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Survey NEURAL CHAPERONES

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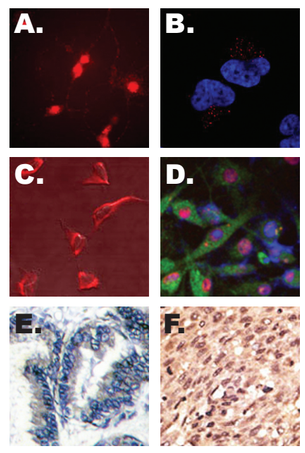
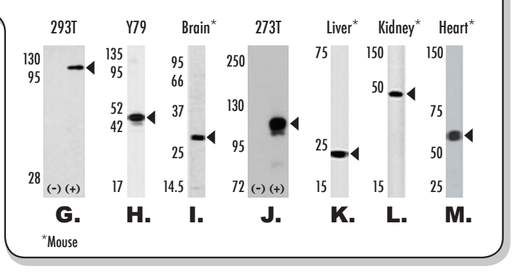
C.	Nestin	Human carcinoma (HeLa)	AP2020b
D.	LRRK2	Tau-stable SY5Y	AP7099h
E.	DCLK1	Human hepatocarcinoma	AP7219b
F.	LRP5	Human hepatocarcinoma	AP6157a
G.	APP	Transfected 293T cells	AP6306a
H.	NSE	Y79 cells	AP2780a
I.	NTF3	Mouse brain	AP7763b
J.	NTRK1	Transfected 293T cells	AP7686d
K.	Neurogenin 3	Mouse liver	AP2024a
L.	PSEN2	Mouse kidney	AP6305b
M.	CERK	Mouse heart	AP7088b





Chaperones

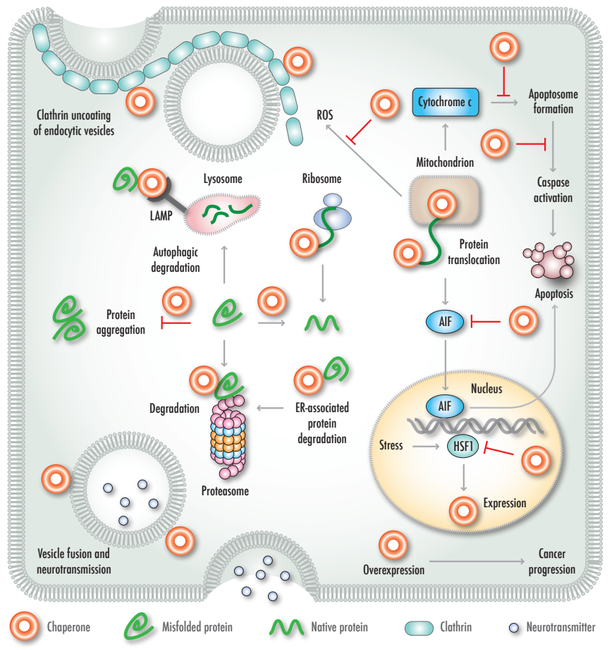


Fig. 1 Role of molecular chaperones in cellular processes. Molecular chaperones facilitate protein folding and prevent protein aggregation. They also regulate autophagy, vesicle fusion, signal transduction, apoptosis and proteasomal degradation. **AIF**, apoptosis inducing factor; **ER**, endoplasmic reticulum; **HSF1**, heat shock transcription factor 1; **LAMP**, lysosomal-associated membrane protein; **ROS**, reactive oxygen species (1).

Molecular chaperones associated with protein-conformational disorders

Neurodegenerative disease	Disease genes	Chaperones involved	Lesions
Parkinson's disease	α-Synuclein, Parkin, UCHL1, PINK1, DJ1, HTRA2, LRRK2	HSP70 , HSP40, HSP90, HSC70, CDC37, TRAP1, 14-3-3, HSPA9, CHIP	Intracellular Lewy bodies
Alzheimer's disease	APP, Presenilin 1, Presenilin 2	HSP72 , HSP28, HSP27 , HSP90	Extracellular senile plaques Intracellular neurofibrilary tangles
Dementia with Lewy bodies	α-Synuclein	HSP27 , HSP40, HSP60, HSP70, HSP90, HSPA5	Intracellular Lewy bodies
Familial amyotropic lateral sclerosis	SOD1	HSC70	Intracellular inclusions
Huntington's disease	Huntingtin	HSP40, HSP70	Mutant huntingtin
Spinocerebellar ataxias	Ataxins	HSP40 , HSP70	Mutant ataxin
Spinal and bulbar muscular atrophy	Androgen receptor	HSP70	Mutant androgen receptor

