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REVIEW

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Linking DJ-1 to neurodegeneration offers novel insights for understanding the pathogenesis of Parkinson's disease

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Abstract Rare monogenic forms of Parkinson's disease (PD) are promoting our understanding of the molecular pathways involved in the common, non-Mendelian forms of the disease. Here, we focus on PARK7, an autosomal recessive form of early-onset parkinsonism caused by mutations in the *DJ-1* gene. We first review the genetics of this form and the rapidly expanding knowledge about the structure and biochemical properties of the DJ-1 protein. We also discuss how DJ-1 dysfunction might lead to neurodegeneration, and the implications of this novel piece of information for the pathogenesis of the common PD forms. Although much work remains to be done to clarify the biology of DJ-1, its proposed activity as a molecular chaperone and/or as oxidative sensor appear intriguing in the light of the current theories on the pathogenesis of PD.

Keywords Parkinson's disease · Genes · Loci · PARK7 · DJ-1

Abbreviations *AR:* Androgen receptor · *DJBP:* DJ-1 binding protein · *GAPDH:* Glyceraldehyde 3-phosphate dehydrogenase · *HDAC:* Histone deacetylase · *PD:* Parkinson's disease · *PIAS:* Protein inhibitor of activated STAT · *STAT:* Signal transducers and activators of transcription · *SUMO:* Small ubiquitin-like modifier

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Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease, with a prevalence of 1–2% in population aged 65 years or older [1]. The disease is clinically defined by the presence of parkinsonism (the combination of akinesia, resting tremor, and muscular rigidity), and a good response to dopaminergic therapy. These features are associated at pathological level with neuronal loss and

Table 1 Current catalogue of genes and loci for PD (*LB* Lewy bodies)

Locus	OMIM	Map position	Gene	Inheritance	Pathology	Reference
PARK1	#168601	4q21-q23	*α-synuclein*	Dominant, high penetrance	LB positive	10
PARK2	#600116	6q25-q27	*parkin*	Recessive	Mostly LB negative	25
PARK3	602404	2p13	Unknown	Dominant-incomplete penetrance	LB positive	108
PARK4	605543	4p15	Unknown	Dominant, high penetrance	LB positive	109
PARK5	191342	4p14	*UCH-L1*	Dominant	Unknown	29
PARK6	605909	1p36-p35	Unknown	Recessive	Unknown	36
PARK7	#606324	1p36	*DJ-1*	Recessive	Unknown	37
PARK8	607060	12p11-q13	Unknown	Dominant-incomplete penetrance	LB negative	110
PARK9	606693	1p36	Unknown	Recessive	Unknown	38
PARK10	606852	1p32	Unknown	Non-Mendelian	Unknown	5
PARK11	607688	2q36-q37	Unknown	Non-Mendelian	Unknown	6
Pending	601828	2q22-q23	*NR4A2*	Dominant	Unknown	111

gliosis, mainly in the *substantia nigra pars compacta* but also in other brain areas, and formation of cytoplasmic inclusions called Lewy bodies and Lewy neurites in the surviving neurons [2].

PD is generally a sporadic disorder, but in a significant proportion of cases (10–15% in most studies) it runs in families without a clearcut Mendelian pattern. More rarely, it segregates as a Mendelian trait with either autosomal dominant or recessive inheritance. The common form of PD is therefore likely to be a complex trait, determined by several genetic as well as nongenetic factors. In the last few years family-based linkage analysis and positional cloning have led to the identification of several loci and genes for the rare monogenic forms (Table 1) [3, 4], and more recently of two loci for the classical, non-Mendelian forms (Table 1) [5, 6]. Although the monogenic forms so far identified explain a very small fraction of PD cases, they are promoting the understanding of the molecular pathways involved in the common forms of PD.

In the light of these genetic studies PD is therefore etiologically heterogeneous. It is possible that PD includes many distinct diseases with distinct molecular mechanisms, but presenting with similar clinical and/or pathological endpoints. However, a more convincing view is that there are common pathways underlying PD, which can be initiated by several causes including rare Mendelian mutations or a combination of multiple genetic and nongenetic factors in the common forms. A possible way to reconcile the evidence from the different monogenic forms and other research lines is to assume that PD is a disorder of the protein quality control system which is associated with neuronal accumulation of misfolded proteins and presence of protein aggregates. This view is supported by the results of studies on two other gene products, which have been firmly associated with PD: α-synuclein and parkin.

