

# SUMO and Parkinson's Disease

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**Abstract** Parkinson's disease (PD) is one of the most common degenerative disorders of the central nervous system that produces motor and non-motor symptoms. The majority of cases are idiopathic and characterized by the presence of Lewy bodies containing fibrillar  $\alpha$ -synuclein. Small ubiquitin-related modifier (SUMO) immunoreactivity was observed among others in cases with PD. Key disease-associated proteins are SUMO-modified, linking this posttranslational modification to neurodegeneration. SUMOylation and SUMO-mediated mechanisms have been intensively studied in recent years, revealing nuclear and extranuclear functions for SUMO in a variety of cellular processes, including the regulation of transcriptional activity, modulation of signal transduction pathways, and response to cellular stress. This points to a role for SUMO more than just an antagonist to ubiquitin and proteasomal degradation. The identification of risk and age-at-onset gene loci was a breakthrough in PD and promoted the understanding of molecular mechanisms in the pathology. PD has been increasingly linked with mitochondrial dysfunction and impaired mitochondrial quality control. Interestingly, SUMO is involved in many of these processes and up-regulated in response to cellular stress, further emphasizing the importance of SUMOylation in physiology and disease.

**Keywords** Parkinson's disease · SUMOylation · Aggregation · Oxidative stress · Mitochondria · Transcription

## Introduction

Parkinson's disease (PD) is the second most common degenerative disorder of the central nervous system worldwide, with an estimated 7–10 million people affected. After its first description in 1817 by James Parkinson as shaking palsy, PD was considered a degenerative disease of the extrapyramidal system and basal ganglia. However, PD as such does not denote a defined disease but rather comprises a variety of syndromes that are categorized into (i) idiopathic/ sporadic syndromes, (ii) familiar/genetic syndromes, (iii) symptomatic/secondary syndromes (e.g., induced by neurotoxins), or (iv) atypical syndromes (within other neurodegenerative diseases such as dementia with Lewy bodies (DLB), multiple-system atrophy, progressive supranuclear palsy, and corticobasal degeneration). Although the incidence of PD increases with age, an estimated four percent of people with PD are diagnosed before the age of 50.

The four cardinal symptoms coincidentally classified as primary motor symptoms are muscular rigidity (stiffness), bradykinesia/akinesia (slowness of movement/inability to initiate movement), resting tremor (involuntary muscle contraction at rest), and postural instability (position instability). Supplementary secondary and non-motor symptoms are dystonia, speech problems, hyposmia (diminished sense of smell), rapid eye movement sleep disturbances, fatigue, depression, fear, anxiety, and cognitive deficits.

On the functional level, the most obvious characteristics are a specific loss of dopaminergic cells, in particular at the

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substantia nigra pars compacta that projects into the corpus striatum (putamen and caudate nucleus), and a hypoproduction of dopamine. The lack of dopamine is accompanied by an excess of the neurotransmitter glutamate in the basal ganglia that eventually causes motor dysfunction and primary motor symptoms.

Lewy bodies (LBs) were considered as a defining pathological characteristic of PD and DLB. However, not all cases of PD are accompanied by the presence of LBs. LBs were also found in other brain disorders such as Alzheimer's disease (Goedert et al. 2013), questioning the specific importance of LBs for PD (Schulz-Schaeffer 2012). The accompaniment of neurofilaments, ubiquitin, and  $\beta$ -amyloid in LBs was demonstrated before the main component  $\alpha$ -synuclein was identified in 1997 (Spillantini et al. 1997). Later, immunoreactivity of LBs for many other proteins, including parkin and synphilin-1, was shown (Shimura et al. 1999; Wakabayashi et al. 2000).

SUMOylation simultaneously discovered by two groups (Matunis et al. 1996; Mahajan et al. 1997) emerged in recent years as a likely candidate mechanism to regulate a plethora of different processes within the cell. It is well known that ubiquitin and the small ubiquitin-related modifier (SUMO) share similarities in respect to the protein's tertiary structure and undergo a similar cycle of conjugation and deconjugation. However, there are important differences between both, such as the expression of different SUMO isoforms in mammals, the existence of a SUMO consensus motif, ubiquitin conjugating enzyme 9 (Ubc9) as exclusive SUMO-E2 conjugation enzyme identified so far, and the dispensability of E3 ligases by direct Ubc9-mediated transfer of SUMO to the target protein (Wilkinson and Henley 2010).

SUMOylation and ubiquitination are regarded as protein modifications that act differentially on a protein's function and fate. Surprisingly, research in recent years uncovered the existence of mixing units such as the SUMO-targeted ubiquitin ligases (STUBLs) or E3 ligases with dual functions for SUMO and ubiquitin (Prudden et al. 2007). The mammalian SUMO paralogs SUMO-1 and SUMO-2/SUMO-3, although partially redundant, may fulfill different functions as suggested by various studies on substrate specificity, mono-/polySUMOylation, expression, and oxidative stress, arguing against a generalizing "SUMO function" (Meulmeester et al. 2008; Saitoh and Hinchey 2000; Tatham et al. 2011; Bossis and Melchior 2006).

In several diseases, SUMO immunoreactivity was detected in brain tissues of patients, emphasizing the importance of SUMO in neurodegeneration. SUMO-1 co-localized with neuronal intranuclear inclusions in the brain of patients with polyglutamine disease dentatorubral-pallidolusian atrophy (DRPLA) (Terashima et al. 2002) and in several cases of sporadic and familial neuronal intranuclear inclusion disease (NIID) (Pountney et al. 2003;

Wiltshire et al. 2010). Intranuclear inclusions were also positive for ubiquitin (Mori et al. 2012), and the SUMO substrates promyelocytic leukemia protein (PML), histone deacetylase (HDAC) 4, and Ran GTPase activating protein 1 (RanGAP1) (Takahashi-Fujigasaki and Fujigasaki 2006). Immunocapturing with anti-SUMO-1 antibody and mass spectrometry from intranuclear inclusion bodies from brain tissue of a case with familial NIID identified chaperone heat shock protein (Hsp) 90 and proteins involved in membrane trafficking (Pountney et al. 2008). Furthermore, SUMO-1 immunoreactivity was observed in cytoplasmic inclusions in tissues of multiple-system atrophy cases as well as in LBs in smears of DLB tissue homogenates (Pountney et al. 2005), and SUMO-1 is present in the halo of LBs co-localizing with  $\alpha$ -synuclein in the brains of PD and DLB patients (Kim et al. 2011).

This review highlights some of the SUMO-mediated mechanisms that are described in recent years. Interconnections of proteins to the SUMO pathway may occur on distinct levels, including (i) direct SUMO modification of proteins via covalent binding or interaction, (ii) regulation of members of the SUMOylation machinery and deSUMOylating enzymes, (iii) regulation of SUMOylated proteins, or (iv) functional interactions including transcriptional regulation, subcellular localization, interference with other posttranslational modifications, proteasomal function, and protein solubility.

### Pathogenesis of Parkinson's Disease

The majority of PD cases are idiopathic without specific known cause. Around 20 % of the PD cases account for familiar forms of the disease with mutations in several genes. To date, more than a dozen different gene loci have been associated with familial PD (Table 1), but not all of them have been validated so far. Mutations in *SNCA* and *lrrk2* are inherited in an autosomal dominant manner, whereas *pink1*, *parkin*, and *dj-1* cause autosomal recessive early-onset Parkinsonism. *SNCA* and *lrrk2* alleles were associated with increased risk of sporadic PD, suggesting that idiopathic and familial disease entities share pathophysiological mechanisms. A number of environmental triggers were associated with an increased risk of PD, including neurotoxins and herbicides.

$\alpha$ -Synuclein, DJ-1, and parkin are subject to direct SUMO conjugation and SUMO interaction and will be presented in more detail in the following sections.

#### $\alpha$ -Synuclein

$\alpha$ -Synuclein is a natively unfolded protein of 140 amino acids (Weinreb et al. 1996) that belongs to the family of

**Table 1** Risk genes associated with Parkinson's disease (PD)

Locus	Chromosomal location	Protein	Function	SUMO relevance
PARK1/4	4q21–q23	SNCA ( $\alpha$ -synuclein)/triplications	Not clear	Direct modification
PARK2	6q25.2–q27	Parkin	Ub E3 ligase	Direct modification/DJ-1
PARK3	2p13	Unknown	–	–
PARK5	4p14	UCH-L1 (ubiquitin carboxyl-terminal hydrolase L1)	Deubiquitination	None
PARK6	1p35–36	PINK1 (PTEN-induced putative kinase 1)	Kinase	Parkin, DJ-1
PARK7	1p36	DJ-1	Multifunctional	Direct modification
PARK8	12p11.2–q13.1	LRRK2 (leucine-rich repeat kinase 2)	Kinase	Several sites/ $\alpha$ -synuclein, tau
PARK9	1p36	ATP13A2 (ATPase type 13A2)	ATPase	K804
PARK10	1p32	Unknown	–	–
PARK11	2q36–2q37	GIGYF2 (GRB10 interacting GYF protein 2)	Unknown	K346, K421, K736, K749, K922
PARK12	Xq21–q25	Unknown	–	–
PARK13	2p12	HTRA2 (Htra serine peptidase 2)	Protease	No
PARK14	22q13.1	PLA2G6 (phospholipase A2 group VI)	Phospholipase	K232, K305, K545, K565
PARK15	22q12–q13	FBXO7 (F-box only protein 7)	E3-Ub ligase complex	No
PARK16	1q32	Unknown	–	–
PARK17	16q11.2	VPS35 (vacuolar protein sorting 35)	Protein sorting	K403, K573, K662
PARK18	3q27.1	EIF4G1 (eukaryotic translation initiation factor 4 $\gamma$ )	Translation	K826, K881, K1222, K1452, K1556
	5q23.1–q23.3	Synphilin-1	$\alpha$ -Synuclein interactor	Several sites/ $\alpha$ -synuclein
	2q22–q23	NR4A2	Nuclear receptor	K11, K28, K456, K514

