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Review

Alpha-synuclein and Parkinson's disease

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Abstract. The involvement of α-synuclein in neurodegenerative diseases was first suspected after the isolation of an α-synuclein fragment (NAC) from amyloid plaques in Alzheimer's disease (AD). Later, two different α-synuclein mutations were shown to be associated with autosomal-dominant Parkinson's disease (PD), but only in a small number of families. However, the discovery that α-synuclein is a major component of Lewy bodies and Lewy neurites, the pathological hallmarks of PD, confirmed its role in PD pathogenesis. Pathological aggregation of the protein might be responsible for neurodegeneration. In addition, soluble oligomers of α-synuclein might be even more toxic than the insoluble fibrils found in Lewy bodies. Multiple factors have been shown to accelerate α-synuclein aggregation in vitro. Therapeutic strategies aimed to prevent this aggregation are therefore envisaged. Although little has been learned about its normal function, α-synuclein appears to interact with a variety of proteins and membrane phospholipids, and may therefore participate in a number of signaling pathways. In particular, it may play a role in regulating cell differentiation, synaptic plasticity, cell survival, and dopaminergic neurotransmission. Thus, pathological mechanisms based on disrupted normal function are also possible.

Key words. Parkinson's disease; α-synuclein; genetics; neurodegeneration; aggregation; inclusions; toxicity.

Introduction

Parkinson's disease (PD) is one of the most frequent neurodegenerative disorders, with a prevalence close to 2% after age 65 [1]. It is characterized by resting tremor, rigidity, and bradykinesia, which respond well to levodopa treatment. The pathological hallmarks are the presence of Lewy bodies (LBs) and massive loss of dopaminergic neurons in the pars compacta of the substantia nigra [2]. The cause of this selective neurodegeneration is still unknown. Genetic factors, although initially underestimated, have become an important issue in PD research. The involvement of susceptibility genes is supported by epidemiological studies that showed a higher frequency of PD in relatives of PD patients than in those of controls [for reviews see refs 3, 4] and by elevated concordance in monozygotic twins [5]. In addition, the observation that the disease is transmitted in some families as an autosomal dominant [6] or an autosomal recessive trait [7] suggests that mutations in single genes can cause some forms of PD in a monogenic manner.

Abbreviations: aa = amino acid(s); AD = Alzheimer's disease; ALS = amyotrophic lateral sclerosis; CK-1 = casein kinase 1; CK-2 = casein kinase 2; CNS = central nervous system; DLB = dementia with Lewy bodies; ERK = extracellular regulated kinase; GCIs = glial cytoplasmic inclusions; LB = Lewy body; LN = Lewy neurite; MSA = multiple system atrophy; NAC = non amyloid component of AD amyloid plaques; NCIs = neuronal cytoplasmic inclusions; PA = phosphatidic acid; PB = pale body; PD = Parkinson's disease; PKA = protein kinase A; PKC = protein kinase C; PLD2 = phospholipase D2; WT = wild type

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α-Synuclein was the first 'PD gene' to be discovered. It is also involved in the pathogenesis of Alzheimer's disease (AD) and multiple system atrophy (MSA). Although a coherent view of its role in normal cell function and in neurodegeneration has not yet emerged, important progress has been made, especially through the identification of a number of partners that interact with α-synuclein. In addition, we are beginning to understand how disruption of normal function and/or protein aggregation may result in neuronal cell death.

The synuclein family

Three members of the human synuclein family have been identified: α-, β-, and γ-synuclein (fig. 1). The genes are located on chromosome 4q21 [8–11], 5q35 [9], and 10q23 [12], respectively.

α-Synuclein (NACP, synelfin)

The name synuclein was first given to a 143-amino acid (aa) neuron-specific protein that was isolated by expression screening from the electric organ of the fish *Torpedo californica*. The name 'synuclein' was chosen because the protein was found in both *syn*apses and in the *nucl*ear envelope [13]. Independently, NACP, the 140-aa *n*on-*A* β *c*omponent of AD amyloid *p*recursor [14], and synelfin [15] were cloned in human and in the zebra finch, respectively. They were found to be orthologs of torpedo fish synuclein and rat brain synuclein [10, 16, 17]. When Jakes et al. [17] cloned β-synuclein as a second member of the human synuclein family (see below), they termed the initially isolated family members 'α-synuclein.' All α-synucleins were shown to be extremely well conserved among distantly related species [15–17]. α-Synuclein was not found in the nucleus in several subsequent studies. It appears therefore to be a purely presynaptic protein [17, 18].

In addition to the 140-aa α-synuclein that is expressed predominantly in the brain, there is a 112-aa splice variant (fig. 1) that is expressed predominantly in heart, skeletal muscle, and pancreas [19].

β-Synuclein (PNP14)

Jakes et al. [14] isolated a second member of the human synuclein family, β-synuclein (fig. 1). Like α-synuclein, this protein is expressed predominantly in the brain,