α-Synuclein is abundant in neurons and enriched in the presynaptic compartment [7]. Although its exact function remains unknown, evidence suggests an involvement in synaptic plasticity, regulation of size, and turnover of synaptic vesicles [7, 8, 9]. Missense variants of α-synuclein are a very rare cause of autosomal dominant PD [10, 11], but wild-type α-synuclein is one of the major components of the Lewy bodies in all PD forms and in other "synucleinopathies" [12]. Overexpressing wild-type or mutant human α-synuclein in transgenic animals yields varying degrees of biochemical, pathological, and clinical abnormalities reminiscent of PD, once again implicating α-synuclein in the pathogenesis of the disease in general [13]. α-Synuclein oligomers [14] are believed to represent the precursors of higher order aggregates (fibrils), which are assembled in vivo in the filamentous structures seen in Lewy bodies. Evidence suggests that in different neurodegenerative diseases the oligomers, and not the mature fibrils, are the real neurotoxic molecules [15, 16, 17, 18]. Regulation of the levels of monomeric and/or oligomeric α-synuclein in neurons therefore appears critical, and this regulation might be altered in the common PD forms due to an increased α-synuclein expression or decreased clearance or both. How monomeric or oligomeric α-synuclein exerts neurotoxicity remains unclear, but several possibilities have been suggested, including a direct inhibition of the proteasome system [19, 20], impairment of mitochondrial function [21], derangement in cellular trafficking [22], and damage [23] or functional impairment [24] of synaptic vesicles.

Mutations in *parkin* are much more frequent than *α-synuclein* mutations [25, 26], and they cause an autosomal recessive form of PD. This gene encodes an ubiquitin ligase [27]. Covalent attachment of the ubiquitin polypeptide (ubiquitylation) tags proteins for proteasomal degradation, and this is a fundamental mechanism for the protein quality control system [28]. The many mutations found in PD in *parkin* and one found in *UCH-L1* [29] (encoding a protein needed for the maintenance of the pool of neuronal ubiquitin) [30] implicate the ubiquitylation pathways in the pathogenesis of PD. Furthermore, parkin and α-synuclein might interact either directly (via a glycosylated isoform of α-synuclein) [31] or indirectly via synphilin-1 (a protein known to interact with both parkin and α-synuclein) [32, 33]. These reactions would link parkin to α-synuclein, the other protein which is central in the pathogenesis of PD. Recent studies have indeed shown that parkin suppresses the α-synuclein induced neurodegeneration in *Drosophila* [34] and in cell cultures [35], again suggesting that parkin is also implicated in the pathogenesis of PD in general.

Here we focus on the *DJ-1* gene and its associated phenotype, an autosomal recessive form of early onset parkinsonism. We also review the evidence on the structure and function of the DJ-1 protein. Last, we discuss how DJ-1 defects might lead to neurodegeneration, and how this novel piece of information can promote our understanding of the pathogenesis of the common PD forms.

Genetics of PARK7/*DJ-1*

Three novel loci for autosomal recessive forms of early onset (PARK6, PARK7, and PARK9) and a locus controlling susceptibility to and/or onset age of late-onset PD (PARK10) have been mapped to chromosome 1p, delineating the short arm of chromosome 1 as a hot spot for PD-related genes [5, 36, 37, 38, 39]. We originally mapped the PARK7 locus with genome-wide significance [37] and later confirmed this linkage in a different dataset [40]. Fine mapping studies and a positional cloning strategy led us to the identification of mutations in the *DJ-1* gene in two families definitely linked to PARK7 [41].

The human *DJ-1* gene spans 24-kb at genomic level, and it contains eight exons, the first two of which are noncoding and subject to alternative splicing [41, 42]. The expression of the *DJ-1* transcripts is ubiquitous in the brain areas and extracerebral tissues [41, 42] and seems more abundant in subcortical than in cortical brain areas [41]. However, this pattern remains to be confirmed at the protein level. The protein encoded by the human *DJ-1* gene possesses 189 amino acids, and its function has remained unknown until recently.

The *DJ-1* gene has been highly conserved in evolution. The structures of the mouse and human *DJ-1* genes are similar, and human and mouse DJ-1 proteins display 90% amino acid identity [42]. The regulation of DJ-1 expression is mostly unknown, but a Specificity Protein 1 (a transcription factor) dependent site at position −100 from the transcription initiation site appears to contribute most of the promoter activity [42].

All patients in the Dutch kindred in whom PARK7 was originally identified carry a homozygous deletion which removes approx. 14 kb of genomic sequence, including 4 kb upstream of the *DJ-1* start codon, and a large part of the coding region (Fig. 1). In the patients of an Italian PARK7-linked family a homozygous missense mutation replaces the highly conserved leucine at position 166 with proline (L166P) in the DJ-1 protein [41].

