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Unraveling the pathogenesis of Parkinson's disease – the contribution of monogenic forms

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Abstract. The field of Parkinson's disease pathogenesis is rapidly evolving from the one of a monolithic and obscure entity into the one of a complex scenario with several known molecular players. The ongoing systematic exploration of the genome holds great promise for the identification of the genetic factors conferring susceptibility to the common non-Mendelian forms of this disease. However, most of the progress of the last 5 years has come from the successful mapping and cloning of genes responsible for rare Mendelian variants of Parkinson's disease. These discoveries are providing tremendous help in understanding the molecular mechanisms of this devastating disease. Here we review the genetics of the monogenic forms of Parkinson's disease. Moreover, we focus on the mechanisms of disease caused by α-synuclein and parkin mutations, and the implications of this growing body of knowledge for understanding the pathogenesis of the common forms of the disease.

Key words. Parkinson's disease; genetics; pathogenesis; α-synuclein; parkin; DJ-1; PINK1.

Introduction

The classical concept of Parkinson's disease (PD), is based on a clinical-pathological triad: the presence of parkinsonism (a combination of akinesia, resting tremor and muscular rigidity), a good response to dopaminergic therapy, and the presence of neuronal loss and gliosis in specific brain areas (the *substantia nigra pars compacta* and other areas), with formation of ubiquitin-positive cytoplasmic inclusions called Lewy bodies (LBs) in the surviving neurons [1, 2].

Most cases of PD present in sporadic form, a minority (~ 15%) are familial and only a few display typical Mendelian patterns of inheritance (either autosomal dominant or recessive) (fig. 1). Today, we understand better the genetic, pathological and clinical features of some Mendelian forms which are responsible for a minority of PD cases, usually of early onset. The majority of PD cases, especially the sporadic, late-onset ones, are still idiopathic in nature.

The classical definition of PD captures some common clinical and pathological features, but none of the triad components is specific for this disease [3, 4]. Parkinsonism can be the clinical correlate of lesions or dysfunctions at various levels in the basal ganglia, including the *substantia nigra* and striatum, which can be caused by neurodegeneration, inflammation, drugs, toxins, tumors and vascular lesions. Response to l-dopa or synthetic dopamine agonists is characteristic, but again, non-specific of PD, resulting from the loss of nigral dopaminergic neurons of any kind, in the presence of spared post-synaptic

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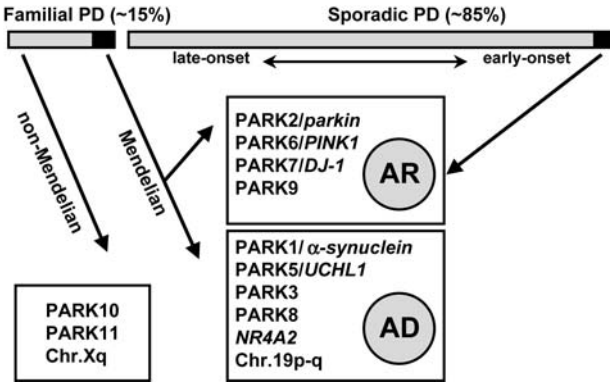

Figure 1. Inheritance patterns and genetic loci for PD. Linkage analysis and positional cloning have recently led to the identification of genes and loci for Mendelian and non-Mendelian forms of PD. AR, autosomal recessive; AD, autosomal dominant.

striatal dopamine receptors. Finally, LB pathology is observed in many conditions, including Alzheimer's disease (AD), Lewy body dementia (DLB), progressive supranuclear palsy, neurodegeneration with iron accumulation type 1, and in a few brains of elderly individuals who died without neurological disease.

The classical concept of PD therefore identifies the area of overlap between two spectra: that of l-dopa-responsive parkinsonian syndromes and that of LB-associated conditions. In the absence of a biological marker, this concept has persisted, also for its usefulness in the clinical workup and treatment of patients. However, the lack of a definition in terms of molecular mechanisms and the lack of biomarkers have hampered research for cause(s) and mechanism(s) of PD.

The discovery of different Mendelian forms has challenged the monolithic concept of PD, initiating a process of disease reclassification based on genetic and molecular biology criteria (fig. 1, table 1). Some of these PD forms of known etiology (such as PARK1/*α-synuclein* and PARK3) are associated with LB pathology, but the resulting clinical and pathological spectrum is broader than that of classical PD, overlapping with DLB and tau-related neurodegeneration [5, 6]. Other monogenic forms (such as PARK2/*parkin* and PARK8) pose even deeper challenges, as they might display differing pathology with or without LBs in different cases [7, 8], yet they might associate with a clinically typical PD phenotype. The pathological spectrum associated with these known Mendelian forms of PD therefore spans different, seemingly disparate conditions of classical neuropathology. The scenario might become even more complex as more Mendelian forms are identified, and a similar revolution in understanding and classification will likely invest a higher percentage of PD cases in the future, as the etiological studies and the identification of biomarkers progress.

The different Mendelian forms of PD do not necessarily fit into a common pathogenetic pathway. However, these rare forms are promoting understanding of the pathogenesis of the common forms of this devastating disease. Extensive investigations on the first of such monogenic forms (PARK1/*α-synuclein* and PARK2/*parkin*) are delineating protein misfolding and a defective protein quality control system as central themes in these rare forms, and in PD in general. For the more recently identified forms, such as PARK7/*DJ-1* and PARK6/*PINK1*, the pathogenetic mechanisms and relationships to common PD are less clear, and these forms might reveal involvement of novel pathways.

In the first section of this paper, the literature about loci and genes for monogenic forms of PD is reviewed. In the second section, the available data on the pathogenesis of PD forms caused by mutations in PARK1/*α-synuclein* and PARK2/*parkin*, and their implications for understanding the mechanisms underlying the common forms of the disease are discussed. We have recently reviewed in detail the PARK7/*DJ-1*-linked form and the genome-wide screens performed in the common forms of PD [9, 10]. Therefore, these topics will be discussed only briefly.

Genetics of the Mendelian forms of PD

Genetics of the autosomal dominant forms of PD

PARK1/*α-synuclein*

A first monogenic PD form was mapped to chromosome 4q21-q23 in a large Italian-American family known as the 'Contursi kindred', with autosomal dominant PD and LB pathology [11–13]. Soon after this locus (PARK1) was identified, the etiological heterogeneity of PD became evident, as linkage to this region was excluded in most autosomal dominant families examined [14, 15].

One year later, a missense mutation, A53T, was identified in the *α-synuclein* gene (*SNCA*), which co-segregated with PD in the Contursi family and three smaller Greek kindreds [16]. Haplotype analysis suggested a founder effect for this mutation [17]. The A53T mutation has been found in about 15 more families, all of Greek ancestry [5, 18–20], and a second mutation, A30P, was identified in one German family [21]. However, mutational analysis in a large series of sporadic and familial PD cases was negative, in keeping with results from linkage studies, and delineating mutations in *α-synuclein* as a very rare cause of PD [22–24]. Nevertheless, the discovery of *α-synuclein* mutations in PARK1 was a major breakthrough. The protein encoded by this gene, α-synuclein, was soon identified as one of the major components of LBs in classical PD [25], DLB [26] and of the glial cytoplasmic inclusions in multiple system atrophy [27], a group of neurodegenerative disorders now also termed