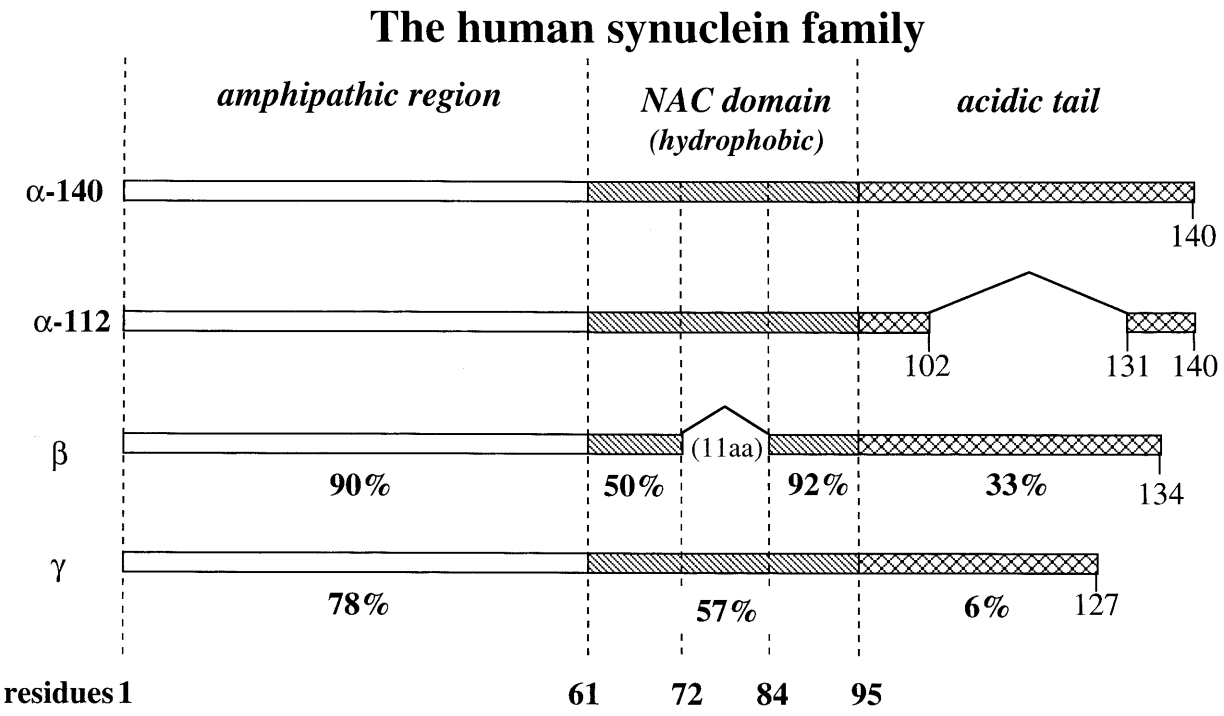

Figure 1. The human synuclein family. The different synucleins are represented as a bar. Amino acid positions are indicated at the bottom. The N-terminal amphipathic region, the hydrophobic NAC region, and the acidic tail are separated by vertical dashed lines and are differently hatched. The α-112 splice variant of α-synuclein (lacking 28 amino acids within the acidic tail) as well as β- and γ-synuclein are shown. The NAC region of β-synuclein lacks 11 central amino acids (residues 73–83). The degree of amino acid identity compared to α-synuclein, according to cross-species consensus sequences [141], is given as a percentage below each domain.

this protein is expressed predominantly in the brain, where it is concentrated in presynaptic nerve terminals. It consists of 134 aa and is the human ortholog of brain-specific bovine phosphoneuroprotein 14 (PNP14) that is also localized in central nervous system (CNS) synapses [20, 21] and seems to be regulated by phosphorylation [22].

γ-Synuclein (BCSG1, persyn)

The third member of the human synuclein family, the 127-aa γ-synuclein (fig. 1), was cloned by homology and is expressed in adult brain, including the substantia nigra, but also in ovarian tumors [12]. Interestingly, γ-synuclein also seems to play a role in developing peripheral nervous tissue, as two orthologs, persyn and a synuclein-like cDNA, were isolated by subtractive cDNA cloning from mouse embryonic trigeminal ganglia [23] and from rat neonatal dorsal root ganglia [24], respectively. Persyn is expressed only in the brain of adult animals but in motor and sensory neurons from embryos and adults. In contrast to the other synucleins, persyn was localized in the cytosol of the cell body and cell processes [23, 25] and was shown to influence the integrity of the neurofilament network [25].

The sequence of γ-synuclein is almost identical to that of BCSG 1 (breast-cancer-specific gene 1) which was found to be overexpressed in infiltrating breast carcinoma [26]. Its overexpression was associated with increased invasiveness of the tumor in vitro and a profound increase in metastases in vivo [27].

Hypotheses for the normal function of α-synuclein

Several hypotheses for the normal function of α-synuclein have been proposed, based on its structure, physical properties, and interacting partners. Understanding the role of α-synuclein in normal cell life might be of critical importance since disruption of its normal function might result in neurodegeneration.

Structure and physical properties of α-synuclein

In aqueous solution, α-synuclein is natively unfolded with an extended structure composed of random coil without a hydrophobic core [28, 29]. This structure is similar to that found in tubulin/actin-binding proteins (e.g., tau), Ca^{2+}/calmodulin-binding proteins, or phosphatase/kinase inhibitors [28] and may be critical for protein-protein interactions.

Histologically, α-synuclein co-localizes with synaptic vesicles [13, 21, 30]. In vivo, it binds to rat brain vesicles via the first four 11-mer N-terminal repeats [31] (fig. 2). In vitro, it binds to monolayer phospholipid membranes, acquiring an α-helical secondary structure [32], probably formed by the seven N-terminal 11-residue repeats containing the conserved core sequence Lys-Thr-Lys-Glu-Gly-Val [15, 32] (fig. 2). Interestingly, α-synuclein binds exclusively to acidic phospholipids, especially phosphatidic acid, and to vesicles with small diameters (fig. 3). This may target the protein to specific subpopulations of membranes or vesicles [32]. Neither of the two described mutations causing PD, changing alanine to proline at residue 30 (A30P) and alanine to threonine at residue 53 (A53T, see below), nor experimentally introduced deletions of the residues 9–41, 43–56 and 58–102 abolish binding to acidic phospholipid vesicles, indicating that lipid binding is a broadly distributed property of α-synuclein [33, 34] (fig. 2). However, not all cellular α-synuclein seems to be linked to membranes, since it can also be purified from the cytosol [15, 16, 35].

In vitro, α-synuclein is constitutively phosphorylated on serine residue 129 (and to a lesser extent on serine residue 87) by casein kinases 1 and 2 (CK-1 and CK-2) but not by protein kinase A (PKA) or C (PKC) [36]. Phosphorylation and dephosphorylation of α-synuclein seem to be tightly regulated in vivo [36] and might influence its binding to lipid membranes or to phospholipase D2 (PLD2) [37] (fig. 3).

Interacting proteins

Another step in elucidating the function of a protein is the identification of its interacting partners. They may, for example, point to signaling pathways or regulatory roles. In addition, the interacting proteins may themselves be candidate genes for PD, since mutations that might influence their interaction with α-synuclein may also cause neurodegeneration.

Physical and functional homology of α-synuclein with 14-3-3 chaperone proteins. The α-, β- and γ-synucleins show homology to a region of the 14-3-3 chaperone proteins and may be members of this superfamily [38]. Like these proteins, α-synuclein binds to extracellular regulated kinase (ERK), dephospho-BAD (a Bcl-2 homolog) and PKC in decreasing order of affinity, but also to the 14-3-3 proteins themselves (fig. 3). The affinity of membrane-bound α-synuclein for different PKC isoforms also differs ($\alpha = \gamma > \varepsilon > \beta = \delta$). Forced expression of wild-type (WT) or A53T α-synuclein in human embryonic kidney (HEK) cells inhibited PKC activity. Although α-synuclein and 14-3-3 both bind to BAD, the interactions are independent, the former binding dephospho-BAD, the latter, phospho-BAD [38]. Since ERK, BAD, and PKC are involved in the regulation of cell viability, α-synuclein may play a role in the same signaling pathways (fig. 3).

