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REVIEW

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Synucleins and their relationship to Parkinson's disease

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Abstract Parkinson's disease (PD) is one of the most common neurodegenerative motor disorders, marked by chronic progressive loss of neurons in the substantia nigra. It has long been believed that PD is caused by environmental factors. The discovery of genetic factors involved in PD has improved the understanding of the pathology of the disease. The first gene found to be mutated in PD encodes for the presynaptic protein α-synuclein. α-Synuclein is a major component of Lewy bodies and Lewy neurites, which represent the morphological hallmarks of the disease. The mechanisms by which α-synuclein is involved in nigral cell death remain poorly understood. Moreover, the factors triggering the formation of α-synuclein-positive inclusion bodies remain enigmatic. Indeed, even the normal cellular functions of α-synuclein and of the other synucleins (β-synuclein and γ-synuclein) are still unknown. Several lines of evidence suggest that they play a role in the regulation of vesicular turnover under normal nonpathological conditions.

Keywords Parkinson's disease · Synuclein (α, β and γ) · Lewy bodies · Substantia nigra · Dopamine · Human · Rodent · Song bird

Introduction

In 1988, a protein was isolated from *Torpedo californica* by using an antiserum against cholinergic vesicles. Since this protein was localized to the nuclear envelope of neurons and to presynaptic nerve terminals, it was named synuclein (Maroteaux et al. 1988).

During investigations of the composition of amyloid plaques of patients with Alzheimer's disease (AD), a peptide was solubilized and termed the non-Aβ-component of AD amyloid (NAC). In 1993, the cDNA of the precursor protein of this peptide (named NACP) was cloned (Ueda et al. 1993). In the early 1990s, a 14-kDa phosphoneuroprotein (PNP 14) was identified; it is expressed by neurons, but not by glial cells (Tobe et al. 1992; Shibayama-Imazu et al. 1993; Nakajo et al. 1993, 1994). NACP and PNP 14 have been identified as two distinct synucleins and termed α-synuclein and β-synuclein, respectively (Jakes et al. 1994).

A further homologue (BCSG1) was isolated in 1997 from breast cancer tissue (Ji et al. 1997) and, because of its homology to α-synuclein and β-synuclein, the terminology γ-synuclein has been proposed (Lavedan 1998). In addition to these three synucleins, other members of the synuclein family have been identified. These include synoretin (Surguchov et al. 1999), a bovine orthologue of γ-synuclein (Surguchov et al. 2001), the avian synelfin, which is a homologue of α-synuclein (George et al. 1995), and persyn (Ninkina et al. 1998; Buchman et al. 1998b), which has subsequently been shown to be identical to γ-synuclein (Tiunova et al. 2000).

Synuclein genes and proteins

The human α-synuclein gene (SNCA or PARK1) maps to chromosome 4q21.3–q22 (Spillantini et al. 1995; X. Chen et al. 1995; Shibasaki et al. 1995). The mouse α-synuclein gene has been mapped to the mouse chromosome six (Touchman et al. 2001). The human NACP/α-synuclein gene is composed of six exons, whereby the translation start codon ATG is encoded by exon 2 and the stop codon TAA is encoded by exon 6 (Xia et al. 2001).

The intron/exon structure of the mouse and human α-synuclein genes are highly conserved (Touchman et al. 2001) and the human and murine α-synuclein protein

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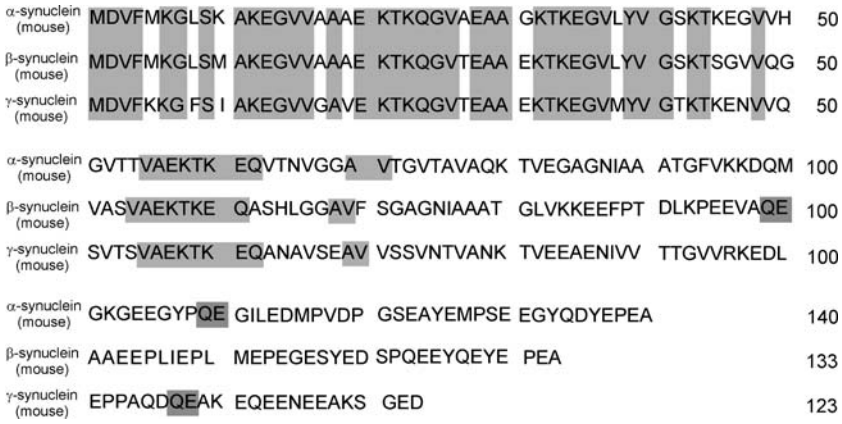

Fig. 1 Amino acid sequence alignment of murine synuclein proteins. The *gray background* highlights amino acid residues conserved between α-synuclein, β-synuclein and γ-synuclein

sequences are 95.3% identical (Lavedan 1998). The amino acid sequences of α-synuclein of mouse and human differ at seven positions. Interestingly, mouse α-synuclein, like the mutated human A53T α-synuclein, which has been observed in some familial cases of Parkinson's disease (PD; Polymeropoulos et al. 1997), contains a threonine residue at position 53 (Rochet et al. 2000).

