α -Synuclein in Parkinson's Disease: Pathogenic Function and Translation into Animal Models

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Key Words

Aggregates · Lewy bodies · Parkinson's disease · Substantia nigra · α -Synuclein

Abstract

Parkinson's disease is a common neurodegenerative disease characterised by the loss of dopaminergic neurons in the substantia nigra pars compacta and the formation of α -synuclein aggregates found in Lewy bodies throughout the brain. Several α -synuclein transgenic mouse models have been generated, as well as viral-mediated overexpression of wild-type and mutated α -synuclein to mimic the disease and to delineate the pathogenic pathway of α -synuclein-mediated toxicity and neurodegeneration. In this review, we will recapitulate what we have learned about the function of α -synuclein and α -synuclein-mediated toxicity through studies of transgenic animal models, inducible animal models and viral-based models.

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bodies (LBs). In patients, these features lead to motor dysfunction, such as bradykinesia, rigidity, tremors and postural instability. Before the occurrence of motor dysfunction, non-motor manifestations, such as reduced olfactory sense, sleep abnormalities, depression and dementia are observed, suggesting that PD is a complex neurodegenerative disease that is not restricted to SNpc. PD exists both as an idiopathic and a familial disorder [1]. So far, there are 18 genes known to be linked to PD, termed PARK genes [2, 3] (table 1). The occurrence of LBs has been described for familial as well as sporadic forms of PD. The main component of these intracellular deposits is aggregated α -synuclein [4]. Cytoplasmic deposition of α -synuclein does not only occur in PD, it is also a common pathological feature of many neurodegenerative diseases, such as multiple system atrophy, dementia with LB and neurodegeneration with brain iron accumulation type 1, which are collectively referred to as synucleinopathies, suggesting a causative role of α -synuclein in these disorders [5].

Parkinson's Disease and Other Synucleinopathies

Parkinson's disease (PD) is one of the most common neurodegenerative diseases, affecting 5 million people worldwide. It is characterised by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the formation of inclusions called Lewy

α -Synuclein: Structural Features and Pathophysiology

α -Synuclein is a 14.46-kDa neuronal protein that is highly expressed in the brain [6, 7]. It was named after its first discovery in the synapse and nucleus [8]. However, later studies described α -synuclein mainly as a cytoplas-

Table 1. Loci and genes associated with PD

Designation	Gene	Locus	Protein function	Inheritance	Ref.	LBs
PARK 1	<i>SNCA</i> point mutation	4q21	among others: synaptic vesicle release and homeostasis	AD	50	+
PARK 2	<i>Parkin</i>	6q25–q27	ubiquitin ligase	AR	206	–/(+)
PARK 3	unknown	2p13	unknown	AD	207	+
PARK 4	<i>SNCA</i> genomic multiplication	4q21	see above	AD	56	+
PARK 5	<i>UCH-L1</i>	4p14–15	ubiquitin-hydrolase/ligase	AD	208	unknown
PARK 6	<i>PINK-1</i>	1q35–36	mitochondrial protein kinase	AR	209	+
PARK 7	<i>DJ-1</i>	1p36	oxidative stress response	AR	210	unknown
PARK 8	<i>LRRK2</i>	12p11.2–q13.1	protein kinase	AD	211, 212	+
PARK 9	<i>ATP13A2</i>	1p36	lysosomal type 5 P-ATPase	AR	213, 214	unknown
Kufor-Rakeb syndrome						
PARK 10	unknown	1p32	unknown	not clear	215	unknown
PARK 11	<i>GIGYF2</i>	2q36–q37	unknown	AD	216	unknown
PARK 12	unknown	Xq21–q25	unknown	X linked	217	unknown
PARK 13	<i>HTRA2/Omi</i>	2p12	HtrA2/Omi stress-regulated serine endoprotease	AD	218	+
PARK 14	<i>PLA2G6</i>	22q13.1		AR	219	unknown
PARK 15	<i>FBXO7</i>	22q11.2–qter		AR	219	unknown
PARK 16	<i>SLC45A3</i> <i>NUCKS1</i> <i>RAB7L1</i> <i>SLC41A1</i> <i>PM20D1</i>	1q32	SLC41A1 (magnesium transporter) RAB7L1 (small GTP-binding protein playing an important role in regulating exo- and endocytotic pathways) NUCKS1 (nuclear protein containing several consensus phosphorylation sites for casein kinase II and cyclin-dependent kinases)	not clear	220	unknown
PARK 17	<i>GAK/DGKQ</i>	chromosome 4p	GAK (cyclin G-associated kinase, a cell cycle regulator) DGKQ (diacylglycerol kinase, theta; involved in the phosphatidylinositol signalling system)	not clear	217	unknown
PARK 18	<i>HLA DRA</i>	6p21.3	class II HLA-DR antigens that are expressed by antigen-presenting cells	not clear	221	unknown

AD = Autosomal dominant; AR = autosomal recessive.

mic protein, which accumulates at presynaptic terminals [7, 9, 10]. Interestingly, α -synuclein expression is not restricted to neuronal cells: haematopoietic cells, including T cells, B cells, natural killer cells and monocytes, as well as cells from the erythroid lineage in murine bone marrow and peripheral erythrocytes have been shown to express α -synuclein ubiquitously [11, 12]. α -Synuclein has been recognised as a very soluble protein with an unfolded tertiary structure, although, recently, it has been suggested that α -synuclein exists in its native conformation as an α -helically folded tetramer [13–15]. However, to date, these findings still lack confirmation [16] and the main physiological function of α -synuclein as a natively unfolded monomer is widely accepted [16]. α -Synuclein is a member of the synuclein family that includes β - and

γ -synuclein [17] and the retina-specific paralogue synoretin [18]. This protein family is specific to vertebrates and has never been discovered in single-cell organisms (including yeast) or invertebrates. The three homologues contain between 127 and 140 amino acids and are about 55–62% identical. α -, β - and γ -synuclein genes are highly expressed in the brain [17]. However, the expression of α -synuclein is not only restricted to the brain; it is also expressed to a medium extent in skeletal muscle and heart, and to a low extent in the pancreas, kidney, lung and placenta [6, 19].