Interaction with synphilin-1. Synphilin-1, which binds to the first 39 residues of α-synuclein (fig. 2), was

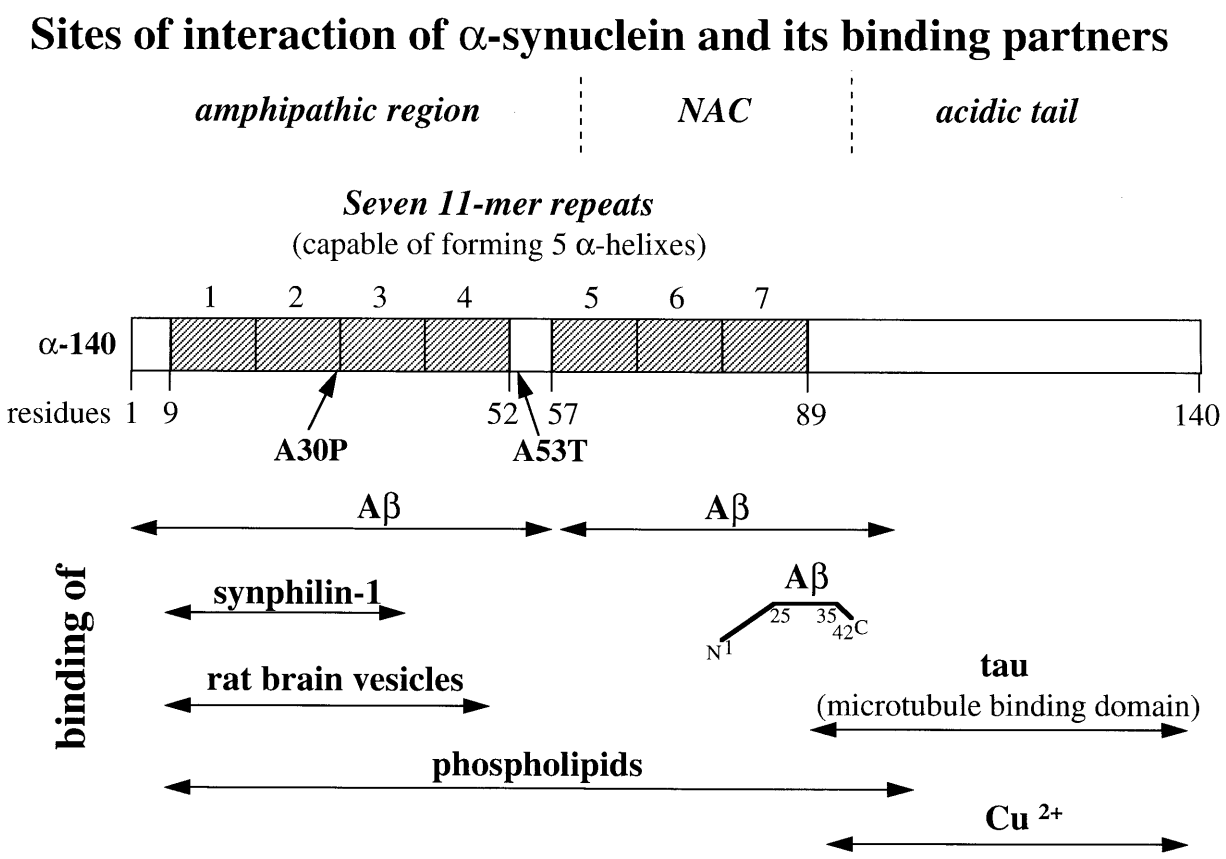

Figure 2. Sites of interaction between α-synuclein and its binding partners. α-Synuclein is represented as a bar and the three major domains are depicted as in fig. 1. The seven 11-mer repeats that are able to form five α-helixes are numbered and hatched. The two PD-linked mutations (A30P and A53T) are indicated. The sites of interaction between α-synuclein and the respective partners are delimited by double-headed arrows. In one study, only the amino acids 25–35 of the Aβ-peptide interacted with the C-terminal part of the NAC domain [45]. This is represented by a broken line, the 'N' and 'C' indicating the N and C terminus of the Aβ-peptide, respectively.

identified in a two-hybrid screen [39] (fig. 3). It is a novel protein with little similarity to other known proteins. It contains several protein-protein interaction domains, such as ankyrin-like repeats and a coiled-coil domain. Thus, synphilin-1 might act as an adaptor protein anchoring α-synuclein to other proteins (fig. 3), such as those involved in vesicle transport or cytoskeletal function. It also contains a predicted ATP/GTP-binding domain. Cytosolic eosinophilic inclusions that resemble LBs and contain NAC and synphilin-1 immunoreactivity formed in cells co-transfected with synphilin-1 and NAC, suggesting that synphilin-1 might modulate this aggregation. In addition, synphilin-1 is present in LBs [39, 40].

Interaction with tau. The hyperphosphorylated microtubule-associated protein tau is the major constituent of insoluble paired helical filaments found in neurofibrillary tangles and plaque neurites in AD [41, 42]. Tau from human brain cytosol binds to α-synuclein in affinity assays [43]. In addition, tau is co-expressed with α-synuclein in axons of embryonic rat hippocampal cell cultures. In a direct binding assay, the microtubule-binding domain of tau bound to the C terminus (residues 87–140) of WT, A30P, and A53T α-synuclein [43] to the same extent (fig. 2). Consequently, only tau that is not bound to microtubules can interact with α-synuclein. Since α-synuclein binds Aβ and brain vesicles via the conserved N-terminal repeat region [31, 44, 45] and binds tau via its C terminus (fig. 2), it may form bridges between these ligands. In addition, α-synuclein stimulates PKA that phosphorylates the Ser262 and Ser356 residues of tau [43] (fig. 3). Since the phosphorylation of Ser262 inhibits tau binding to microtubules [46], α-synuclein may modulate tau function (fig. 3).

Putative physiological roles of α-synuclein

In addition to the described interactions with different proteins, converging evidence from complementary ex-

perimental approaches forms the basis for the following hypotheses for the physiological role of α-synuclein.