A variety of risk genes linked to PD were identified since the first description of the *SNCA* locus (Polymeropoulos et al. 1997). Only in a few cases, the loci have been validated. The table indicates which of the candidate proteins either are directly SUMO-modified or carry motifs with high probability for SUMOylation and which of them were shown to interact with SUMOylated proteins

synucleins, which also includes  $\beta$ - and  $\gamma$ -synucleins. Alternative splicing of exon 3 and/or 5 of the  $\alpha$ -synuclein encoding *SNCA* gene results in truncated forms of 112 or 126 amino acids (Beyer 2006).  $\alpha$ -Synuclein is abundantly expressed in human brain. The protein was first isolated in 1988 using antiserum against cholinergic vesicles. The protein was found to be localized to nuclear envelope and presynaptic terminals of neurons, thus named synuclein (Maroteaux et al. 1988). Later, a non-A $\beta$  component of Alzheimer's disease amyloid (NAC) was found in amyloid plaques in Alzheimer's disease patients and identified as homolog of rat synuclein, thus named SNCA (Ueda et al. 1993; Jakes et al. 1994).  $\alpha$ -Synuclein is involved in synaptic processes and synaptic vesicle trafficking although the exact physiological function remains to be determined (Chandra et al. 2004, 2005). Structurally,  $\alpha$ -synuclein can be divided into 3 domains, an amphipathic N-terminal domain, a central hydrophobic NAC domain with propensity to form  $\beta$ -sheet, and a proline-rich C-terminal tail.

A mutation in the *SNCA* gene on chromosome 4q21, causing an A53T amino acid substitution, was described in 1997 and identified as first PD gene (PARK1) (Polymeropoulos et al. 1997). Two other missense mutations (A30P

(Kruger et al. 1998) and E46 K (Zarranz et al. 2004)) as well as gene deletions were observed in familial PD. The PD gene PARK4 was described in a large family of causative PD and later identified as gene triplication of *SNCA* (Farrer et al. 1999; Singleton et al. 2003). Identification of *SNCA* gene duplication (Chartier-Harlin et al. 2004) followed, suggesting the existence of a gene dosage effect.  $\alpha$ -Synuclein is a major constituent of LBs, a hallmark of PD and DLB (Spillantini et al. 1997), giving rise to the term “synucleinopathies”.  $\alpha$ -Synuclein undergoes various types of post-translational modifications such as phosphorylation, acetylation, nitration, O-glycosylation, ubiquitination, and SUMOylation (Fujiwara et al. 2002; Kang et al. 2012; Giasson et al. 2000; Kubo et al. 2001; Tofaris et al. 2003; Dorval and Fraser 2006). The functional consequences of these modifications and their contribution to  $\alpha$ -synuclein-mediated toxicity are contrarily discussed.

The SUMOylation of  $\alpha$ -synuclein was shown in cultured cells and mammalian brain in vivo (Dorval and Fraser 2006; Krumova et al. 2011). SUMO can be targeted to several lysine residues within the molecule although lysine residues 96 and 102 were identified as major monoSUMOylation sites of  $\alpha$ -synuclein. In vitro experiments

showed that SUMOylation of  $\alpha$ -synuclein significantly delayed or even blocked the aggregation and fibrillation of  $\alpha$ -synuclein by an unknown mechanism. Conversely, SUMO deficiency leads to more inclusions within cells. Moreover, preventing  $\alpha$ -synuclein from SUMO modification enhanced apoptosis in cells and, even more importantly, resulted in significant less dopaminergic, vesicular monoamine transporter (VMAT)-positive cells in the substantia nigra pars compacta of rats within 12 weeks (Krumova et al. 2011). These results suggest that SUMOylation enhances the solubility of  $\alpha$ -synuclein and that aggregation of  $\alpha$ -synuclein contributes to its toxicity.

In another study, impaired proteasomal function resulted in the enhanced formation of high molecular weight  $\alpha$ -synuclein aggregates containing both ubiquitinated and SUMOylated species (Kim et al. 2011). A follow-up study suggested that human polycomb protein triggered the SUMOylation and subsequent aggregation of  $\alpha$ -synuclein, thereby reducing the vulnerability of cells in response to staurosporine (Oh et al. 2011). Hence, the causal relationship between SUMOylation and aggregation and the relevance to  $\alpha$ -synuclein toxicity need to be further investigated.

## DJ-1

DJ-1 was initially discovered in 1997 as an oncogene that acts cooperatively with H-Ras and whose expression is increased in several types of cancer (Nagakubo et al. 1997) and later as a regulatory subunit of an RNA-binding protein (Hod et al. 1999). The DJ-1 gene is located on chromosome 1p36 and encodes a protein of 189 amino acids length. The solution of the DJ-1 crystal structure revealed that the protein exists as a homodimer (Tao and Tong 2003; Wilson et al. 2003). DJ-1 belongs to the peptidase C56 family of proteins but possesses no protease activity due to a distorted catalytic triad and the occlusion of the putative active site by an additional C-terminal helix (Shendelman et al. 2004). DJ-1 cDNA is ubiquitously expressed in various human tissues such as pancreas, kidney, skeletal muscle, liver, brain and heart (Nagakubo et al. 1997; Zhang et al. 2005). DJ-1 protein is expressed in all brain regions with immunoreactivity in neurons and glial cells/astrocytes. In situ hybridization revealed that DJ-1 mRNA is not expressed specifically in dopaminergic neurons but instead generally in neurons (Galter et al. 2007). DJ-1 is predominantly localized in the cytoplasm, but also in the nucleus and associated with mitochondria (Nagakubo et al. 1997; Hod et al. 1999; Canet-Aviles et al. 2004). Recent evidence was provided for extracellular localization and function of DJ-1 (Kim et al. 2012).

DJ-1 is a multifunctional protein that, under oxidative stress, exerts profound cytoprotective functions in its

capacity as redox-dependent molecular chaperone, transcriptional co-activator, and regulator of androgen receptor signaling.

The involvement of DJ-1 with neurodegeneration became apparent when the gene locus was identified as PARK7 causing early-onset autosomal recessive Parkinsonism (van Duijn et al. 2001; Bonifati et al. 2003). Data regarding the cellular expression of DJ-1 mRNA and protein in the brain of PD patients compared to control cases remain inconsistent (Galter et al. 2007; Olzmann et al. 2007a; Kumaran et al. 2009). DJ-1 protein does not accumulate in LBs or Lewy neurites. Instead, it is strongly expressed in reactive astrocytes of sporadic PD cases (Bandopadhyay et al. 2004; Rizzu et al. 2004). In this context, it is interesting that DJ-1 is also strongly expressed in reactive astrocytes adjacent to brain infarcts, indicating its importance in oxidative stress conditions (Neumann et al. 2004; Mullett et al. 2009).

Several causative mutations were identified in the DJ-1 gene that account for 1–2 % of early-onset cases of PD. The most severe missense mutation L166P is located near the dimer interface and disrupts  $\alpha$ -helix G of the structurally important C-terminal helix–kink–helix motif (Bonifati et al. 2003; Gorner et al. 2007), leading to a dramatic destabilization of the homodimer and functional loss of DJ-1L166P. Other sequence alterations such as M26I, E64D, and E163K result in only slight disturbances of the DJ-1 structure, and their effects on DJ-1 function are less clear.

DJ-1 is posttranslationally modified by oxidation, S-nitrosylation, and SUMOylation (Canet-Aviles et al. 2004; Akhtar et al. 2012; Tao and Tong 2003). DJ-1 is considered as an atypical peroxiredoxin-like peroxidase that scavenges peroxides by oxidizing cysteine residue 106 (Andres-Mateos et al. 2007) and enabling mitochondrial translocation. The role of SUMO modification with respect to DJ-1 function involves both the SUMOylation of DJ-1 itself and DJ-1 regulation of other proteins' SUMOylation, thereby regulating their activities. DJ-1 protein was shown to be covalently modified by SUMO-1 at lysine residue 130 as the main SUMOylation site. Already before PARK7 was identified as DJ-1, the SUMO-1 modification of DJ-1 at K130 was found to be necessary for DJ-1 to manifest full activities for cell transformation and stimulation of cell growth (Takahashi et al. 2001). UV irradiation stimulates the expression and SUMOylation of DJ-1, as well as its oxidation and shuttling into the nucleus, suggesting that DJ-1 SUMOylation is important for its protective effect from UV-induced apoptosis. Mutation of K130 to arginine has a minimal impact on the structure of the protein (Tao and Tong 2003), but leads to multi-/polySUMOylation elsewhere in the DJ-1 protein. Mutated DJ-1 recovers in insoluble fractions and results in sensitization of cells against UV irradiation (Shinbo et al. 2006). This suggests

that improper SUMOylation rather than structural alterations is responsible for the dysfunction of DJ-1. Intriguingly, also the dysfunctional disease-associated mutant DJ-1L166P showed improper SUMOylation (Shinbo et al. 2006). These studies illustrate that mutation of a physiological SUMOylation site might lead to improper SUMOylation at other sites within the target protein. Mutations of any amino acid, not necessarily a lysine residue, in a disease-related protein, might result in altered SUMOylation.