The sequence of α -synuclein can be subdivided into three distinct domains (fig. 1): the N-terminal domain, the non-amyloid component of plaques (NAC) domain and the C-terminal domain.

Fig. 1. Schematic representation of the human α -synuclein sequence and domains. The N-terminal domain of α -synuclein with copies of the KTKEGV consensus sequence. KTKEGV consensus sequence is represented in blue. The five α -synuclein mutations found in PD patients are represented in the N-terminal part of the protein. The NAC and C-terminal domains are illustrated in red and green, respectively. The phosphorylation sites of α -synuclein are also depicted. Colors refer to the online version only.

N-Terminal Domain

The highly conserved N-terminal domain (amino acids 1–65) contains 6 copies of an imperfect 11-amino-acid repeat that displays a KTKEGV consensus sequence [20]. This domain is unstructured in solution and allows the binding to phospholipid vesicles and plasma membranes, possibly via lipid rafts [21]. In neurons, α -synuclein is associated with presynaptic vesicles [22, 23]. This association is modulated by synaptic activity whereby α -synuclein dissociates from vesicles after electrical stimulation of the neuron and only slowly re-associates [24]. Upon binding to membranes, the unstructured α -synuclein shifts to an α -helical conformation. The amphipathic α -helices are reminiscent of the lipid-binding domains of class A₂ apolipoproteins [25].

NAC Domain

The central hydrophobic domain of α -synuclein (amino acids 66–95) is known as the NAC domain [6]. Interestingly, this domain was first isolated from senile plaques of Alzheimer patients. Later on, it turned out to be an essential part of α -synuclein that is responsible for its biochemical and biophysical properties [6]. The hydrophobic NAC domain is the highly amyloidogenic part of the molecule and is essential for aggregation [26]. Sequence analysis of different amyloidogenic proteins showed that they all harbour a common and very similar hydrophobic region with a consensus sequence (⁶⁶VGGAVVAGV⁷⁴) [27–31]. Several studies have suggested that this conserved region is essential for protein aggregation [32–36].

C-Terminal Domain

The C-terminal domain of α -synuclein (amino acids 96–140) contains mainly amino acids with an acidic pH and is, therefore, negatively charged [37]. It has been suggested that the negatively charged C-terminus inhibits aggregation by shielding the NAC region, because C-terminal truncated forms of α -synuclein have a higher propensity to aggregate [38–41] and metal-induced aggregation of α -synuclein also impacts the C-terminus [42–45]. Interestingly, the SN contains elevated levels of iron [46–49] and is most affected by the progressive loss of dopaminergic neurons in PD. Furthermore, dysregulation of iron metabolism also results in neurodegeneration in the SN [49].

Pathophysiology of α -Synuclein and Its Aggregation

With the discovery of a mutation in the α -synuclein gene (A53T) [50] that leads to the development of the familial form of PD, the first association of α -synuclein with the development of PD was made. In the same year, Spillantini et al. [51] identified α -synuclein as the main component of LBs. Up to now, there are 5 known point mutations in the α -synuclein gene (A30P [52], E46K [53], H50Q [54], G51D [55] and A53T [50]), which all lead to the development of the familial form of PD.

The next milestone in unraveling the mystery of PD was the discovery of chromosomal triplications [56] and duplications [57, 58] in the α -synuclein gene in PD families. Carriers of triplications have an earlier age of PD onset than carriers of duplications in the α -synuclein gene. Thus, the amount of α -synuclein mainly determines both the age of onset and the severity of the disease. Also, polymorphisms in the promoter region of the α -synuclein gene that result in increased expression of the protein lead to a higher vulnerability to developing PD [59–62]. Therefore, wild-type (WT) α -synuclein is also capable of inducing PD. It has been suggested that high amounts of α -synuclein trigger the aggregation of the protein and this might be a PD-inducing factor [63].

Fibrillar α -synuclein is found in LBs. It is assumed that fibrils develop from oligomers upon further aggregation. One possible model for the formation of oligomers and fibrils is shown in figure 2. The aggregation of monomeric α -synuclein to form fibrils is a stepwise process running through different stages, whereby different intermediates either further aggregate to form protofibrils and insoluble fibrils, or remain as oligomeric α -synuclein and end up as amorphous aggregates [64–66]. The formation of oligo-

Fig. 2. Schematic representation of possible α -synuclein aggregation. Possible model of the process of α -synuclein dimerisation, oligomerisation and fibrillisation. Under physiological conditions, α -synuclein is mainly natively unfolded or bound to vesicles. Natively unfolded or partially folded α -synuclein can aggregate to form dimers, and further aggregation leads to small and large oligomers that can further aggregate to form protofibrils and insoluble protofibrils, leading to LB formation.

mers and fibrils is accompanied by dramatic conformation changes from an unfolded to a β -sheet structure. In vitro studies have shown that the aggregation of α -synuclein is a nucleation-dependent process: pre-aggregated seeds attract monomeric α -synuclein whereby the aggregation is significantly accelerated [67, 68]. Notably, disease-associated mutations seem to increase the propensity to aggregate: the A53T and E46K mutations seem to favour the formation of fibrils, while the A30P mutation is more likely to lead to the accumulation of oligomers [67, 69–77].