Regulation of synaptic plasticity and neuronal differentiation. The involvement of α-synuclein in synaptic plasticity is suggested by the findings that (i) α-synuclein is a presynaptic protein that interacts with brain vesicles and phospholipid membranes [17, 18, 31, 32] (figs 2, 3), (ii) 'synelfin,' the bird ortholog of α-synuclein, was upregulated in vivo during the critical period of song learning, a phase of maximal neuronal plasticity in the zebra finch [15], (iii) α-synuclein mRNA was highly expressed in early postnatal rat brain. During this developmental period, target contact, sprouting, and synapse formation take place and the amount of α-synuclein per synapse was higher than in adulthood [47, 48]. α-synuclein may also be involved in synaptic development and maintenance, since, in rat embryonic hippocampal cells in culture, α-synuclein was detected in axons and developing presynaptic terminals after their formation [30]. Finally, α-synuclein seems to contribute to neuronal differentiation, because (i) treatment of rat pheochromocytoma cells (PC12) with nerve growth factor, which induces a neuronal phenotype, caused a sustained increase in α-synuclein levels [49], (ii) in vivo, α-synuclein is localized in the cell body of neuronal precursors during early embryonic development in mice [50] and humans [51], but in presynaptic terminals in postnatal and adult cortex [50, 51].

The involvement of α-synuclein in synaptic plasticity and neuronal differentiation may be mediated by the selective inhibition of PLD2 by α-synuclein [37] (fig. 3), since isoforms of phospholipase D were shown to be implicated in cell growth and differentiation [52]. In addition, phosphatidic acid (PA), a metabolite of PLD2, to which α-synuclein can bind, may mediate changes in cell morphology [37] (fig. 3).

Interestingly, the involvement of α-synuclein in neuronal and synaptic development could not be confirmed in mice lacking the α-synuclein gene homozygously, since these mice were behaviorally normal and showed neither macroscopic nor microscopic changes in their nervous system [53].

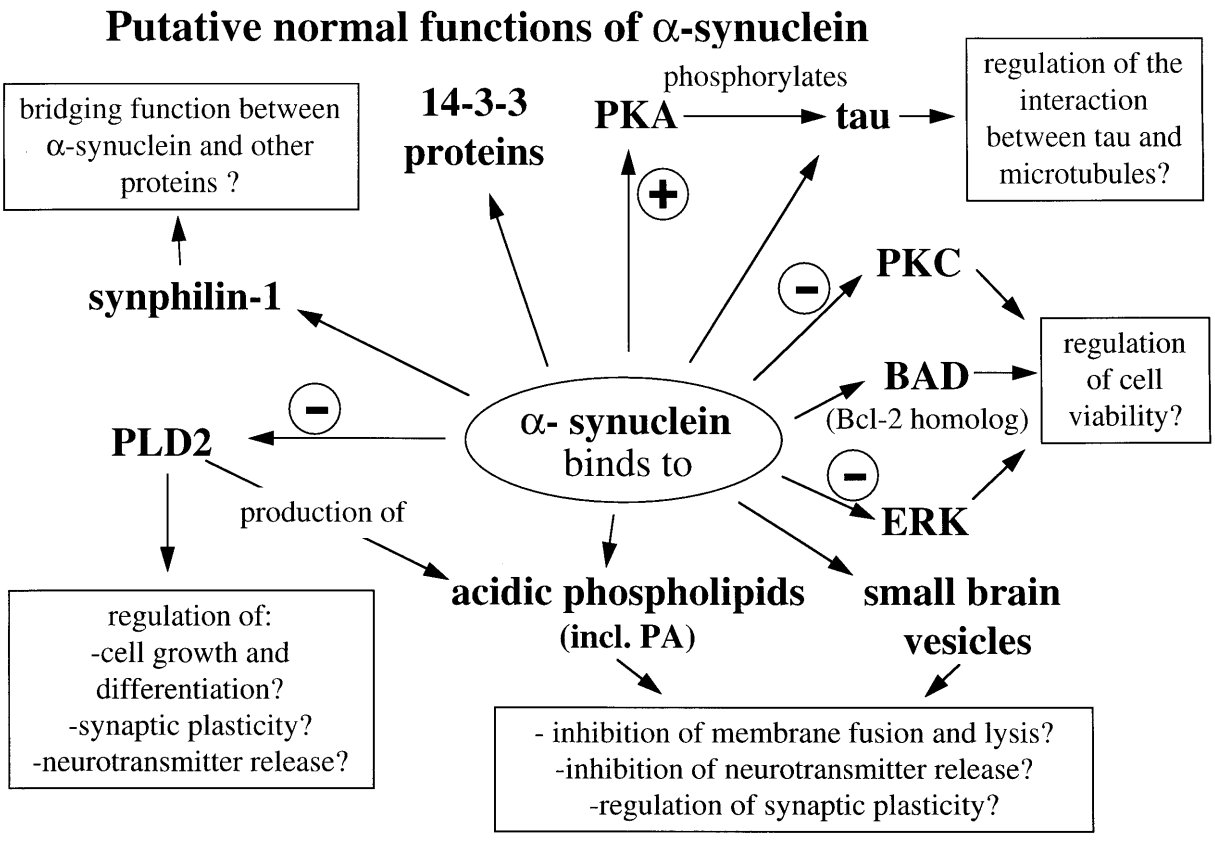

Figure 3. Putative normal functions of α-synuclein. The binding partners of α-synuclein are indicated by arrows. '−' and '+' indicate enzyme inhibition or activation by α-synuclein respectively. The boxes describe potential functions of α-synuclein interacting with the respective partner. PA, phosphatidic acid; PLD2, phospholipase D2; PKC, protein kinase C; PKA, protein kinase A; ERK, extracellular-regulated kinase.

Regulation of dopamine release. There is electrophysiological evidence that α-synuclein may regulate dopamine neurotransmission. Paired stimulus depression of dopamine release, thought to be involved in working memory, learning, and reward, is shortened in α-synuclein knock-out mice [53]. The mechanism is independent of the dopamine D2 receptor, suggesting that α-synuclein might negatively regulate dopamine release by modulating the releasable dopamine pool through its interactions with vesicle membranes (fig. 3). Two metabolites of PLD2 (PA and diacylglycerol) are both known to increase neurotransmitter release [52, 53]. Thus, α-synuclein might exert, by inhibition of PLD2, negative feedback regulation of PA synthesis and neurotransmitter release (fig. 3) [37]. A second explanation of the downregulation of neurotransmitter release by α-synuclein could be that class A α helices, such as those formed by α-synuclein in association with membranes, are reported to stabilize membranes and to inhibit membrane fusion and lysis [32] (fig. 3).