Table 1. Neurodegenerative disorders and related chaperones. Many neurodegenerative disorders are associated with degeneration and death of neuronal populations due to the accumulation of aggregated or misfolded proteins (14). Molecular chaperones may provide the first line of defence against protein aggregate formation. **UCHL1**, ubiquitin carboxy-terminal hydrolase L1; **PINK1**, PTEN-induced kinase 1; **DJ1**, Parkinson disease (autosomal recessive, early onset) 1; **HTRA2**, serine peptidase 2; **LRRK2**, leucine-rich repeat kinase 2); **HSP**, heat shock protein; **HSC70**, heat shock cognate 70; **CDC37**, cell division cycle 37 homolog (S. cerevisiae); **TRAP1**, TNF receptor-associated protein 1; **14-3-3**, 14-3-3 protein beta/alpha; **CHIP**, STIP1 homology and U-box containing protein 1; **APP**, amyloid precursor protein; **SOD1**, superoxide dismutase 1 (1-11).

Mitochondrial kinase PINK1

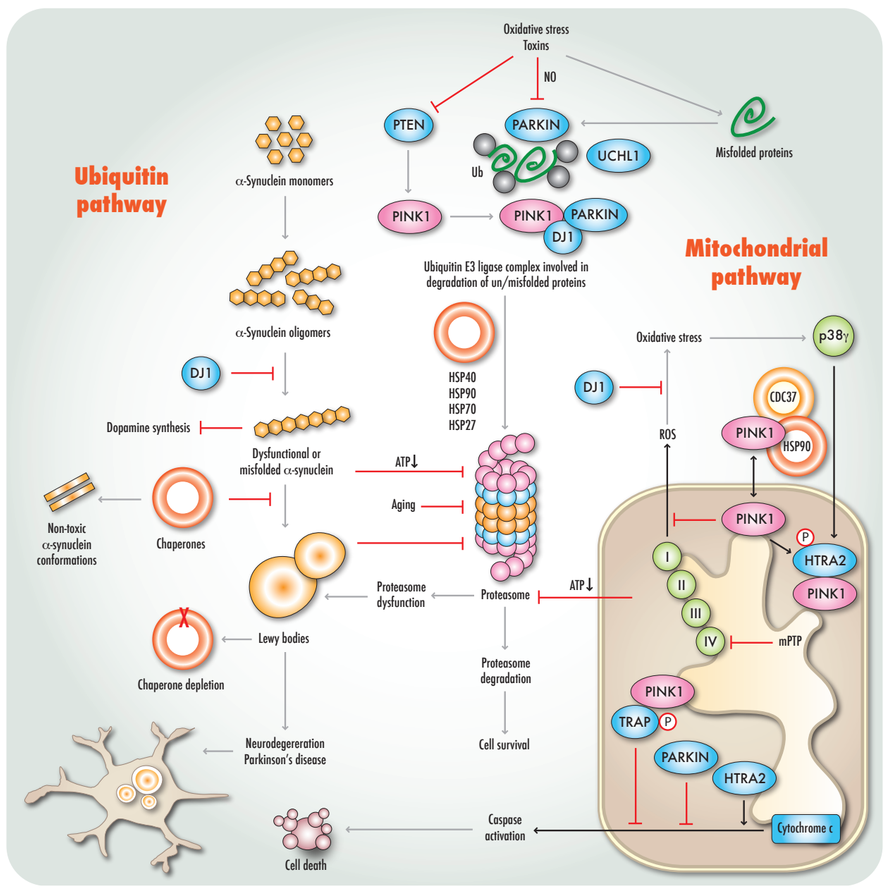


Fig. 2 Role of PINK1 in the pathogenesis of Parkinson's disease. The PINK1 gene (PTEN-induced kinase 1) encodes a serine/threonine protein kinase that localizes to mitochondria. Mutations in PINK1 cause PARK6 familial Parkinson's disease (PD) and provide the most direct link between PD and mitochondria (4-6, 8, 12, 13).