Since our initial report a total number of 11 different mutations in *DJ-1* have been identified, including missense, truncating, splice site mutations, and large deletions (Fig. 1, Table 2) [41, 43, 44] (K. Hedrich et al., submitted). In particular, an additional patient with early-onset PD carrying compound heterozygous *DJ-1* mutations (including one truncating and one splice site mutation; case #8 in Table 2) provides strong support to the contention that *DJ-1* mutations are pathogenic in this disease. For other mutations, especially the single

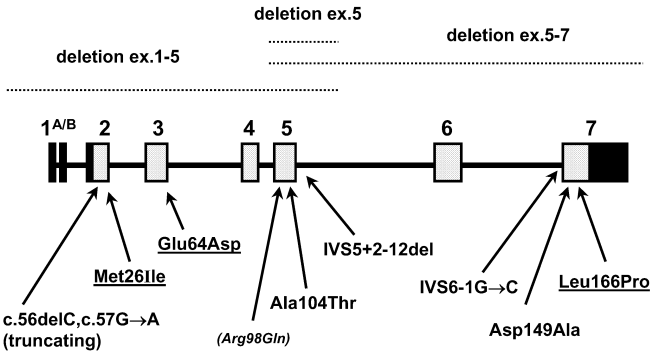

Fig. 1 Genomic structure of the *DJ-1* gene and mutations identified in PD. *Dark boxes* Noncoding exonic sequence; *light boxes* coding exonic sequence. Three missense mutations observed in homozygous form are underlined. The Arg98Gln change is a rare polymorphic variant observed in approx. 1.5% of control chromosomes in whites (K. Hedrich et al., submitted)

heterozygous ones, a pathogenic role remains to be demonstrated. On the basis of the first screening in large series of patients, *DJ-1* mutations are the second most frequent identifiable genetic cause of PD after *parkin*, but the frequency of *DJ-1* mutations seems rather low, being estimated at about 1–2% in early-onset PD [43, 44] (K. Hedrich et al., submitted).

The occurrence of heterozygous genomic rearrangements in *DJ-1* emphasizes the importance of gene dose assays for a sensitive screening. This has been performed in only one study so far (K. Hedrich et al., submitted), and further work, including gene dose assays and promoter analysis, are therefore needed to determine accurately the frequency of this form among early-onset PD forms and to characterize the associated phenotype. That the DJ-1 protein exists as a dimer (see below) suggests the possibility of dominant-negative mutations, and therefore a dominant pattern of inheritance should not preclude testing the *DJ-1* gene until there is more clarity on how mutations in DJ-1 lead to disease. The identification of additional disease-linked missense mutations will help pinpointing functionally important domains in the DJ-1 protein and will promote understanding of the role of DJ-1 and the mechanisms of DJ-1 related disease.

The similarities between *DJ-1* and PARK6-associated phenotypes and the proximity of the two loci on chromosome 1p raise the question of whether mutations in similar or functionally related genes underlie the two forms. An alternative possibility is that, due to a large genomic rearrangement occurring in one of the families in which these loci have been originally mapped [36, 37], linkage is found in seemingly different regions, while there is only one underlying defective gene. To explore this possibility a mutational analysis of the *DJ-1* gene should also be performed in the original PARK6-linked family.

Evidence for linkage to the PARK7 region was not found in genome scans for late-onset familial PD [5, 45, 46], suggesting that *DJ-1*, as with the other known genes

Table 2 Clinical and genetic features in patients with DJ-1 mutations

Code	Presentation	Origin	Sex	Onset[a]	Duration[b]	First mutation	Second mutation	Reference
#1	Familial	Dutch	F	31	17	Δ exon 1–5	Δ exon 1–5	41
#2	Familial	Dutch	M	40	10	Δ exon 1–5	Δ exon 1–5	41
#3	Familial	Dutch	M	<40	Na	Δ exon 1–5	Δ exon 1–5	41
#4	Familial	Dutch	M	27	11	Δ exon 1–5	Δ exon 1–5	41
#5	Familial	Italian	M	28	18	L166P	L166P	41
#6	Familial	Italian	F	35	21	L166P	L166P	41
#7	Familial	Italian	M	27	32	L166P	L166P	41
#8	Sporadic	Hispanic	F	24	5	IVS6–1G→C	c.56delC, c.57G→A	43 [c]
#9	Sporadic	Hispanic	M	35	9	A104T	–	43 [d]
#10	Sporadic	Jewish	na	39	na	M26I	M26I	44
#11	Sporadic	Afro-Caribbean	na	36	na	D149A	–	44 [d]
#12	Sporadic	South Tyrolean	M	42	17	Δ exon 5–7	–	–[e]
#13	Sporadic	Russian	F	17	na	IVS5 +2–12del	–	–[e]
#14	Sporadic	Serbian	M	45	8	Δ exon 5	–	–[f]
#15	Sporadic	Turkish	M	<40	na	E64D	E64D	–[g]

[a] Age (years) at disease onset
[b] Disease duration at the time of the last examination
[c] The c.56delC, c.57G→A mutation leads to frameshift and truncation of DJ-1 protein after the first 18 amino acids
[d] Gene copy dose analysis not performed
[e] K. Hedrich et al., submitted
[f] Personal communication, C. Klein and V. Kostic, September 2003
[g] Personal communication, R. Krüger and O. Riess, October 2003

for Mendelian PD, is not a major locus in common familial forms. However, whether genetic variation in *DJ-1* modifies the susceptibility to or modulates the expression of sporadic late-onset PD or other neurodegenerative diseases remains to be explored.