### Parkin

Parkin is a ubiquitin ligase of 465 amino acids that is encoded in humans by the *PARK2* gene on chromosome 6q (Kitada et al. 1998; Shimura et al. 2000). Parkin belongs to the RBR family (RING, in between RING, RING) of atypical E3 ligases. An ubiquitin-like domain (UBL) is located at the amino terminus of parkin. The C-terminal half of the protein comprises two RING finger motifs (RING1 and RING2). Both flank the cysteine-rich in between RING fingers (IBR) motif (Morett and Bork 1999). The atypical E3 ligase character refers to the fact that parkin binds the E2-conjugating enzyme UBE2L3 via the first RING domain, but requires a conserved cysteine residue in the second RING domain in order to transfer ubiquitin in a HECT-type E3-like manner (Wenzel et al. 2011). Alternative splicing results in at least 7 different parkin isoforms (Beyer et al. 2008; Kitada et al. 1998, 2000) diverging for the presence or absence of the above-mentioned domains (Dagata and Cavallaro 2004). Parkin is present in tissues such as heart, testis, and skeletal muscle and is highly expressed in the brain. Intracellularly, parkin is localized in the cytosol (Shimura et al. 1999) and associated with the Golgi apparatus (Kubo et al. 2001). A defined portion of parkin can be recruited to mitochondria and the nucleus (Stichel et al. 2000; Cookson et al. 2003). Parkin is a component of a multiprotein E3 ubiquitin ligase complex that targets misfolded proteins for degradation and protects cells against various forms of cellular stress. The subcellular localization of parkin was suggested to define its specific functions such as gene regulation, mitochondrial quality control, neutralization of oxidative stress, and ER stress (Narendra et al. 2008; Chen et al. 2010a). Parkin can promote monoubiquitination of substrates as well as polyubiquitination linked via K27, K48, or K63 of ubiquitin (Doss-Pepe et al. 2005; Geisler et al. 2010). Polyubiquitin chains are built of at least four K48-linked ubiquitin molecules attached to a target protein, are recognized by the 26S proteasome subunit, and promote degradation. Monoubiquitination and polyubiquitination via K63 or K27 direct the localization of proteins. Intriguingly, parkin undergoes autoubiquitination in an

E2-dependent manner, leading to its own degradation (Um and Chung 2006; Lim et al. 2005; Choi et al. 2000).

Mutations in the *PARK2* gene are the most common autosomal recessive cause of early-onset PD and typically not associated with  $\alpha$ -synuclein and ubiquitin-positive LBs (Kitada et al. 1998; Abbas et al. 1999; Hattori et al. 1998; Leroy et al. 1998). However, parkin immunoreactivity was present in the central core of a majority of LBs from sporadic and non-parkin familial PD cases, suggesting that parkin is a key factor in the biogenesis of LB inclusions (van de Warrenburg et al. 2001; Schlossmacher et al. 2002; Shimura et al. 2000; Bandopadhyay et al. 2005).

About 50 % of the mutations in the *PARK2* gene account for genomic rearrangements such as duplications and deletions (Elfferich et al. 2011). The remaining 50 % represent small point mutations, most of which located within the RING domains. Two mutations each were found in the UBL domain (P37L and R42P) and IBR motif (G328E and R334C) (Geisler et al. 2010).

Parkin selectively interacts with SUMO-1, but not with SUMO-2. The interaction between parkin and SUMO-1 was observed in mammalian central nervous system and in neuronal cells. The association of SUMO-1 with parkin results in increased autoubiquitination and ubiquitin–proteasome system (UPS)-mediated degradation. SUMO-1 promotes the shuttling of a small pool of parkin protein from the cytosol into the nucleus. Proteasome inhibition resulted in the enhanced nuclear shuttling of parkin in the presence of SUMO-1 (Um and Chung 2006). Numerous putative substrates and interaction partners of parkin have been identified in recent years, which will be discussed in more detail below.

### Molecular Mechanisms for Parkinson' Disease

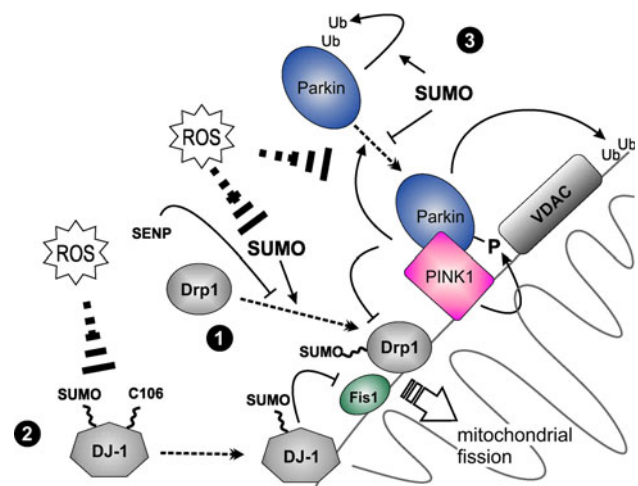
The majority of PD cases are idiopathic, which means “a disease of its own”, of unknown causes. Indeed, the cause and pathogenesis of PD remain elusive. However, starting with the discovery of environmental triggers such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or herbicides through to the discovery of risk genes in familial PD, various mechanisms have been suggested in recent years that might be involved in or are even responsible for the pathology of PD in all entities, independent of their origin. Recent findings implicate mitochondrial dysfunction, oxidative damage, subcellular mislocalization of proteins, and abnormal protein accumulation as key molecular mechanisms compromising dopamine neuronal function and survival (Pils and Winklhofer 2012; Hauser and Hastings 2013; Patel and Chu 2011; Kalia et al. 2013; Cuervo et al. 2010; Michel et al. 2013). This review focuses on PD pathology in light of SUMOylation-dependent processes.

## Mitochondrial Homeostasis and Function

Mitochondria supply most of the energy for the brain and are of peculiar interest in neurodegeneration. Contrary to the obsolete view of mitochondria as static bodies, these are highly dynamic organelles that undergo constant cycles of fission and fusion to maintain their morphology and function (Chen and Chan 2009; Youle and van der Bliek 2012; Zungu et al. 2011). The process of mitochondrial fusion allows DNA replication, complementation of protein/lipid contents, and distribution of metabolic intermediates. Fission is necessary for the redistribution of mitochondrial DNA during cell division. It increases the number and capacity of mitochondria and enables to segregate damaged mitochondrial parts for autophagy by the lysosomal system, known as “mitophagy”. There is growing evidence that SUMO plays a role in regulating mitochondrial fission and fusion (Fig. 1).

Dynamin-related protein 1 (Drp1) is a key regulator of mitochondrial fission and the first identified mitochondrial SUMO target. Drp1 is recruited from the cytosol to the mitochondrial outer membrane to coordinate membrane scission (Shaw and Nunnari 2002). Drp1 interacts with the SUMO-conjugating enzyme Ubc9 via multiple regions and is a direct target of SUMO modification by all three SUMO isoforms at non-canonical conjugation sites. The activity of Drp1, rather than the mitochondrial recruitment, seems to be linked to SUMOylation (Figueroa-Romero et al. 2009). SUMO-1 overexpression dramatically increases mitochondrial fragmentation, while Drp1 is protected from degradation under the same conditions, resulting in a more stable active pool of Drp1 (Harder et al. 2004). A cytosolic pool of sentrin-/SUMO-specific protease (SENP) 5 catalyzes the cleavage of SUMO-1 from a number of mitochondrial substrates, including Drp1. Overexpression of SENP5 rescues SUMO-1-induced mitochondrial fragmentation. By contrast, the silencing of SENP5 results in fragmented and altered mitochondrial morphology (Zunino et al. 2007). These studies suggest that SUMOylation of Drp1 leads to increased Drp1-mediated fission (Fig. 1). The mitochondrial fission enables the cell to isolate defective segments and to prevent further damage as response to minor cell stress.

PTEN-induced putative kinase 1 (PINK1) is an atypical member of the family of serine/threonine kinases and encoded by the early-onset PD gene *PARK6* (Valente et al. 2004). The 581-amino-acid-long protein is characterized by an N-terminal mitochondrial targeting sequence, a transmembrane helix, a central kinase domain, and a C-terminal domain with possibly autoregulatory function (Beilina et al. 2005). PINK1 mRNA is expressed in the brain with highest levels in the hippocampus, substantia nigra, and cerebellar Purkinje cells (Blackinton et al.



**Fig. 1** SUMO is involved in mitochondrial processes. Cellular stress leads to the formation of ROS and up-regulation of global SUMOylation, which in turn influences various mitochondrial processes. Three pathways are exemplarily shown and indicated by white numbers on black circles. (1) Drp1 translocation from the cytosol to the mitochondrial membrane is necessary for Drp1 to exert its function as mediator of mitochondrial fission. SUMOylation promotes and deSUMOylation blocks the translocation of Drp1. Moreover, SUMO-1 overexpression results in a more stable active pool of Drp1. (2) SUMOylation of the cytoprotective protein DJ-1 facilitates its subsequent oxidization at C106 and translocation to the mitochondrial membrane where it inhibits Fis1, another key player in mitochondrial fission. (3) PINK1 accumulates at the outer membrane of depolarized damaged mitochondria and recruits the E3 ligase parkin. Parkin-mediated ubiquitination of proteins such as the pore-forming VDACS promotes mitophagy. Ubiquitination of the parkin substrate Drp1 promotes Drp1 degradation and counteracts mitochondrial fission. SUMOylation of parkin reduces substrate ubiquitination in favor of parkin autoubiquitination and drives parkin translocation into the nucleus. ROS reactive oxygen species, SUMO small ubiquitin-related modifier, SENP sentrin-/SUMO-specific protease; PINK1 PTEN-induced putative kinase 1, Drp1 dynamin-related protein 1, Fis1 fission protein 1, VDACS voltage-dependent anion channel, Ub ubiquitin

2007). PINK1 mRNA expression is abundantly found in neurons, but not in glial cells, whereas immunoreactivity of the protein was also shown in astrocytes (Wilhelmus et al. 2012). PINK1 is considered as key regulator of mitochondrial homeostasis and mitochondrial quality control on molecular and organellar levels.