Table 1. Current catalogue of genes and loci for PD

Locus	Position	Gene	Inheritance pattern	Pathology	Clinical features
PARK1	4q21–q23	***SNCA (α-synuclein)***	dominant, high penetrance	LB tau pathology	early onset, aggressive course, dementia, severe autonomic disturbances in some cases
PARK3	2p13	unknown	dominant, incomplete penetrance	LB β-amyloid tau pathology	late onset, classical PD, dementia in some cases
PARK5	4p14	***UCHL1***	likely dominant	unknown	classical PD
PARK8	12p11–q13	unknown	dominant, incomplete penetrance	LB negative LB tau pathology	classical PD, dementia and amyotrophy in some cases
Pending	2q22–q23	***NR4A2 (NURR1)***	likely dominant	unknown	classical PD
PARK2	6q25–q27	***parkin***	recessive	mostly LB negative LB (1 case) tau pathology	early onset slow progression good, prolonged response to l-dopa, dystonia at onset, sleep benefit
PARK6	1p36–p35	***PINK1***	recessive	unknown	early onset, slow progression
PARK7	1p36	***DJ-1***	recessive	unknown	early onset, slow progression
PARK9	1p36	unknown	recessive	unknown	juvenile onset, multisystemic involvement, l-dopa response
PARK10	1p32	unknown	non-Mendelian	unknown	classical PD (Icelandic population)
PARK11	2q36–q37	unknown	non-Mendelian	unknown	classical PD (sib pairs study)
Pending	Xq	unknown	non-Mendelian	unknown	classical PD (sib pairs study)

Parkinsonism might sometimes be the prominent clinical feature in other inherited neurodegenerative diseases, which are usually associated with multisystemic neurological phenotypes such as spinocerebellar ataxia type 2, type 3, type 6, and X-linked dystonia parkinsonism (lubag). The PARK4 locus (chromosome 4p15) is removed from this catalogue because a mutation at the PARK1 locus (whole *SNCA* locus triplication) is associated with PD in the family which initially provided suggestive evidence for linkage to PARK4 [42].

alpha-synucleinopathies [28]. In recent years, several transgenic models have been generated in rodents, flies and worms expressing the human wild-type or mutant *α-synuclein* gene carrying the A53T or A30P mutation [29–35]. These models show varying degrees of biochemical, pathological and behavioural abnormalities reminiscent of PD, and further support the contention that derangements in the α-synuclein pathways are important in the pathogenesis of PD. Although mutations in its gene are very rare, the α-synuclein protein therefore plays a central role in PD.

Polymorphisms in the promoter and in a regulatory element 10 kb upstream of the gene influence *α-synuclein* expression in cell culture systems. Alleles conferring increased expression levels could conceivably act as risk factors for PD [36, 37]. However, most of the studies published so far have found no association between these polymorphisms and PD, while the finding of *α-synuclein* haplotypes associated with PD awaits replication in independent datasets [38]. In comparison with classical PD, the clinical phenotype associated with mutations in *α-synuclein* is characterized by an earlier onset (on average in the mid-forties), and reduced prevalence of tremor [5, 12, 19, 20]. Occurrence of cases with rapid progression, dementia, myoclonus and severe autonomic dysfunction indicates that PARK1 can present as a more widespread neurodegeneration than classical PD [5, 12], as confirmed by recent autopsy studies [39]. However, in Greek patients carrying the A53T mutation, and in the German family with the A30P mutation, the clinical picture appears similar to classical PD [19, 20, 40]. The phenotypic spectrum associated with mutations in *α-synuclein* therefore appears broad, and a wide variability of onset ages is observed in the same family [12, 18, 40], suggesting the existence of genetic and/or non-genetic modifiers.

Five years after identification of the mutation in the Contursi kindred, two reports have renewed interest into PARK1. A third missense mutation (E46K) associated with a phenotype ranging from PD to DLB was identified in one Spanish kindred [41]. Moreover, a triplication of the *α-synuclein* locus was found to cosegregate with PD in an Iowan kindred previously linked (though not significantly) to another region (PARK4, chromosome 4p15) [42, 43]. The discovery of the *α-synuclein* locus tripli-

cation, already found in a second family [44], extends the results of the studies in transgenic animal models, showing that overexpression of wild-type α-synuclein protein is associated with human neurodegeneration. In the Iowan kindred the phenotype is characterized by early-onset and rapidly progressive parkinsonism, dementia, autonomic dysfunction and body weight loss. The pathology shows widespread LBs [45–47]. As in the Contursi kindred, also in this family the clinical and pathological spectrum is therefore closer to DLB than to classical PD.

PARK3

A second locus for autosomal dominant PD was mapped to chromosome 2p13 in a genome-wide linkage search using large kindreds of European ancestry [6]. The phenotypic spectrum is wide, encompassing typical PD of late onset (average onset age of 59 years) and LB pathology, but also cases with dementia in addition to parkinsonism, and presence of neurofibrillary tangles and senile plaques in addition to LB pathology [48–50]. On the basis of haplotype analysis, a low penetrance (40%) was estimated for the mutation [6]. Linkage to the 2p13 region has not been replicated in other large kindreds. Fine mapping studies refined the PARK3 critical region to 2.5 Mb, but mutational screening of the genes contained in the region has been negative so far [51–53]. More recently, a genome-wide scan of affected sib-pairs analyzed the onset age of PD as the phenotype of interest [54]. In this study, suggestive evidence for linkage was detected to the PARK3 region, confirming that an important genetic determinant of PD risk and/or modifier of disease onset age might reside in this region. A recent report has suggested a role for the sepiapterin reductase gene, located within the PARK3 region and implicated in dopamine synthesis, in modifying the age at onset of PD [55].

PARK5/*ubiquitin C-terminal hydrolase-L1* (*UCHL1*)

The *UCHL1 g*ene encodes a protein which catalyses the hydrolysis of ubiquityl-peptide conjugates in vitro [56]. However, the UCHL1 function in vivo seems rather to stabilize the neuronal mono-ubiquitin levels by binding mono-ubiquitin and preventing its degradation [57]. UCHL1 is abundant in neurons, and is present in LBs [58]. Moreover, a mouse with a homozygous *UCHL1* intragenic deletion develops neurodegeneration with ubiquitylated inclusions [59].

UCHL1 was therefore considered a candidate gene for PD, but linkage to its region has so far not emerged in genome-wide scans performed in PD. Direct sequencing of the gene revealed a single missense mutation (I93M) in two German sibs with classical PD and family history, suggesting autosomal dominant inheritance with incomplete penetrance [60]. Pathological studies have not been reported in this family. Subsequent screening of this gene has consistently been negative, suggesting that I93M is either a rare cause of PD or a rare neutral polymorphism [61–64]. Involvement of UCHL1 in the pathogenesis of PD is also supported by the fact that a different polymorphism in this gene (S18Y) seems inversely associated with PD [65–68], and that the UCHL1 protein displays ubiquitin ligase activity for α-synuclein in vitro [69]. Although the genetic evidence is not entirely convincing, the function of UCHL1 makes it likely that this protein is involved in the pathogenesis of PD.

PARK8

This locus was identified in a large pedigree named the Sagamihara family from the region of origin in Japan [8]. Clinical features in affected individuals of the kindred resemble classical PD, with an average onset at 51 ± 6 years [70]. Yet nigral degeneration without LBs was found at autopsy. In this family, a genome-wide scan yielded significant evidence for linkage of PD to the centromeric region of chromosome 12, and haplotype analysis suggested incomplete penetrance of the mutation. Linkage to the same region was found in a genome scan for AD, the locus being termed AD5, but this was mostly supported by the subset of families containing at least one affected individual with LB pathology [71]. Very recently, linkage to PARK8 was confirmed in two families of caucasian ancestry with dominantly inherited neurodegeneration, suggesting this to be an important locus and refining the PARK8 critical region [72]. A wide clinical-pathological spectrum is shown in these families, including typical PD with LBs, DLB, tau pathology, nigral degeneration without inclusions and atypical, ubiquitin-positive inclusions [72]. Cloning the gene defective at the PARK8 locus might provide insights for understanding the pathogenesis of PD and links between seemingly different neurodegenerative spectra.

The *NR4A2* gene

The *NR4A2* (*NURR1*) gene encodes a member of the nuclear receptor superfamily of transcription factors [73] which is important for the genesis of dopaminergic neurons [74]. *NR4A2* has been considered a candidate for PD, but linkage to its chromosomal region has not been found in genome scans, and the *NR4A2* locus was excluded by linkage and haplotype analysis in PD families with recessive inheritance [75].