Oligomers and Fibrils of α -Synuclein

Although LBs contain fibrillar α -synuclein, there is a growing body of evidence suggesting that oligomeric and pre-fibrillar forms of α -synuclein, rather than mature fibrils, are the pathogenic species in PD [73, 78, 79]. Overexpression of α -synuclein in cell culture leads to apoptosis and damage of cell organelles without the appearance of fibrils [80]. Co-expression of heat shock protein (Hsp70) and α -synuclein in a *Drosophila* model ameliorated cell death without a change in the number of α -synuclein inclusions [81]. Interestingly, Hsp70 can reduce the amount of misfolded, aggregated α -synuclein species in vivo and in vitro, and can protect from α -synuclein-dependent toxicity [82, 83].

The fibrillar form of α -synuclein can be detected during autopsy in PD patients [51, 84] and could therefore also be neuroprotective. Recently, Bodner et al. [85] have identified a compound that promotes inclusion formation in cellular models of both Huntington's disease and PD, with a reduction in α -synuclein-mediated toxicity.

Research on α -synuclein oligomer formation was first only restricted to in vitro studies. However, in the last years, new cellular and in vivo models have confirmed the existence of α -synuclein oligomers in a cellular environment, too [86]. A novel protein fragment complementation assay has allowed the detection of oligomeric α -synuclein in living cells and neurons [87–90]. Recent in vivo studies have provided evidence for α -synuclein oligomers in brain lysates in rodent models [86, 91].

During the last decade, a number of mechanisms have been suggested as potential consequences of α -synuclein aggregates or oligomers. Amongst those are increased endoplasmic reticulum stress [92], oxidative stress [93], modified chaperone activity [94], dysfunction of the ubiquitin proteasome system [95–98], defects in the mitochondria and Golgi apparatus [80, 99–101], defective axonal transport [102–104], vesicle permeabilisation [105, 106] and lysosomal defects [96, 107]. Some of these mechanisms have so far only been investigated in a cell-free system [96, 105–107], and others have only been identified for mutated α -synuclein [95, 96, 107]. We have previously shown that a heterogeneous population of an

α -synuclein oligomeric species may exist in equilibrium, causing cell death either directly or indirectly. One type of oligomer triggers calcium entry and toxicity, whereas other oligomeric forms induce intracellular seeding in neurons and SH-SY5Y cells [108, 109]. All of these consequences have the assumption in common that α -synuclein aggregates play a key role in the progression of neurodegeneration. However, it remains to be identified which oligomeric species are responsible for α -synuclein-mediated toxicity.

Extracellular α -Synuclein

Because the α -synuclein inclusion body pathology associated with PD occurs in a hierarchical distribution, with its epicentre in the brainstem then extending to the mesolimbic cortex and associated areas, Braak et al. [110] have suggested that α -synuclein pathology spreads gradually throughout the neuraxis as PD progresses. However, as yet, the underlying mechanism of disease progression in PD remains to be determined. Recent studies showing that grafted healthy neurons gradually develop the same pathology as the host neurons in PD brains [111–113] suggest that the pathology arises as a consequence of factors inherent in the PD brain, and aging of transplanted cells promotes the propagation of α -synuclein aggregation from host to graft. The presence of LBs in neurons that were transplanted years previously, but not in recently transplanted neurons, has highlighted the possibility that α -synuclein is released from neurons to the extracellular space and is taken up by other cells. The potential release of α -synuclein into the extracellular space would also be consistent with the fact that measurable quantities of α -synuclein are detected in cerebrospinal fluid and plasma [114, 115].

In vitro experiments have shown that fibrillar and oligomeric forms of α -synuclein can be taken up from the extracellular space by neurons, possibly through an endocytic pathway [116] or via an as yet undetermined route. Furthermore, α -synuclein has been reported to be taken up through Rab5A-dependent endocytosis and to form intra-cytoplasmic inclusions in cultured neurons [117].

With the appearance of aggregated α -synuclein in naïve transplanted embryonic stem cells in PD brains [111, 112], the possibility of neuron-to-neuron transmission of misfolded α -synuclein in PD has been highlighted. Recombinant α -synuclein oligomers are taken up by cultured neurons and trigger cell death [108, 109]. Furthermore, Desplats et al. [118] recently demonstrated that

α -synuclein can be directly transmitted from neuronal cells overexpressing α -synuclein to transplanted embryonic stem cells both in tissue culture and in transgenic animals, supporting the idea that a prion-like mechanism could be responsible for the host-to-graft transfer of PD pathology [119]. These data raise the possibility that a specific conformation of α -synuclein is transmitted from host cells that promotes the aggregation of α -synuclein and triggers toxicity in adjacent neurons. Understanding the sequence of events in the pathological processes underlying the formation and toxicity of α -synuclein oligomers inside and outside neurons in PD will be crucial to the understanding of PD aetiology. Suitable animal models will be necessary for a better understanding of the α -synuclein-linked pathophysiology.

Physiology of α -Synuclein: Lessons Learned from α -Synuclein Knockout Models

The physiological function of α -synuclein is not fully understood to date. It has been suggested that α -synuclein plays a role in synaptic plasticity [120], dopamine release [121] and the generation of synaptic vesicles [122]. Moreover, it has been suggested that α -synuclein possesses a chaperone function [123]. α -Synuclein seems to be important for the maintenance of vesicle homeostasis, vesicle pool, storage of dopamine as well as regulation of vesicle size [124–126]. These findings point to a role of α -synuclein in synaptic transmission [127–130]. Furthermore, this assumed function is underlined by the fact that α -synuclein inhibits the activity of the dopamine transporter [131]. α -Synuclein knockout (KO) mice are an interesting model to study the loss of function of α -synuclein and offer new information concerning its physiological role. Although a large variety of models that overexpress α -synuclein have been developed [reviewed in ref. 132], only a few KO models have been generated (table 2). To date, only 2 different KO mice have been developed: Abeliovich et al. [127] and Schluter et al. [133] targeted exons 1 and 2, whereas Cabin et al. [128] deleted exons 4 and 5. These α -synuclein KO mice are viable with a normal lifespan, are fertile and show normal brain organisation, suggesting that α -synuclein is not an essential gene that is required for vital function. The absence of neurodegeneration in any brain area indicates that α -synuclein deletion is not toxic. Concerning the motor phenotype, α -synuclein KO mice displayed reduced locomotor activity in response to amphetamine, reduced rearing activity in the open field along with reduced dopamine levels in the striatum [127].