The homozygous deletion found in the patients of the Dutch PARK7 family represents a natural knockout of *DJ-1*, indicating that the loss of function of DJ-1 is pathogenic [41]. Our recent findings indicate that the L166P mutation also induces a loss of DJ-1 function, because the mutant DJ-1^{L166P} protein is unstable and rapidly degraded, resulting in much lower steady state levels in both transfected cells and patient lymphoblasts [47]. The instability of DJ-1^{L166P} has also been reported in an independent study [48]. Taken as a whole this evidence suggests that the mutant is misfolded and underlies rapid degradation, as observed in many genetic diseases [49].

In addition to having a low steady-state level, the subcellular localization of the mutant DJ-1^{L166P} protein in transfection experiments is changed in comparison to the pattern seen with the wild-type DJ-1, suggesting a further pathogenic mechanism. While the wild-type DJ-1 shows uniform localization in the cytosol and nucleus [41, 50, 51], the DJ-1^{L166P} mutant retains the nuclear localization but has lost the uniform cytosolic distribution and is mostly colocalized with mitochondria [41]. We have recently confirmed this finding using untagged *DJ-1* constructs, therefore excluding tagging effects on the conformation of the mutant protein [47]. However, due to the high levels of expression in cell systems analyzed [41, 47] we could not exclude that a fraction of wild-type DJ-1 also localizes to mitochondria, as recently suggested by others [48]. This possibility warrants further investigations. The mutant DJ-1 could therefore be targeted to mitochondria as a result of abnormal structure, or the reduced steady state levels could lead DJ-1 to occupy only its sites of highest affinity binding, which may include mitochondria and the nucleus. Further studies are needed to investigate the subcellular localization of DJ-1. However, the fact that the mutant (or perhaps also the wild-type) DJ-1 is colocalized with mitochondria suggests links to the function of these organelles, such as energy production, oxidative stress, and apoptosis. Mitochondrial abnormalities have been described in classical PD [52], and assessing the mitochondrial function in the patients with *DJ-1* mutations is warranted.

Pathology studies

Pathological analysis of brains from patients with *DJ-1* related forms is of great importance, but brain material is not currently available. Moreover, investigating the presence of the DJ-1 protein in brain from patients with Lewy body disease and other neurodegenerative diseases might provide clues on the involvement of *DJ-1* in common forms of neurodegeneration. While we have not found evidence of DJ-1 immunoreactivity in Lewy bodies with the available antibodies, we have found that the DJ-1 immunoreactivity colocalizes within a subset of pathological tau inclusions in various neurodegenerative disorders, including Pick's disease, Alzheimer's disease, Lewy body dementia, progressive supranuclear palsy, and frontotemporal dementia with parkinsonism linked to chromosome 17 [53]. This supports the view that different neurodegenerative diseases may have similar pathogenetic mechanisms, which likely include a role for DJ-1. This finding raises the question of whether tau pathology is also present in the brain of patients with DJ-1 mutations. Interestingly, tau pathology has been found in patients

with parkin disease [54, 55]. Therefore, if this would also be the case in DJ-1 related disease, it would suggest the existence of a common pathological signature in DJ-1 and parkin-related forms. Investigating the expression of the DJ-1 protein in the brain of patients with parkin disease and vice versa is also warranted.

Clinical aspects

Early onset of parkinsonism, slow disease progression, and good response to levodopa are uniform clinical features in different autosomal recessive forms of early onset, including *parkin* -related, *DJ-1*-related, and PARK6-linked families [26, 37, 40, 56, 57, 58]. This makes their differentiation difficult on clinical grounds, indicating the importance of genetic testing. The average age at onset is in the early 30s in the *parkin* and *DJ-1* related families whereas onset in PARK6-linked families might be slightly later (late 30s–early 40s). Interestingly, psychiatric disturbances (including severe anxiety in three cases, and psychotic episodes in one), early behavioral disturbances (one case), and dystonic features (including blepharospasm in two cases) are reported in both the original *DJ-1* related families, and they might be clinically useful for suspecting *DJ-1* involvement [37, 40, 58]. The presence of severe anxiety and panic attacks has indeed been noted in two further *DJ-1* related patients identified in a recent screen [44]. However, a note of caution is warranted as these psychiatric disturbances are nonspecific; they occur in PD in general [59] and have also been reported in patients with mutations in the *parkin* gene [60, 61]. Analyzing larger case series is therefore needed to investigate whether psychiatric disturbances are more frequent in the *DJ-1* related form than in other PD forms.

