial localization of DJ-1 in response to oxidative stress is a means for this protein to exert its cell-protective effect, although the mechanism by which this is accomplished remains to be fully elucidated.

Discussion

In this report, we provide evidence that DJ-1 translocates to mitochondria and nucleus in response to oxidative stress with different time frames, and that this translocation does not require oxidation of its cysteine residues. Although mitochondrial and nuclear localization of DJ-1 had been demonstrated (Canet-Aviles et al. 2004; Taira et al. 2004a; Zhang et al. 2005), the relationship between its sub-cellular localization and cell protective activity had not been addressed. To study whether these translocations of DJ-1 are required for its cytoprotective role, we constructed mitochondria-targeted DJ-1 (DJ-1^{Mito}) and nucleus-targeted DJ-1 (DJ-1^{Nuc}), and tested their effect on H₂O₂-induced cell death. DJ-1^{Mito} inhibited cell death more effectively than DJ-1^{Nuc} or wild-type DJ-1, suggesting that oxidative stress-induced early translocation of DJ-1 from the cytoplasm to mitochondria is involved in its protective role against oxidative stress.

Mitochondria have several compartments including its outer membrane, inner membrane, inter-membrane space and matrix. Each compartment has distinct protein components supporting different roles. We confirmed that DJ-1 is localized in the outer membrane of mitochondria as reported previously (Canet-Aviles et al. 2004). However, Zhang et al. reported DJ-1 to be localized in the inter-membrane space and matrix of mitochondria, but its role in these compartments has not been explained (Zhang et al. 2005). Discrepancies in the sub-mitochondrial localization of DJ-1 may be explained by the presence or absence of oxidative stress as well as due to possible methodological differences.

Since endogenously expressed DJ-1 is localized to the outer membrane of mitochondria upon oxidative stress, we fused the mitochondrial targeting signal of Bcl-X_L protein to the C-terminus of DJ-1 in order to construct DJ-1^{Mito}. Bcl-X_L is specifically targeted to the mitochondrial outer membrane, a process that requires the COOH-terminal transmembrane domain flanked at both ends (Kaufmann et al. 2003). This sequence has been shown as a *bona fide* targeting signal for the mitochondrial outer membrane as it confers soluble EGFP specific mitochondrial localization (Kaufmann et al. 2003). The mitochondrial outer membrane is an important site for apoptosis regulation. Permeabilization of this membrane is regarded as the 'point of no return' in executing the apoptotic cascade in a variety of cell death pathways (Chipuk et al. 2006). This permeabilization step is directly regulated by a subset of the BCL-2 family of proapoptotic proteins, which induce disruptions in the outer mitochondrial membrane and subsequent release of death-promoting proteins such as cytochrome c. Considering the cytoprotective activity of DJ-1 and particularly its mitochondrial localized fraction, it remains to be determined whether DJ-1 in the mitochondrial outer membrane can inhibit the activity of proapoptotic BCL-2 family proteins and prevent mitochondrial outer membrane permeabilization as another mechanism of cytoprotection.

Our data suggest that the localization of DJ-1 to the mitochondria or nucleus in response to H₂O₂ does not necessarily require oxidation of its cysteine residues. In contrast, Canet-Aviles et al reported that translocation of DJ-1 to mitochondria requires cysteine 106 oxidation in response to paraquat (Canet-Aviles et al. 2004). Several factors may account for this discrepancy. First, they presented immunocytochemical data, which are difficult to provide quantitative information. Second, they employed cysteine to alanine mutants rather than cysteine to serine substitution used in our present study; serine is closer to cysteine in terms of molecular weight and structure than alanine is. Third, the cell lines used and oxidative stress conditions applied were different in the respective experiments. In fact, it is expected that the anti-oxidant function of DJ-1 in cell death signaling events in the mitochondria is carried out by its non-oxidized form, whereas the oxidized protein is nonfunctional in this regard.

The differential time course for the translocation of cytoplasmic DJ-1 first to the mitochondria and at a later point to the nucleus is another indication for the multi-faceted functions of DJ-1, all of which have a cytoprotective role. While the mitochondrial localized DJ-1 appears to be primarily responsible for protection against oxidative stress in the short term, nuclear DJ-1 also protects against oxidant damage but clearly through different mechanisms. For example, the ability of DJ-1 to interact with and sequester the death protein Daxx in the nucleus, and prevent it from gaining access to the cytoplasm and activating apoptosis signal regulating kinase 1 is a potent mechanism for cytoprotection (Junn et al. 2005). Additionally, the transcriptional activity of DJ-1 can confer anti-apoptotic properties (Xu et al. 2005).