Drp1 can be phosphorylated by PINK1, which in turn results in reduced mitochondrial scission possibly via decreased GTPase activity of Drp1. Drp1 overexpression exaggerates PINK1 deficiency phenotypes, which are characterized by enhanced mitochondrial fragmentation and increased amounts of dephosphorylated Drp1. Interestingly, Drp1 and fission protein 1 (Fis1) expression influence PINK1 expression possibly to provide a regulatory feedback mechanism (Mai et al. 2010; Taguchi et al. 2007; Sandebring et al. 2009). Parkin interacts with and

subsequently ubiquitinates Drp1 for promoting its proteasome-dependent degradation. Pathogenic mutation or knockdown of parkin inhibits the ubiquitination and degradation of Drp1, leading to increased levels of Drp1 for mitochondrial fragmentation (Wang et al. 2011). DJ-1 exerts its cytoprotective function to oxidative stress either directly or by the regulation of antioxidative gene induction. Early studies by Canet-Aviles et al. (2004) in cells showed that cytosolic DJ-1 translocates to the outer mitochondrial membrane in response to oxidative stress and compromises Fis1-mediated mitochondrial fission by promoting its proteasomal degradation (Zhang et al. 2012). Shuttling to mitochondria is driven by the oxidization of the cysteine residue 106, which scavenges hydrogen peroxide and leads to a pI shift in DJ-1 to a more acidic point (Kinumi et al. 2004; Taira et al. 2004; Andres-Mateos et al. 2007). UV irradiation induces the SUMOylation of DJ-1, which is then oxidized and recruited to mitochondria. The K130 SUMOylation site of DJ-1 is located in close proximity to His126, which functions together with C106 as catalytic diad in a C-terminally cleaved and activity-enhanced fragment of DJ-1 (Chen et al. 2010b), further emphasizing the importance of SUMOylation for DJ-1 activity.

Taken together, the presented data suggest that Drp1 itself is modified and regulated by SUMOylation. Oxidative stress promotes the expression of SUMO and the translocation of Drp1, resulting in enhanced mitochondrial fission. Cytoprotective DJ-1 and parkin counteract SUMOylation and inhibit Drp1- and Fis1-mediated fragmentation (Fig. 1).

Full-length PINK1 is localized to mitochondria and partially imported through the outer and inner membranes in that the kinase domain faces the cytoplasm (Zhou et al. 2008). PINK1 is constitutively proteolyzed at the mitochondrial membrane of healthy mitochondria, keeping the levels of PINK1 low. The cleavage is catalyzed by the mitochondrial processing peptidase (MPP) and PARL/AFG3L2 (Meissner et al. 2011; Greene et al. 2012), leading to the formation of a 52-kDa fragment that can be further degraded by the proteasome (Lin and Kang 2008). Mitochondrial membrane depolarization leads to the accumulation of unprocessed PINK1 due to impaired mitochondrial import and reduced proteasomal degradation (Zhou et al. 2008; Narendra et al. 2010; Becker et al. 2012; Greene et al. 2012). The stabilized PINK1 directly phosphorylates parkin, which is required for efficient mitochondrial translocation of parkin (Shiba-Fukushima et al. 2012). The E3 ubiquitin ligase parkin catalyzes ubiquitination of outer mitochondrial membrane proteins. Subsequently, damaged mitochondria are transported to the perinuclear region and undergo mitophagy (Fig. 1). Intriguingly, SUMO-1 modification of parkin enhances its autoubiquitination and drives

parkin translocation into the nucleus (Um and Chung 2006). By this means, SUMOylation of parkin might also diminish the pool of parkin that is available for the mitochondrial recruitment. PINK1-dependent phosphorylation of other mitochondrial proteins such as the mitochondrial chaperone TRAP1/Hsp75 or HtrA2, a mitochondrial serine protease encoded by PARK13, is likewise implicated in the protection against oxidative stress (Pridgeon et al. 2007; Plun-Favreau et al. 2007).

A surprising number of SUMO-1 conjugates were observed in mitochondrial fractions, hypothesizing that SUMO may play a central role in regulating mitochondrial processes. Several proteins are recruited to mitochondria in order to fulfill their specific function. SUMOylation could be a posttranslational switch that allows the dynamic and reversible regulation of mitochondrial homeostasis and function in response to various cellular stressors.

### Response to Cellular Stress

Response to cellular stress can be regulated in several ways within the cell. This section highlights SUMO-regulated signal transduction events that are involved in cell survival and apoptosis. Signal transduction cascades can be initiated by extracellular stimuli and intrinsic factors. Extracellular signals such as growth factors, hormones, cytokines, and toxins must be transmitted via receptor mediation or by crossing the plasma membrane. Nuclear receptors such as androgen receptor, peroxisome proliferator-activated receptor (PPAR), and retinoid X receptor  $\alpha$  (RXR $\alpha$ ) represent a specific class of proteins that directly bind to DNA response elements and regulate the expression of specific genes. Intrinsic factors such as caspases and proapoptotic and antiapoptotic proteins play an important role in the regulation of apoptosis.

Rearranged during transfection (RET) belongs to the family of receptor tyrosine kinases that are expressed on the cell surface. Binding of glial cell-derived neurotrophic factor (GDNF) to RET is neuroprotective for dopaminergic neurons. The signal is intracellularly transduced through the activation of the phosphatidylinositol 3-kinase (PI3K) and the Akt/protein kinase B pathway. The PI3K class IA phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-trisphosphate (PIP3). The phosphatase PTEN (phosphatase and tensin homolog) hydrolyzes PIP3 to PIP2, thus acting as the catalytic antagonist of PI3K and inhibitor of the Akt pathway (Stambolic et al. 1998). The activity of PTEN requires its association with the inner face of the plasma membrane via electrostatic interactions. SUMO-1 modification of PTEN at K266 within the CBR loop facilitates the cooperative binding between PTEN and the plasma membrane (Huang et al. 2012). This SUMO effect might not only be relevant in tumorigenesis but also have a broad

impact on cell survival in general. DJ-1 is a key negative regulator of PTEN function. DJ-1 overexpression, which is found in various carcinoma samples, leads to hyperphosphorylation of Akt/protein kinase B and increased cell survival, whereas DJ-1 knockdown has opposite effects (Kim et al. 2005; Davidson et al. 2008). Additionally, DJ-1 prevents the down-regulation of RET mRNA and RET receptor by hypoxia-inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ) (Foti et al. 2010). Recently, SUMOylation of Akt itself was shown to be essential for its activation (Li et al. 2013), demonstrating a variety of regulatory mechanisms through SUMOylation.

Activation of the nuclear factor-kappaB (NF- $\kappa$ B) signaling pathway is widely connected to cell survival. NF- $\kappa$ B activity is tightly regulated by several factors including posttranslational modifications such as phosphorylation, acetylation, and ubiquitination. The transcription factor NF- $\kappa$ B, in its most abundant form a heterodimer of the p50/p65 (RelA) subunits, is retained in a cytosolic complex with I $\kappa$ B $\alpha$ . Phosphorylation and ubiquitination of I $\kappa$ B $\alpha$  release NF- $\kappa$ B from the complex and enable NF- $\kappa$ B translocation into the nucleus and transcriptional regulation. The phosphorylation of I $\kappa$ B $\alpha$  is catalyzed by the I $\kappa$ B kinase (IKK), a complex of the two catalytic subunits IKK $\alpha$  and IKK $\beta$  and the regulatory subunit IKK $\gamma$ /NEMO (NF- $\kappa$ B essential modulator).

Growing evidence suggests that several proteins in the NF- $\kappa$ B pathway are subject to SUMOylation (Girdwood et al. 2003). One of the first proteins that were shown to be competitively modified by ubiquitin and SUMO is I $\kappa$ B $\alpha$ . I $\kappa$ B $\alpha$  is conjugated to SUMO on the same lysine residue K21 that also serves as ubiquitin target. In contrast to ubiquitination, SUMOylation neither requires prior phosphorylation nor results in I $\kappa$ B $\alpha$  proteasomal degradation (Desterro et al. 1998). NF- $\kappa$ B activity is inhibited by the SUMO E3 ligases protein inhibitor of activated signal transducers and activators of transcription (PIAS) 1 and 3 that bind to the C-terminal transactivation domain and N-terminal DNA binding domain of the RelA subunit, respectively (Jang et al. 2004). The RelA subunit of NF- $\kappa$ B is, in addition, covalently SUMOylated by PIAS3. SUMO-1 modification of NEMO is critical for stress-mediated NF- $\kappa$ B activation although the underlying mechanisms remain unclear. The SUMO E3 ligase PIAS $\gamma$  seems to mediate NEMO SUMOylation with substrate specificity for SUMO-1, but not for SUMO-2 and SUMO-3, thereby contributing to NF- $\kappa$ B activation (Mabb et al. 2006).

The PD-associated proteins DJ-1, parkin, and ubiquitin C-terminal hydrolase L1 (UCH-L1) are modulators of NF- $\kappa$ B activity. The deubiquitinating enzyme cellular zinc finger anti-NF-kappaB (Cezanne) inhibits NF- $\kappa$ B translocation by stabilizing the inhibitor I $\kappa$ B $\alpha$ . DJ-1 interacts with Cezanne, inhibits its deubiquitination activity, promotes

NF- $\kappa$ B nuclear translocation, and enhances cell survival (McNally et al. 2011). Parkin mediates neuroprotection through activation of NF- $\kappa$ B signaling (Henn et al. 2007). UCH-L1 inhibits NF- $\kappa$ B activation possibly through reversal of I $\kappa$ B $\alpha$  ubiquitination (Takami et al. 2007). SUMOylation of members of the NF- $\kappa$ B pathway (e.g., I $\kappa$ B $\alpha$ ) and binding of SUMO E3 ligases to nuclear NF- $\kappa$ B/RelA seem to prevent full NF- $\kappa$ B activation and cell survival. SUMOylation of NEMO though contributes to NF- $\kappa$ B activation. It remains to be determined whether and how SUMOylation of these proteins might provide a general mechanism to regulate cell survival depending on upstream signals or intrinsic factors.