The **mitochondrial pathway** is a main pathway to parkinsonism. Impaired oxidative phosphorylation and decreased complex I activity in PD leads to reactive oxygen species formation and oxidative stress. In addition, there is loss of mitochondrial membrane potential. This leads to opening of the mitochondrial permeability transition pore (mPTP), release of cytochrome c in the cytosol, and activation of mitochondrial dependent apoptosis resulting in caspase activation and cell death. Recessive-inherited genes, such as PINK1, DJ1 (Parkinson's disease protein 7) and HTRA2 (HtrA serine peptidase 2), might all have neuroprotective effects against the development of mitochondrial dysfunction. Parkin has also been shown to inhibit the release of cytochrome c.

Another main pathway of cell toxicity is the **ubiquitin pathway**. It involves α-synuclein, misfolded proteins and aggregation. These proteins are ubiquitinated and initially degraded by the ubiquitin–proteasome system, in which Parkin has a crucial role. The proteins PINK1, Parkin, and DJ1 form functional ubiquitin E3 ligase complex to promote degradation of un/misfolded proteins (7). There is accumulation and failure of clearance by the ubiquitin–proteasome system over time, which leads to the formation of fibrillar aggregates and Lewy bodies. α-Synuclein protofibrils can also be directly toxic, leading to oxidative stress that can further impair the ubiquitin–proteasome system by reducing ATP levels, inhibiting the proteasome, and by oxidation of Parkin. This processes accelerate the accumulation of aggregates. Phosphorylation of α-synuclein-containing aggregates might have a role in their pathogenicity and formation.

Dysfunction of both pathways leads to oxidative stress, leading to irreversible cellular damage and death. **I–IV**, mitochondial electron transport chain complexes I–IV; **NO**, nitric oxide; **ROS**, reactive oxygen species; **UCHL1**, ubiquitin carboxy-terminal esterase L1; **HTRA2**, serine peptidase 2; **TRAP1**, TNF receptor-associated protein 1; **DJ1**, Parkinson disease (autosomal recessive, early onset) 1.