Recent positron emission tomography studies in *DJ-1* related patients showed more uniform patterns of caudate/putamen dopaminergic terminal dysfunction than that observed in classical PD, and a greater dopaminergic dysfunction than the one expected on the basis of clinical severity [58]. These features also resemble the pattern observed in *parkin* -related [62] and PARK6-linked [57] disease, suggesting that these three autosomal recessive forms of early onset are similar to each other on pathophysiological grounds.

Linking DJ-1 to neurodegeneration

The exact role of the DJ-1 protein remains mostly unknown, but several pieces of information are available in the literature which provide clues to a possible function and allow hypotheses linking DJ-1 to neurodegeneration to be formulated (Fig. 2). The current evidence suggests an involvement of DJ-1 in processes as different as cell cycle regulation and oncogenesis, sperm maturation and fertilization, control of gene transcription, regulation of mRNA stability, and response to cell stress.

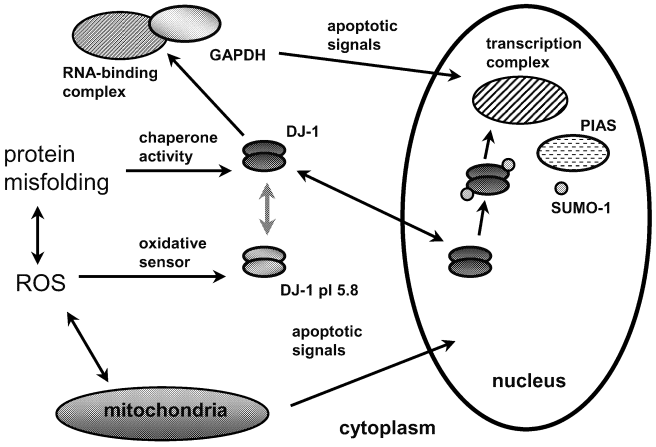

Fig. 2 Model of DJ-1 as a stress-responsive, multifunctional protein. DJ-1 might have chaperone activity, which helps refolding misfolded proteins induced by oxidative and other cell-stress conditions. DJ-1 itself might sense oxidative signals via oxidation of the Cys 106 residue into sulfinic acid. Furthermore, DJ-1 might influence the expression of genes for the stress response at transcriptional and post-transcriptional levels by interacting with PIAS and other nuclear cofactors and with cytosolic RNA-binding protein complexes. The latter complexes may also be associated with GAPDH, a protein with functional links to apoptosis and PD

A human cDNA termed *DJ-1* was first cloned in 1997 in a yeast two-hybrid screen for human proteins interacting with the protein encoded by the oncogene c-*myc*. The DJ-1 protein was reported to have oncogenic properties in cooperation with the oncogene ras [50]. Recent proteomic-based studies found increased expression of the DJ-1 protein in various human tumors, again suggesting involvement of DJ-1 in oncogenesis [63, 64, 65].

In 1999 a human protein named RS that was identical to DJ-1 was identified as the regulatory subunit of a RNA-binding protein complex [51]. The authors suggested that DJ-1 is a multifunctional protein involved in cytoskeleton-coupled RNA sorting, RNA degradation and functions in the nucleus as well. Interestingly, in the latest study [51] DJ-1 was copurified with glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Whether GAPDH is a genuine DJ-1 interactor remains to be explored. However, this is a first, potential link between DJ-1 and neurodegeneration (Fig. 2). GAPDH is increasingly recognized as a multifunctional protein which is involved not only in glycolysis but also in other processes, which include the induction of a neuronal apoptotic pathway [66, 67, 68]. Evidence also links GAPDH to the pathogenesis of classical PD, as nuclear translocation of GAPDH has been detected in nigral neurons in postmortem PD brains, and GAPDH colocalizes with α-synuclein in Lewy bodies [69]. In the light of this evidence the possible interaction between DJ-1 and GAPDH warrants further investigation. Interestingly, the yeast genes encoding DJ-1 and GAPDH homologs are induced during cell stress, together with chaperones, antioxidants, and other stress-response genes [70].

Two independent groups have identified a rat protein called CAP1 (contraception-associated protein 1) or SP22 (sperm protein 22), putatively involved in spermatogenesis and fertilization, as the rat DJ-1 homolog [71, 72]. Whether DJ-1 is involved in these processes in humans remains unclear. However, DJ-1 is present in human sperm, mainly as a flagellum protein, whereas in rats the homolog CAP1 or SP22 is also abundant in sperm heads [73]. In the tail DJ-1 colocalizes with β-tubulin, a major axoneme component. The issue of fertility has not been studied in men with DJ-1 related parkinsonism, and this should be actively explored in the future.