Table 2. Recapitulation of the main α -synuclein KO mice or α -/ β -/ γ -synuclein KO mice that have been generated and their main characteristics

Model	Deleted exons	Behavioural symptoms	Tissues	Cellular observations	Ref.
α -syn. KO	exons 1–2	amphetamine-stimulated activity decreased	striatum	no changes in synaptic density normal DA re-uptake, normal DA release, decreased DA contents DA release altered under-stimulation	127
α -syn. KO	exon 1	no obvious phenotype	striatum	no decreased abundance of TH or DA transport no changes in synaptic protein levels of NT normal, levels of DA uptake ok	133
α -syn. KO	exons 4–5	no obvious phenotype anxiety	hippocampus	decreased pool of undocked vesicles	128
α -syn. KO	all genes	no obvious phenotype	CNS	no compensation of β -/ γ -synuclein	222
Double α -/ β -syn. KO	exon 1 α -syn. β -syn. KO	no obvious phenotype	hippocampus and striatum	up-regulation of γ -syn. (compensatory mechanisms) normal synaptic vesicles pool normal synapse structure 20% DA decrease in double α -/ β -synuclein KO	136
Double α -/ γ -syn. KO	exons 1–2 of α -syn. exons 1–3 of γ -syn.	no obvious phenotype	striatum SNpc	reduced number of dopaminergic neurons in the SNpc no decrease in DA levels	137
Double α -/ γ -syn. KO	exons 1–2 of α -syn. exons 1–3 of γ -syn.	hyperdopaminergic-like behavioural phenotype	striatum	elevated levels of electrically evoked DA in the caudate putamen no decrease in DA levels	142
Double α -/ γ -syn. KO	exon 1–2 of α -syn. exons 1–3 of γ -syn.	no obvious phenotype	striatum SNpc	reduced DA content in the striatum of aging α -synuclein no progressive loss of dopaminergic neurons in SNpc of aging α -/ γ -synuclein null mice down-regulation of dopaminergic and synaptic markers in the striatum	134
Triple α -/ β -/ γ -syn. KO	exon 1 of α -syn. β -syn. KO exons 1–3 of γ -syn.	no obvious phenotype in young mice hyperdopaminergic-like behavioural phenotype	brain lysate	absence of neurodegeneration in triple KO mice or changes in synaptic strength decrease in synaptobrevin-2 and large increase in CSP levels triple KO mice exhibited an age-dependent decrease in SNARE complex assembly	123
Triple α -/ β -/ γ -syn. KO	exons 1–2 of α -syn. β -syn. KO exons 1–3 of γ -syn.	hyperdopaminergic-like behavioural phenotype	striatum	no TH-positive neuron loss decreased DA levels increased DA release upon electrical stimulation	141
Triple α -/ β -/ γ -syn. KO	exon 1 α -syn. β -syn. KO exons 1–3 of γ -syn.	mortality after age 3 months	hippocampus and striatum	mild decrease in TH neurons alteration in synaptic structure and transmission	139

DA = Dopamine; NT = neurotransmitter.

Eighteen-month-old α -synuclein KO mice displayed a reduction in striatal dopamine and tyrosine hydroxylase (TH)-positive fibres in the striatum, but there was no progressive loss of TH-positive neurons in the SN in aged α -synuclein-deficient animals [134]. Ultrastructural analysis of synaptic terminals revealed a decrease in the pool of undocked vesicles in both hippocampal tissue and

hippocampal neuronal cell culture [128, 135], suggesting that α -synuclein may regulate presynaptic function by controlling the ‘reserve’ or ‘resting’ pool of vesicles. Altogether, these data strongly suggest a role for α -synuclein in vesicle trafficking and dopamine release. However, a lack of strong effects from α -synuclein deletion and the highly homologous structure of all members of the synu-

clein family suggest that both β -synuclein and γ -synuclein could compensate for the lack of α -synuclein. To prove this theory, several α -, β - and γ -synuclein KO mice have been crossbred. Double α -/ β -synuclein KO mice did not present any obvious phenotype but showed reduced dopamine levels [136]. In contrast, γ -synuclein deletion led to an early loss of TH-positive neurons in the SNpc in the absence of any dopamine level reduction [137]. Double α -/ γ -synuclein deletion did not worsen the phenotype or cellular defects of γ -synuclein KO mice. The absence of up-regulation of β -synuclein expression [138] suggests that β -synuclein does not take over certain functions of the missing synuclein. However, mice lacking all 3 α -/ β -/ γ -synuclein family members showed alterations in synaptic structure and transmission, age-dependent neuronal dysfunction as well as diminished survival [139]. From a molecular point of view, these alterations might be explained by a total disorganisation of the soluble N-ethylmaleimide-sensitive attachment receptor (SNARE) protein complex in the α -/ β -/ γ -synuclein null mice. Interestingly, overexpression of α -synuclein in triple KO mice was able to rescue this phenotype, thus strongly indicating a role for α -synuclein in SNARE complex assembly [123]. Further evidence for a role for α -synuclein in SNARE assembly comes from the study of Chandra et al. [94]. Cysteine string protein α (CSP α) is a co-chaperone essential for synaptic transmission [140]. CSP α -deficient mice showed severe progressive neurodegeneration coherent with a significant reduction in synaptic SNARE complexes [94]. SNARE proteins play an important role in vesicle release in the synaptic cleft, vesicle recycling and synaptic integrity. CSP α is a key factor in the folding and refolding of SNARE complexes. Interestingly, overexpression of α -synuclein in CSP α -deficient mice abolished defective SNARE assembly, thus providing evidence that α -synuclein is able to take over the function of CSP α . The study of Chandra et al. [94] is, therefore, a strong indicator of the potential chaperone function of α -synuclein and conclusively demonstrates that α -synuclein is able to function as a co-chaperone.