Evidence for association between an intronic polymorphism (IVS6+18 insG) and PD remains controversial [76–78]. More recently, two heterozygous mutations (–291Tdel and –245T → G) in the non-coding region (exon 1) of *NR4A2* were identified in 10 out of 107 patients with familial PD and classical onset [79]. The inheritance pattern appears autosomal dominant, and haplotype analysis suggests a founder effect for the –291Tdel mutation in families of German ancestry. The associated pathology is unknown. These mutations seem associated

with dramatic decreases of expression of *NR4A2* itself and tyrosine hydroxylase, one of its target genes [79]. However, some of the reported functional findings, in particular the fact that heterozygous mutations lead to more than 50% reduction in *NR4A2* messenger RNA (mRNA) levels, are difficult to explain. Furthermore, the evaluation of this gene in independent, large cohorts of PD families has so far been negative, suggesting that *NR4A2* mutations, at least those localized in exon 1, are very rare in PD [80–82].

Synphilin-1 and susceptibility to PD

Synphilin-1 was identified in a yeast two-hybrid screen as an interactor of α-synuclein [83]. Its function remains unknown, but, like α-synuclein, synphilin-1 is enriched in presynaptic terminals [84]. Synphilin-1 is encoded by the *SNCAIP* gene [85]. Immunoreactivity for synphilin-1 is present in LBs and in glial cytoplasmic inclusions, suggesting that deposition of synphilin-1 is a feature of synucleinopathies [86, 87]. Overexpression of synphilin-1 alone or in combination with parkin or α-synuclein yields ubiquitin-positive, cytoplasmic inclusions, suggesting a role for synphilin-1 in promoting protein aggregation [83, 88].

Suggestive evidence for linkage to the chromosomal region containing the *SNCAIP* gene (5q23) has been detected in different genome screens of late-onset PD [89–91], making *SNCAIP* a candidate susceptibility gene for classical PD. Earlier mutation screenings of the *SNCAIP* gene in PD were negative, but recently, a single heterozygous missense mutation (R621C) was identified in two sporadic patients with late-onset PD [92]. Further work is needed to clarify whether genetic variation in *SNCAIP* influences susceptibility to PD.

Further dominant loci

It is likely that other autosomal dominant forms of PD will be identified in the future, as most of the previous loci have been excluded in additional pedigrees [93–95]. We have recently obtained suggestive evidence for linkage to chromosome 19 in a Cuban family segregating clinically typical PD of late onset [96]. The pathology of this form remains unexplored.

Genetics of the autosomal recessive forms of PD

PARK2/*parkin*

An autosomal recessive form of juvenile parkinsonism (AR-JP) was first described in Japanese families [97–99], and a genetic locus (PARK2) was mapped to chromosome 6q25-q27 [100]. Subsequent studies confirmed this linkage in families from several ethnic groups, delineating PARK2 as an important locus [101, 102]. The defective gene was identified by positional cloning, and termed *parkin* [103]. The gene extends over more than 1 Mb and contains 12 exons, which are expressed ubiquitously in the brain and many extra-cerebral tissues. Splice variants have been described for the human and mouse *parkin* genes [103, 104]. More than 70 mutations in the *parkin* gene have been identified so far in different populations (reviewed in [105]). In addition to point mutations, large genomic rearrangements (leading to exon deletions and multiplications) have frequently been detected, in both homozygous and heterozygous state, indicating the importance of gene-dosage techniques for sensitive screening of *parkin* [106–108]. Haplotype analyses suggest the occurrence of a founder effect for some of the recurrent point mutations [109, 110]; on the contrary, the exon rearrangements seem to have arisen from independent mutational events [109].

Large and comprehensive studies indicate that mutations in this gene are found in ~50% of the familial PD cases compatible with recessive inheritance and onset before age of 45 years, and in ~15–20% of the sporadic cases with onset before 45 [106, 108, 111]. Most of the sporadic PD cases with *parkin* mutations have a very early onset (before age of 30), whereas mutations in this gene are rare among the sporadic cases of later onset [106, 111]. All of these studies were hospital based, and the frequency of *parkin* mutations might be lower in population-based series of PD cases [112]. Nevertheless, a *parkin* mutation is a major cause of early-onset PD, which in turn represents 5–10% of all PD cases [113].

A few studies suggest that parkinsonism due to a *parkin* mutation might sometimes be dominantly inherited [114–116]. However, the possibility of pseudo-dominant inheritance must be considered as an alternative explanation of the disease in multigeneration families. Examples of pseudo-dominant inheritance of *parkin*-related disease have been shown in Japanese and Italian families [117–120], suggesting that *parkin* gene mutations might be frequent in some populations.

Involvement of the *parkin* gene in late-onset PD has been explored to a lesser extent, as patients with early onset and/or recessive inheritance were pre-selected in most of the mutational screens performed so far [106, 111, 121]. However, a recent study of PD sib pairs supporting linkage to the PARK2 locus found *parkin* mutations in 11% of the pairs with onset after age 50 [108]. In another study, *parkin* mutations were found in 28% of the recessive families with onset between 46 and 55 years [122]. Taken together, these data implicate the *parkin* gene in a substantial fraction of late-onset families.

The *parkin* gene promoter has been characterized [123, 124], and single-nucleotide polymorphisms (SNPs) have been identified [125, 126]. One of these variants (–258 T/G) is located in the core promoter region and the 'G' allele is associated with decreased gene expression in cell culture assays [125]. Moreover, the frequency of

the G allele was increased among patients with classical, late-onset PD with borderline significance, suggesting that this variant can be a risk factor for the common form of PD [125].

Several other polymorphisms have been identified in the *parkin* gene, including missense changes [127–129]. The allelic frequencies show here wide variations between ethnic groups, with the Ser167Asn and Val380Leu polymorphisms being most frequent among Orientals and Caucasians, respectively [128, 129]. Other variants are virtually population specific, such as the Arg366Trp in Orientals and the Asp394Asn in Caucasians [128, 129]. The results of allelic association studies of these polymorphisms in classical PD remain rather conflicting, and none of the analyzed variants seems to have strong effects on the risk of classical PD [130, 131].

A different gene named *pacrg* (*pa*rkin co-regulated gene) lies in close proximity to *parkin*, but on the opposite DNA strand, and it shares with *parkin* a bi-directional promoter [132]. This gene, also termed *glup* (gene located upstream of *parkin*) is expressed in many tissues, including the brain. The encoded ~30 kDa protein has an unknown function, but it forms a complex with molecular chaperones and might promote the formation of intracellular inclusions [133]. Immunoreactivity for the PACRG protein has also been found in LBs [133]. Mutation screenings of the *pacrg/glup* gene in PD have so far not been published.

Do single heterozygous parkin mutations cause PD?

In several early-onset PD cases a single heterozygous *parkin* mutation is found, even after gene dosage analysis or sequencing of the promoter region. Moreover, the age of PD onset is later in these patients than in those with mutations identified in both *parkin* alleles [112, 121, 122, 134]. This raises the questions whether single heterozygous *parkin* mutations are sometimes sufficient to cause early-onset PD. Mechanisms to explain the disease in these cases might involve haploinsufficiency, dominant-negative or dominant gain-of-function mechanisms. Positron emission tomography (PET) studies showing that heterozygous carriers of *parkin* mutations have mild nigrostriatal dysfunctions also suggest that these mutations might be harmful, at least at the subclinical level [135, 136].

However, mutations found in the single heterozygous state were also found in the homozygous state, or in compound heterozygosity with a second mutation in other, unrelated early-onset cases [134]. Moreover, the absence of symptoms in the vast majority of heterozygous relatives of patients with parkin-related disease who carry two mutations [122] strongly suggests that most *parkin* mutations are recessive, but in some cases the second mutation escapes detection by current methods. Mutations in the large introns, large inversions or large deletions in the promoter can also be envisaged, and the possibility that the second mutation is present in a different gene encoding a protein involved in the same pathway cannot be excluded. Screening the *DJ-1* gene in a few cases carrying single heterozygous *parkin* mutations failed to identify any mutations [137, 138]. Instead, the –258 T/G polymorphism in the *parkin* promoter was much overrepresented in cases with single mutations, and it might also be pathogenic [122, 125].