Regulation of cell viability. Whether α-synuclein induces apoptosis or protects cells against apoptosis is a matter of debate. The findings that (i) serum deprivation increased α-synuclein expression in HEK 293 cells [38] and that (ii) α-synuclein expression was increased in dopaminergic neurons of the substantia nigra pars compacta (SNpc) following chronic 1-methyl-4-phenyl-1, 2, 3, 6-tetrohydropyridin intoxication in mice, a treatment that causes apoptotic dopaminergic cell death [54], are suggestive of a pro-apoptotic role for α-synuclein, although its expression in cells with apoptotic morphology has not been demonstrated. Thus, the observed upregulation of α-synuclein could be a compensatory protective mechanism. Consistent with this hypothesis are (i) upregulation of α-synuclein in healthy but not in apoptotic neurons in the rat SNpc after induction of apoptosis by striatal injury [48], (ii) in human AD frontal cortex, an increase in the amount of α-synuclein per synapse in early stages of the disease [35, 55], but decrease in moderate and severe AD [56]. No alterations in α-synuclein expression were observed in cultures of rat pheochromocytoma cells (PC12) deprived of serum, which induces apoptosis [49], or in rat brain lesioned with 6-hydroxydopamine [57]. The regulation of cell viability might be mediated by the interaction of α-synuclein with proteins such as PKC, ERK, or BAD (fig. 3).

α-Synuclein and neurodegeneration

Genetics

Linkage of the PARK1 locus on chromosome 4q21, where the α-synuclein gene is located, to autosomal dominant PD in a large Greek family [58] suggested that α-synuclein might be implicated in the pathogenesis of PD. Screening of the PARK1-linked family for mutations in the α-synuclein gene revealed a missense mutation that changed a G to an A at position 209 (G209A), resulting in an Ala53Thr (A53T) exchange [59]. This mutation co-segregated with the disease in three additional, apparently unrelated, PARK1-linked families, and was absent in 314 control chromosomes. α-Synuclein was therefore considered to be the first pathogenic 'PD gene.' However, the identified mutation corresponds to the WT sequence in the rat, raising questions about its pathogenicity [60]. The discovery of another missense mutation in the α-synuclein gene [nucleotide change G88C, resulting in an Ala30Pro (A30P) exchange] associated with autosomal dominant PD in a German family [61] reinforced the hypothesis that these mutations are involved in the pathogenesis of PD.

Subsequently, however, searches for these two mutations in the α-synuclein gene in many familial (dominant and recessive), sporadic, and early-onset PD patients have proven unsuccessful [62–69]. Other mutations in the α-synuclein gene have also been sought: the entire coding region of α-synuclein (using as templates either cDNA or genomic DNA including exon/intron boundaries) was sequenced in 61 autosomal dominant PD families [64, 70, 71], in 21 PD patients with at least one affected first-degree relative [72, 73], in 7 isolated PD cases [73], and in 24 pathologically proven sporadic PD cases [74]. Very recently, 28 autosomal dominant PD families and 158 cases with at least one affected relative were screened by single-strand conformational polymorphism (SSCP) [75]. Furthermore, β- and γ-synuclein were analyzed in both familial and isolated PD cases [76–79]. Despite these efforts, only a dozen autosomal dominant PD families with α-synuclein mutations have been identified worldwide [59, 61, 75, 80, 81]. All but one of these families carried the A53T mutation, and according to haplotype analysis, probably descended from a common founder in Greece [82]. Reduced penetrance of the A53T mutation was described in some of the families [80, 81]. The remaining family carried the A30T mutation. No other mutations have been detected. Mutations in the α-synuclein gene account therefore for only a very few cases of autosomal dominant PD.

Members of the synuclein family were also screened for mutations that may be involved in other neurodegenerative diseases. To date, no mutations have been detected in the α-synuclein gene in 26 index patients with familial early-onset AD [10] or in 7 histologically confirmed MSA cases [83]. In amyotrophic lateral sclerosis (ALS) patients, only the γ-synuclein gene was examined and no mutations were found [79].

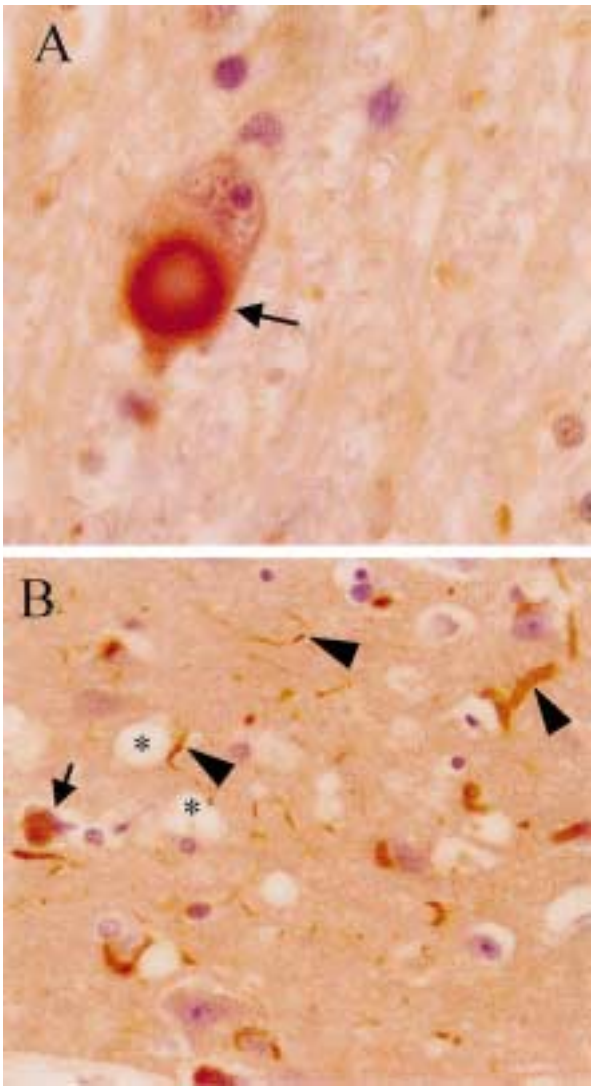

Figure 4. α-Synuclein immunoreactivity in Lewy bodies and Lewy neurites. A polyclonal anti-α-synuclein antibody against residues 111–131 (Chemicon) was used with diaminobenzidine as the chromogen (brown). (*A*) Lewy body with intensively stained halo (arrow) in a dopaminergic neuron in the substantia nigra of a PD patient. (*B*) Cortical Lewy body (arrow) and Lewy neurites (arrowheads) in a patient with Lewy body dementia (DLB). Note spongiosis (asterisk) typical of DLB.