The human α-synuclein gene encodes a small (140 amino acid) protein characterized by repetitive imperfect repeats (KTKEGV) distributed throughout most of the amino-terminal half of the polypeptide, a hydrophobic middle region (NAC region) and an acidic carboxy-terminal region (Eliezer et al. 2001). The highly conserved amino-terminal repeat domain of α-synuclein is thought to mediate both lipid binding and dimerization (Jensen et al. 1997). In addition, this domain shares a common "natively unfolded" tertiary structure that has been implicated in protein-protein interactions (Weinreb et al. 1996; Davidson et al. 1998). The C-terminal acidic tail (residues 96–140) appears to be primarily involved in the solubilization of the high molecular weight complexes (S. M. Park et al. 2002). Thus, the carboxy-terminus of α-synuclein may regulate the aggregation of full-length α-synuclein and determine the diameter of α-synuclein filaments (Murray et al. 2003). In addition, α-synuclein has chaperone activity, which is lost upon removing its C-terminal acidic tail (Kim et al. 2002).

Under physiological conditions in vitro, α-synuclein is characterized by the lack of a rigid well-defined structure and thus it belongs to the class of intrinsically unstructured proteins. Intriguingly, α-synuclein is characterized by a remarkable conformational plasticity, adopting a series of different conformations depending on the environment (Uversky 2003).

The human β-synuclein gene (SNCB) maps to chromosome 5q35 (Spillantini et al. 1995). The mouse β-synuclein gene (Sncb) has been localized to mouse chromosome 13 (Sopher et al. 2001). Like the human β-synuclein gene, Sncb appears to consist of six exons separated by five introns. β-Synuclein is composed of 134 amino acids in humans (Jakes et al. 1994) and 133 amino acids in mice (Fig. 1). Mouse and rat β-synuclein are identical and share 97.8% identity with the human protein (Lavedan 1998). The human γ-synuclein gene (SNCG) is located on chromosome 10q23 (Lavedan et al. 1998b), whereas the mouse γ-synuclein gene lies on mouse chromosome 14 (Alimova-Kost et al. 1999). The human γ-synuclein gene is composed of five exons that encode a 127-amino-acid protein (Lavedan et al. 1998b), whereas the mouse γ-synuclein gene encodes a 123-amino-acid protein (Fig. 1). Human γ-synuclein is 87.7% and 83.8% identical to the mouse and rat proteins, respectively (Lavedan 1998).

Concerning the structure of these synucleins, β-synuclein, which lacks 11 central hydrophobic residues compared with its homologues, exhibits the properties of a random coil, whereas the α-synucleins and γ-synucleins are slightly more compact and structured (Uversky et al. 2002). γ-Synuclein, unlike its homologues, forms soluble oligomers at relatively low concentrations (Uversky et al. 2002). Evidence suggests that the synuclein family members constitute a novel class of substrate for G-protein-coupled receptor kinases (GRKs). Thus, GRK2 preferentially phosphorylates the α and β isoforms, whereas GRK5 prefers α-synuclein as a substrate (Pronin et al. 2000).

Localization within the central nervous system

All three synucleins are expressed in the brain of humans and rodents (Galvin et al. 2001; Giasson et al. 2001; J. Y. Li et al. 2002b).

α-Synuclein

α-Synuclein is expressed throughout the brain at high levels, whereby the most prominent expression of α-synuclein mRNA is found in the hippocampus of rats (Maroteaux and Scheller 1991) and mice (Hong et al. 1998). The protein, at least in the rat hippocampus, seems to be localized in nerve terminals rather than in somata (Withers et al. 1997; Mori et al. 2002).

In rats, α-synuclein is located, for example, within nerve terminals of the caudate putamen and ventral pallidum, in the locus coeruleus, in the oculomotor, facial, hypoglossal, accessory and ambiguous nuclei, in spinal

chord laminae I and II and in preganglionic sympathetic nuclei (J. Y. Li et al. 2002b). α-Synuclein-positive neurons have been detected, for example, within the olfactory bulb, in deeper layers of the cerebral neocortex and in the pontine nucleus (Mori et al. 2002).

In humans, α-synuclein is predominantly localized at presynaptic terminals but has also been identified in the perikarya of neurons, such as those in the raphe nuclei and the hypoglossal and arcuate nuclei, and in neurons of the vagus nucleus (Giasson et al. 2001; J. Y. Li et al. 2002b).