In conclusion, the present observations highlight the cytoprotective effect of DJ-1 present as a dimer in the outer membrane of mitochondria without necessarily being oxidized at its cysteine residues. Elucidating the processes that lead from its mitochondrial translocation to cell survival requires additional investigations.

Acknowledgments

The authors thank Dr. Jae-Kyun Ko for helpful discussions. This work was supported by National Institutes of Health Grants NS053517 and NS059869 to M.M.M. and by research grants from the American Parkinson Disease Association and the Foundation of UMDNJ to E.J. M.M.M. is the William Dow Lovett Professor of Neurology.

References

- Bandopadhyay R, Kingsbury AE, Cookson MR, Reid AR, Evans IM, Hope AD, Pittman AM, Lashley T, Canet-Aviles R, Miller DW, McLendon C, Strand C, Leonard AJ, Abou-Sleiman PM, Healy DG, Ariga H, Wood NW, de Silva R, Revesz T, Hardy JA, Lees AJ. The expression of DJ-1 (PARK7) in normal human CNS and idiopathic Parkinson's disease. *Brain*. 2004; 127(Pt 2):420–430. [PubMed: 14662519]
- Baulac S, LaVoie MJ, Strahle J, Schlossmacher MG, Xia W. Dimerization of Parkinson's disease-causing DJ-1 and formation of high molecular weight complexes in human brain. *Mol Cell Neurosci*. 2004; 27(3):236–246. [PubMed: 15519239]
- Bonifati V, Rizzu P, Squitieri F, Krieger E, Vanacore N, van Swieten JC, Brice A, van Duijn CM, Oostra B, Meco G, Heutink P. DJ-1 (PARK7), a novel gene for autosomal recessive, early onset parkinsonism. *Neurol Sci*. 2003; 24(3):159–160. [PubMed: 14598065]
- Canet-Aviles RM, Wilson MA, Miller DW, Ahmad R, McLendon C, Bandyopadhyay S, Baptista MJ, Ringe D, Petsko GA, Cookson MR. The Parkinson's disease protein DJ-1 is neuroprotective due to cysteine-sulfinic acid-driven mitochondrial localization. *Proc Natl Acad Sci U S A*. 2004; 101(24):9103–9108. [PubMed: 15181200]
- Chipuk JE, Bouchier-Hayes L, Green DR. Mitochondrial outer membrane permeabilization during apoptosis: the innocent bystander scenario. *Cell Death Differ*. 2006; 13(8):1396–1402. [PubMed: 16710362]
- Cookson MR. Pathways to Parkinsonism. *Neuron*. 2003; 37(1):7–10. [PubMed: 12526767]
- Hod Y, Penttyala SN, Whyard TC, El-Maghrabi MR. Identification and characterization of a novel protein that regulates RNA-protein interaction. *J Cell Biochem*. 1999; 72(3):435–444. [PubMed: 10022524]
- Honbou K, Suzuki NN, Horiuchi M, Niki T, Taira T, Ariga H, Inagaki F. The crystal structure of DJ-1, a protein related to male fertility and Parkinson's disease. *J Biol Chem*. 2003; 278(33):31380–31384. [PubMed: 12796482]
- Huai Q, Sun Y, Wang H, Chin LS, Li L, Robinson H, Ke H. Crystal structure of DJ-1/RS and implication on familial Parkinson's disease. *FEBS Lett*. 2003; 549(1-3):171–175. [PubMed: 12914946]
- Inden M, Taira T, Kitamura Y, Yanagida T, Tsuchiya D, Takata K, Yanagisawa D, Nishimura K, Taniguchi T, Kiso Y, Yoshimoto K, Agatsuma T, Koide-Yoshida S, Iguchi-Ariga SM, Shimohama