In contrast, Anwar et al. [141] described normal abundance of SNARE protein complexes in α -/ β -/ γ -synuclein KO mice, but demonstrated elevated dopamine release upon electrical stimulation. Furthermore, double α -/ γ -synuclein KO leads to a hyperactive phenotype, including increased dopamine release under electrical stimulation in the striatum [142]. Altogether, these studies suggest that synucleins participate in the regulation of dopamine transmission by regulating the readily releasable pool of vesicles.

Interestingly, α - and γ -synuclein, and double α -/ γ -synuclein KO mice are resistant to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism [133, 137, 143–145] and mitochondrial toxins, such as malonate and 3-nitropropionic acid [146]. This strongly indicates that α -synuclein participates in MPTP-induced toxicity through the modulation of oxidative stress.

Genetic Mouse Models: Lessons Learned from α -Synuclein-Overexpressing Animal Models

To date, many mouse models overexpressing α -synuclein using different promoters and mutations have been generated (table 3). However, a large number of them failed to reproduce the characteristic features observed in PD patients. The use of TH and Thy-1 promoters to overexpress α -synuclein in neurons does not lead to progressive loss of dopaminergic neurons, despite the occurrence of aggregation of α -synuclein in Thy-1 α -synuclein mice. However, the double expression of both A30P and A53T α -synuclein mutations under the TH promoter induced a moderate dopaminergic neuronal loss in the SN associated with the accumulation of α -synuclein [147, 148]. Expression of mutated A53T α -synuclein under the mouse prion promoter in mice led to a striking and fatal motor phenotype [149] and α -synuclein aggregates were found throughout the brain except for the SN. However, only a moderate motor phenotype characterised by dystonic posture and tremors, but not associated with α -synuclein aggregates, was found in mice overexpressing the WT and A30P α -synuclein under the mouse or hamster prion promoter [150–153]. The lack of nigrostriatal pathology in these mouse models suggests that mild overexpression of α -synuclein is not sufficient to promote neurodegeneration of dopaminergic neurons. Nevertheless, expression of α -synuclein under the platelet-derived growth factor (PDGF)- β promoter, which resulted in a higher α -synuclein expression in neurons and a moderate α -synuclein expression in glial cells [154], induced dopaminergic neuronal loss and inclusion body formation [78]. Comparison of the activity of Thy-1 or PDGF- β promoters showed that α -synuclein expression under Thy-1 was higher in neurons and allowed α -synuclein expression in a more widespread neuronal population than when α -synuclein was expressed under the PDGF- β promoter [155]. However, Thy-1 α -synuclein mice did not develop dopaminergic neurodegeneration, while PDGF- β α -synuclein mice developed nigrostriatal pathology. Such differences could be ex-

Table 3. Recapitulation of the main genetic α -synuclein-overexpressing mice that have been generated

Model	Promoter (species)	Behavioural symptoms	Age at phenotype onset	Nigro-striatal damage	Aggregation	Other observations	Ref.
m- α -syn.	(m)Thy1	mild impairment in motor performance	6 months	no	non-fibrillar aggregates	ubiquitin immunopathology in spinal cord and brainstem synaptic defects in the neuromuscular junction abnormal mitochondria, demyelination, axonal loss	223
h- α -syn.	(m)Thy1	olfactory deficits	3 months	no	proteinase K-resistant α -syn. inclusions		224
h-A53T α -syn.	(m)Thy1	decreased motor performance	2 months	no	Lewy-like pathology in the spinal cord	neuromuscular degeneration dystrophic neurites and cell bodies occasionally stained intensely for ubiquitin	225
h-A30P α -syn.	(m)Thy1	NA	1 month	no	detergent insoluble α -syn.	neither endogenous mouse α -syn. nor β -syn. were detected in detergent-insoluble fractions	226
h-A30P/A53T α -syn.	(rat)TH	NA	NA	no	no	Lewy-type inclusion did not develop in the nigrostriatal system normal DA levels	227
h-A30P A53T α -syn. (double mutation)	(rat)TH	reduced locomotor activity amphetamine-stimulated activity increased	2–3 months	loss of TH neurons	no	increased density of the dopamine transporter reduction in DA levels	147, 148
h-A53T α -syn.	(m)PrP	progressive reduction in vertical motor activity	3 months	no	accumulation in the neurites and soma of neurons	A53T mutant human α -syn. does display more frequent and widespread accumulation in the neurites and soma of neurons increased DA levels in old mutant mice	228, 229
h-A53T α -syn.	(m)PrP	motor impairments death	7 months	no	α -syn. inclusions	wallerian degeneration in ventral roots absence of α -syn. inclusions in TH-positive neurons	149
h- α -A53T syn.	(m)PrP	sustained posturing reduced amplitude increased spontaneous activity death	10 months	no	α -syn. accumulation associated with ubiquitin	no loss of striatal DA absence of striatal pathology	150
h- α -syn.		no neuronal dysfunction		no	α -syn. accumulation	NA	
h-A30P α -syn.	(hamster) PrP	progressive motor disorders (rigidity, dystonia, gait impairments, tremors)	2 weeks	no	no	no loss of TH-positive neurons normal TH immunoreactivity normal DA levels	151, 152
h-A30P α -syn.	(m)PrP	decreased locomotion impaired motor coordination	6 months	no	no LB	reduced size of the DA pool storage decreased response to amphetamines normal levels of dopamine higher dopamine levels	152, 153
h- α -syn.	(h)PDGF β	mild motor deficit thigmotaxis	2 months	yes	nuclear and cytoplasmic inclusions	TH-positive neurons displayed abnormal accumulation of α -syn. and ubiquitin decreased DA levels in the caudoputamen region	78, 155
h- α -A53T syn.	(h)PDGF β	progressive motor deficits neurodegeneration	NA	NA	very few inclusions	NA	230