Controversial results have been obtained with respect to the substantia nigra (SN). α-Synuclein-positive neurons have been reported to occur within the SN (Mori et al. 2002). However, α-synuclein might be redistributed within the SN during development. Thus, in humans, α-synuclein immunoreactivity can be detected during 15 and 18 weeks of gestational age (GA) in the perikarya of SN neurons. A redistribution of α-synuclein from the perikarya to the processes of SN neurons occurs by 18 weeks GA in parallel with synaptophysin (Galvin et al. 2001). In rodents, a high level α-synuclein protein within cell bodies of the pars compacta of the SN (SNpc) can be detected in the first postnatal week, followed by a decrease of immunoreactive cells between postnatal days 7 and 14 (Jakowec et al. 2001). There are also reports that α-synuclein-positive signals can be detected in the SNpc of normal adult rat brains, but not in the perikarya of SNpc neurons (J. Y. Li et al. 2002b). However, the use of monoclonal antibodies (termed Syn-1) instead of polyclonal anti-α-synuclein antibodies has recently demonstrated that α-synuclein is also present in the soma of specific neuronal populations in the rat brain, as, for example, in the SNpc (Andringa et al. 2003).

β-Synuclein

Both α-synuclein and β-synuclein are expressed predominantly in brain, being concentrated in presynaptic nerve terminals (Jakes et al. 1994). In the rat brain, β-synuclein immunoreactivity can be observed, for example, in the cerebellar cortex, granular cell layer of the olfactory bulb, hippocampus, striatum, cerebral cortex, caudate putamen, thalamic reticular nuclei and brain stem, including the oculomotor, facial, hypoglossal, accessory and ambiguous nuclei (Nakajo et al. 1994; Mori et al. 2002; J. Y. Li et al. 2002b). β-Synuclein immunoreactivity appears to be weak in the locus coeruleus and very weak or virtually absent from the perikarya of the neurons in the SNpc (J. Y. Li et al. 2002b).

β-Synuclein immunoreactivity is mainly found in the cytoplasmic matrix in the presynaptic axon terminals (Nakajo et al. 1994). Within hippocampal neurons, β-synuclein co-localizes almost exclusively with synaptophysin in presynaptic terminals (Murphy et al. 2000). However, β-synuclein is not exclusively found in nerve terminals but is also present in the perikarya of neurons, such as those in dorsal root ganglia (Giasson et al. 2001) and the hippocampus (Mori et al. 2002).

γ-Synuclein

γ-Synuclein is principally expressed in the brain, particularly in the human SN (Lavedan et al. 1998b) and cortex, whereas it is present only at low levels in mouse and rat cortex (Giasson et al. 2001). In addition, γ-synuclein has not been found in the caudate putamen and only very weak γ-synuclein immunoreactivity has been observed in the SN pars reticularis of the rat brain, although sparsely distributed γ-synuclein-containing neurons have been detected in the SNpc (J. Y. Li et al. 2002b). γ-Synuclein is localized in the terminals and in cell bodies of the Edinger–Westphal nucleus, the red nucleus, the locus coeruleus, most cranial nerve-related nuclei and the spinal cord (J. Y. Li et al. 2002b). However, a large proportion of γ-synuclein-immunoreactive cells seems to represent glia cells (Brenz Verca et al. 2003). In contrast to α-synuclein or β-synuclein, which co-localize almost exclusively with synaptophysin in presynaptic terminals, γ-synuclein does not co-localize with synaptophysin (Murphy et al. 2000). Instead, γ-synuclein immunoreactivity, at least in peripheral neurons, is distributed diffusely throughout cell bodies and axons (Buchman et al. 1998a).

The subcellular localization of the synucleins has not been established in detail; suggested sites of action include the presynaptic terminals, the nuclear envelope and the cytoplasm (Maroteaux and Scheller 1991; Lavedan 1998). Thus, α-synuclein has been shown to co-localize with synaptophysin-immunoreactive presynaptic terminals, although their synaptic targets are unknown (Iwai et al. 1995).

α-Synuclein protein function

Ostrerova and colleagues (1999) have demonstrated that α-synuclein is able to inhibit protein kinase C activity. In addition, the use of α-synuclein-transfected B103 neuroblastoma cells has shown that α-synuclein up-regulates the expression of caveolin-1 and down-regulates extracellular signal-regulated kinase (ERK) activity (Hashimoto et al. 2003). ERK plays a central role in various neuronal functions, including survival (Subramaniam et al. 2003), neuronal growth, synapse formation, synaptic plasticity and long-term potentiation (Impey et al. 1999). Therefore, modulation of the ERK-signaling pathway may represent an important mechanism in normal α-synuclein function.

The idea that α-synuclein has a role in synaptic plasticity is supported from studies in song birds. The avian homologue of α-synuclein is synelfin. Synelfin mRNA has been found to be up-regulated during a critical period of song learning in the lateral magnocellular nucleus of the anterior neostriatum (lMAN) of zebra finches, *Taeniopygia guttata* (George et al. 1995). Interestingly, the synelfin protein is found in the presynaptic terminals of lMAN neurons, projecting to the robust nucleus of the archistriatum (Jin and Clayton 1997), another brain nucleus in birds that is crucially involved in song learning. Moreover, pronounced up-regulation of the

mRNA has been found in area X (a further brain nucleus involved in song control) in response to exogenously applied testosterone (Hartman et al. 2001).