- S, Ariga H. PARK7 DJ-1 protects against degeneration of nigral dopaminergic neurons in Parkinson's disease rat model. *Neurobiol Dis.* 2006; 24(1):144–158. [PubMed: 16860563]
- Ito G, Ariga H, Nakagawa Y, Iwatsubo T. Roles of distinct cysteine residues in S-nitrosylation and dimerization of DJ-1. *Biochem Biophys Res Commun.* 2006; 339(2):667–672. [PubMed: 16316629]
- Junn E, Taniguchi H, Jeong BS, Zhao X, Ichijo H, Mouradian MM. Interaction of DJ-1 with Daxx inhibits apoptosis signal-regulating kinase 1 activity and cell death. *Proc Natl Acad Sci U S A.* 2005; 102(27):9691–9696. [PubMed: 15983381]
- Kaufmann T, Schlipf S, Sanz J, Neubert K, Stein R, Borner C. Characterization of the signal that directs Bcl-x(L), but not Bcl-2, to the mitochondrial outer membrane. *J Cell Biol.* 2003; 160(1): 53–64. [PubMed: 12515824]
- Kim RH, Smith PD, Aleyasin H, Hayley S, Mount MP, Pownall S, Wakeham A, You-Ten AJ, Kalia SK, Horne P, Westaway D, Lozano AM, Anisman H, Park DS, Mak TW. Hypersensitivity of DJ-1-deficient mice to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and oxidative stress. *Proc Natl Acad Sci U S A.* 2005; 102(14):5215–5220. [PubMed: 15784737]
- Kinumi T, Kimata J, Taira T, Ariga H, Niki E. Cysteine-106 of DJ-1 is the most sensitive cysteine residue to hydrogen peroxide-mediated oxidation in vivo in human umbilical vein endothelial cells. *Biochem Biophys Res Commun.* 2004; 317(3):722–728. [PubMed: 15081400]
- Kotaja N, Karvonen U, Janne OA, Palvimo JJ. PIAS proteins modulate transcription factors by functioning as SUMO-1 ligases. *Mol Cell Biol.* 2002; 22(14):5222–5234. [PubMed: 12077349]
- Lee SJ, Kim SJ, Kim IK, Ko J, Jeong CS, Kim GH, Park C, Kang SO, Suh PG, Lee HS, Cha SS. Crystal Structures of Human DJ-1 and Escherichia coli Hsp31, Which Share an Evolutionarily Conserved Domain. *J Biol Chem.* 2003; 278(45):44552–44559. [PubMed: 12939276]
- Macedo MG, Anar B, Bronner IF, Cannella M, Squitieri F, Bonifati V, Hoogveen A, Heutink P, Rizzu P. The DJ-1L166P mutant protein associated with early onset Parkinson's disease is unstable and forms higher-order protein complexes. *Hum Mol Genet.* 2003; 12(21):2807–2816. [PubMed: 12952867]
- Martinat C, Shendelman S, Jonason A, Leete T, Beal MF, Yang L, Floss T, Abeliovich A. Sensitivity to oxidative stress in DJ-1-deficient neurons: An ES-derived cell model of primary parkinsonism. *PLoS Biology.* 2004; 2(11):e327. [PubMed: 15502868]
- Menzies FM, Yenissetti SC, Min KT. Roles of Drosophila DJ-1 in survival of dopaminergic neurons and oxidative stress. *Curr Biol.* 2005; 15(17):1578–1582. [PubMed: 16139214]
- Meulener M, Whitworth AJ, Armstrong-Gold CE, Rizzu P, Heutink P, Wes PD, Pallanck LJ, Bonini NM. Drosophila DJ-1 mutants are selectively sensitive to environmental toxins associated with Parkinson's disease. *Curr Biol.* 2005; 15(17):1572–1577. [PubMed: 16139213]
- Miller DW, Ahmad R, Hague S, Baptista MJ, Canet-Aviles R, McLendon C, Carter DM, Zhu PP, Stadler J, Chandran J, Klinefelter GR, Blackstone C, Cookson MR. L166P mutant DJ-1, causative for recessive Parkinson's disease, is degraded through the ubiquitin-proteasome system. *J Biol Chem.* 2003; 278(38):36588–36595. [PubMed: 12851414]
- Mitsumoto A, Nakagawa Y. DJ-1 is an indicator for endogenous reactive oxygen species elicited by endotoxin. *Free Radic Res.* 2001; 35(6):885–893. [PubMed: 11811539]
- Mitsumoto A, Nakagawa Y, Takeuchi A, Okawa K, Iwamatsu A, Takanezawa Y. Oxidized forms of peroxiredoxins and DJ-1 on two-dimensional gels increased in response to sublethal levels of paraquat. *Free Radic Res.* 2001; 35(3):301–310. [PubMed: 11697128]
- Moore DJ, Zhang L, Dawson TM, Dawson VL. A missense mutation (L166P) in DJ-1, linked to familial Parkinson's disease, confers reduced protein stability and impairs homo-oligomerization. *J Neurochem.* 2003; 87(6):1558–1567. [PubMed: 14713311]
- Niki T, Takahashi-Niki K, Taira T, Iguchi-Arigo SM, Ariga H. DJBP: A Novel DJ-1-Binding Protein, Negatively Regulates the Androgen Receptor by Recruiting Histone Deacetylase Complex, and DJ-1 Antagonizes This Inhibition by Abrogation of This Complex. *Mol Cancer Res.* 2003; 1(4): 247–261. [PubMed: 12612053]
- Olzmann JA, Brown K, Wilkinson KD, Rees HD, Huai Q, Ke H, Levey AI, Li L, Chin LS. Familial Parkinson's disease-associated L166P mutation disrupts DJ-1 protein folding and function. *J Biol Chem.* 2003; 279(9):8506–8515. [PubMed: 14665635]