Daxx was identified in 1997 as Fas-death-domain-associated protein that activates the JNK pathway and potentiates Fas-mediated apoptosis (Yang et al. 1997; Torii et al. 1999). Later studies showed that Daxx is involved in UV-induced cell death (Khelifi et al. 2005). Daxx possesses both cytoplasmic and nuclear functions. Cytosolic localization of Daxx is required for the binding and activation of the proapoptotic factor apoptosis signal-regulating kinase 1 (ASK-1) (Chang et al. 1998). DJ-1 binding to Daxx blocks the nucleocytoplasmic translocation of Daxx. Retention of Daxx in the nucleus prevents it from binding to ASK-1 (Junn et al. 2005). Although recent findings focus on nuclear functions of Daxx, the role of SUMO-interacting motifs (SIM) for cytosolic functions of Daxx will be of additional interest.

Hypoxia and the formation of reactive oxygen species (ROS) stabilize HIF-1 $\alpha$ . Under physiological conditions, HIF-1 $\alpha$  is constitutively ubiquitinated and degraded by the proteasome, keeping the levels of HIF-1 $\alpha$  low. Oxidative stress blocks the degradation of HIF-1 $\alpha$ , which in turn shuttles into the nucleus and induces the transcription of proapoptotic genes. Hypoxic stress promotes the SUMOylation of HIF-1 $\alpha$ . SUMOylated HIF-1 $\alpha$  binds to the ubiquitin ligase von Hippel-Lindau (VHL) and undergoes ubiquitination and degradation. In this case, SUMOylation serves as a direct signal for ubiquitin-dependent degradation. The SUMO isopeptidase SENP1 removes SUMO from HIF-1 $\alpha$  and enhances its stability. Hypoxia-induced transcription of HIF-1 $\alpha$ -dependent genes such as vascular endothelial growth factor, erythropoietin, and glucose transporter-1 is markedly reduced in SENP1-deficient mice, indicating that SENP1 contributes to the regulation of the hypoxic stress response (Cheng et al. 2007). In one report, Seliger et al. (2007) suggested that the PD-associated protein UCH-L1 may counteract VHL-driven HIF-1 $\alpha$  ubiquitination, stabilizing the factor for subsequent transcriptional activity.

Oxidative stress within a certain range has been shown to induce global protein modification by SUMO-2/SUMO-3 rather than SUMO-1 (Bossis and Melchior 2006).



Furthermore, the free pool of SUMO-2/SUMO-3 ready for conjugation to target proteins is much bigger than the amount of free SUMO-1. This led to the hypothesis that cells may use SUMO-2/SUMO-3 modification as a protective response to various cellular stress stimuli. Only a few proteins have been identified as exclusive targets for specific SUMO isoforms so far. Functional differences between SUMO paralogs remain elusive. Taken together, SUMO-mediated regulation in response to cellular stress appears veritable. The limited number of studies and the fragmentary knowledge do not allow a general concept of SUMO under stress.

### SUMO: Nuclear Functions and Gene Regulation

Gene regulation is a complex interplay between various regulatory factors. It requires the availability of specific transcription factors, by either surface-receptor-mediated signaling and nuclear translocation or internalization of nuclear receptor proteins or shuttling of other proteins into the nucleus. Histones play a key role in the accessibility of DNA regions, a prerequisite for transcriptional regulation. Histones undergo posttranslational modification, especially acetylation. The addition of acetyl to the  $\epsilon$ -amino group of lysine residues neutralizes the positive charge of histones and decreases the interaction with the negative-charged DNA. Highly acetylated histones form more accessible chromatin, which in general is associated with active transcription. The action of the responsible histone acetyltransferases (HATs) is counteracted by HDACs. Deacetylation of histones allows the negative-charged DNA to wrap more tightly.

The nuclear receptors PPAR family of transcription factors, their co-activators (e.g., PPAR $\gamma$  co-activator 1 $\alpha$  (PGC-1 $\alpha$ ) or RXR $\alpha$ ), and their co-repressors (e.g., nuclear receptor co-repressor (NCoR)) are key gene regulators in mitochondrial biogenesis, energy metabolism, and apoptosis (Wadosky and Willis 2012). SUMOylation of the PPAR isoforms  $\alpha$ ,  $\gamma$ 1, and  $\gamma$ 2 blocks their transcriptional activities through the recruitment of co-repressors (e.g., NCoR). SUMO thereby inhibits PPAR-mediated apoptosis (Pourcet et al. 2010; Ohshima et al. 2004; Yamashita et al. 2004; Chung et al. 2011).

SUMOylation also suppresses the transcriptional activity of RXR $\alpha$ , which is reversed through specific deSUMOylation of RXR $\alpha$  by SENP6 (also named SUMO-specific isopeptidase (SUSP1)) (Choi et al. 2006).

The oncogene mouse double minute 2 homolog (MDM2) is a RING type E3 ubiquitin ligase, which mediates autoubiquitination as well as the ubiquitination of other substrates, including the p53 tumor suppressor protein. The p53 tumor suppressor has been identified as a key effector protein in familial as well as sporadic cases of PD

(Alves da Costa and Checler 2011). p53-mediated death of dopaminergic neurons was shown in a zebrafish model (Bretaud et al. 2007). p53 is tightly regulated by MDM2 in two ways. First, polyubiquitination of p53 by MDM2 targets p53 to proteasomal degradation (Honda et al. 1997; Haupt et al. 1997; Kubbutat et al. 1997). Second, interaction of MDM2 with the p53 transcription activation domain blocks transcriptional activities of p53 (Chen et al. 1995).

MDM2 is SUMO-1-modified by the SUMO E3 ligases PIAS1 and PIAS $\beta$ . Upon SUMOylation, the E3 ligase activity of MDM2 is shifted from self-ubiquitination toward substrate ubiquitination (Buschmann et al. 2001). Consistently, SUSP4 promotes MDM2 self-ubiquitination and positively regulates p53 (Lee et al. 2006).

Interestingly, p53 itself undergoes SUMO-1 and SUMO-2/SUMO-3 modification that is believed to increase p53 transcriptional activities (Gostissa et al. 1999; Rodriguez et al. 1999; Stindt et al. 2011). Likewise, a SUMOylation-defective mutant of *Drosophila* p53 showed markedly less activity than the wild type (Mauri et al. 2008). Modifications of mammalian p53 are catalyzed by the SUMO ligase activity of MDM2 (Chen and Chen 2003; Stindt et al. 2011). Alternate reading frame (ARF) tumor suppressor stimulates MDM2-mediated SUMOylation but blocks ubiquitination of p53 by MDM2.

Homeodomain-interacting protein kinase 2 (HIPK2), a member of the nuclear serine/threonine kinases, directly phosphorylates and activates p53. HIPK2 itself can be modified by SUMOylation and acetylation. HIPK2 localizes with p53 into nuclear bodies and shows only low basal acetylation. SUMOylation of HIPK2 enhances its association with HDAC3 and subsequent HIPK2 deacetylation. ROS trigger the deSUMOylation of HIPK2 that is paralleled by HIPK2 acetylation and significantly enhance cell survival (de la Vega et al. 2012). Consistently, HIPK2-null mice showed a loss of about 40 % of dopaminergic neurons in the substantia nigra and Parkinson-like motor symptoms (Zhang et al. 2007). In this context, it is interesting that SUMOylation mediates the recruitment of the HDAC6 and HDAC2, leading to SUMO-dependent transcriptional repression (Girdwood et al. 2003; Yang and Sharrocks 2004). Parkin is reversibly recruited to the centrosome by direct binding with HDAC6 through multiple interaction domains (Jiang et al. 2008).

The first DJ-1-interacting protein identified was PIAS $\alpha$ , also known as androgen-receptor-interacting protein 3, which suppresses androgen receptor reporter activity (Takahashi et al. 2001). DJ-1 restores the activity of the androgen receptor reporter by sequestering its negative regulator PIAS $\alpha$  (Tillman et al. 2007). Due to the nature of PIAS $\alpha$  as SUMO E3 ligase, it remains to be determined whether SUMOylation might have an impact on androgen receptor activity or not. Interestingly, androgen

response elements are present in the promoter region of tyrosine hydroxylase (TH), the rate-limiting enzyme for catecholamine biosynthesis (Jeong et al. 2006). The influence of SUMOylation on the TH promoter will be discussed below. Similarly, DJ-1 antagonizes the inhibitory effect of the DJ-1 binding protein (DJBP) on the androgen receptor by abrogation of the DJBP–HDAC complex (Niki et al. 2003). DJ-1 mediates the induction of the glutamate cysteine ligase, the rate-limiting enzyme for the biosynthesis of glutathione, an antioxidant that prevents damage caused by ROS (Zhou and Freed 2005).

SUMO has diverse effects on transcriptional activity. In many cases, SUMOylation represses transcription either by modifying transcription factors or by the recruitment of HDACs. But there are also some exceptions to the rule with SUMO-mediated transactivation. In the case of p53, SUMO seems to promote p53-mediated cell death.

### SUMO and Aggregation

Brain tissues from PD patients show characteristic cytoplasmic eosinophilic inclusions, the LBs, and dense structures in neuronal processes, the Lewy neurites. LBs are considered the hallmark of idiopathic PD and DLB, but they are also detectable in cases of Pick's disease, corticobasal degeneration, tauopathies, and multiple-system atrophy. LBs are particularly abundant in the substantia nigra but also present in the cortex, amygdala, and locus coeruleus (Braak et al. 2003).

Although  $\alpha$ -synuclein is considered the primary structural component of LBs, immunoreactivity against a variety of other proteins such as ubiquitin,  $\beta$ -amyloid, synphilin-1 (Wakabayashi et al. 2000), neurofilaments, lipofuscin, and neuromelanin was observed in the deposits. LBs seem to be a consequence of excess protein misfolding, failure of intracellular clearance/recycling systems, protein accumulation, and segregation. Despite marked abnormalities, neurons with inclusions may survive for years and preserve sufficient functional integrity prior to cell death.