Since mammalian α-synuclein is predominately found in areas of the adult central nervous system (CNS) that display synaptic plasticity, including the cerebral cortex, the hippocampus, the amygdala and the olfactory bulb (Maroteaux and Scheller 1991; Iwai et al. 1995), α-synuclein could also be involved in synaptic plasticity in the mammalian brain. However, α-synuclein knockout mice do not show any altered long-term potentiation in area CA1 of the hippocampus on tetanic stimulation (Abeliovich et al. 2000), nor do they show altered spatial learning (P. E. Chen et al. 2002).

Furthermore, under normal non-pathological conditions, α-synuclein binds to small unilamellar phospholipid vesicles containing acidic phospholipids, but not to vesicles with a net neutral charge (Davidson et al. 1998). In the same year, Jenco and coworkers (1998) reported that α-synuclein and β-synuclein may be able to inhibit phospholipase D2, possibly by direct interaction at the membrane surface. Therefore, synucleins might be important regulatory components for synaptic vesicles (Jenco et al. 1998). Indeed, α-synuclein antisense oligonucleotide-treated cultures display a significant reduction in the distal pool of synaptic vesicles (Murphy et al. 2000). Hippocampal synapses of α-synuclein-deficient mice exhibit a selective deficiency of undocked vesicles, with no affect having been seen with respect to docked vesicles (Cabin et al. 2002). These mice also exhibit significant impairment in synaptic response to a prolonged train of repetitive stimulation capable of depleting docked and reserve pool vesicles (Cabin et al. 2002). Moreover, the replenishment of the docked vesicles by reserve pool vesicles after depletion is slower in the mutant synapses (Cabin et al. 2002). Since α-synuclein is synthesized in the cell soma, it has to be transported to its target sites. At least in peripheral nerves, α-synuclein is almost exclusively transported by the slow component of axonal transport (W. Li et al. 2004). Interestingly, the axonal transport of α-synuclein slows significantly with aging (W. Li et al. 2004).

α-Synuclein can be phosphorylated at serine residues (Okochi et al. 2000; Hasegawa et al. 2002). In pathological cases in which abnormal α-synuclein deposits can be found, α-synuclein is extensively phosphorylated at Ser-129 (Hasegawa et al. 2002). In addition, α-synuclein can be phosphorylated on tyrosine residues (Ellis et al. 2001) directly by Src or Fyn (Ellis et al. 2001; Nakamura et al. 2002). Mutation analysis has revealed that activated Fyn specifically phosphorylates tyrosine residue 125 of α-synuclein (Nakamura et al. 2001). Since Fyn regulates various signal transduction pathways in the CNS and plays an essential role in neuronal cell differentiation, survival and plasticity, the phosphorylation of α-synuclein might be involved in one of the Fyn-mediated signalling pathways in neurons.

Synuclein and PD

Mutations in synuclein genes

Although candidate-gene approaches to linkage in PD families were initially unsuccessful, a genome-wide scan in some forms of familial PD has mapped PD to 4q21–23 (Polymeropoulos et al. 1996) in which a candidate gene, α-synuclein, resides. In 1997, a missense mutation in the α-synuclein gene was identified. This mutation (A53T) is characterized by a change from alanine to threonine at position 53 in Italian–American and Greek families with autosomal dominant inheritance for the PD phenotype (Polymeropoulos et al. 1997). In 1998, a further mutation (A30P) was identified in a family of German origin with an autosomal dominant form of PD (Kruger et al. 1998). These mutant proteins, especially the A53T mutated α-synuclein, were natively unfolded (J. Li et al. 2002a) and displayed the tendency to form non-fibrillar aggregates (J. Li et al. 2002a) and Lewy-bodies-like fibrils in vitro (Conway et al. 1998).

It should be pointed out that the autosomal dominant mode of inheritance in the cases with α-synuclein mutations suggests a toxic gain of function as being the underlying pathomechanism. Interestingly, recent data indicate that there is haploinsufficiency at the α-synuclein gene and that the ratio of expression of the wild-type to mutant alleles (Ala30Pro and Ala53Thr) correlates with the severity of the clinical phenotype (Kobayashi et al. 2003).

Additionally, a further mutation in the α-synuclein gene (E46K) has recently been discovered in a Spanish family with autosomal dominant parkinsonism (Zarranz et al. 2004). Moreover, triplication of the α-synuclein locus has recently been shown to cause PD (Singleton et al. 2003; Bradbury 2003; Farrer et al. 2004); this is associated with a doubling of α-synuclein expression in the brain (Farrer et al. 2004). In contrast to the discoveries made in the case of α-synuclein, no mutations in the β-synuclein and γ-synuclein genes in familial PD have been detected so far (Lavedan et al. 1998a; Flowers et al. 1999; Lincoln et al. 1999a, 1999b). In addition, antibodies directed against β-synuclein (Jakes et al. 1994) and γ-synuclein (Ji et al. 1997) fail to stain Lewy bodies or Lewy neurites.