Recent studies show that a few *parkin* mutations, including R275W (associated with LB pathology in one PD case), induce the formation of aggresome-like inclusions when overexpressed in cell cultures [139, 140]. This suggests that some *parkin* mutants are misfolded and rapidly degraded, thereby inducing a loss of function. However, the fact that the parkin^{R275W} mutant retains ubiquitin-ligase activity in vitro [88] suggests that the pathogenic mechanism of R275W and few other *parkin* mutations is not the loss of function, but the gain of a toxic function, mediated by the misfolding of the mutant protein [140]. These observations also support the argument that few *parkin* mutations might be dominant and cause disease in heterozygous state.

A distinct question is whether single heterozygous *parkin* mutations might increase the risk of late-onset PD, as suggested recently [108, 141]. A note of caution is warranted here, as gene-dosage analysis was not performed in one study, which has probably missed many heterozygous exon rearrangements [141]. On the other hand, some of the heterozygous variants identified might also represent rare harmless polymorphisms [108]. More genetic and functional studies are thus required to clarify the role of single heterozygous *parkin* mutations in both early- and late-onset PD.

Pathology studies

Only a few brains from patients with *parkin* mutations have been examined so far [7, 98, 115, 142–146]. The commonly observed pathological features were neuronal loss and gliosis in the *substantia nigra pars compacta* and *locus coeruleus*. In single cases, neurodegeneration was more widespread, including the *substantia nigra pars reticulata* [142, 146], and the spinocerebellar system [144]. LBs were absent in all but one case, who was a compound heterozygous carrier of a deletion within exon 3 and the missense mutation R275W [115]. The parkin^{R275W} mutant protein has residual ubiquitin-ligase activity [88], and it might thus be pathogenic by a different mechanism (see previous paragraph) [140].

Tau-positive inclusions were found in neurons and astrocytes in some parkin brains [7, 142, 144], and recently a distinct tauopathy, progressive supranuclear palsy, was reported in a carrier of a single heterozygous *parkin* mutation [145]. Whether these represent coincidental findings or whether tau pathology is in the spectrum of parkin disease remains unclear.

Neuroimaging studies

Several studies with fluorodopa and PET in parkin disease have been based on a small number of cases, and one report on a larger series was published recently [147]. These studies confirm the presence of presynaptic dopaminergic dysfunction, as in common PD. Moreover, PET abnormalities in parkin patients display some degree of left-right asymmetry, perhaps less pronounced than in classical PD, and a clear rostro-caudal gradient (the putamen being more severely affected than caudate), as in classical PD [147]. Mild abnormalities were observed in asymptomatic heterozygous carriers, suggesting the presence of subclinical disease [135, 136]. PET studies also confirmed that the progression in parkin patients was slower than in classical PD [136]. Results using the raclopride tracer suggest that the postsynaptic dopamine receptors might also be abnormal in parkin patients [135].

Clinical features

The clinical phenotype associated with *parkin* mutations is characterized by early-onset parkinsonism, good and prolonged response to levodopa and a benign, slow course. The average onset age was in the early thirties in European patients, but late-onset cases have been described up to 70 years of age [122]. Motor fluctuations and levodopa-induced dyskinesias are present, whereas marked cognitive or vegetative disturbances seem rare. The age of disease onset is the most important predictor of *parkin* mutations in that the earlier the onset, the more frequent the mutations [106, 111, 122]. There are no specific clinical features that identify patients with *parkin* gene mutations from other early-onset forms [106, 111, 122]. However, symmetrical onset, dystonia at onset and hyperreflexia, slower progression of the disease and a tendency towards a greater response to levodopa might be more frequent among patients with *parkin* mutations [122]. The phenotypic spectrum overlaps with classical PD for late-onset cases [122], and with dopa-responsive dystonia for early-onset cases [148]. Rare atypical presentations have also been described, and a wide variability in onset age and phenotype is observed even within the same families [110, 116, 122, 144, 149], suggesting the existence of genetic and/or non-genetic modifiers.

The importance of parkin in the clinical practice

Mutations in the *parkin* gene represent a frequent cause of early-onset PD, and they must therefore be considered in the diagnostic workup. Mutation screening of *parkin* is difficult, and it should always include gene copy analysis in addition to genomic sequencing. The genetic counselling of patients with *parkin* mutations and their relatives is difficult because of the current uncertainties about the role of single heterozygous mutations, and the broad phenotypic spectrum associated, including a very large intra-familial variance. Genotype-phenotype correlations for parkin disease are poorly understood because the spectrum of mutations is wide and probably incomplete, and it is often difficult to establish whether and how a given mutation has a biological effect.

PARK7/*DJ-1*

We detected linkage of early-onset parkinsonism to chromosome 1p36 (the PARK7 locus) by genome-wide homozygosity mapping in a large Dutch family with autosomal recessive PD originating from a genetically isolated population [150], and later confirmed this linkage in a second Italian family [151]. By positional cloning within the refined PARK7 critical region, we identified mutations in the *DJ-1* gene in these two families [152]. Subsequent mutational screenings in a large series of patients with early-onset PD identified further mutations, but they have suggested *DJ-1* mutations to be a rare cause of the disease, being implicated in about 1–2% of the early onset cases [138, 153, 154]. However, it is still early to accurately estimate the frequency of *DJ-1* involvement in PD, and to delineate its mutational and phenotypic spectrum. An extensive review on the genetics and the molecular biology of PARK7/*DJ-1* was recently published [9]. The most significant findings from the recent studies include resolution of the crystal structure of the human DJ-1 protein and the discovery that it exists as homo-dimer [155–157]; and the discovery that the L166P mutation found in the Italian PARK7 family confers instability to the DJ-1 protein, leading to very low steady-state levels in cells [158–160]. Together with the complete absence of protein in the patients of the Dutch PARK7 family, this indicates that the loss of function of DJ-1 leads to neurodegeneration. The function of DJ-1 remains largely unknown, but genetic and biochemical studies suggest a role as an antioxidant and/or a molecular chaperone (reviewed in [9]). These putative functions would make DJ-1 a candidate player in the current pathogenetic scenarios of classical PD as well. However, known DJ-1 interactions with cytosolic RNA-binding protein complexes and nuclear transcriptional cofactors might also reveal the involvement of novel mechanisms in the survival of dopaminergic neurons, and in the pathogenesis of neuronal cell death in PD [9].

The pathology of PARK7 remains unknown, and LBs from classical PD stained negative for DJ-1; however, DJ-1 immunoreactivity co-localizes with pathological tau inclusions in different neurodegenerative disorders known as tauopathies [161], and with the α-synuclein-positive glial inclusions in multiple system atrophy [162], suggesting further links between seemingly different diseases and suggesting a role of DJ-1 in their pathogenesis. Elucidating the role of DJ-1 might therefore lead to a better understanding of the pathogenesis of Parkinson's disease and other common neurodegenerative disorders.

PARK6/*PINK1*

A genome-wide scan and homozygosity mapping in a large consanguineous kindred from Sicily with early-onset PD (range: 32–48 years) yielded significant linkage to the 1p36-p35 region [163]. This linkage was subsequently confirmed in an independent dataset of European families [164]. The pathology of the PARK6-linked form remains unknown. A PET study showed a pattern similar to parkin-related disease, including evidence for subclinical dopaminergic dysfunction in heterozygous carriers [165]. The clinical features in PARK6-linked families are those of early-onset PD, but dystonia at onset has not been observed [166].