Neuropathology

α-Synuclein in PD. α-Synuclein has been localized with antibodies against the N-terminal, C-terminal, and the central domain of the protein in brainstem and cortical LBs and Lewy neurites (LNs), now considered to be the two pathological hallmarks of both PD and dementia with Lewy bodies (DLB) [84–87] (fig. 4). Western blots showed that they contained both partially truncated α-synuclein and insoluble aggregates of both full-length and truncated protein [88, 89]. LBs and LNs did not contain β- or γ-synuclein immunoreactivity [88]. Some extracellular α-synuclein-positive LBs were also observed [90]. In addition to LBs and LNs, α-synuclein-positive pale bodies (PBs), thought to be precursors of LBs [91], and perikaryal threads [86] have been described. The latter consist of α-synuclein-positive LB filaments and might represent the initial stage of PB and LB formation.

On ultrastructural examination, a major component of LBs were straight or twisted α-synuclein-positive LB filaments, 50–700 nm long and about 5–10 nm wide [86, 90]. α-Synuclein was hypothesized to assemble into 5-nm protofilaments, two of which might interact to form a LB filament [90].

Van Duinen et al. [92] described an asymptomatic case with cortical and subcortical PBs and LBs, the number of which largely exceeded the diagnostic criteria for DLB. Surprisingly, the inclusions were α-synuclein negative and there was no neuronal loss, consistent with the absence of clinical symptoms. This suggests that the inclusions are associated with neural degeneration only in the presence of α-synuclein.

In addition to intra-neuronal accumulation of α-synuclein, pathologic α-synuclein-positive argyrophilic inclusions have been described in both astrocytes and oligodendrocytes, suggesting that the pathological process in PD involves more than neuronal cells [93, 94].

α-Synuclein and NAC in AD. NAC (fig. 1) was first isolated from a preparation of AD amyloid plaques [14]. It was also found immunohistochemically in association with amyloid plaques in some studies [55, 95], but not all [89, 96]. The proteolytic process resulting in cleavage of NAC from α-synuclein is not yet known. Interestingly, full-length α-synuclein was not found in AD amyloid plaques [14, 35, 96], whereas it accumulates in dystrophic neurites, in hippocampal neuritic tangles, and in presynaptic terminals in AD brains [55, 97, 98].

α-Synuclein in MSA. In brains of MSA patients, argyrophilic α-synuclein- and ubiquitin-positive cytoplasmic inclusions are found in numerous oligodendrocytes (GCIs, glial cytoplasmic inclusions) and scattered neurons (NCIs, neuronal cytoplasmic inclusions) [97, 99–102]. α-Synuclein-positive neuronal intranuclear inclusions have also been described [103]. Biochemical analysis showed that GCIs contain αβ-crystallin and tubulins in addition to high-molecular-weight aggregates of possibly truncated α-synuclein [104]. Ultrastructurally, both GCIs and NCIs contain 15 to 30-nm-wide fibrils that are irregular on their outer surface [102]. Some are tubular in appearance, perhaps due to incorporation of other proteins or entrapment of microtubules in bundles of α-synuclein filaments.

GCIs are found with similar distributions in striatonigral degeneration and olivopontocerebellar atrophy [105], two subtypes of MSA with different patterns of neuronal loss. It is therefore not clear what role GCIs play in the pathogenesis of these diseases.

α-Synuclein in ALS and other neurodegenerative disorders. α-Synuclein deposits have also been described in astrocytes of the spinal cord and in Schwann cells of spinal nerves in ALS [97]. Neurofibrillary tangles and glial inclusions in progressive supranuclear palsy and in corticobasal degeneration, and Pick bodies in Pick's disease were labeled with an antibody against the C terminus, but not with an antibody against the N terminus of α-synuclein [106]. In addition, α-synuclein-positive LBs and LNs were detected in a case of Hallervorden-Spatz disease [107].

Putative pathological mechanisms

The clinical and pathological picture is similar in PD cases that are caused by α-synuclein mutations and those without mutations. Since PD comprises a group of disorders with probably multifactorial (both genetic and non-genetic) etiology, α-synuclein as an essential component of the LB might contribute to a final common pathogenic pathway of the disorders. Thus, elucidation of the functional consequences of the causal mutations may also help in understanding the pathogenesis of sporadic PD. A wide range of in vitro and in vivo studies has attempted to shed light on this process.

α-Synuclein aggregation. Because of its 'natively unfolded' structure, α-synuclein might be especially prone to self-aggregate or to cause the aggregation of other proteins or intracellular structures (fig. 5). In vitro, both full-length WT and mutant α-synuclein (A30P and A53T) were capable of time-, temperature-, pH- and concentration-dependent self-aggregation into fibrils, when present in supersaturating conditions [108, 109]. These fibrils were similar to those isolated from LBs of patients with PD and DLB, or from the filamentous inclusions characteristic for MSA [110]. Fibrillization was accompanied by a change in the secondary structure of α-synuclein from an unfolded random coil to an antiparallel β sheet [111–113]. In a similar time-, con-