It is still a matter of debate whether fibrils and aggregates are contributors to  $\alpha$ -synuclein neurotoxicity or not. Analog questions were stated in tauopathies or aggregopathies regarding the role of key proteins such as Tau, amyloid precursor protein, huntingtin, and ataxin. It was hypothesized that aggregates and fibrils are harmful due to occlusion of the cell, blockage of the proteasome, and segregation of protein, thereby diminishing the pool of functional protein or nucleation of aggregation in a prion-like manner. On the other hand, aggregates were considered as beneficial for cells in terms of protection from aberrant misfolded protein, neutralization of toxic intermediate species, or deposition in order to preserve cellular

function (Gosavi et al. 2002; Masliah et al. 2000; Sharon et al. 2003). There is growing evidence that inclusions do contain not only ubiquitinated species but also SUMOylated species. With certainty, SUMOylation can enhance the solubility of a protein as shown in numerous studies.

A recent study investigated the direct effect of SUMOylation on the aggregation propensity of  $\alpha$ -synuclein in vitro. SUMOylation of a small portion of  $\alpha$ -synuclein was sufficient to prevent  $\alpha$ -synuclein from aggregation. In vivo experiments suggested that SUMO-deficient  $\alpha$ -synuclein is more prone to aggregate and exaggerates  $\alpha$ -synuclein-mediated toxicity, implicating a detrimental role of  $\alpha$ -synuclein aggregation (Krumova et al. 2011). Seemingly contradictory conclusions were drawn from another study claiming that proteasomal dysfunction results in the aggregation of SUMOylated species (Kim et al. 2011). However, the presented data do not necessarily exclude each other.

Several studies investigated the relationship between  $\alpha$ -synuclein and other PD-associated proteins such as parkin, DJ-1, UCH-L1, and synphilin-1. Intriguingly, many of these proteins are involved in ubiquitination processes and their action may converge at several junctions. Interference between ubiquitination and SUMOylation, although manifested in rare cases only, might be a general concept for cytoprotection.

Parkin and DJ-1 seem to be protective against  $\alpha$ -synuclein toxicity on TH-positive neurons and  $\alpha$ -synuclein fibrillation (Shendelman et al. 2004; Petrucelli et al. 2002). Interaction of  $\alpha$ -synuclein and synphilin-1 via their C-termini promotes the formation of cytosolic inclusions (Engelender et al. 1999; Kawamata et al. 2001). Hsp70 inhibits  $\alpha$ -synuclein fibril formation (Dedmon et al. 2005). Parkin, PINK1, and DJ-1 form a ubiquitin E3 ligase complex (the so-called PPD complex) that promotes unfolded protein degradation (Xiong et al. 2009). The E3 ubiquitin ligase tumor necrosis factor receptor-associated factor 6 (TRAF6) binds and ubiquitinates misfolded mutant DJ-1 and  $\alpha$ -synuclein and promotes their accumulation into cytoplasmic aggregates. Parkin mediates the ubiquitination and targeting of misfolded DJ-1 into aggregates (Olzmann et al. 2007b). Parkin ubiquitination of the  $\alpha$ -synuclein-interacting protein synphilin-1 (Kawamata et al. 2001; Chung et al. 2001) may be involved in the formation of LB inclusions associated with PD (Lim et al. 2005). Excess  $\alpha$ -synuclein worsens the disease in mice lacking the neuronal deubiquitinating enzyme UCH-L1 (Shimshak et al. 2012).

Finally, an exploratory quantitative proteomic study has isolated potential SUMO substrates that are specifically up-regulated in response to proteasome inhibition, a common feature in brain aging and neurodegeneration (Abbas et al. 1999).

## Regulation of Subcellular Localization and Translocation

To fulfill their specific function, proteins need to be translocated to or localized at their correct site of action. Therefore, the regulation of the subcellular localization has a major impact on the protein's function. Additionally, the function of a protein might be dependent on the regulation of potential interaction partners.

Several studies implicate that SUMOylation is able to drive the subcellular localization, translocation, or interaction of proteins. Molecular consequences of SUMOylation, such as creating a new binding site for interaction, inhibiting interaction, and inducing a conformational change, have been described previously (Geiss-Friedlander and Melchior 2007). The initial discovery of SUMOylation as a posttranslational modification was shown for RanGAP1, a protein that, depending on its SUMOylation status, shuttles from the cytosol to the nuclear pore complex and interacts with the Ran-binding protein 2 (RanBP2) (Mahajan et al. 1997; Matunis et al. 1996).

RanBP2, a member of the nucleoporin family with SUMO E3 ligase activity (Pichler et al. 2002), is localized to the cytoplasmic fibers of the nuclear pore complex and involved in the regulation of nucleocytoplasmic shuttling of proteins. RanBP2 is a substrate of parkin, leading to ubiquitination and proteasomal degradation of RanBP2 (Um et al. 2006). RanBP2 binds to both parkin and HDAC4. HDAC4 belongs to class II HDACs (also HDAC5, 6, 7, 9, and 10) that are able to shuttle between the cytoplasm and nucleus through the nuclear pore complex. HDAC4 is a substrate of RanBP2 and conjugated to SUMO-1 at a single lysine residue. SUMOylation seems to be important for the full activity of HDAC4 (Kirsh et al. 2002), implicating consequences for histone deacetylation and transcriptional repression. Parkin can control the levels of SUMOylated and functional HDAC4 as a consequence of RanBP2 ubiquitination and degradation. Similarly, parkin may also regulate the SUMOylation or translocation of other RanBP2 substrates. Considering additionally the SUMO-dependent shuttling of parkin itself into the nucleus, SUMOylation might be one molecular mechanism for parkin-mediated transcriptional control.

TRAF- and TNF-receptor-associated protein (TTRAP) was identified as a novel effector protein for signal transduction by distinct members of the TNF receptor family (Pype et al. 2000). TTRAP inhibits the transcriptional activation of NF- $\kappa$ B, is involved in DNA repair, and may associate with PML nuclear bodies. The TTRAP sequence contains a SIM that is required for its binding to SUMO-2/SUMO-3 and TTRAP nucleolar localization (Hecker et al. 2006; Vilotti et al. 2012). Thus, SUMOylation controls TTRAP nuclear activity in response to cellular stressors.

TTRAP was identified as a novel DJ-1 interactor with a stronger binding to the PD-associated DJ-1 mutants M261 and L166P over DJ-1 wild type (Zucchelli et al. 2009).

The multifunctional protein Daxx acts as a transcriptional co-regulator and modulator of apoptosis. Daxx has a nuclear export signal (NES)-like motif and two nuclear localization signal (NLS) motifs, one in the center and one near the carboxy terminus. The C-terminal NLS ranging from amino acid 627 to 634 includes the two major covalent SUMOylation sites K630 and K631 (Jang et al. 2002; Yeung et al. 2008). In addition, Daxx has two SUMO interacting motifs, one at the very N-terminus and one at the very C-terminus, named SIM-N and SIM-C, respectively. The two SIMs feature differences in affinities and binding modes to SUMO probably due to differences in charged residues that flank the identical hydrophobic core sequences (I-I-V-L) of the two SIMs. SIM-N shows a higher affinity toward SUMO than SIM-C and binds SUMO-1 predominantly in parallel orientation, whereas SIM-C interconverted between parallel and antiparallel binding to SUMO-1. Furthermore, SIM-N binds intramolecularly to an adjacent  $\alpha$ -helical bundle, suggesting an autoregulatory mechanism for Daxx (Lin et al. 2006; Santiago et al. 2009; Escobar-Cabrera et al. 2011). SUMO interacting motifs in the protein Daxx are important for the subcellular localization and function of Daxx (Chen et al. 2006). Nuclear localization of Daxx and its interaction with p65 inhibit the acetylation of p65, which is required for the full activation of NF- $\kappa$ B-mediated gene expression (Croxtton et al. 2006; Park et al. 2007; Rothgiesser et al. 2010). Interaction of Daxx with DJ-1 prevents the nucleocytoplasmic translocation of Daxx and sequesters Daxx in the nucleus, thereby blocking cytosolic functions of Daxx (e.g., in apoptosis). Similarly, the subcellular localization and gene expression of the *Drosophila* homolog of Daxx, the Daxx-like protein (DLP), is regulated by DJ-1 $\beta$ , a *Drosophila* homolog of DJ-1. DJ-1 $\beta$  protects against oxidative stress and UV-induced apoptosis, whereas loss-of-function DJ-1 $\beta$  mutants or overexpression of DLP induces apoptosis via the c-Jun N-terminal kinase (JNK)/*Drosophila* fork head box subgroup O (dFOXO) pathway (Hwang et al. 2013). Daxx associates via SIMs with several SUMOylated transcription factors and acts as transcriptional co-repressor of antiapoptotic genes (e.g., via the recruitment of HDACs) (Chang et al. 2005; Lin et al. 2006; Kuo et al. 2005; Mizuta and Kuroda 2004). PML oncogenic domains (PODs), also called PML nuclear bodies (PML-NBs) or nuclear domain 10 (ND10), are discrete interchromosomal accumulations of several proteins, including PML and Sp100. Daxx can be recruited to PODs, a process that requires SUMO modification of PML and a SIM within Daxx (Ishov et al. 1999; Lin et al. 2006). A SUMO-defective PML mutant that cannot be

SUMOylated is no longer able to interact with Daxx (Lehembre et al. 2001; Li et al. 2000). Sequestration of Daxx to PODs can attenuate the transcriptional co-repressor activity/transrepression (Betarbet et al. 2000; Chang et al. 2005; Lin et al. 2006).

Under stress conditions such as UV irradiation, Daxx seems to be recruited to PIAS1 foci and interact with PIAS1-SUMOylated substrates via its C-terminal SIM. Thereby, Daxx appears to act as proapoptotic downstream effector of PIAS1. The SUMO E3 ligase activity of PIAS, but not SUMOylation of Daxx itself, seems to be necessary for the recruitment of Daxx- and PIAS-mediated UV-induced apoptosis (Sudharsan and Azuma 2012).