Very recently, a truncating (W437Stop) and a missense (G309D) mutation have been identified in the *PINK1* gene in the original PARK6-linked family and two additional smaller kindreds [167]. The loss of function of the PINK1 protein therefore causes this Mendelian form of PD. *PINK1* is ubiquitously expressed, and it encodes a 581-amino acid protein which possesses a protein kinase domain and is targeted to mitochondria (fig. 2) [167]. The localization of the PINK1 protein to mitochondria links a primary defect of these organelles to the pathogenesis of parkinsonism, with possible important implications for further understanding the role of mitochondria in the pathogenesis of common forms of PD. Future studies will reveal the frequency of involvement of this gene in PD and the associated phenotypic spectrum.

PARK9

This locus was mapped to the 1p36 region by genome-wide linkage analysis in a single consanguineous family from Jordan [168]. Five siblings were affected with a multisystemic neurodegenerative disease (Kufor-Rakeb syndrome) clinically quite far from PD, and more closely resembling pallido-pyramidal degenerations, with juvenile onset (below age 20), akinetic-rigid parkinsonism (no tremor), pyramidal tract dysfunction, supranuclear gaze paresis and cognitive deterioration. The parkinsonism was levodopa responsive, but the progression was rapid, and neuroimaging showed progressive brain atrophy starting from the pallidum and pyramids [169]. The pathology of this form remains unexplored.

Genome-wide screens in the common forms of PD

Four genome-wide scans in a large series of small families, each containing at least one pair of relatives affected with classical PD, have been completed; others are in progress [89–91, 170]. The most important result of these studies has been the detection of a significant linkage to three novel regions, on chromosomes 1p32 (PARK10), 2q36-q37 (PARK11) and on the X chromosome [91, 171, 172]. These regions might therefore harbour susceptibility genes for classical late-onset PD. The same region on 1p32 was significantly linked to onset age of PD in another study [173]. In addition to these significant findings, a series of chromosomal regions with interesting or suggestive positive LOD scores has been generated. Though none of these regions achieved statistical significance, the analysis of a larger dataset might identify further genuine linkages. The results of genomic screens in classical PD were recently reviewed [10].

Pathogenesis of Parkinson's disease – the contribution of α-synuclein and parkin

The contribution of α-synuclein

The first monogenic form of PD (PARK1) led to a landmark discovery: α-synuclein is a key player in the pathogenesis of both autosomal dominant PD and idiopathic PD. While missense variants (A53T, A30P, E46K) [16, 21, 41] and overexpression of this protein [42, 44] are a rare cause of autosomal dominant PD, wild-type α-synuclein is one of the major components of the LBs in all forms of PD and other synucleinopathies [174]. In the last 3 years, several transgenic animal models have been generated that overexpress wild-type or mutant human α-synuclein. These models display varying degrees of biochemical, pathological and clinical abnormalities reminiscent of PD, and further support the contention that α-synuclein is primarily implicated in the pathogenesis of PD in general [175].

α-Synuclein is a 140-amino acid protein which has been highly conserved in evolution; it is abundant in neurons and enriched in the presynaptic compartment [176]. Although its exact function remains unknown, involvement in synaptic plasticity and in regulation of size and turnover of synaptic vesicles has been suggested. Mice in which the *α-synuclein* gene has been knocked out possess a normal number of dopaminergic neurons and synapses but show mild reduction in striatal dopamine levels and abnormalities in amphetamine-induced responses, suggesting a role for α-synuclein in the regulation of the dopaminergic neurotransmission [177]. *α-Synuclein* knockout mice do not develop PD-like pathology, in keeping with the role of this protein in PD being due to a gain of function rather than a loss of function. However, the possibility that loss of the normal function of α-synuclein might contribute to disease progression cannot be excluded [178].

The α-synuclein protein contains a N-terminal amphipathic region; a central region, which includes the amyloidogenic peptide NAC (*n*on-*aβ c*omponent of Alzheimer's disease amyloid); and a C-terminal acidic region (fig. 2). Six imperfect repeats of 11 amino acids, containing the KTKEGV consensus motif, are present within the first 95 amino acids. These repeats confer a variation in hydrophobicity which is typical of the amphipathic

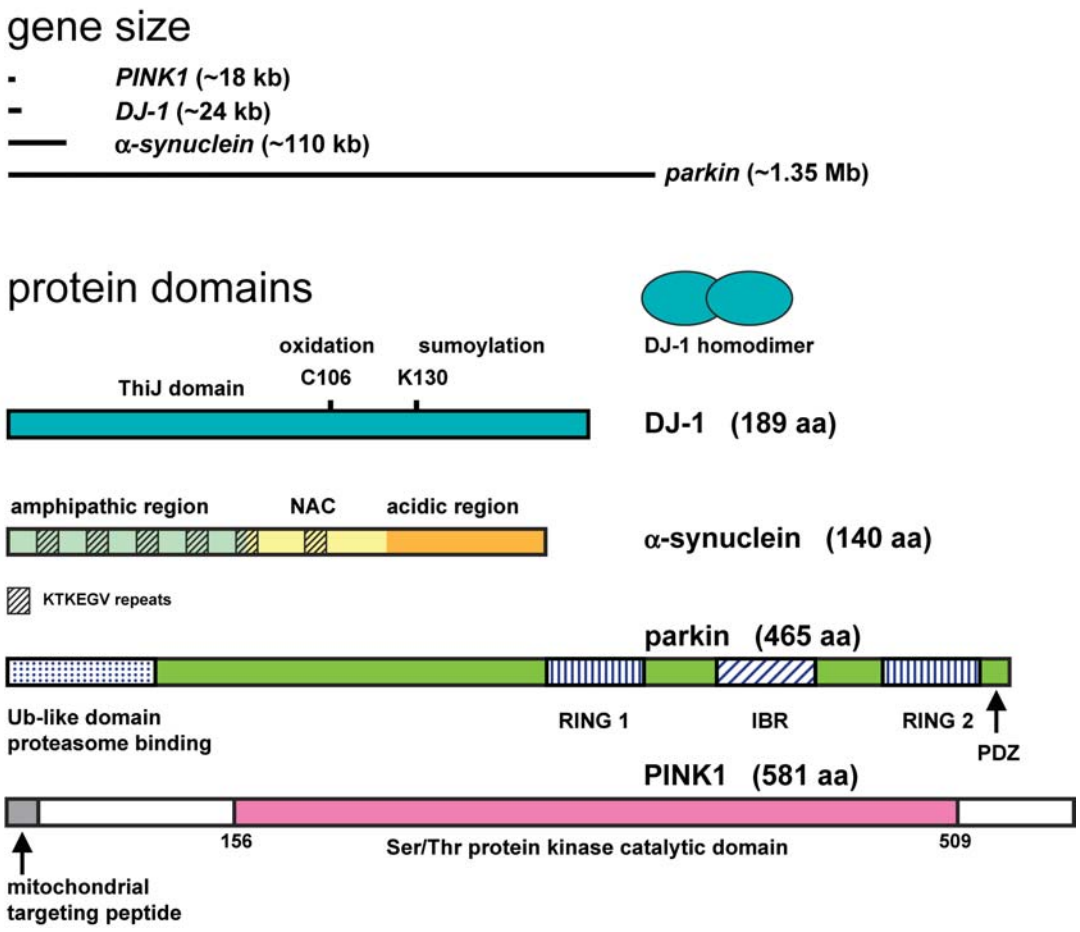

Figure 2. PD-associated genes and their products. Comparison of the genomic size of four genes, which are firmly implicated in Mendelian forms of PD, and primary structure of the encoded proteins. The DJ-1 protein forms homodimers in solution.

α-helix of the lipid-binding domain in apolipoproteins [176, 179]. And indeed, α-synuclein shows homology with fatty acid binding proteins, but also with the 14-3-3 family of molecular chaperones, and it displays inhibitory activity on phospholipases. α-Synuclein is natively unfolded, and its secondary structure is critically dependent on the interaction with lipid membranes, which markedly increase the α-helix content [180].