In a yeast two-hybrid system the protein inhibitor of activated STAT (PIAS) xα/ARIP3 has been detected as a DJ-1 binding protein [74]. PIASxα/ARIP3 is expressed predominantly in the testis and is a modulator of the androgen receptor (AR) transcriptional activity [75]. In cell cultures the effects of PIASxα/ARIP3 on AR activity depend from the cell lines and the reporter genes used [76]. However, in most cell lines, including Sertoli cells, PIASxα/ARIP3 is a negative modulator of the AR transcriptional activity [74]. DJ-1 might therefore positively regulate the AR-mediated transcriptional activity by recruiting PIASxα and thereby removing its inhibitory activity [74]. PIASxα/ARIP3 belongs to a family of proteins that modulate the activity of transcription factors by functioning as small ubiquitin-like modifier (SUMO) 1 ligases [77]. Interestingly, a DJ-1 mutant at Lys130 (K130R) was unable to regulate the AR activity [74]. As the lysine 130 of DJ-1 seems to be sumoylated [74], it is possible that this modification is necessary for the full activity of DJ-1.

More recently another DJ-1 binding protein, DJBP, was identified [78]. DJBP is also an AR-binding protein which is specifically expressed in the testis and appears to inhibit AR activity by recruiting a histone deacetylase (HDAC) complex [78]. The binding of DJ-1 to the DJBP-AR complex abrogates the HDAC-DJBP interaction, resulting in the enhancement of the AR activity. DJ-1 appears therefore to positively regulate AR by antagonizing the inhibitory effects of PIASxα and DJBP [74, 78].

The fact that DJBP is expressed exclusively [78] and PIASxα/ARIP3 predominantly in the testis [75] suggests that these proteins are not relevant for the neuronal function of DJ-1. However, other members of the PIAS family, PIAS3 and PIASy, have also been shown to bind DJ-1 [74]. These interactions remain to be characterized and could be more relevant for the effects of DJ-1 on gene expression in neurons.

Recent observations suggest that sumoylation plays an important role in brain function and neurodegeneration [79, 80] Most of the known substrates for sumoylation are nuclear proteins, and sumoylation might influence protein function by changing the substrate localization, by competing with ubiquitylation, thereby inhibiting substrate degradation, and by directly modulating the functional properties of the substrate [81]. Intriguingly, sumoylation regulates the activity not only of the steroid receptor superfamily but also of transcription factors mediating the heat-shock response, such as HSF1 and HSF2 [81]. It will be interesting to see whether these transcription factors are also targeted by DJ-1 either directly or through interaction with PIAS. Future studies will reveal the real targets of DJ-1 activity in the nucleus, but perhaps an important contribution of the discovery of *DJ-1* mutations in PARK7 is the focusing to the nuclear mechanisms and the control of gene expression in PD pathogenesis.

Sumoylation can also influence synaptic function via regulation of calcium/calmodulin-dependent kinase II CaMKII, [79], suggesting yet another possible link between DJ-1 and neuronal function. There is evidence that sumoylated proteins are increased in the brain of patients with polyglutamine diseases [82], and altered sumoylation increases neurodegeneration in *Drosophila* models of polyglutamine disease [80]. Whether these findings are relevant for other neurodegenerative diseases is unknown. However, by its link to sumoylation, DJ-1 might extend the involvement of this ubiquitin-like protein-conjugation system in other neurodegenerative diseases such as PD.

Important clues to a possible role of DJ-1 in neurodegeneration have come from the evidence that human DJ-1 is converted into a variant having more acidic pI in response to exogenous oxidative stress or endogenous reactive oxygen species, suggesting a role for DJ-1 as an antioxidant, or a sensor of oxidative stress (Fig. 2) [83, 84]. Furthermore, the transcription of the yeast *DJ-1* homolog *YDR533C* is upregulated together with many chaperones and antioxidants during cell stress, notably the stress induced by weak acids [70], external pH changes [85], or protein misfolding [86], which in turn are associated with oxidative stress, raising the question of whether DJ-1 also plays a role as a molecular chaperone. Another, recently identified member of the DJ-1–ThiJ-PfpI superfamily, the *Escherichia coli* EcHsp31, is a stress-inducible chaperone [87]. A recent study has indeed provided the first in vitro evidence for a role of DJ-1 as molecular chaperone (see below) [88].

On the basis of the available evidence we have proposed that DJ-1 is involved in the cellular response to stress at multiple levels (Fig. 2) [41]: (a) First, it might directly react to stress signals (e.g., redox changes, misfolded proteins) being an antioxidant and/or a molecular chaperone. (b) Second, DJ-1 might modulate the gene expression of the stress response at the post-transcriptional level by the known interaction with RNA-binding protein complexes. Interestingly, the post-transcriptional regulation of gene expression is important for both neuronal function and spermatogenesis. (c) Third, DJ-1 might translocate to the nucleus in response to stress signals. In the nucleus it might interact with PIAS or other cofactors and modulate the gene expression at the transcriptional level.