Abnormal accumulation of α-synuclein is a morphological hallmark of PD

Morphological hallmarks of PD include not only losses dopaminergic (DAergic) neurons in the SNpc and tyrosine hydroxylase (TH)-immunoreactive fibers in the striatum, but also abnormal accumulations of α-synuclein in neuronal cell bodies located in the SNpc (Spillantini et al. 1998).

The presence of Lewy bodies is the diagnostic hallmark of PD; they can be recognized by their eosinophilic bodies and unstained halos. These eosinophilic bodies are found in specific regions of the nervous system but differ in their

frequency, size, shape and structure (Gibb et al. 1991). The major component of Lewy bodies is an aggregated form of the presynaptic protein α-synuclein. Hence, an abnormal accumulation of α-synuclein in the cell bodies of the SNpc is a hallmark of PD (Lotharius and Brundin 2002). Although the reason for α-synuclein gradually being transformed into virtually insoluble Lewy bodies or Lewy neurites is still unknown, this feature serves as the best marker available for the visualization of PD-related lesions (Spillantini et al. 1998). Since α-synuclein is one of the major components of Lewy bodies (Iwatsubo 2003), the process of Lewy body formation can be divided into several stages (Wakabayashi et al. 1998; Takahashi and Wakabayashi 2001):

Stage 1:
Diffuse cytoplasmatic anti-α-synuclein staining
Stage 2:
Occurrence of irregularly shaped staining of moderate intensity
Stage 3:
Discrete staining corresponding to "pale bodies"
Stage 4:
Ring-like staining of a typical Lewy body with a central core and a surrounding halo

Within the SN, neurons exhibiting stage 1 or stage 2 structures are more numerous in patients with a shorter disease duration than in those with a longer duration (Wakabayashi et al. 1998). Such neurons are also observed in incidental subclinical PD patients (Wakabayashi et al. 1998). The pale bodies seen in stage 3 are considered to represent precursors of Lewy bodies or may represent a region of Lewy body formation (Takahashi and Wakabayashi 2001).

Abnormal aggregation of α-synuclein

Neither wild-type α-synuclein nor mutated α-synuclein fold into structured globular forms in vitro (Weinreb et al. 1996; Conway et al. 1998). The transformation of α-synuclein from its unfolded structure to a folded stage involves changes in its conformation and its quaternary structure (Goldberg and Lansbury 2000). This change causes the abnormal deposits of aggregated α-synuclein. There is no evidence for a stable structured α-synuclein monomer, i.e. the β-sheet conformation is not long-lived in the absence of oligomerization (Rochet and Lansbury 2000). At high concentrations, wild-type α-synuclein can self-aggregate in solution to form Lewy-body-like fibrils and discrete spherical assemblies, a process that is accelerated in mutated forms of α-synuclein (Conway et al. 1998). Several agricultural chemicals, such as rotenone and paraquat, when administered systemically, can induce specific features of PD (Betarbet et al. 2002), including losses of DAergic neuron in the SN (Brooks et al. 1999; McCormack et al. 2002). Moreover, these pesticides can induce a conformational change in α-synuclein and thus accelerate the rate of the formation of α-synuclein fibrils in vitro (Uversky et al. 2001).

Currently, the factors that induce the change from the soluble form to the aggregated form of α-synuclein found in Lewy bodies or Lewy neurites remain unknown. Within the inclusion bodies, α-synuclein is present in nitrated (Giasson et al. 2000; Ischiropoulos 2003) and hyperphosphorylated (Neumann et al. 2002) forms. Based on this, it has been speculated that phosphorylation of α-synuclein at its serine residue Ser129 may be crucially involved in synucleopathy (Fujiwara et al. 2002). Use of specific antibodies raised against phosphorylated α-synuclein has revealed a staining pattern in human brain (Saito et al. 2003) resembling the stages of Lewy body formation as proposed by Wakabayashi et al. (1998; see above).

Formation of inclusion bodies: α-synuclein and associated molecules

The first signs of the formation of Lewy bodies in the human SN are evident as punctate α-synuclein-stained perikaryal material that, via incorporation of ubiquitin, yields pale-body-like structures from which Lewy-body-type inclusions are formed (Kuusisto et al. 2003). Thus, a hallmark of Lewy body formation is the appearance of abnormal accumulations of α-synuclein. Ubiquitin-positive Lewy-body-like inclusions are formed when α-synuclein, synphilin-1 and parkin are co-expressed (Chung et al. 2001b). Synphilin-1 seems to represent a direct α-synuclein interaction partner. Thus, synphilin-1 associates with α-synuclein and promotes the formation of cytosolic inclusions (Engelender et al. 1999). Fluorescence resonance energy transfer has demonstrated that the C-terminus of α-synuclein is closely associated with the C-terminus of synphilin-1 (Kawamata et al. 2001). Moreover, an intermolecular interaction has been shown to exist between the N-terminus of α-synuclein and ubiquitin (Sharma et al. 2001). Recent data suggest that casein-kinase-II-mediated phosphorylation of synphilin-1 is required for the interaction of synphilin-1 and α-synuclein (G. Lee et al. 2004).