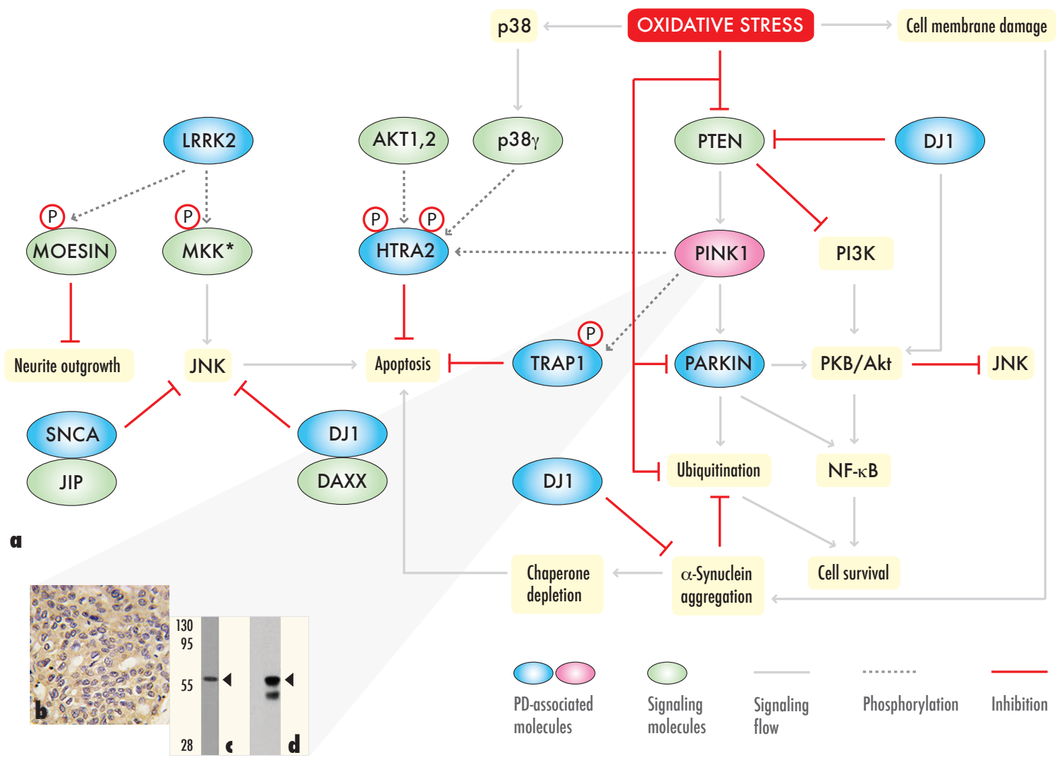


Fig.3 Protein signaling and neuroprotective mechanisms of PINK1. The signaling pathways and biological processes are highlighted in yellow **(a)**. **PTEN**, phosphatase and tensin homolog; **AKT**, v-akt murine thymoma viral oncogene homolog; **JNK**, mitogen-activated protein kinase 8; **MKK***, mitogen-activated protein kinase kinases 3, -4, -6, -7. **PI3K**, phosphatidylinositol 3-kinase; **p38**γ, p38 mitogen-activated protein kinase, isoform gamma; **MKK***, mitogen-activated protein kinase kinase 3, -4, -6, -7; **JIP1**, JNK-interacting protein 1; **DAXX**, death-domain associated protein; **NF-κB**, nuclear factor kappa-B. ABGENT's PINK1 monoclonal antibody # AM6406a was applied for immunohistochemistry of hepatocarcinoma tissue **(b),** PINK1 detection in brain tissue **(c)** and in transfected 293 cells **(d)**. For more PINK1-related products, visit www.abgent.com.

PINK1 in Parkinson's Disease

Gene locus	Chromosome	Gene name	Association	Form of Parkinsonism
PARK1 & PARK4	4q21.3-q22 & 4p15	SNCA (α-Synuclein)	Mutations	Autosomal dominant
PARK2	6q25.2-q27	PARK2 (Parkin)	Mutations	Autosomal recessive early-onset
PARK3	2p13	SPR (Sepiapterin reductase)	DNA polymorphism	Autosomal dominant
PARK5	4p14	UCHL1 (Ubiquitin thiolesterase)	Mutations	Autosomal dominant
PARK6	1p36.12	PINK1 (PTEN-induced kinase 1)	Mutations	Autosomal recessive early-onset
PARK7	1p36	DJ1 (PD protein 7)	Mutations	Autosomal recessive early-onset
PARK8	12q12	LRRK2 (Leucine-rich repeat kinase 2)	Mutations	Autosomal dominant
PARK9	1p36	ATP13A2 (ATPase type 13A2)	Mutations	Autosomal recessive
PARK10	1p	HIVEP3 (HIV enhancer binding protein 3)	DNA polymorphism	Autosomal dominant
PARK11	2q37.1	GIGYF2 (GRB10 interacting GYF protein 2)	Mutations	Autosomal dominant
PARK12	Xq21-q25	PARK12	DNA polymorphism	X-linked
PARK13	2p13.1	HTRA2 (Serine peptidase 2)	Mutations	Autosomal dominant
PARK14	22q13.1	PLA2G6 (Phospholipase A2)	Mutations	Idiopathic
PARK15	22q11.2-qter	FBXO7 (F-box protein 7)	Mutation	Autosomal recessive

Table 2. Loci and genes associated with Parkinson's disease (PD). In addition to the table, polymorphisms or mutations in **NR4A2** (nuclear receptor subfamily 4, group A, member 2), **NDUFV2** (NADH dehydrogenase flavoprotein 2), **ADH3** (alcohol dehydrogenase 1C), **FGF20** (fibroblast growth factor 20), **GBA** (β-glucosidase), and **MAPT** (microtubule-associated protein tau) genes have been associated with susceptibility to PD (OMIM, 4).

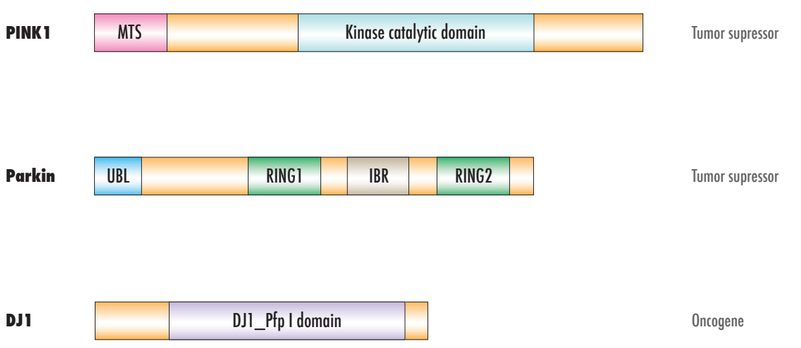


Fig. 4 Domain architecture of PINK1, Parkin and DJ1 proteins. In PD, these autosomal recessive genes are linked to oxidative stress or mitochondrial dysfunction. **MTS**, mitochondrial targeting sequence; **UBL**, ubiquitin-like domain; **RING**, RING finger motif; **IBR**, in between ring fingers (4).