Biochemical studies have shown that α-synuclein displays a concentration-dependent property to form oligomeric species (also called protofibrils) and amyloid β-sheet fibrils in vitro [181, 182]. Oligomers are believed to represent the precursors of higher-order aggregates in vivo, which are assembled in the filamentous structures seen in LBs and Lewy neurites (fig. 3). Evidence suggests that in different neurodegenerative diseases, the oligomers are the neurotoxic molecules [183–187]. In support of this view, increased oligomerization, not fibrillization, is the shared property of the first two PD-linked α-synuclein missense mutations [188, 189]. The effect of the third, recently identified mutation (E46K) remains to be investigated in this regard. However, studies in transgenic animals suggest that the fibrillar form of α-synuclein is the primary toxic moiety [33, 34].

Several biophysical and biochemical studies investigated α-synuclein aggregation, but only recently has the oligomerization process been specifically addressed, and the factors involved are just beginning to be characterized. It is possible that small amounts of α-synuclein oligomers are normally formed in the neuron as well, and evidence for the existence of oligomers in normal conditions has indeed been obtained recently [190].

Factors enhancing α-synuclein fibrillization in vitro include heavy metals and pesticides (implicated in PD on the basis of epidemiological studies), oxidative stress and heparin, but how these factors relate to the formation of oligomers is unknown. On the other hand, it is known that molecular crowding, interaction with calcium, possibly via calmodulin, interaction with lipid membranes and polyunsaturated fatty acids promote and/or enhance oligomerization, whereas saturated fatty acids decrease the oligomerization [190].

Furthermore, studies in cell culture and animal models have shown that mitochondrial inhibitors such as rotenone

and paraquat [191–193], oxidative [194, 195] and nitrative [196] conditions, and proteasomal inhibition [197] are all associated with increased α-synuclein fibrillization with formation of intracellular aggregates.

Post-translational modifications of α-synuclein, including transglutaminase-mediated cross-linking [198], phosphorylation [199], nitration [200] and mono- and diubiquitylation [201] likely contribute to the formation and/or stabilization of aggregates. On the other hand, magnesium [202], β-synuclein and γ-synuclein[203, 204] appear to inhibit the fibrillization of α-synuclein.

Intrinsic biophysical properties of α-synuclein probably confer a high neurotoxic potential to this protein, in analogy with proteins that are pivotal in other neurodegenerative diseases, such as tau and β-amyloid for AD, or PrP for prion disease. Recent evidence suggests that interactions between tau and α-synuclein can synergistically promote the polymerization of both proteins [205], in keeping with the co-localization of α-synuclein and tau epitopes in LBs [206, 207], and the overlapping tau and α-synuclein pathology in different PD monogenic forms, including PARK1 and PARK8 [39, 72].

Regulation of α-synuclein neuronal levels is likely subject to tight control, and a major hypothesis is that the primary abnormality in PD is abnormally increased α-synuclein expression. The factors controlling expression of *α-synuclein* in human neurons remain largely unexplored, and this appears a very promising area for future studies.

Studies of the human *α-synuclein* promoter in cell systems suggest that high- and low-expression alleles exist [37], but so far clear evidence of overrepresentation of the high-expression alleles in PD is lacking. Increased expression of *α-synuclein* and decreased expression of *β-synuclein* have been reported in brains from patients with diffuse LB disease, suggesting that an imbalance between the expression of the different synucleins might be pathogenic [208]. However, another study did not find changes in α-synuclein expression in PD brain [209].

Another possibility is that in PD there is a primary decrease in the clearance of α-synuclein and/or its oligomers. Direct interaction between α-synuclein and a proteasomal subunit has been reported [210, 211]. However, whether α-synuclein is physiologically degraded by the proteasome or by different systems remains controversial, with evidence in favour [212, 213], including ubiquitin-independent proteasomal processing [214], and evidence against it [213, 215]. Other studies suggest that calpain (a cysteine protease) [216], neurosin/kallikrein-6 (a serine protease) [217] and the lysosomes [196, 213] are also involved in cleavage and degradation of α-synuclein. Intriguingly, in an embryonic hippocampal cell line, overexpression of parkin stimulated the calpain-mediated degradation of α-synuclein and protected from α synuclein-induced citotoxicity, suggesting that α-synuclein and parkin are functionally linked through non-proteasomal proteolytic pathways [218]. Whether α-synuclein (or a modified form) is a parkin substrate in vivo in the human brain remains controversial. Elucidating the physiological pathways of α-synuclein degradation is another very important area of current investigation.

How is α-synuclein neurotoxic?

Apart from the question of how abnormal oligomerization or fibrillization of α-synuclein is determined in PD, another central problem is how monomeric, oligomeric, or fibrillar α-synuclein exerts its toxicity. This remains unclear, but several possibilities have been suggested, including direct inhibition of the proteasome system [211, 219, 220], impairment of mitochondrial function [221] and derangement in cellular trafficking [222]. As a further mechanism, it has been proposed that α-synuclein oligomers form pore-like structures similar to bacterial toxins, which are able to damage synaptic vesicles [223]. In the case of the dopaminergic neuron, the release of dopamine in the presynaptic cytosol would lead to oxidative stress (fig. 3).

Any kind of selective interaction between α-synuclein and dopamine has the potential to explain the relative selectivity of the PD neurodegenerative process for the dopaminergic neurons. In this regard, it is interesting that in a neuronal culture system, the toxicity of α-synuclein requires endogenous dopamine production, as it is abolished by tyrosine hydroxylase inhibition and seems mediated by reactive oxygen species [224].

Other studies suggest that α-synuclein interacts with and enhances the activity of the dopamine transporter [225], and it inhibits the monoamine vesicular transporter [226]. Together with direct damage to vesicles by pore-like oligomers, these effects would converge to the common endpoint of increasing the cytosolic levels of free dopamine, leading to oxidative stress. Moreover, the oxidative metabolite dopamine quinone can form adducts with the same α-synuclein, and these adducts inhibit the conversion of α-synuclein oligomers to higher aggregates, further reinforcing the oligomer-mediated toxicity (fig. 3) [227].

The links between α-synuclein and dopaminergic neurons are also highlighted by evidence that *α-synuclein* overexpression might enhance the toxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [MPTP] [228], whereas *α-synuclein* knockout mice are resistant to the toxicity of MPTP [229, 230], a selective dopaminergic neurotoxin, which inhibits mitochondrial complex I but also leads to dopamine redistribution from vesicles to cytosol and dopamine-mediated oxidative stress [231].

Several studies in cell culture systems have linked the overexpression of wild-type and especially mutant *α-synuclein* to mitochondrial dysfunction [221], oxidative