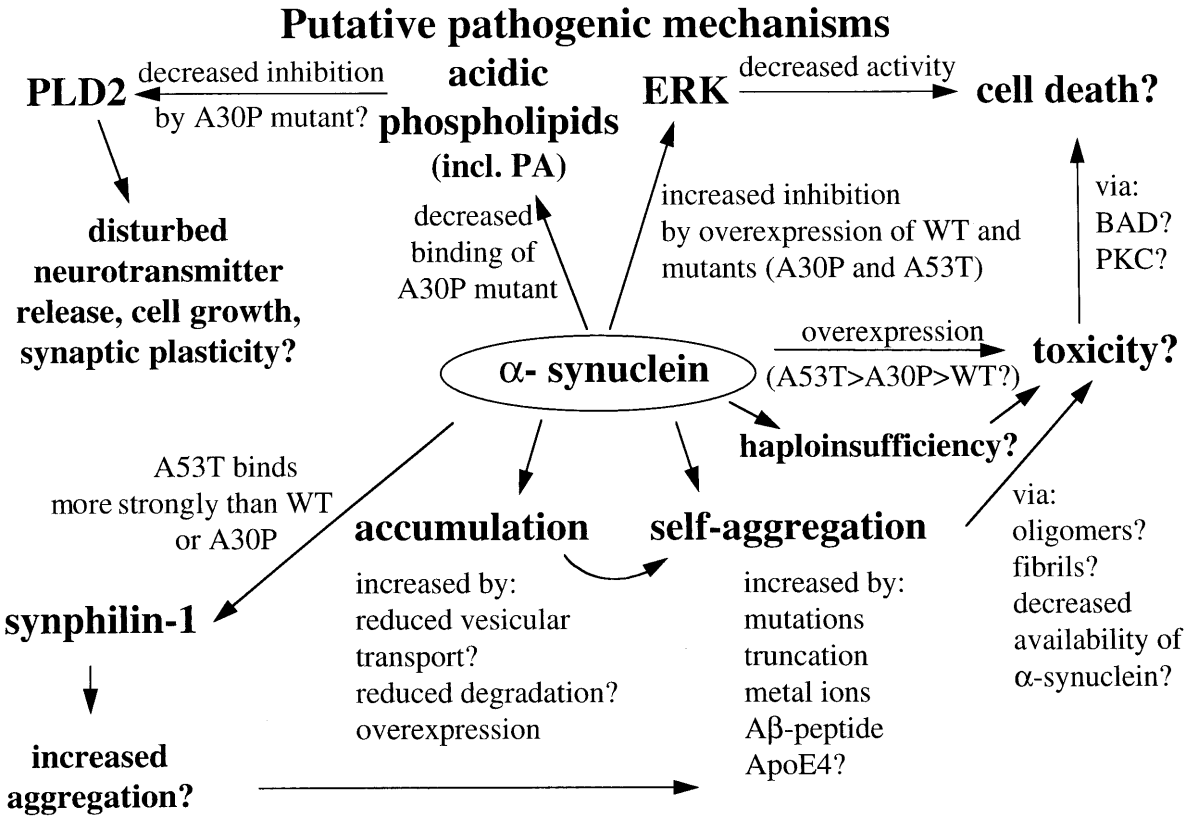

Figure 5. Putative pathological mechanisms. The binding partners and hypothetical steps of the different pathogenic mechanisms are indicated by arrows. PA, phosphatidic acid; PLD2, phospholipase D2; ERK, extracellular regulated kinase; WT, wild type; A30P and A53T, described α-synuclein mutations; >, possibly more toxic than.

centration-, and temperature-dependent manner, the α-synuclein fragment NAC self-aggregated into amyloid-like filaments with a thioflavin-positive β-sheet structure [95, 114], residues 1–18 of the NAC peptide being sufficient for this aggregation [115]. Solutions containing aggregated α-synuclein, NAC(1–35) and NAC(1–18) were toxic to SH-SY5Y cells in vitro [115]. Fibril formation from WT and mutated (A53T and A30P) α-synuclein involves nucleation-dependent elongation [113, 116] in which the protein aggregates into seeds and then accelerates fibril formation in a dose-dependent manner. Heterologous seeding of WT α-synuclein by A53T-mutated seeds also occurred, a possible explanation for the dominant effect of this mutation.

Factors accelerating α-synuclein aggregation (fig. 5).

1. *Mutations*. The A30P and A53T mutations did not change the secondary structure of the protein, its interaction with itself [110], or the intracellular distribution of α-synuclein in cell culture [34]. In addition, the WT and mutated forms could be stabilized to a similar degree by the anionic detergent sodium dodecyl sulphate (SDS) [110]. In one study, the time lag before aggregation (not fibrillization) was shortest for the A53T mutant, intermediate for the A30P mutant, and longest for WT α-synuclein [111]. In another study, however, no difference was observed between the aggregation of WT and A30P α-synuclein [109]. Very recently, α-synuclein with the A30P mutation was shown to form fibrils more slowly than WT and A53T α-synuclein [113]. But the A30P mutation facilitated the formation of non-fibrillar α-synuclein oligomers: spheres, chains of spheres (protofibrils), and rings resembling circularized protofibrils [110, 113]. Given the pathogeneity of the A30P mutation, these oligomers might be as or even more toxic than the α-synuclein fibril. Drugs that prevent fibrillization but not oligomerization of α-synuclein might, therefore, like the A30P mutation, accelerate disease progression [113]. The ultrastructure differed between filaments originating from WT α-synuclein (mainly 6 to 8-nm-wide unbranched filaments) compared to the A30P and A53T filaments (mainly mature filaments with diameters of 10–12 nm) [117]. The latter form β sheets more easily, possibly explaining the earlier onset of the disease in patients carrying these mutations [117].
2. *Truncation*. Truncated α-synuclein, consisting only of residues 1–120, was more prone to form filaments in vitro than full-length α-synuclein [118]. These filaments were very similar to those found in vivo. It would therefore be possible that as yet unidentified proteases may create a pool of these shorter products initiating α-synuclein aggregation. This hypothesis is supported by observations that a large proportion of the α-synuclein extracted from partially purified LBs appeared truncated on Western blots [88] and that full-length WT or A53T α-synuclein, in contrast to NAC, did not induce inclusion formation when co-transfected with synphilin-1 [39].
3. *Concentration*. The A30P mutation reduced transport of α-synuclein from the cell body to axon terminals by reducing its capacity to bind to vesicles [31]. This might increase the concentration of α-synuclein in the cell body up to a critical level leading to aggregation and eventually to the formation of LBs and LNs. For PD not linked to α-synuclein mutations, unknown modifications of α-synuclein or its putative 'receptor' molecules in the axonal transport apparatus may have similar effects [31]. Alternatively, transient physiological supersaturating concentrations of α-synuclein in some neuronal cell compartments may lead to pathological aggregation in the presence of factors that accelerate seeding and, thus, aggregate formation [116].
   Increased expression or decreased degradation of α-synuclein would also increase its concentration. The co-existence of α-synuclein with ubiquitin in the LBs [90] suggests that alterations of α-synuclein catabolism by the proteasome might contribute to disease pathogenesis. Whereas WT and mutant (A30P and A53T) α-synuclein were reported to be ubiquitinated to the same extent in rabbit reticulocyte lysates and in COS-7 cells [119], no ubiquitination of α-synuclein was detected in TSM1 neuronal cells [120]. Decreased proteasome-dependent degradation of the A53T α-synuclein mutant was described in SH-SY5Y cells [121], but was not confirmed in HEK 293 and TSM1 cells [120]. Furthermore, ubiquitination does not seem to be a prerequisite for LB formation, since some LBs are α-synuclein positive but ubiquitin negative [90].
4. *Interaction with metal ions*. Ferric iron that was found to be increased in the substantia nigra of PD patients [122–127] caused aggregation of α-synuclein in a dose-dependent manner in vitro. This aggregation resulted in fibrils similar to those found in LBs and could be partially blocked by desferoxamine, a high-affinity iron chelator [128]. Under the same conditions, the 112-residue splice variant of α-synuclein also aggregated whereas β-synuclein did not, indicating that the aggregation caused by ferric iron might take place in the NAC region, which is deleted in β-synuclein (fig. 1) [128]. Ferric iron may be produced by oxidative stress, e.g., in vitro in the presence of cytochrome c and H_2O_2 [129].
   Cu^{2+}, at subneutral pH (6.5), is a potent inducer of α-synuclein oligomerization [130]. Oligomerization is mediated by the C-terminal acidic region of the protein (fig. 2), since it did not occur in C-terminally

truncated proteins. Aluminum had a double effect on α-synuclein. It induced its oligomerization [130] as well as formation of an α-helix resistant to proteases such as trypsin, α-chymotrypsin, and calpain [131], possibly increasing the intracellular concentration of α-synuclein.