- Park J, Kim SY, Cha GH, Lee SB, Kim S, Chung J. Drosophila DJ-1 mutants show oxidative stress-sensitive locomotive dysfunction. *Gene*. 2005; 361:133–139. [PubMed: 16203113]
- Shendelman S, Jonason A, Martinat C, Leete T, Abeliovich A. DJ-1 is a redox-dependent molecular chaperone that inhibits alpha-synuclein aggregate formation. *PLoS Biol*. 2004; 2(11):e362. [PubMed: 15502874]
- Taira T, Iguchi-Ariga SM, Ariga H. Co-localization with DJ-1 is essential for the androgen receptor to exert its transcription activity that has been impaired by androgen antagonists. *Biol Pharm Bull*. 2004a; 27(4):574–577. [PubMed: 15056870]
- Taira T, Saito Y, Niki T, Iguchi-Ariga SM, Takahashi K, Ariga H. DJ-1 has a role in antioxidative stress to prevent cell death. *EMBO Rep*. 2004b; 5(2):213–218. [PubMed: 14749723]
- Takahashi K, Taira T, Niki T, Seino C, Iguchi-Ariga SM, Ariga H. DJ-1 positively regulates the androgen receptor by impairing the binding of PIASx alpha to the receptor. *J Biol Chem*. 2001; 276(40):37556–37563. [PubMed: 11477070]
- Takahashi-Niki K, Niki T, Taira T, Iguchi-Ariga SM, Ariga H. Reduced anti-oxidative stress activities of DJ-1 mutants found in Parkinson's disease patients. *Biochem Biophys Res Commun*. 2004; 320(2):389–397. [PubMed: 15219840]
- Tao X, Tong L. Crystal structure of human DJ-1, a protein associated with early onset Parkinson's disease. *J Biol Chem*. 2003; 278(33):31372–31379. [PubMed: 12761214]
- van Duijn CM, Dekker MC, Bonifati V, Galjaard RJ, Houwing-Duistermaat JJ, Snijders PJ, Testers L, Breedveld GJ, Horstink M, Sandkuijl LA, van Swieten JC, Oostra BA, Heutink P. PARK7, a Novel Locus for Autosomal Recessive Early-Onset Parkinsonism, on Chromosome 1p36. *Am J Hum Genet*. 2001; 69(3):629–634. [PubMed: 11462174]
- Ved R, Saha S, Westlund B, Perier C, Burnam L, Sluder A, Hoener M, Rodrigues CM, Alfonso A, Steer C, Liu L, Przedborski S, Wolozin B. Similar patterns of mitochondrial vulnerability and rescue induced by genetic modification of alpha-synuclein, parkin, and DJ-1 in *Caenorhabditis elegans*. *J Biol Chem*. 2005; 280(52):42655–42668. [PubMed: 16239214]
- Wilson MA, Collins JL, Hod Y, Ringe D, Petsko GA. The 1.1-A resolution crystal structure of DJ-1, the protein mutated in autosomal recessive early onset Parkinson's disease. *Proc Natl Acad Sci U S A*. 2003; 100(16):9256–9261. [PubMed: 12855764]
- Xu J, Zhong N, Wang H, Elias JE, Kim CY, Woldman I, Pifl C, Gygi SP, Geula C, Yankner BA. The Parkinson's disease-associated DJ-1 protein is a transcriptional co-activator that protects against neuronal apoptosis. *Hum Mol Genet*. 2005; 14(9):1231–1241. [PubMed: 15790595]
- Yang Y, Gehrke S, Haque ME, Imai Y, Kosek J, Yang L, Beal MF, Nishimura I, Wakamatsu K, Ito S, Takahashi R, Lu B. Inactivation of Drosophila DJ-1 leads to impairments of oxidative stress response and phosphatidylinositol 3-kinase/Akt signaling. *Proc Natl Acad Sci U S A*. 2005; 102(38):13670–13675. [PubMed: 16155123]
- Yokota T, Sugawara K, Ito K, Takahashi R, Ariga H, Mizusawa H. Down regulation of DJ-1 enhances cell death by oxidative stress, ER stress, and proteasome inhibition. *Biochem Biophys Res Commun*. 2003; 312(4):1342–1348. [PubMed: 14652021]
- Zhang L, Shimoji M, Thomas B, Moore DJ, Yu SW, Marupudi NI, Torp R, Torgner IA, Ottersen OP, Dawson TM, Dawson VL. Mitochondrial localization of the Parkinson's disease related protein DJ-1: implications for pathogenesis. *Hum Mol Genet*. 2005; 14(14):2063–2073. [PubMed: 15944198]
- Zhou W, Freed CR. DJ-1 up-regulates glutathione synthesis during oxidative stress and inhibits A53T alpha-synuclein toxicity. *J Biol Chem*. 2005; 280(52):43150–43158. [PubMed: 16227205]
- Zhou W, Zhu M, Wilson MA, Petsko GA, Fink AL. The oxidation state of DJ-1 regulates its chaperone activity toward alpha-synuclein. *J Mol Biol*. 2006; 356(4):1036–1048. [PubMed: 16403519]