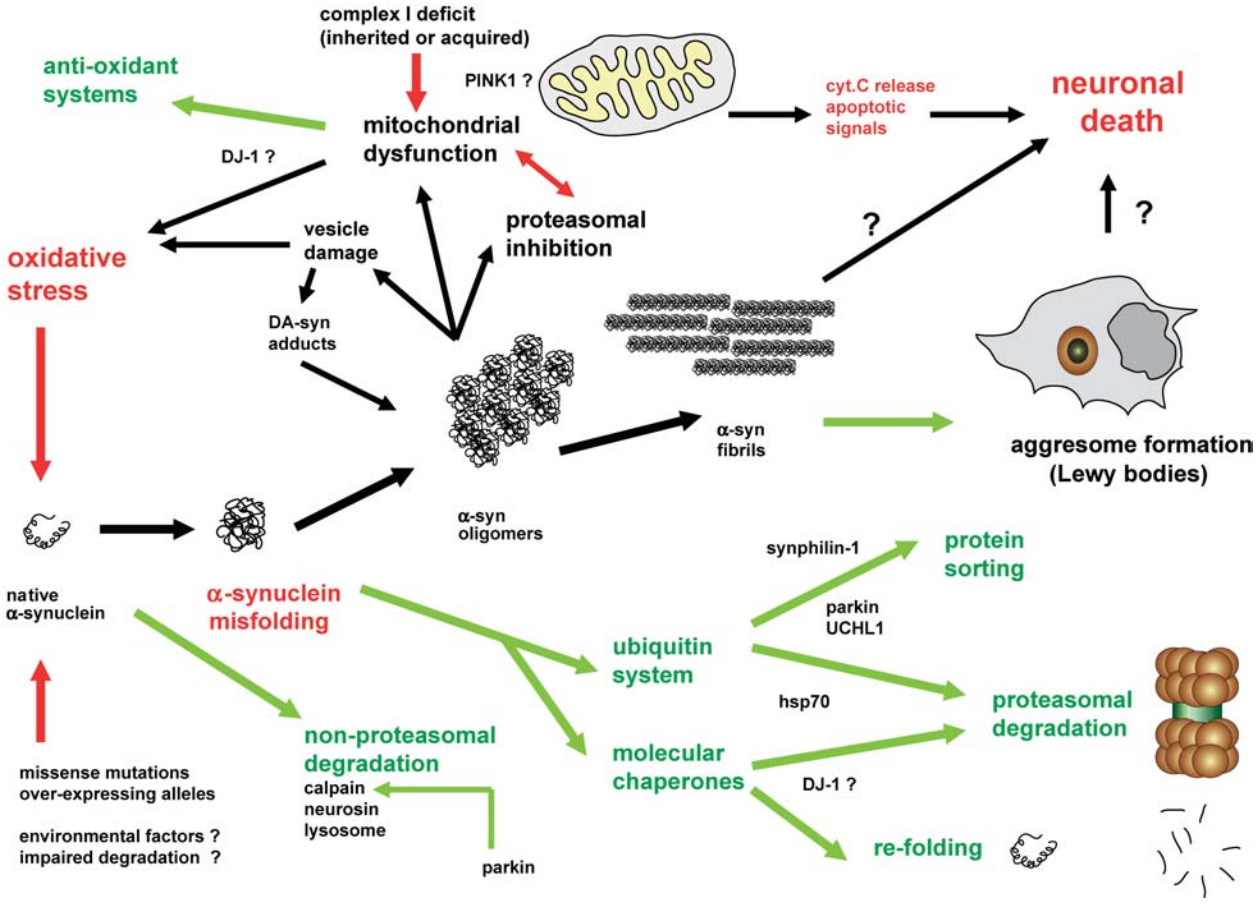

Figure 3. Model of the molecular pathogenesis of PD. The Mendelian forms of PD are unraveling the role of key proteins, such as α-synuclein and parkin, which also play important roles in the pathogenesis of the common forms of the disease. α-Synuclein misfolding and aggregation are central in the pathogenesis of PD. α-Synuclein oligomers and/or fibrils are suspected to be neurotoxic through a cascade of self-perpetuating mechanisms, which include mitochondrial and proteasomal inhibition, vesicle damage and oxidative stress, ultimately leading to cell death. In rare families, the cascade is activated by mutations in the *α-synuclein* gene, but the primary cause of α-synuclein misfolding and aggregation in the common form of PD remains unknown. A primary role for mitochondrial defects is supported by the discovery that mutations in PINK1, a mitochondrial protein kinase, cause PARK6. In response to oxidative stress and/or protein misfolding, the systems of antioxidants and molecular chaperones (which might include DJ-1) attempt to repair the damaged proteins. The proteins which cannot be efficiently repaired are tagged for degradation though the ubiquitin-proteasome system and other non-proteasomal pathways. If these systems are also insufficient, the excess of misfolded proteins is sequestered in an insoluble, inert form (aggresomes). Parkin might promote the degradation of α-synuclein and other misfolded proteins through proteasomal and non-proteasomal pathways, and it might also be involved in the formation of aggresomes.

stress [221, 232–234], susceptibility to dopamine-mediated toxicity [235, 236], proteasomal inhibition [220, 234] and apoptosis [220, 234]. It is of interest that in other cell systems, *α*-synuclein exerted protection against oxidative stress [237] and anti-apoptotic properties [238], and protected mice from paraquat-induced neurodegeneration [239]. These findings illustrate the complexity of the *α*-synuclein pathways and the possible confounding effects of the different cell types, levels of expressions, and experimental paradigms.

The observation that systemic administration of the mitochondrial complex I inhibitor rotenone, a substance not selectively uptaken by dopamine neurons, causes selective dopamine neuronal loss with LB-like inclusions in rodents [193], is compatible with the argument that dopamine neurons are particularly vulnerable to systemic complex I dysfunction and that mitochondrial defects might be a primary event in common PD. The recent discovery of mutations in PINK1, a mitochondrial putative protein kinase, in PARK6, strengthens the argument of mitochondrial defects as primary event [167]. The lack of complex I impairment in cells of patients with PD caused by *α-synuclein* mutations also suggests that the *α*-synuclein cascade might be downstream of the mitochondrial defects in common PD [240].

However, many reciprocal influences are known between mitochondrial dysfunction, oxidative stress, protein misfolding and oligomerization, proteasome inhibition and activation of unfolded protein response, leading to many possible vicious cycles and ultimately to neuronal cell

death. All of these mechanisms have been implicated in the pathogenesis of common PD, but it is difficult to disentangle the primary and secondary events (fig. 3).

The role of the LBs and the molecular chaperones in PD

The role of the LBs is controversial, as they might be neurotoxic, inert or protective for the neuron. Inclusions could be detrimental by sequestering molecular chaperones, proteasomal subunits and other important molecules. However, a growing body of evidence suggests that inclusions function to eliminate toxic soluble species by sequestering them in insoluble form. This mechanism could compensate in situations where the protein quality control system (chaperones, proteasome and other systems) is insufficient or overloaded (fig. 3). This view is strongly supported by the fact that LBs resemble the so-called aggresomes [241], which are believed to be part of the cell repertoire of responses to protein misfolding and aggregation [242]. LBs display many similarities to aggresomes, including the morphology, presence of chaperones, proteasomal subunits, and other aggresomal markers such as γ-tubulin and pericentrin [241, 243].

Molecular chaperones assist the proper folding of nascent polypeptides, refolding of damaged proteins and delivering of proteins for proteasomal degradation (fig. 3) [244]. Studies in transgenic animals and cell models of neurodegeneration show that manipulation of the chaperone and of the ubiquitin systems markedly influences pathogenesis. In rodent and fly models of different neurodegenerative diseases (including α-synuclein), the overexpression of chaperones reduces, whereas interference with chaperones aggravates neurotoxicity [175, 245]. These effects are not accompanied by visible modification of aggregates. On the contrary, interference with ubiquitylation enhances pathogenesis but also markedly reduces the formation of aggregates [246]. Taken as a whole, these studies support the contention that inclusions are not primarily pathogenic, and they might actually be protective.

The contribution of parkin

The discovery of *parkin* mutations in PARK2 provided a second landmark contribution to the understanding of PD, highlighting the role of the ubiquitin-proteasome system in the pathogenesis of both parkin-linked and classical PD.

The function of the parkin protein

The *parkin* gene encodes a 465-amino acid protein with an N-terminal domain homologous to ubiquitin (Ub-L), and two RING finger domains separated by an in-between-RING (IBR) domain in the C-terminal part (fig. 2) [103, 247]. The parkin protein is widely expressed in neurons and glial cells, and it has been localized at many levels, including the cytosol, endoplasmic reticulum, Golgi complex, synaptic vesicles, postsynaptic densities, nuclear matrix, and the outer mitochondrial membrane. The Ub-L domain of parkin adopts the ubiquitin fold [248, 249] and shares a motif with proteins interacting with the proteasome [250]. Recent evidence indeed suggests that parkin binds proteasomal subunits, such as Rpt6 [251] and Rpn10 [249], linking this structure to the ubiquitylation machinery.