Although an involvement of human DJ-1 in the oxidative stress response, or in the response to misfolded protein stress remains to be proven, and the exact function

of DJ-1 remains unclear, the proposed model is intriguing in the light of the evidence of oxidative stress and protein misfolding documented in the brains of patients with PD [13, 89]. Recent studies have shown that mutations in *α-synuclein* and *parkin*, two other PD-related genes, might also be linked to oxidative stress [90, 91]. A redox regulation of sperm maturation and motility has been proposed [92, 93], and such a mechanism of "physiologically controlled oxidative stress" could explain why DJ-1 is involved in the spermatogenesis and fertilization.

One can also speculate that DJ-1 signals in a specific pathway regulated by redox state. Reactive oxygen species can also be viewed as regulators of cellular functions by the effect that redox status has on key proteins such as c-Jun N-terminal kinase, JNK, and mitogen-activated protein kinases, MAPK, and transcription factors which are activated in response to redox changes [94]. The c-Jun N-terminal kinase signaling pathway plays an important role in the neuronal apoptotic response, dopamine- and excitotoxicity-induced apoptosis, and the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of PD [95, 96].

It is therefore clear that much work is still ahead to clarify the biology of DJ-1 and the mechanisms of DJ-1 related neurodegeneration.

Structure and biochemical properties of DJ-1

DJ-1 belongs to the ThiJ/PfpI superfamily of proteins (Pfam01965) which contain a highly conserved domain (ThiJ) and include members in all kingdoms of life. Prokaryotic members of this family with known function include: ThiJ, involved in the thiamine synthetic pathway, PfpI and other proteases, araC and other transcription factors, the glutamine amidotransferase family, which includes some bacterial catalases, and a recently recognized family of bacterial chaperones (EcHsp31). This functional divergence makes it impossible to predict the function of human DJ-1 only on the basis of sequence homology.

Important insights about the function of DJ-1 are coming from structural biology studies. We developed a computer-assisted model of the DJ-1 protein [41], and five independent groups have recently resolved the crystal structure of human DJ-1 at resolutions of 1.95–1.1 Å [88, 97, 98, 99, 100]. These structures are very similar to each other and to our model [41], with the DJ-1 monomer assuming an α/β sandwich fold similar to the so-called flavodoxin-like, or Rossmann fold that is conserved in the DJ-1–ThiJ-PfpI superfamily. An important finding in all these crystal studies is that DJ-1 exists as dimer, a finding that we and others independently observed in gel filtration experiments [47, 48, 88, 97, 99]. Most of the residues involved in the dimerization are highly conserved, but DJ-1 and its closest homologs share a peculiar dimerization pattern in the superfamily, which is partly determined by the presence of an additional C-terminal helix. A putative active site has been identified close to the dimer interface, with some similarities to the active site of cysteine proteases, the residues Cys 106, His 126, and perhaps Glu 18 being likely involved [97, 98, 99]. However, these residues do not show the orientation required for the proton transfer which is typical of the cysteine protease catalysis [99]. In keeping with this, biochemical attempts to detect protease [88, 98, 99] or kinase [99] activity of DJ-1 have been unsuccessful so far.

Moreover, the crystal studies confirm our prediction that the residue mutated in the Italian PARK7 patients (Leu 166) is located in a C-terminal α-helix [88, 97, 98, 99, 100] and show that this helix is part of a hydrophobic core formed by three helices (two contributed by the C-terminal and one by the N-terminal part of the monomer), which is involved in the dimerization [88, 97, 99]. The L166P mutation appears to disrupt the C-terminal domain and the dimerization capability, suggesting that the dimerization is functionally important. A novel disease-linked missense mutation (M26I) has been recently reported [44]. Intriguingly, the residue Met26 is located in the N-terminal helix which contributes to the same hydrophobic core and is spatially close to Leu 166; furthermore, this N-terminal helix contributes to the putative active site of DJ-1 [97]. Our findings in gel filtration studies also suggest that the L166P mutant does not form dimers but adopts a different higher order structure or complexes with other proteins [47]. Interestingly, a deletion mutant lacking residues 173–189 is also reported to form higher aggregates [97]. Taken as a whole, these findings suggest that the dimeric structure is important for the function of DJ-1.

The rapid progress in purification and crystallization of DJ-1 allows testing the protein for the several proposed activities, including protease, hydrolase, kinase, amidotrasferase, catalase, chaperone, and transcription factor. Initial biochemical studies suggest that protease, kinase, and amidotransferase activities are unlikely, whereas catalase or other catalytic activities remain unexplored and still intriguing possibilities. Very recently the first in vitro evidence for a role of DJ-1 as molecular chaperone was obtained [88]. In this study purified human DJ-1 dose-dependently prevented the heat-induced aggregation of two classical chaperone substrates, citrate synthase and luciferase. The activity of DJ-1 was ATP independent, and it was maintained after H_2O_2 treatment, supporting the idea that DJ-1 functions under oxidative conditions. A hydrophobic groove which is created at the molecular interface of the DJ-1 dimer is likely the structural correlate of the chaperone activity [88]. This is potentially an important finding, which remains to be confirmed in vivo. Moreover, it remains to be seen whether the dimer represents a natively active form, or whether in response to specific signals DJ-1 undergoes other structural changes that make it functionally active. It has been reported that replacement of the Cys 53 residue, located in the dimer interface, abolishes the pI shift of DJ-1 in response to oxidative stimuli, suggesting that this shift is mediated by the oxidative conversion of the sulfydrylic group of cysteine to cysteine sulfinic acid [98]. It has been