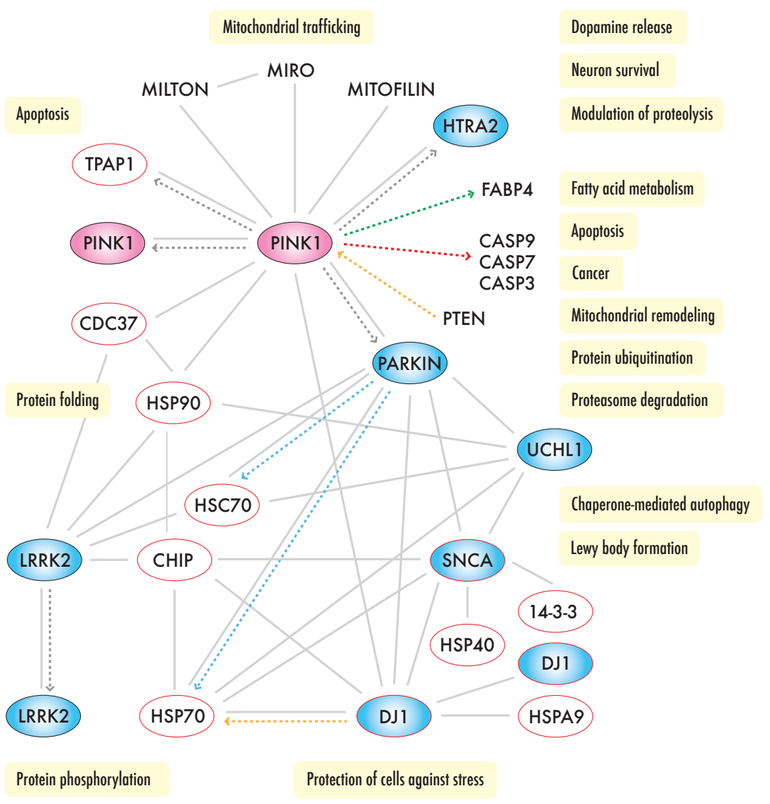


Fig. 5 Schematic representation of molecular interactions between PINK1, its functional partners and chaperones in Parkinson's disease. The biological processes, related to these protein interactions, are highlighted in yellow. **CASP**, caspase; **Miro**, mitochondrial GTPase; **PTEN**, phosphatase and tensin homolog; **Milton**, adapter protein; **FABP4**, fatty acid binding protein 4 ; **Mitofilin**, inner membrane protein, mitochondrial; **14-3-3**, 14-3-3 protein beta/alpha; **HSP40**, DnaJ (Hsp40) homolog, subfamily B, member 6 (8).

Product Abbreviations

CLCN4: chloride channel 4
MIB1: mindbomb homolog 1; DAPK-interacting protein 1; ubiquitin ligase mind bomb; ubiquitin ligase protein MIB1
LRRK2: leucine-rich repeat kinase 2; augmented in rheumatoid arthritis 17; PARK8
DCLK1: doublecortin-like kinase 1; doublecortin and CaM kinase-like 1
LRP5: low density lipoprotein receptor-related protein 5; exudative vitreoretinopathy 1
APP: amyloid beta (A4) precursor protein; A4 amyloid protein; amyloid-beta protein; beta-amyloid peptide
NSE: enolase 2 (gamma, neuronal); 2-phospho-D-glycerate hydrolyase; neurone-specific enolase
NTF3: neurotrophin 3
NTRK1: neurotrophic tyrosine kinase, receptor, type 1; oncogene TRK; tyrosine kinase receptor A
PSEN2: presenilin 2 (Alzheimer disease 4); Alzheimer's disease 3-like
CERK: ceramide kinase; lipid kinase LK4

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