DA = Dopamine; NA = not available; m = murine; h = human; PrP = prion.

plained by the fact that: (1) α -synuclein immunoreactivity was denser in neurons and (2) α -synuclein was also detected in glial cells due either to α -synuclein expression driven by the PDGF- β promoter in glial cells and/or α -synuclein spreading from neurons [155]. Altogether, these findings suggest that PDGF- β promoter-mediated expression of α -synuclein mimics some aspects of PD pathogenesis and also supports a role for non-neuronal cells in the nigrostriatal pathology.

Contribution of Non-Neuronal Cells in α -Synuclein Pathology

The fact that expression in both neurons and glia resulted in neurodegeneration suggests that α -synuclein overexpression restricted to neurons is not sufficient to induce degeneration of dopaminergic neurons in vivo [155]. Indeed, high levels of α -synuclein aggregates in neurons can be secreted and transmitted to surrounding cells such as non-neuronal cells. Restricted overexpression of human mutated A53T α -synuclein in astrocytes driven by the GFAP (glial fibrillary acidic protein) promoter led to motor dysfunction, cell death, and dopaminergic cell loss in the midbrain and spinal cord [156]. The expression of α -synuclein restricted to oligodendrocytes, using the proteolipid protein promoter, led to detergent-insoluble α -synuclein aggregates [157]. Microglia could also play a role in PD pathogenesis, since microglial activation has been detected in the brains of PD patients, and a correlation between secreted pro-inflammatory cytokines and glial accumulation of α -synuclein has been reported in mice [158, 159]. Altogether, these studies argue for a non-cell autonomous mechanism for dopaminergic cell loss in PD. Further work on non-neuronal cells (such as specific microglial expression of α -synuclein in vivo) or co-cultures is required to explore the contribution of α -synuclein-mediated toxicity in dopaminergic neurons.

Basic genetic mouse models display different disadvantages. Using constitutively active promoters, α -synuclein overexpression occurs already in the early stages of embryogenesis [160, 161], and even though α -synuclein overexpression has been reported to impair neurogenesis, foetal brain development appears normal in mice overexpressing α -synuclein [162]. Thus, establishment of compensatory mechanisms could probably reduce the effect of α -synuclein overexpression in mice [163]. Furthermore, stable cell lines overexpressing α -synuclein are continuously less sensitive to α -synuclein-mediated toxicity than cells transiently transfected with α -synuclein [own unpubl. data]. The use of an inducible system such as tet-off conditional transgenic approaches and viral vectors to in-

duce α -synuclein overexpression in adult mice abolishes such probable compensatory mechanisms.

Conditional Mouse Models

tet-off conditional approaches have been used in the context of PD [164, 165] (table 4). Due to the addition of the tetracycline transactivator (tTA) domain, which suppresses transgene expression by administration of tetracycline or doxycycline, conditional overexpression of α -synuclein is possible [166]. This allows the detailed analysis of the progression of α -synuclein pathology and motor phenotypes and potential reversal of observed effects when the expression of α -synuclein is turned off. To date, only a few studies have employed this system (table 4). Activation of α -synuclein expression using the calcium/calmodulin-dependent protein kinase II α (CaMKII α)-tTA promoter led to high expression of α -synuclein in the olfactory bulb and forebrain, and weaker expression in the basal ganglia, cortex, cerebellum and brainstem [165, 167]. This mouse model developed nigral and hippocampal neuropathology, including reduced neurogenesis and neurodegeneration in the absence of fibrillary inclusions, leading to cognitive impairment and progressive motor decline. There was also a strong trend to a reduction in TH-positive neurons in the SNpc, although statistical significance was not reached [165]. Inducible A53T α -synuclein mice developed age-dependent progressive neurodegeneration starting at 20 months of age, with massive neuronal loss in the frontal cortex and dorsal striatum [168], while human A30P α -synuclein expression led to impaired olfactory neurogenesis [167]. Interestingly, turning off transgene expression stopped disease progression [165]. Another mouse line generated with the same promoter overexpressing either WT α -synuclein or A53T α -synuclein showed massive degeneration of post-mitotic neurons in the dentate gyrus during post-natal development [164]. Bypassing these post-natal developmental defects by suppression of the transgene until post-natal day 21 led to abnormal accumulation of α -synuclein in the hippocampus, which correlated with memory impairment and structural synaptic deficits. Interestingly, when α -synuclein expression was suppressed, partial pre-clearing of existing α -synuclein aggregates and improvement in memory function were observed [169]. While α -synuclein overexpression driven by the CaMKII α -tTA promoter reproduced only a few features of PD, the use of the GFAP-tTA promoter led to astrocytic expression of A53T α -synuclein and resulted in a 60% loss of TH-positive neurons in the SN [156]. This α -synuclein-overexpressing mouse model provides strong evidence for the involvement of astrocytes in the patho-

Table 4. Recapitulation of the models overexpressing α -synuclein tissue specifically and conditionally