Parkin possesses ubiquitin-ligase activity [252–254]. Covalent attachment of the ubiquitin (Ub) polypeptide (ubiquitylation) tags proteins for proteasomal degradation, and this is a fundamental mechanism for the protein quality control system [255]. Parkin interacts through its RING-IBR-RING domains with different Ub-conjugating enzymes, including the cytosolic UbcH7 and UbcH8 [252– 254], and with the endoplasmic reticulum-associated UBC6 and UBC7 [256]. Parkin also ubiquitylates itself and promotes its own proteasomal degradation [254, 257]. Much work has explored the physiological and pathological role of parkin, assuming a link to the ubiquitin-proteasome system. However, ubiquitin conjugation is a signalling system which regulates a broad range of cellular processes, including gene transcription, endocytosis and protein sorting [255, 258]. Parkin might also be linked to neurodegeneration via a different ubiquitin-mediated pathway.

Parkin knockout mice have recently been generated [259, 260]. These mice are viable and fertile; they develop and maintain a normal number and morphology of dopaminergic neurons, normal brain morphology and normal general behaviour. However, they show some biochemical, electrophysiological and behavioural abnormalities, which indicates subclinical dysfunction in the dopaminergic nigrostriatal pathways [259, 260]. These findings suggest that parkin plays a role in the regulation of dopaminergic neurotransmission. However, the *parkin* knockout mice failed to reproduce the parkinsonian syndrome and the loss of dopaminergic neurons, the main pathological features of PD and of parkin disease in humans, suggesting that the role of parkin might differ among species, and indicating that this role is not essential for the development and survival of dopaminergic neurons in mice [259, 260]. In keeping with this view, species-specific differences in the biochemical properties of parkin between rodents and humans, and variation of the biochemical profile of human (but not murine) parkin with age have recently been reported [261].

Parkin has many putative interactors

PARK2 is in most cases a classical recessive disease, suggesting that the loss of parkin function is pathogenic. Moreover, several disease-causing missense mutations in parkin abolish Ub-ligase activity [88, 252, 254]. To the

extent that loss of this function is the culprit in parkin disease, accumulation of non-ubiquitylated substrates is important in the pathogenesis. The identification of parkin substrates is therefore of interest.

Yeast two-hybrid screens and other approaches have provided several putative candidates (fig. 4), including CDCrel-1, a synaptic vesicle-associated protein [254] and its close homolog CDCrel-2; [262], synaptotagmin XI, implicated in regulation of synaptic vesicle trafficking [263]; the putative G-protein-coupled transmembrane PAEL-receptor (*p*arkin-*a*ssociated *e*ndothelin receptor-*l*ike receptor) [256]; synphilin-1, an α-synuclein-interacting protein [88]; αSp22, a brain-specific glycosylated isoform of α-synuclein [264]; calcium/calmodulin-dependent serine protein kinase (CASK), a postsynaptic protein [265]; cyclin-E, a protein linked to cell cycle regulation and neuronal apoptosis [266]; p38, a component of the transfer RNA (tRNA)-aminoacyl synthetase complex [267]; and α- and β-tubulins [268]. These putative interactors suggest the involvement of different pathways in the pathogenesis of parkin disease, including synaptic derangement, endoplasmic reticulum stress and the unfolded protein response, and induction of apoptosis (fig. 4). However, whether the accumulation of one or several of these putative substrates is neurotoxic remains unclear. The interaction between parkin and the chaperone Hsp70 [251, 269] could mediate the interaction with a variety of misfolded proteins, explaining the apparent broad substrate specificity of parkin (fig. 4). Accumulation of parkin putative substrates in the brain of patients with parkin disease is so far limited to single reports [256, 264, 266], and this has not been observed in *parkin* knockout mice [260].

The reported interactions between parkin and α-synuclein, either direct via native α-synuclein [218], the αSp22 isoform [264], or indirect via synphilin-1 [88], would link parkin to α-synuclein, another central protein in the pathogenesis of PD. These interactions might underlie the deposition of ubiquitylated α-synuclein in LBs [88, 264]. Other studies showed that parkin overexpression protects from α-synuclein-induced neurotoxicity in cell cultures [218, 270], and from α-synuclein-induced neurodegeneration in *Drosophila* [271], also suggesting that parkin is implicated in the pathogenesis of PD in general.

The absence of LBs in most patients with parkin disease is compatible with the idea that the Ub-ligase activity of parkin is important for the formation of these inclusions. The report of LBs in a PD patient carrying compound heterozygous *parkin* mutations is in agreement with this view [115], as one of these mutants (R275W) retains E3 activity [88]. However, a different explanation is that the pathogenesis of the parkin disease differs from that of classical PD with LB pathology. The recent finding that parkin is recruited in aggresomes in cell cultures in response to proteasomal and other cell stresses is compatible with an active role of parkin in the formation of aggresomes and of LBs [243, 272, 273].

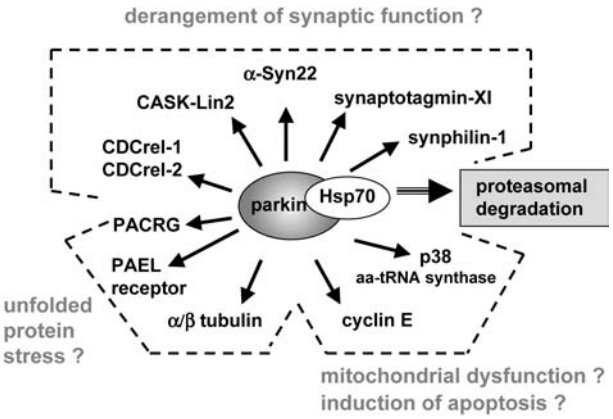

Figure 4. Interactions of the parkin protein. The interaction between parkin and the chaperone Hsp70 might mediate some of the other interactions of parkin. Parkin also directly interacts with the proteasome, linking this structure with the ubiquitylation machinery. The accumulation of non-ubiquitylated parkin substrates might cause neurodegeneration in parkin disease. The current scenario suggests that derangement of the synaptic function, protein misfolding, mitochondrial dysfunction and activation of apoptotic pathways play a role in the pathogenesis of this Mendelian PD form.

Parkin, mitochondria and apoptosis

A link between parkin, mitochondria and apoptosis is suggested by the *Drosophila parkin* knockout model [274]. These flies show reduced longevity, male sterility, locomotor defects and mild, age-related structural changes in the dorsomedial cluster of dopaminergic brain neurons [274]. The male sterility is due to mitochondrial defects in spermatogenesis, and the locomotor defects are caused by a mitochondrial myopathy leading to apoptotic cell death [274]. This model failed to reproduce dopaminergic neuronal loss typical of human parkin disease. However, its findings are important in the light of mitochondrial defects observed in classical PD [275]. Recently, parkin has also been linked to protection from mitochondria-dependent cell death in a cell culture model [276]. Oxidative stress and nitric oxide production have been observed after overexpression of disease-linked *parkin* mutations in cell cultures [277]. A recent proteomic study of brain from *parkin* knockout mice revealed abnormalities in the levels of several mitochondrial proteins and evidence of oxidative damage [278], strongly suggesting that mitochondrial defects and oxidative damage are central in the pathogenesis of parkin-linked disease.

The parkin ubiquitin-ligase activity could protect cells in several ways, including tagging the unfolded substrates for proteasomal degradation, promoting formation of aggresome-like inclusions and perhaps by signalling in

other ubiquitin-mediated pathways related to cell death. Whatever the initial mechanism is, the final endpoint in parkin disease shows evidence of mitochondrial derangement, oxidative stress and apoptosis [274, 276–278], suggesting further links to the pathogenesis of classical PD.

Conclusion

Molecular genetics has provided tremendous contributions to our understanding of the pathogenesis of PD. Most of this progress has come from analysis of rare inherited forms of the disease, and identification of the genes involved in other forms holds the promise of yielding further significant steps forward. Yet we are just beginning to disentangle the complexity of the common forms of the disease, and the genome-wide linkage screens have recently produced the first significant results. Perhaps the next few years will also see the shift from the current 'anatomical' screening of the genome to a more functional analysis exploring quantitative changes in gene and protein expression. Profiling the transcriptome and the proteome in human tissue, animal and cell-based models might provide functional signatures of the disease process, allowing recognition of pathways and development of integrative views.

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