Normal wild-type α-synuclein seems not to interact with parkin directly (Shimura et al. 2001). Parkin seems to interact either directly with glycosylated α-synuclein (Shimura et al. 2001) or indirectly with α-synuclein, by interacting with (or ubiquitinating) the α-synuclein-interacting protein synphilin-1 (Chung et al. 2001b).

Parkin is involved in the ubiquitin-proteosome pathway in which proteins are targeted for degradation by covalent ligation of multiple ubiquitin molecules (Hershko and Ciechanover 1998). Misfolded proteins that have been tagged by ubiquitin chains are targeted to the proteosomes for destruction (Larsen et al. 1998). Failure of the ubiquitin-proteosome pathway in the degradation of abnormal proteins is thought to lead to nigral degeneration. Indeed, there is evidence that impaired protein clearance can induce cell death of DAergic ventral mesencephalic neurons (McNaught et al. 2002).

Since not all α-synuclein-positive inclusions contain ubiquitin (Goedert 2001), ubiquitination of α-synuclein is probably not a prerequisite for inclusion formation. Indeed, ubiquitination of α-synuclein is not required for inclusion formation (Sampathu et al. 2003) but α-synuclein can be ubiquitinated either directly or indirectly within the inclusions. Misfolded α-synuclein, which escapes ubiquitination, may lead to the death of DAergic neurons.

α-Synuclein and its interaction with β-synuclein and γ-synuclein

β-Synuclein and γ-synuclein have been localized within the CNS. Despite no mutation in the β-synuclein or γ-synuclein gene having been linked to PD, and despite the absence of β-synuclein and γ-synuclein in Lewy bodies or Lewy neurites (Jakes et al. 1994; Ji et al. 1997), both these synucleins may have a role in inclusion body formation or in the formation of α-synuclein-positive fibrils.

Although β-synuclein and γ-synuclein possess similar biophysical properties to α-synuclein, they inhibit α-synuclein fibril formation, whereby complete inhibition of α-synuclein fibrillation has been observed at a 4:1 molar excess of β-synucleins and γ-synucleins (Uversky et al. 2002). Moreover, transgenic mice that express human α-synuclein develop a Parkinsonian movement disorder concurrent with α-synuclein inclusions and the loss of DAergic fibers, whereas double-transgenic mice, expressing human α-synuclein and β-synuclein, show significant amelioration of the α-synuclein-induced phenotype (Windisch et al. 2002; J. Y. Park and Lansbury 2003).

Are α-synuclein accumulations predictive for DAergic cell death?

Although intracellular aggregates might be cytotoxic (Bence et al. 2001), evidence indicates that the presence of inclusion bodies may also be protective (Chung et al. 2001a). The presence of α-synuclein-positive aggregates in the cytoplasm of neurons seems not to be predictive for neurodegeneration. In PD, the prefibrilar α-synuclein intermediate might be more toxic than fibrilized α-synuclein aggregates (Goldberg and Lansbury 2000). Therefore, α-synuclein protofibrils, rather than the fibrils commonly found in Lewy bodies, might represent the deleterious species (Lotharius and Brundin 2002). Thus, in PD brains, the majority of SNpc neurons undergoing apoptotic-like cell death do not appear to contain somal Lewy bodies (Tompkins and Hill 1997). Perhaps they die before Lewy body formation has occurred (Tompkins and Hill 1997).

Moreover, DAergic neurons of the SN containing Lewy bodies appear to be "healthier" than neighbouring neurons with regard to morphological and biochemical criteria (Goldberg and Lansbury 2000). Further support for this view originates from a recent publication by Tanaka and coworkers (2004). In this report, the authors have investigated the role of aggresomes in cell viability by using 293T cells overexpressing α-synuclein and its interacting partner synphilin-1; inhibition of proteasome activity elicits the formation of aggresomes. Interestingly, these aggresomes are found in 60% of the non-apoptotic cells but only in 10% of the apoptotic cells, supporting a cytoprotective role for these inclusions (Tanaka et al. 2004).

Therefore, the inclusion bodies might have a protective role, the inclusions possibly sequestering toxic species and/or diverting α-synuclein from toxic assembly pathways (Goldberg and Lansbury 2000). Thus, the proteins that have not been sequestered in the inclusion bodies may lead to cell death (Ciechanover 2001). Indeed, a combination of 1-methyl-4-phenylpyridinium ion (MPP+) or rotenone (two chemical compounds used for modeling PD in rodents) and proteasome inhibition causes the appearance of α-synuclein-positive inclusion bodies. Unexpectedly, however, proteasome inhibition blocks MPP+-induced or rotenone-induced DAergic neuronal death (Sawada et al. 2003). This indicates that the proteasome mediates DAergic neuronal degeneration and that its inhibition causes α-synuclein inclusions. According to these results, α-synuclein inclusion body formation is not predictive for DAergic cell death.