Model	Promoter (species)	Behavioural symptoms	Nigrostriatal damage	Aggregation	Other observations	Ref. No.
h- α -syn.	<i>CaMKIIα-tTA</i>	cognitive impairment, progressive motor decline	yes (bordering significant levels)	Accumulation of α -syn. in nigral axons	nigral and hippocampal neuropathology reduced neurogenesis and neurodegeneration strong tendency to a reduction in DA neurons bordering significant levels	165
h- α -syn. h-A53T- α -syn.	<i>CaMKIIα-tTA</i>	memory impairment	no	abnormal accumulation of α -syn. in the limbic system, particularly in the hippocampus	massive cell loss in the developing hippocampal DG absence of fibrillar inclusions α -syn. accumulation impaired survival of neural precursor cells A53T α -syn. caused more severe neurodegeneration suppression of α -syn. expression partially clears the pre-existing α -syn. pathology and reverses structural synaptic defects	164, 169
h-A53T- α -syn.	<i>CaMKIIα-tTA</i>	increases in ambulatory activities elevated rearing activities	no	α -syn. aggregates	widespread neurodegeneration was evident in the brains of 20-month-old A53T transgenic mice reduction (30%) in striatal neurons reduction in α -syn. phosphorylation at serine 129	168
h- α -syn. h-A53T- α -syn.	<i>GFAP-tTA</i>	progressed paralysis death.	yes	accumulation of α -syn. aggregates	astrocytosis microglial activation mid-brain dopaminergic and spinal cord motor neuron degeneration 60% loss of (TH)-positive dopaminergic neurons in the SNpc	156
h-A30P- α -syn.	<i>CaMKIIα-tTA</i>	NA	NA	NA	impaired olfactory neurogenesis	167

NA = Not available; DG = dentate gyrus.

genesis of PD. Altogether, tet-off conditional systems offer a promising approach to modelling α -synuclein-induced pathology in mice.

Viral-Vector-Mediated Overexpression of α -Synuclein

Viral vector systems have been widely used to promote α -synuclein overexpression in rodents (table 5). They offer an efficient way to promote PD-like cellular and axonal pathology in the nigrostriatal system, with features commonly found in human patients, such as protein aggregation, neuronal dysfunction, reduced dopamine levels, inflammatory response and the loss of TH-positive neurons. The use of adeno-associated virus (AAV) appears more efficient than the lentiviral system for inducing such a phenotype [170–175]. A recent comparison of different AAVs in the rat nigrostriatal system revealed that the newer rAAV serotypes 2/1, 2/5 and 2/8 had marked increase in transduction and spread of the reporter green fluorescent protein expression in dopaminergic nigrostriatal neurons and projections to the striatum and

globus pallidus compared with rAAV2/2 2 weeks after injection [176]. To date, most of the studies have been performed in rats and, depending on the promoter used to drive α -synuclein expression, neuronal loss occurred faster or slower: α -synuclein expression driven by the chicken β -actin promoter led to higher levels of α -synuclein and cell loss occurred 8 weeks after AVV- α -synuclein infection, with a 30–80% loss of TH-positive neurons [170], whereas the use of the cytomegalovirus promoter induced a PD pathology 4 months after AVV- α -synuclein infection, with a 50% loss of dopaminergic neurons [172, 177].

Post-translational modifications of α -synuclein are vast and include phosphorylation, nitration, oxidation, truncation, ubiquitination, sumoylation and glycosylation [reviewed in ref. 178–180]. It has recently been shown that phosphorylation at S129 and/or S87 alters the conformation of monomeric α -synuclein and impacts the oligomerisation properties of α -synuclein [181]. Immunohistochemical and biochemical studies revealed that the majority of α -synuclein is phosphorylated at S129 (S129-P) within inclusions isolated from patients with PD and other synucleinopathies [182–187]. To dissect the

Table 5. Recapitulation of α -synuclein-overexpressing animal models using viral vectors

Model	Promoter (species)	Injection site	Species	Behavioural symptoms	Nigro-striatal damage	Aggregation	Other observations	Ref. No.
AAV h- α -syn.	chicken β -actin promoter	SN	rat	motor impairment	yes	α -syn.-positive cytoplasmic and axonal inclusions	overexpression of WT or mutant A53T-neurodegenerative pathology in the nigrostriatal DA neurons dystrophic and fragmented neurites	170
AAV h- α -syn. h-A30P- α -syn.	CMV/chicken β -actin promoter	SN	rat	lack of behavioural deficits	yes	α -syn. cytoplasmic aggregates	Lewy-like dystrophic neurites in the SN and striatum loss of SN neurons	172
AAV h- α -syn.	CMV promoter	SN	rat	mild deterioration in the motor phenotype	yes	α -syn. aggregates	50% loss of dopaminergic neurons formation of aggregates containing phospho- α -syn. caspase-9 activation	177
AAV h- α -syn.	synapsin-1 promoter	SN	rat	impairment in both drug-induced and spontaneous motor behaviour	yes	α -syn. aggregates	reduced striatal innervation progressive loss of TH-positive terminals in the striatum reduction in DA reuptake and release axonal swelling reduced striatal innervation	231, 232
AAV h- α -syn. AAV h-A30P- α -syn.	pgk-1 promoter	SN	rat	motor asymmetries	yes	NA	WT α -syn. induces higher loss of dopaminergic markers than A30P α -syn. reduction in DA and DA release decreased density of DA vesicles and synaptic contacts in striatal terminals	121
AAV h- α -syn.	CMV promoter	SN	mice	NA	yes	α -syn. aggregates reminiscent of LB	25% reduction in DA neurons caspase-9 activation	175

CMV = Cytomegalovirus; DA = dopamine; pgk-1 = phospho-glycerate kinase 1.