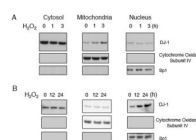


Fig.1. Sub-cellular distribution of DJ-1 in human neuroblastoma cells SK-N-BE(2)C in response to hydrogen peroxide

Cells were treated with 0.1 mM H₂O₂ for up to 3 h (A) and up to 24 h (B), and then fractionated to cytosol, mitochondria and nucleus. Fractionated samples were subjected to Western blot analysis with anti-DJ-1 (Stressgen), anti-cytochrome oxidase subunit IV (Molecular Probe) and anti-Sp1 (Santa Cruz Biotechnology). This figure demonstrates that DJ-1 moves to mitochondria within 3 h of H₂O₂ stimulation and to the nucleus by 12 h. A representative Western blot of three independent experiments is shown.

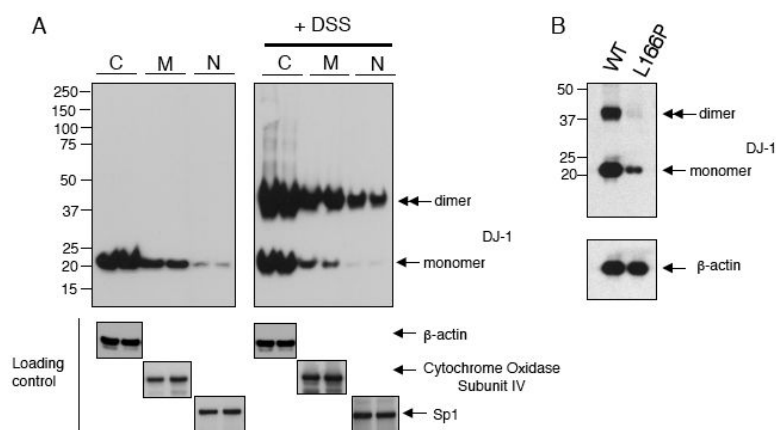


Fig.2. Presence of DJ-1 dimer in mitochondria and nucleus

(A) SK-N-BE(2)C cells exposed to 0.1 mM H₂O₂ for 3h (cytosol, mitochondria fraction) or 24h (nucleus fraction) and then treated with 100 μM disuccinimidyl suberate (DSS) for 30 min. Fractionated samples were subjected to SDS-PAGE and Western blot analysis with anti-DJ-1 (Stressgen) and with antibodies for several loading controls such as anti-β-actin (Sigma), anti-cytochrome oxidase subunit IV and anti-Sp1. DJ-1 dimers are detected in mitochondrial, nuclear as well as cytosolic fractions. The experiment was run in duplicates. (B) SK-N-BE(2)C cells were transiently transfected with Flag-tagged DJ-1, wild-type or L166P mutant, and treated with DSS. Samples were subjected to SDS-PAGE and Western blot analysis.