Interactions of synuclein and the DAergic system

α-Synuclein is not toxic in non-DAergic human cortical neurons, but rather exhibits neuroprotective activity (Xu et al. 2002). However, accumulation of α-synuclein in cultured human DAergic neurons results in apoptosis. The DA-dependent neurotoxicity is thought to be mediated by protein complexes that contain α-synuclein and 14-3-3 protein, which are elevated selectively in the SN in PD. Thus, accumulation of soluble α-synuclein protein complexes can render endogenous DA toxic, suggesting a potential mechanism for the selectivity of neuronal loss in PD (Xu et al. 2002).

Co-localization of α-synuclein with TH, the rate-limiting enzyme in DA biosynthesis, in DAergic cells has been confirmed by immunoelectron microscopy and a dose-dependent inhibition of TH by α-synuclein has been observed in cell-free systems (Perez et al. 2002).

Nevertheless, overexpression of wild-type or A53T mutant α-synuclein in DAergic cells in culture does not significantly alter TH protein levels. However, synuclein overexpressing cell lines have significantly reduced TH activity and a corresponding reduction in DA synthesis. These data suggest that α-synuclein plays a role in the regulation of DA biosynthesis, acting to reduce the activity of TH (Perez et al. 2002). In addition, α-synuclein forms complexes with the presynaptic human DA transporter (hDAT), both in neurons and in co-transfected cells, through direct binding of α-synuclein to the carboxyl-terminal tail of hDAT. This complex formation facilitates the membrane clustering of DAT, thereby accelerating

cellular DA uptake and DA induced cellular apoptosis (F. J. Lee et al. 2001).

High levels of α-synuclein induce loss of DAergic neurons

Recently, a genomic triplication of the α-synuclein gene has been shown to be associated with familial PD, the disease being caused by overexpression of α-synuclein (Singleton et al. 2003, 2004; Bradbury 2003; Farrer et al. 2004). This indicates that high levels of α-synuclein may provoke the loss of DAergic neurons.

Overexpression of wild-type and mutant α-synuclein (A53T; A30P) in the human cell-line SH-SY5Y leads to an increase in reactive oxygen species (ROS). Furthermore, cells overexpressing different α-synucleins (normal and mutated forms) display reduced viability, compared with control cells. These findings indicate that a functional link to α-synuclein overexpression and apoptosis of neurons may exist, caused by a breakdown of the intracellular steady-state levels of ROS (Junn and Mouradian 2002). Further, primary cultures of embryonic human mesencephalon, overexpressing wild-type or mutant human α-synuclein (A53T), show a 27% or 49% loss of DAergic neurons, respectively (Zhou et al. 2002). Moreover, overexpression of α-synuclein in the nigrostriatal system of rats induces cellular and axonal atrophy, including α-synuclein-positive cytoplasmic inclusions, which are accompanied by a loss of 30%–80% of the nigral DAergic neurons and a 40%–50% reduction in striatal DA (Kirik et al. 2002). Thus, nigral DAergic neurons are vulnerable to high levels of α-synuclein, supporting a role for α-synuclein in the pathogenesis of PD. Unfortunately, these experiments do not allow conclusions to be drawn regarding whether the high levels of soluble α-synuclein or the α-synuclein-positive inclusion bodies cause the cell death in the SN.

Synuclein mouse models

Transgenic *Drosophila* flies (Feany and Bender 2000; Auluck and Bonini 2002; Pendleton et al. 2002), *Caenorhabditis elegans* (Lakso et al. 2003) and rats overexpressing human α-synuclein (Kirik et al. 2002; Klein et al. 2002; Lo et al. 2002) are available; however, genetically manipulated mice represent one of the most widely used animal models of synucleopathies. Two different strategies are available for examining α-synuclein function in genetically manipulated animals. One strategy takes advantage of the finding that mice with a target deletion of the α-synuclein gene are viable. The other strategy involves the generation of transgenic mice. These mice either overexpress "normal" α-synuclein or express one of the mutated α-synuclein forms or both A30P and A53T mutated α-synuclein. Recently, γ-synuclein null mutant mice have been generated; these are viable and fertile and do not display evident phenotypical abnormalities (Ninkina et al. 2003).

α-Synuclein knockout mice

Mice with a target deletion of the α-synuclein gene are viable and develop normally (Abeliovich et al. 2000); the concentrations of DA and DA metabolites are unchanged in these animals (Schluter et al. 2003). However, they display a reduction in striatal DA and an attenuation of DA-dependent locomotor response in response to amphetamine (Abeliovich et al. 2000). Nevertheless, unlike Parkinson's brains, the brains of these mice do not contain Lewy bodies, nor do they show signs of major neuronal loss (Goldberg and Lansbury 2000). MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a neurotoxin that inhibits mitochondrial complex I; it has been found to induce α-synuclein aggregation in the SN (Kowall et al. 2000; Kuhn et al. 2003). The α-synuclein knockout mice display resistance to MPTP-induced degeneration of DAergic neurons and DA release. This resistance is not attributable to abnormalities of the DA transporter, which appears to function normally in α-synuclein null mutant mice (Dauer et al. 2002).