5. *Interaction with Aβ*. The Aβ25–35 fragment triggers α-synuclein self-oligomerization in vitro [132]. Furthermore, recombinant α-synuclein was shown to bind to amyloid plaques in AD brains [44]. The C-terminal part of the NAC region binds to residues 25–35 of Aβ-peptide (fig. 2) [45]. However, the presence of Aβ25–35 has not yet been described in vivo, thus the relevance of this fragment for the pathological process still needs to be established. Additional binding domains for Aβ are α-synuclein residues 1–56 and 57–97 [44] (fig. 2). In addition, transglutaminase catalyses the formation of covalent NAC polymers in vitro as well as polymers between NAC and Aβ-peptide, suggesting that this enzyme may be involved in amyloid formation [133].
6. *Interaction with apolipoprotein E*. The NAC fragment interacts specifically with apolipoproteins E3 and E4 forming SDS-resistant complexes. Apolipoproteins E3 and E4 could thus facilitate the aggregation of Aβ and NAC in the AD plaques [134]. Whether this interaction is important for the pathogenesis of PD still needs to be established. However, a recent association study in patients with sporadic PD suggested that a combination of apolipoprotein E4 and a polymorphism in the α-synuclein promotor may be pathogenic in PD [135].

Toxicity of α-synuclein and its mutant forms. Since α-synuclein interacts with three proteins known to affect cell viability (BAD, PKC, ERK; fig. 3) and is upregulated under cell stress induced by serum deprivation in HEK 293 cells, the effects of α-synuclein overexpression were investigated in vitro [38]. α-Synuclein was toxic in both HEK 293 and SK-N-SH cells, in a dose-dependent manner. The A53T and A30P mutations increased cell toxicity, the former more than the latter [38]. In rat mesencephalic cells, only overexpression of the human A53T mutant α-synuclein resulted in cell death [136]. Conversely, transfection with an α-synuclein antisense construct was cytoprotective under conditions of serum deprivation [38]. However, overexpression of WT or mutant α-synuclein was not toxic in BE(2)-M17 or HEK 293 cells in another study [137]. Since overexpression of α-synuclein (WT, A30P, and A53T) inhibits ERK, a MAP kinase activated in some cell survival transduction pathways, excessive inhibition of ERK may result in loss of cell viability [138] (fig. 5). The A30P mutant bound less effectively to acidic phospholipids and was less prone to form α helixes in its lipid-bound conformation [33]. This could lower the putative feedback regulation of PA synthesis by α-synuclein-mediated PLD2 inhibition [33] (fig. 5). The interaction between synphilin-1 and the A53T mutant was twice as strong as that of WT or A30P (fig. 5) [39] and might favor α-synuclein aggregation.

In vivo, transgenic mice overexpressing human α-synuclein, in addition to endogenous mouse α-synuclein, showed marked loss of dopaminergic neurons in different brain regions (including the SNpc), and formation of inclusion bodies containing human α-synuclein. Interestingly, these inclusions were composed of granular, not fibrillar, α-synuclein aggregates [139], which might be the possibly more toxic non-filamentous aggregates observed in vitro by Conway et al [113]. In *Drosophila* also, that overexpress human WT or mutant α-synucleins, dopaminergic cell loss, filamentous intraneuronal inclusions and age-dependent locomotor dysfunction (premature loss of climbing ability) were observed [140]. Onset of the movement disorder was earliest with the A30P mutation and identical for the A53T and WT protein [140]. This would be consistent with the in vitro observation that the A30P mutation results in a higher concentration of toxic, soluble α-synuclein oligomers [113]. However, further studies in these animal models will be needed to determine whether neurodegeneration is due to α-synuclein aggregation or to interference with a function of the protein.

Haploinsufficiency of α-synuclein in PD. In contrast to hypotheses linked to overexpression and aggregation of α-synuclein, a limited body of evidence suggests that haploinsufficiency might be the disease mechanism. In lymphoblastoid cell lines from members of a family carrying the A53T mutation, the mutant allele was less or not at all expressed in most of the affected mutation carriers [80]. Interestingly, mutant α-synuclein could not be detected in three asymptomatic heterozygotes, suggesting that lack of the protein was not an immediate cause of PD. However, longitudinal follow-up of an asymptomatic mutation carrier who still expresses the mutated allele will be necessary to demonstrate whether decreased expression of the mutant mRNA precedes disease onset.

The notion of haploinsufficiency was also evoked in a second study showing reduced α-synuclein expression in the substantia nigra of nine histologically proven PD patients without known α-synuclein mutations [73]. Aggregation of α-synuclein might also cause α-synuclein deficiency by entrapping the protein, thus reducing the amount of available functional α-synuclein (fig. 5).

However, one affected mutation carrier of the above-mentioned family did express the mutant allele [80] which argues against haploinsufficiency. Furthermore, haploinsufficiency would not be consistent with the absence of a parkinsonian phenotype in mice lacking

α-synuclein [53], although the life span of these mice is possibly too short to permit the development of overt symptoms.

Conclusions

α-Synuclein is a major component of histopathological hallmarks of several neurodegenerative disorders collectively designated as synucleinopathies: LBs in PD, DLB, and AD, oligodendral and neuronal cytoplasmic inclusions in MSA, and aggregates in astrocytes and Schwann cells in ALS. However, whether these markers are the cause or a consequence of the disease is not yet established. The only proof of a direct involvement of α-synuclein in PD is the identification of a few PD cases with mutations in this gene, but the mechanism by which these mutations lead to selective neurodegeneration has not been elucidated. It is tempting to postulate that α-synuclein mutations are toxic simply by accelerating the aggregation of the protein into protofibrils, fibrils and, finally, their deposition in LBs. But mutations could also be toxic because they impair the numerous potential normal functions of the protein or decrease its availability by favoring fibril formation. Inhibitors of α-synuclein oligomerization and aggregation would help to resolve this question. The answer might also have implications for PD that is not caused by α-synuclein mutations, but in which the α-synuclein aggregation is caused by other factors.

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