proposed that this modification leads to functional activation of the protein in response to oxidative stress. [98]. However, the observation that another conserved cysteine (Cys 106) displays extreme sensitivity to radiation damage, suggests that Cys 106 also mediates the oxidative conversion of DJ-1 [99]. Indeed the analysis of crystal structure of oxidized DJ-1 confirmed that Cys 106 undergoes modification to sulfinic acid [88]. The presence of one or more cysteines which react to oxidative conditions supports the idea that DJ-1 is an oxidation-response protein.

The *E. coli* stress-inducible chaperone EcHsp31belongs to the DJ-1–ThiJ-PfpI superfamily [87] and possesses protease activity as well [88]. It has been suggested that it switches from chaperone to protease function on the basis of the temperature shift [87], as observed with another bacterial protein [101]. Similarly, one could speculate that DJ-1 has also dual function of chaperone and enzyme depending on the cell stress level. Despite many questions remain not answered, the resolution of the structure of DJ-1 has been an important step forward to clarify the exact function of the protein.

The function of DJ-1 as molecular chaperone is intriguing in the light of the role of the molecular chaperones in the pathogenesis of PD and other neurodegenerative diseases. These proteins function in assisting the proper folding of nascent polypeptides and the refolding of damaged proteins; they are also involved in targeting and/or delivering of protein to the proteasomal system for degradation [102]. Studies in transgenic animal models of disease and cell systems provided convincing evidence that manipulation of the chaperone system influences markedly the pathogenesis [13]. In rodent and fly models of different neurodegenerative diseases (including α-synuclein), the overexpression of chaperones (such as Hsp70) reduces, whereas interference on the chaperones aggravates neurotoxicity [103, 104, 105, 106]. Now it is urgent to test whether these effects are replicated by manipulating the *DJ-1* gene.

Prospects for future studies

The effect of the loss of DJ-1 function, either alone or in combination with mutations in other PD-related genes, can now be investigated in cell culture and animal models, including models of PD induced by dopaminergic neurotoxins, including1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and rotenone. In transgenic flies it will be possible to rapidly screen for genetic modifiers, either enhancers or suppressors of any resulting *DJ-1* related phenotype.

Transgenic mice overexpressing the human *DJ-1* gene have been generated and show no influence on fertility in comparison to the wild type animals [107]. Expression of the transgene was high in testis but also in the brain, and these mice might also be useful to investigate putative brain functions of DJ-1, including resistance to dopaminergic toxins, oxidative stress and protein misfolding.

Expression profiling in cell cultures in which the *DJ-1* gene is manipulated or in patient-derived cells might facilitate the characterization of *DJ-1* related pathways. Biochemical and cell biology studies have also been initiated to understand further the function of DJ-1, identify its interacting partners, and explore possible relationships between DJ-1 and the proteins encoded by genes which are firmly implicated in PD and other common neurodegenerative disease. Initial studies in our laboratory suggest that DJ-1 does not directly interact with α-synuclein and tau [47].

Conclusions

The *DJ-1* gene has been highly conserved in evolution and is abundantly and ubiquitously expressed in the brain and other body tissues. However, its function has remained elusive, and no one has previously linked the DJ-1 protein to brain function or brain disorders. The discovery that mutations in *DJ-1* cause autosomal recessive early-onset forms of PD establishes that the loss of the DJ-1 function leads to human neurodegeneration. Clarifying the mechanisms of *DJ-1* related disease might also potentially shed light on novel mechanisms of brain neuronal maintenance and promote the understanding of pathogenesis of common forms of PD. Although much work remains to be done to clarify the biology of this protein, the chaperone activity of DJ-1 and/or its possible role as oxidative sensor are intriguing in the light of the current pathogenetic scenarios for PD. However, the importance of identifying a novel gene causing PD is even greater if this leads to innovative ideas about pathogenesis. In the case of DJ-1 potentially novel insights are the focus on the nuclear and cytoplasmic control of gene expression in PD pathogenesis.

Note added in proof. Very recently, a triplication of the α-synuclein locus was found to cosegregate with PD in the kindred which previously yielded suggestive evidence for linkage to the PARK4 locus. This important finding strongly suggests that the overexpression of wild type α-synuclein leads to neurodegeneration in humans [112]

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