UV irradiation leads to SUMOylation and subsequent oxidization of DJ-1, followed by translocation to the nucleus. Interestingly, disease-associated mutant DJ-1L166P shows improper SUMOylation and is more sensitive to UV-induced apoptosis. Besides that, DJ-1L166P recovers in insoluble fractions and shows partial degradation by the proteasome (Shinbo et al. 2006). The DJ-1 mutant is strongly associated with the chaperones Hsp70 and CHIP compared to DJ-1wt (Li et al. 2005). BAG1 (Bcl-2-associated athanogene 1), a cochaperone of HSP70 and DJ-1 interacting protein, is able to compensate for mutant DJ-1L166P by restoring DJ-1 subcellular distribution, dimer formation, and chaperone activity and by ameliorating cell survival (Deeg et al. 2010).

#### Interaction Networks of Posttranslational Modifications

Proteins can be subject to various posttranslational modifications. Some of them target the same amino acid residues within a protein, but a cross-talk between the modifications is not necessarily restricted to direct mechanisms. The discovery of SUMO opened the doors to speculations that ubiquitination and SUMOylation act competitively regarding the targeted lysine residue and the fate of a protein. Indeed, some studies showed that proteins such as NF- $\kappa$ B, yeast PCNA, and SHMT1 were competitively modified by ubiquitin or SUMO (Desterro et al. 1998; Hoege et al. 2002; Anderson et al. 2012). Surprisingly, SUMOylation was identified as a targeting signal for ubiquitination and ubiquitin-dependent degradation (Uzunova et al. 2007; Cheng et al. 2007). The ubiquitinating enzymes were designated as STUbLs (Prudden et al. 2007). Several E3 ligases such as topoisomerase I binding arginine/serine-rich (TOPORS), MDM2, tripartite motif containing protein 27 (TRIM27), and ubiquitin-like with PHD and ring finger domains 2 (UHRF2) have dual functions as ubiquitin and SUMO E3 ligases, depending on the specific substrate (Weger et al. 2005; Chen and Chen 2003; Chu and Yang 2011; Oh and Chung 2013). A common perception especially in neurodegeneration suggested that

failure of the proteasomal system leads to the accumulation of ubiquitinated species that might harm the cell, whereas SUMOylation may enhance protein solubility. However, inhibition of the proteasomal function also results in the accumulation of SUMOylated proteins, indicating that the interplay between SUMOylation, ubiquitination, and the UPS appears to be more complex than anticipated (Tatham et al. 2011; Kim et al. 2011).

Lysine residues of transcription factors can be post-translationally modified by acetylation, a process known to modulate protein-protein interaction, enhance DNA binding, and regulate transactivation. SUMOylation of these transcription factors seems to play opposing roles as shown for p65. In addition, several enzymes of the HATs and HDAC families were shown to be modified by SUMO, thereby regulating their localization and activity (David et al. 2002; Kirsh et al. 2002; Girdwood et al. 2003; Cheng et al. 2004). Finally, acetylation has been suggested to enhance SUMOylation of histone H4 (Shiio and Eisenman 2003). Site-specific acetylation of SUMO paralogs (SUMO-1 at lysine 37 and SUMO-2 at lysine 33) was suggested as a regulatory principle in the control of SUMO–SIM interactions. Acetylation of SUMO prevents binding to several SIM-containing proteins such as PML, Daxx, and PIAS, thereby modulating SUMO-dependent transcriptional repression (Ullmann et al. 2012). A recent study reported that in vitro N-terminally acetylated  $\alpha$ -synuclein primarily presents as monomeric protein and does not form dimers, tetramers, or higher molecular weight species. N-acetylated  $\alpha$ -synuclein forms similar fibrils as the non-modified form but shows lower fibril assembly rates (Kang et al. 2012).

Phosphorylation attained interest through the identification of phosphorylation-dependent SUMOylation motifs (PDSMs) in proteins such as heat shock factors (HSFs) and MEF2A (Hietakangas et al. 2006). Similarly, the phosphorylation of proteins might be dependent on their SUMOylation status (Yao et al. 2011). Phosphorylation of serine residues flanking SIM-C of Daxx increased the local negative charge and thereby facilitated electrostatic interactions with a conserved lysine residue in the basic interface of SUMO. Moreover, SIM phosphorylation markedly enhanced the binding affinity of SUMO-1 over SUMO-2/SUMO-3, thus enabling SUMO-paralog-specific selectivity (Chang et al. 2011).

In conclusion, posttranslational modifications are likely to orchestrate the harmonic interplay of proteins and may act as molecular switches that determine the specific function of proteins at a given time and place. This applies to the physiological situation and may be even more important in diseases. Conversely, disharmony of interaction networks might account for cellular insult and cell death.

## Vulnerability of Dopaminergic Cells

Many efforts were made to elucidate the cause for the specific vulnerability of dopaminergic neurons in PD. Moreover, similar to other neurodegenerative diseases, the idiopathic pathology initiates in a small region of the brain and spreads according to a predestined sequence to other areas (Braak et al. 2003). Dopamine neuron loss and depletion of the neurotransmitter dopamine are hallmarks of PD. However, many PD symptoms only manifest when at least 55–60 % of the dopaminergic neurons are gone. Dopamine is synthesized from the amino acid tyrosine, which is converted to L-dihydroxyphenylalanine (L-DOPA) by the enzyme TH, the rate-limiting enzyme for catecholamine biosynthesis. L-DOPA is further converted to dopamine by the enzyme DOPA decarboxylase (or aromatic amino acid decarboxylase), which is found in the cytoplasm. After neurotransmitter release, dopamine can be taken up from the synaptic cleft via the dopamine active transporter DAT. Dopamine can be metabolized by the monoamine oxidase, an enzyme located in the outer mitochondrial membrane.

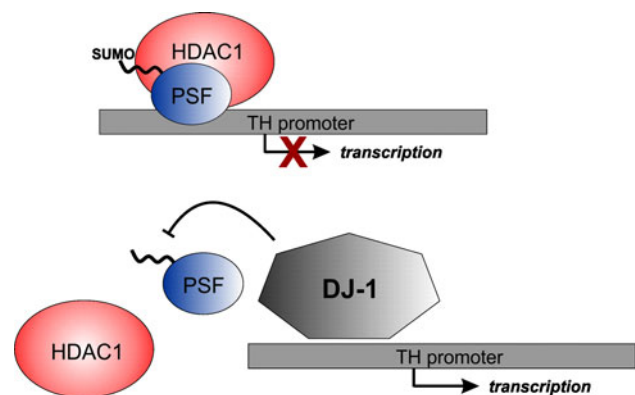
TH is depleted in PD, and there is evidence of an increase in oxidative and inflammatory nigral environment (Jeong et al. 2006). Although rare, mutations of PD-associated proteins were helpful to unravel potential pathological mechanisms in the disease. Loss of function might lead to selective neurodegeneration of nigrostriatal dopaminergic neurons. PET neuroimaging in ante mortem studies demonstrated severe dopamine depletion and reduced dopamine uptake in homozygous DJ-1 mutation carriers (Dekker et al. 2004). DJ-1 is one candidate protein that seems to prevent the specific and slowly progressive loss of nigrostriatal dopamine neuron function, while the underlying molecular mechanisms still remain to be elucidated.

A very interesting study of Zhong et al. (2006) showed that DJ-1 transcriptionally up-regulates the human TH, whereas pyrimidine tract-binding protein-associated splicing factor (PSF) acts as a transcriptional repressor (Fig. 2). PSF is SUMOylated at K338, which facilitates the recruitment of HDAC1 and enables transcriptional repression. SUMO mutation of PSF as well as the presence of DJ-1 that prevents the posttranslational modification of PSF interrupts the interaction between PSF and HDAC1 and relieves the transcriptional repression of the TH promoter. Down-regulation of DJ-1 results in lower acetylation levels of TH-promoter-bound histones H2A, H2B, H3, and H4. A similar involvement was shown for the transcriptional up-regulation of MnSOD (Zhong and Xu 2008).

The synaptic calcium-/calmodulin-dependent serine protein kinase (CASK) belongs to the membrane-

associated guanylate kinase protein family (Hata et al. 1996). CASK functions as a multidomain scaffolding protein and interacts among others with several cell surface proteins, including amyloid precursor protein, neuroligins, and syndecans. It regulates calcium/calmodulin serine/threonine kinase II (CaMKII), a central molecule in mechanisms of synaptic plasticity and memory (Malik et al. 2013). Conjugation of SUMO-1 to CASK promotes the dissociation of CASK from the membrane and reduces the interaction between CASK and protein 4.1. The function of protein 4.1 is to bind spectrin, a postsynaptic density protein required for synapse formation (Sytnyk et al. 2006). The interaction between CASK and protein 4.1 is required for CASK function in spinogenesis. Overexpression of a CASK–SUMO-1 fusion construct, which mimics CASK SUMOylation, impairs spine formation (Chao et al. 2008). CASK interacts with parkin but is not a substrate for parkin-mediated ubiquitination. The authors speculated that CASK may be involved in trafficking parkin to the appropriate subcellular compartments (Fallon et al. 2002).

Ras homolog enriched in striatum (Rhes) is a small GTP binding protein whose expression is highly enriched in striatum. Signaling by 7 transmembrane receptors through heterotrimeric G proteins is inhibited by Rhes. Rhes is involved in cAMP/protein kinase A signaling pathway. Rhes is required for a correct dopamine-mediated GTP binding, a function mainly associated with the stimulation of dopamine D2 receptors, indicating that Rhes is an important modulator of dopaminergic transmission in the striatum (Errico et al. 2008). Rhes is a physiological regulator of SUMOylation. Rhes binds directly to both E1 and



**Fig. 2** SUMOylation regulates transcriptional activity. TH is the rate-limiting enzyme for catecholamine synthesis. Both DJ-1 and PSF can bind to the TH promoter. SUMOylation of PSF facilitates the recruitment of HDAC1 and enables transcriptional repression of the TH promoter by PSF. DJ-1 prevents the SUMO modification of PSF and thereby interrupts the interaction between PSF and HDAC1. Subsequently, the transcriptional repression of the TH promoter is relieved. PSF: pyrimidine tract-binding protein-associated splicing factor; HDAC1 histone deacetylase 1, SUMO small ubiquitin-related modifier, TH tyrosine hydroxylase

Ubc9, enhancing cross-SUMOylation as well as thioester transfer from E1 to Ubc9 and is therefore considered to act as E3 ligase for the attachment of SUMO (Subramaniam et al. 2010). Incidentally, Rhes induces the SUMOylation of mutant huntingtin, which leads to enhanced cytotoxicity (Subramaniam et al. 2009).