functional consequences of phosphorylation at serine, threonine or tyrosine residues, most cellular and in vivo studies have employed genetic approaches either to block phosphorylation [replacement of serine or threonine with alanine (S/T into A) or tyrosine with phenylalanine (Y into F)] or to mimic constitutive phosphorylation [e.g. replacement of serine with glutamate or aspartate (S into E/D)]. Interestingly, in rodents S129A mutants form five times more insoluble proteinase-K-resistant inclusions but fewer oligomers than WT and S129D [188, 189]. However, overexpression of S129D and S129A α -synuclein using AAV-mediated gene transfer led to contrary results. While McFarland et al. [190] described α -synuclein-positive deposits in dopaminergic neurons and a similar aggregation pattern for WT, S129D and S129A. Azeredo da Silveira et al. [191] demonstrated more thioflavin-S-positive, proteinase-K-resistant aggregates for S129A α -synuclein compared with WT and S129D α -synuclein. On the contrary, Gorbatyuk et al. [192] reported that S129D α -synuclein had heterogeneous immunostaining in the neuronal cytoplasm, whereas WT and S129A α -synuclein had more homogeneous immunolabelling in the cytoplasm. This discrepancy regarding

the aggregation properties of WT, S129D and S129A α -synuclein in vivo might be due to different assays used to determine the aggregation of α -synuclein, different expression levels of α -synuclein and different time points for immunohistological analysis. Mimicking α -synuclein phosphorylation at serine 87 using S87E mutants inhibited the aggregation of α -synuclein and protected against its toxicity in a rat model [181]. Interestingly, overexpression of WT or S87A α -synuclein induced α -synuclein aggregation, loss of dopaminergic neurons and fibre pathology [181]. Oueslati et al. [181] demonstrated with these results that phosphorylation at this residue plays an important role in controlling α -synuclein neuropathology.

AAV-mediated α -synuclein overexpression has been reproduced in mice; however, less neuronal loss and a milder motor phenotype were observed [175, 193]. Differences in viral vector-mediated α -synuclein overexpression effects in mice and rats could result either from variations between species or technical aspects of viral particle delivery into the SN. Altogether, these studies strongly suggest that AAV-mediated α -synuclein expression in the SN in rats leads to the development of many of the characteristic features of PD.

Table 6. Recapitulation of truncated α -synuclein-overexpressing mouse models using AAV vectors

Model	Promoter (species)	Behavioural symptoms	Nigro-striatal damage	Aggregation	Other observations	Ref. No.
truncated 1–120 h- α -syn. in syn. null mice	(rat) TH	progressive reduction in spontaneous locomotion increased response to amphetamine	no	α -syn. aggregates formation of inclusions in SN and olfactory bulbs	decreased DA contents reduction in the number of DA cells from embryonic ventral mesencephalic cell cultures (in vitro) reduced striatal DA levels	202, 233, 234
truncated 1–130 h- α -syn.	(rat) TH	decreased spontaneous locomotor activity	yes	no	treatment with L-dopa improved phenotype absence of aggregated α -syn. in the brain	204
truncated 1–119 h- α -syn.	murine ROSA26 promoter (Cre-lox)	no gross behavioural or phenotypic abnormalities	no	no	reduction in the levels of striatal DA and its metabolites	203
truncated 1–110 h- α -syn. in rat	(chicken) β -actin promoter (AVV) injection in SN	mild behavioural impairment	yes	NA	h- α -syn. 1–110 overexpression sufficient to induce dopaminergic cell loss in the SN	205

DA = Dopamine; NA = not available.

Overexpression of Truncated α -Synuclein

The C-terminal truncation of α -synuclein appears to be another factor contributing to α -synuclein-mediated toxicity (table 6). Indeed, several proteases cleave α -synuclein, such as calpain [194, 195], neurosin [196], cathepsin D [197, 198], the matrix metalloproteinases MMP-1 and MMP-3 [199, 200] and the 20S proteasome [199]. α -Synuclein fragments resulting from these truncations co-aggregate with full-length α -synuclein in both transgenic mice overexpressing human A53T α -synuclein and in patients with PD and dementia with LBs [201]. Overexpression of truncated 1–120 α -synuclein driven by the TH promoter in α -synuclein KO mice led to the formation of pathological inclusions in the SN and olfactory bulb, and to a reduction in striatal dopamine levels [202]. Similar results were obtained using a conditional mouse model expressing truncated 1–119 α -synuclein [203]. In another study, Wakamatsu et al. [204] showed that overexpression of truncated 1–130 α -synuclein in mice led to a severe loss of dopaminergic neurons in the SN and motor impairment, while mice expressing the same levels of full-length α -synuclein under the same promoter did not develop any nigrostriatal pathology. Co-expression of C-terminal truncated 1–110 human α -synuclein enhanced full-length α -synuclein-induced pathology in a rat AAV model, including enhanced neuronal loss of dopaminergic neurons accompanied by the formation of larger pathological accumulations of

α -synuclein [205]. Altogether, these data strongly suggest that truncated α -synuclein promotes its toxic aggregation.

Conclusions

α -Synuclein aggregation plays a central role in the development and progression of PD and other α -synuclein-linked diseases. Although transgenic mice overexpressing WT or mutated α -synuclein did not demonstrate loss of dopaminergic neurons in the SN, as observed in PD patients, the available animal models still recapitulate some aspects of the human disease, including aggregation of α -synuclein in various cell populations and behavioural abnormalities. Alterations in the nigrostriatal system, with reduced levels of dopamine, are often observed in these models, demonstrating functional changes in dopaminergic neurons without apparent neuronal loss. This might reflect an early event in the cascade of neurodegeneration before massive cell death occurs. Recently, increasing evidence has suggested oligomeric α -synuclein as the pathogenic species in PD and α -synuclein-linked diseases. Oligomers have been proven to be important key players in neuronal toxicity and, therefore, their role in neurodegeneration warrants further investigation in vivo. In our opinion, more emphasis should be placed on the generation of animal models that specifically tackle oligomeric α -synuclein to fully understand the mecha-

nisms involved in α -synuclein-oligomer-induced neurodegeneration. Disease-modifying approaches that lower the burden of α -synuclein oligomers will open new avenues for the development of pharmacological targets and pharmacological interventions.

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