Interestingly, a chromosomal deletion of the α-synuclein locus has been detected in a specific sub-strain of C57BL/6J mice. This strain has been designated C57BL/6S (Specht and Schoepfer 2001). These C57BL/6S animals appear phenotypically normal and the expression of β-synuclein or γ-synuclein and the expression of synphilin-1 seems to be unaffected (Specht and Schoepfer 2001). Analysis of these α-synuclein knockout mice provided the first data regarding the normal functions of α-synuclein in the brain. Thus, investigations into these α-synuclein knockout mice indicate that α-synuclein plays a role in synaptic vesicle recycling (Cabin et al. 2002).

Transgenic α-synuclein mice

The first transgenic mice to express human α-synuclein were generated in 2000 (Masliah et al. 2000). These mice displayed a progressive accumulation of α-synuclein and ubiquitin-immunoreactive inclusions in neocortical, hippocampal and nigral neurons. These alterations were associated with a loss of DAergic terminals in the basal ganglia and with motor impairments (Masliah et al. 2000). Since then, various transgenic α-synuclein mice have been generated, overexpressing wild-type α-synuclein and mutated forms of human α-synuclein. Among these transgenic α-synuclein mice are those with a mutation in A53T (van der Putten et al. 2000; Giasson et al. 2002; M. K. Lee et al. 2002) or A30P (Rathke-Hartlieb et al. 2001; M. K. Lee et al. 2002) or mutations at both positions (Richfield et al. 2002). Controversial results have been obtained by using transgenic α-synuclein (A53T) mice. Thus, expression of A53T mutant human α-synuclein

under the control of mouse Thy1 regulatory sequences in the nervous system of transgenic mice generates animals with neuronal α-synucleinopathy (van der Putten et al. 2000). Despite a lack of transgene expression in the DAergic neurons of the SNpc, the features displayed by these mice are strikingly similar to those observed in human brains with Lewy body pathology, since neuronal degeneration and motor defects have been found (van der Putten et al. 2000). However, even overexpression of wild-type α-synuclein under the control of the platelet-derived growth factor β promotor leads to selective nigrostriatal damages (Masliah et al. 2000).

In contrast to this, transgenic mice expressing mutant A53T human α-synuclein show neuronal dysfunction in the absence of aggregate formation (Gispert et al. 2003). In addition, mice that over-express both mutant α-synuclein variants (A30P and A53T) under the control of the promoter from the TH gene show no nigrostriatal degeneration (Matsuoka et al. 2001).

These controversial results concerning the formation of synuclein-positive inclusion bodies in transgenic mice expressing the mutant α-synuclein variant A53T might arise because normal mouse α-synuclein, like the mutated human A53T α-synuclein, contains a threonine residue at position 53 (Rochet et al. 2000). Thus, the antibodies used might not be able to distinguish between the normal mouse α-synuclein and the human mutated α-synuclein variant A53T. However, the transgenic mice expressing the human A53T probably do not develop all symptoms of PD but are more vulnerable to neurotoxins, e.g. MPTP. This has been recently shown by Song and coworkers (2004) who have compared MPTP-treated mice with MPTP-treated human α-synuclein transgenic mice; the transgenic mice exhibit extensive mitochondrial alterations, increased mitochondrial size, filamentous neuritic aggregations, axonal degeneration and the formation of electron-dense perinuclear cytoplasmic inclusions in the SN (Song et al. 2004).

Furthermore, a double transgenic mouse has been generated that expresses, in its neuronal tissues, human α-synuclein and human beta-amyloid. The functional and morphological alterations in these double transgenic mice resemble the Lewy body variant of AD. In addition, these mice exhibit deficits in learning and memory and prominent age-dependent degeneration of cholinergic neurons. These double mutant animals show higher numbers of α-synuclein-immunoreactive neuronal inclusions compared with single α-synuclein transgenic mice. These data indicate that beta-amyloid may also contribute to the development of Lewy body diseases by promoting the aggregation of α-synuclein (Masliah et al. 2001).

Final remarks

The discovery of mutations in the α-synuclein gene in early-onset familial PD and the identification of α-synuclein as a major component of Lewy bodies have led to substantial progress in the understanding of PD. Despite evidence for synuclein-interaction partners and a possible mechanism involved in the degradation of abnormal accumulated α-synuclein, the pathophysiological and physiological functions of α-synuclein remain largely unknown. The specific physiological roles of α-synuclein and of the other synucleins are largely enigmatic. An understanding of their normal function in the brain will certainly illuminate the processes that are related to their malfunctions. Since synucleins are found in synaptic terminals, they may contribute to signalling processes.

α-Synuclein is involved in PD but how can the misfolding of α-synuclein occur and what triggers this misfolding? In PD, α-synuclein at least aggregates in the soma of specific neuronal populations. Whether this in turn leads to disturbances in synapse formation, vesicle transport and/or release of transmitter or neuropeptides, thereby altering synaptic transmission, remains unknown.

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