Parkin-associated endothelin-receptor-like receptor (Pael-R)/GPR37 is a G-protein-coupled orphan receptor that is highly expressed in dopaminergic neurons in the substantia nigra and accumulates in LBs (Murakami et al. 2004). The membrane protein Pael-R is a substrate for parkin. Accumulation of Pael-R in the ER results in ER stress-induced death of dopaminergic neurons. Correct folding of Pael-R protein is difficult, and approximately half of newly synthesized Pael-R becomes misfolded and is ubiquitinated by parkin and degraded via the ubiquitin–proteasome system (Imai et al. 2001; Kitao et al. 2007).

### PD Models and Neurotoxins

Milestones in PD were the identification of neurotoxins such as MPTP, 6-hydroxydopamine (6-OHDA), rotenone, and N,N'-dimethyl-4,4'-bipyridinium dichloride (trade name paraquat), which induce Parkinson-like syndromes. Neurotoxins are widely used in animal studies to selectively mimic Parkinsonian mechanisms in dopaminergic neurons. These models serve to identify key players in pathological mechanisms and to investigate the specific role of PD-associated proteins.

MPTP appeared as a lipophilic by-product of an analog of the narcotic meperidine (Davis et al. 1979; Langston et al. 1983). The toxicity of MPTP is due to its metabolite MPP<sup>+</sup>, formed in glial cells in the reaction catalyzed by the mitochondrial monoamine oxidase B. When taken up by dopaminergic neurons, MPP<sup>+</sup> accumulates in mitochondria and inhibits complex I (Sayre 1989). Cellular damage caused by MPP<sup>+</sup> is primarily due to inhibition of NAD-linked mitochondrial respiration (Javitch et al. 1985) and energy depletion and secondarily by ROS production and oxidative insult (Cleeter et al. 1992). DJ-1 knockout mice do not show overt neurodegeneration under basal conditions (Andres-Mateos et al. 2007; R. H. Kim et al. 2005). However, additional MPTP treatment greatly enhanced the loss of nigral dopaminergic neurons concomitant with striatal denervation. Protective effects of overexpressed DJ-1 are noteworthy and not restricted to dopaminergic neurotoxicity and seem to act more generally against oxidative stress (R. H. Kim et al. 2005).

6-OHDA shares structural similarities with dopamine and noradrenaline and therefore can be taken up by both the dopamine (DAT) and the noradrenaline transporters

(NET). 6-OHDA poorly crosses the blood–brain barrier and has to be stereotactically injected into the substantia nigra, the medial forebrain bundle (nigrostriatal tract), or the striatum.

The neurotoxin rotenone is naturally occurring in plant roots and was used as an insecticide, piscicide, and pesticide. Unlike MPP<sup>+</sup> or 6-OHDA, rotenone is extremely lipophilic, by which it freely crosses the blood–brain barrier and biological membranes, thus reaching the brain rapidly. Rotenone inhibits mitochondrial complex I and causes oxidative insult via the formation of intracellular ROS. Strikingly, in vivo rotenone administrations to rodents have mimicked typical histopathological features of PD, including the formation of LBs (Sherer et al. 2003). Heikkila et al. (1985) reported a substantial depletion of striatal dopamine and its metabolites after acute stereotaxic injection of rotenone into the median forebrain bundle of rats. In a *Drosophila* model, chronic exposure to sublethal doses of rotenone is accompanied by the selective loss of dopaminergic neurons and locomotor deficits (Sherer et al. 2002). SUMO immunoreactivity was found within and surrounding glial inclusion bodies in atypical PD such as multiple-system atrophy and progressive supranuclear palsy. SUMO-1 showed punctate co-localization with the lysosomal marker cathepsin D in affected brain regions. Association of SUMO-1 with lysosomes was also detected in glial cells bearing  $\alpha$ -synuclein aggregates in a rotenone-lesioned rat model, suggesting a role for SUMO-1 in lysosome function (Wong et al. 2013). Increased levels of SUMO-1 and  $\alpha$ -synuclein-specific species ranging from 12 to 190 kDa were observed in the lesioned brain hemisphere in a unilateral rotenone-lesioned mouse model, with 6–12-month-old mice showing proportionately greater increases in SUMO-1 than mice older than 21 months (Weetman et al. 2013).

Today, paraquat is among the most commonly used herbicides worldwide. It is possibly taken up into the brain by the neutral amino acid transport system and then transported into cells in an Na<sup>+</sup>-dependent manner (Shimizu et al. 2001). Paraquat is an inhibitor of complex I that undergoes redox cycling, being reduced and oxidized to produce superoxide, a major ROS (Bus and Gibson 1984). Paraquat-induced toxicity has been linked to Parkinson-like neurodegenerative phenotype in mice, rats, and primates (Betarbet et al. 2000). *Drosophila melanogaster* as a model organism has proven to be invaluable to dissect molecular determinants of PD-associated protein function (Botella et al. 2009). As in knockout mice and zebrafish, DJ-1 deletions in *D. melanogaster* did not lead to dopaminergic neurodegeneration under basal conditions. However, DJ-1 knockout flies were specifically sensitized to neurotoxin-mediated oxidative stress and have been very

informative for the elucidation of the role of DJ-1 in the antioxidative defense mechanisms implicated in PD (Melener et al. 2005; Menzies et al. 2005).

### Many Pieces: Few Conjunctions

Hundreds of SUMOylated substrates were identified since the discovery of SUMO as a ubiquitin-like posttranslational modification. Several mechanisms were described and a variety of diverse SUMO functions were revealed that ultimately distinguish this modification from ubiquitination.

However, our knowledge is only in part. Single pieces of the whole were investigated, but more is needed to fit them together. In some cases, bridging parts are still missing. For this reason, it is important to search for other SUMO targets or SUMOylating enzymes. Due to the subtle and highly dynamic character of SUMOylation, the identification of new targets turned out to be difficult. Basic approaches are essential and were already introduced in order to identify endogenous SUMO substrates (Tirard et al. 2012; Becker et al. 2013; Lamoliatte et al. 2013). In some cases, it is known that SUMOylation is “somehow” influencing cellular processes, but the exact mechanisms are difficult to comprehend. Therefore, SUMOylated proteins need to be further characterized in terms of SUMOylation sites and molecular consequences. Here, the use of SUMO-defective mutants might be helpful. Although the pathology in PD and other neurodegenerative diseases is rather complex, the development of simple models and organisms may be advantageous to dissect molecular pathways. Likewise, these models can serve to specifically target enzymes of the SUMO pathway such as E3 ligases and SUMO isopeptidases in therapeutical approaches (Yang et al. 2013). To find the missing pieces to the puzzle might require thinking “outside the box”. In earlier times, aggregates were considered as a principal disease-causing species, whereas at present, oligomer species take the center stage. LBs were recognized as defining features of PD and DLB but lack in rare cases of PD. The long-held dogma that  $\alpha$ -synuclein, but not  $\beta$ -synuclein, is toxic to cells needs to be questioned in a similar way (Taschenberger et al. 2013).

### Conclusion

$\alpha$ -Synuclein, DJ-1, and parkin, three representatives of typically PD-associated proteins, are modified by SUMO. The consequences of SUMOylation for each protein seem to be quite diverse. SUMOylation of  $\alpha$ -synuclein might modulate the solubility and toxicity of the protein although possible mechanisms remain elusive due to an indistinct  $\alpha$ -synuclein function. The multifunctional character of the

DJ-1 protein might be causal for the diverse aspects of DJ-1 SUMOylation. SUMOylation appears to be supportive for DJ-1 activity and cytoprotective function as a molecular chaperone and transcriptional regulator. SUMOylation of parkin shifts the equilibrium toward autoubiquitination and nuclear shuttling of parkin. Whether SUMOylation is intended to block parkin function as a major player in mitochondrial quality control or to support parkin function in transcriptional regulation remains to be answered.

The impact of SUMOylation on protein aggregation is controversially discussed. Moreover, aggregation might be beneficial or detrimental for the cells, as suggested for  $\alpha$ -synuclein or huntingtin, respectively (Krumova et al. 2011; Steffan et al. 2004). Lastly, SUMO interferes with ubiquitin and the UPS. This suggests that SUMO may act as regulatory switch in concert with ubiquitin depending on the cellular context.

Apart from its direct modification of disease-associated proteins, SUMO is involved in many other cellular processes. In general, cellular stress results in increased levels of SUMO and SUMOylated species. Therefore, SUMOylation might be involved in stress response as well as mitochondrial homeostasis. SUMO increased mitochondrial fragmentation, whereas rather protective players such as DJ-1, PINK1, and parkin counteracted fission.

SUMO is an important regulator of transcriptional activity in several ways. In the cytosol, SUMO interfered with several members of signaling pathways such as HIF-1 $\alpha$ , IKK/NEMO, I $\kappa$ B $\alpha$ , and Daxx, resulting in degradation, stabilization, or translocation of the targeted proteins. Nuclear regulation of SUMO included the modulation of transcription factor activity or recruitment of HDACs. The consequences were shown to be very diverse, either promoting cell survival or inducing apoptosis. Therefore, SUMOylation might provide regulatory mechanisms for the fine-tuning of cellular processes in order to enable a dynamic response to various stress stimuli. The ability of SUMO to modulate transcriptional activity applied also to regulatory elements of the TH promoter. Therefore, research on SUMO targets and function is of particular interest to answer to the vulnerability of dopaminergic cells in PD.

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