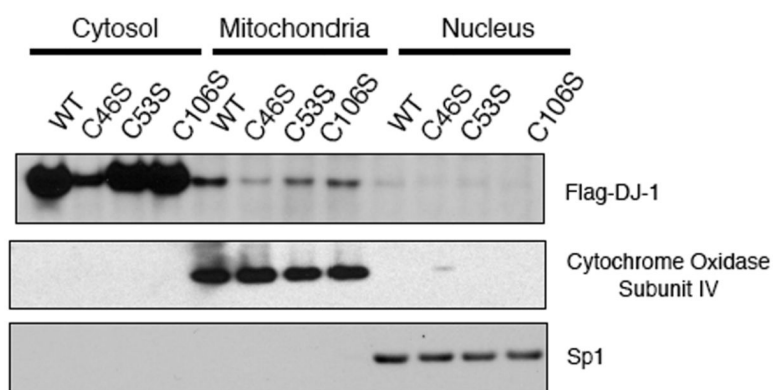


Fig.3. Cysteine oxidation is not involved in the translocation of DJ-1 in response to H₂O₂

SK-N-BE(2)C cells were transfected with plasmids encoding Flag-tagged DJ-1 harboring cysteine to serine substitutions at residues 46, 53 or 106, treated with H₂O₂ for 3 h (cytosol and mitochondria fraction) or 24 h (nuclear fraction), followed by sub-cellular fractionation. Fractionated samples were subjected to Western blot analysis with anti-Flag M2 (Sigma). A representative Western blot of three independent experiments is shown.

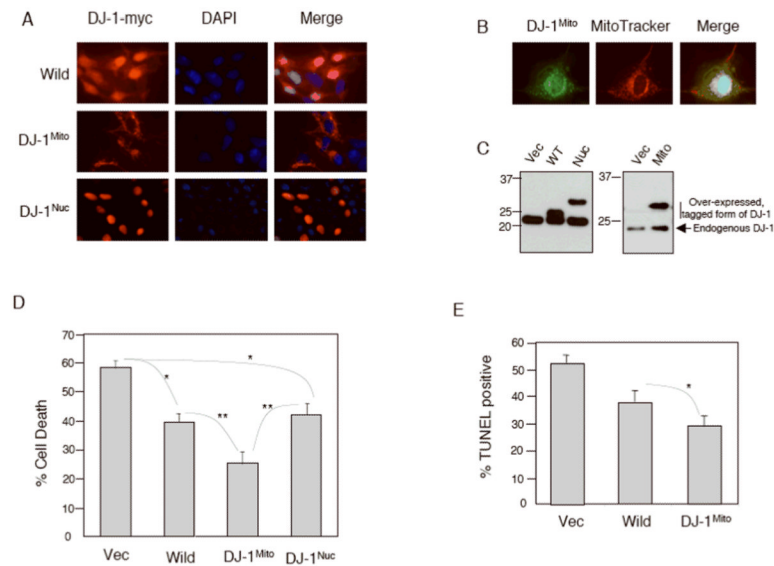


Fig.4. Mitochondrial localized DJ-1 has better neuroprotective activity

(A) SK-N-BE(2)C cells were transfected with wild-type DJ-1, DJ-1^{Mito} or DJ-1^{Nuc} constructs and immunostained with anti-Myc-Rhodamine (Santa Cruz Biotechnology). Nuclei were counter-stained blue with DAPI. (B) SK-N-BE(2)C cells were transfected with DJ-1^{Mito} construct and immunostained with anti-Myc-FITC and MitoTracker Red (Molecular Probes). (C) SK-N-BE(2)C cells were engineered to stably express wild-type DJ-1, DJ-1^{Mito} and DJ-1^{Nuc}, and expression was confirmed by Western blot analysis with anti-DJ-1 (Stressgen). This figure shows over-expressed tagged DJ-1 as well as the endogenous native protein. (D) Stably transformed SK-N-BE(2)C cells described in (B) were challenged with 0.2 mM H₂O₂ for 24 h, and cell death was measured with LDH Cytotoxicity Detection Kit (Roche). DJ-1^{Mito} over-expressing cells fared significantly better compared to cells over-expressing wild-type DJ-1 or DJ-1^{Nuc}. Values are means \pm S.E.M. for triplicates. * ANOVA $p < 0.05$; ** $p < 0.01$. (E) SK-N-BE(2)C cells stably transfected with DJ-1 isoforms were challenged with 0.1 mM H₂O₂ for 12 h, and apoptotic cells were detected by TUNEL staining. TUNEL-positive cells were counted in 8 randomly selected fields. Each microscopic field had 10-30 cells. The data represent means \pm S.E.M. * ANOVA $p < 0.05$.