Pathological roles of α-synuclein in neurological disorders

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Substantial genetic, neuropathological, and biochemical evidence implicates the presynaptic neuronal protein α-synuclein in Parkinson's disease and related Lewy body disorders. How dysregulation of α-synuclein leads to neurodegeneration is, however, unclear. Soluble oligomeric, but not fully fibrillar, α-synuclein is thought to be toxic. The major neuronal target of aberrant α-synuclein might be the synapse. The effects of aberrant α-synuclein might include alteration of calcium homoeostasis or mitochondrial fragmentation and, in turn, mitochondrial dysfunction, which could link α-synuclein dysfunction to recessive and toxin-induced parkinsonism. α-Synuclein also seems to be linked to other genetic forms of Parkinson's disease, such as those linked to mutations in *GBA* or *LRRK2*, possibly through common effects on autophagy and lysosomal function. Finally, α-synuclein is physiologically secreted, and this extracellular form could lead to the spread of pathological accumulations and disease progression. Consequently, factors that regulate the levels, post-translational modifications, specific aberrant cellular effects, or secretion of α-synuclein might be targets for therapy.

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Introduction

α-Synuclein is an abundant 140-residue neuronal protein, that, under physiological conditions, is found mainly in neuronal presynaptic terminals, close to synaptic vesicles. It is a member of a conserved family of proteins that also includes β-synuclein and γ-synuclein, and was originally described as the precursor protein for the non-amyloid component of Alzheimer's disease senile plaques.[1] The protein is intrinsically unfolded, which means that in the purified form at neutral pH it lacks an ordered secondary or tertiary structure. Upon binding to membranes or synthetic vesicles containing acidic phospholipids, however, it assumes an α-helical structure.[2] Although its exact functions are unknown, α-synuclein is assumed to help in the regulation of synaptic-vesicle release and to provide a stabilising effect on complexes of SNARE family proteins.[3–6] Three missense point mutations (Ala53Thr, Ala30Pro, and Glu46Lys) and multiplications of the gene locus have been identified in *SNCA*, which encodes α-synuclein, in families with autosomal dominant Parkinson's disease (PD).[7] Importantly, genome-wide association studies have shown clearly that *SNCA* is also linked to sporadic PD,[8] and indicate a possible link to multiple system atrophy.[9]

Deposits of α-synuclein have been identified in pathological aggregates, such as Lewy bodies, Lewy neurites, and oligodendroglial inclusions in patients with PD and several other neurodegenerative disorders, such as dementia with Lewy bodies and multiple system atrophy.[10] These disorders are termed synucleinopathies. In dementia with Lewy bodies, in which dementia and motor deficits are linked closely in time, accumulation of α-synuclein is seen throughout the brain, including the cortex. In multiple system atrophy, which can involve extrapyramidal, cerebellar, pyramidal, and autonomic dysfunction, the predominant pathological feature is oligodendroglial α-synuclein inclusions. Other neurological disorders, including Alzheimer's disease and neurodegeneration with brain iron accumulation, can also manifest with abnormal α-synuclein deposition, and can thus be viewed as being within the range of synucleinopathies.

Abnormal deposition of α-synuclein occurs early in the disease process, at least in PD, and seems to follow a sequence of ascension from lower brainstem centres to limbic and wide cortical association areas.[11] The more widespread accumulation of α-synuclein is thought to underlie, at least in part, the cognitive and behavioural deficits in PD with dementia. The mechanisms that underlie the aberrant functions of α-synuclein and how these impact on disease pathogenesis remain poorly understood, but some possibilities have been suggested. In this Review we focus on such pathogenic pathways in neurological disorders and highlight recent developments and potential links to other genetic defects associated with PD, which is the most common pure synucleinopathy.

Concentration-dependent α-synuclein oligomerisation and aggregation

Recombinant α-synuclein incubated under certain conditions in vitro assumes an oligomeric conformation and is gradually converted to β-sheet-rich, fibrillar structures that resemble the Lewy bodies and neurites found in human neuropathological samples. This process is termed aggregation and is thought to underlie the toxic potential of α-synuclein.[2] In transgenic mice over-expressing human α-synuclein, coexpression of β-synuclein, the non-amyloidogenic homologue of α-synuclein, inhibits aggregation and is associated with improvements in motor deficits and neurodegenerative alterations, and reduced accumulation of α-synuclein in neurons.[12] Furthermore, overexpression of α-synuclein lacking the central hydrophobic non-amyloid component domain in *Drosophila* abolishes the aggregation and at the same time mitigates the neurotoxic effects seen upon wild-type α-synuclein overexpression in this model.[13]

Which particular species of α-synuclein are toxic has been debated. Some evidence favours fully fibrillar or the intermediate soluble oligomeric species.[14,15] Two recent studies have supported the latter theory. In the first study, artificial α-synuclein proline mutations that increased the speed of oligomerisation but did not lead to fibrillisation yielded variants of α-synuclein that were

more toxic than the wild-type protein.[16] In the second study, mutations that favoured oligomerisation in a rat viral model of α-synuclein nigral overexpression led to greater toxic effects than wild-type protein or variants that led to quick formation of fibrils.[17] In rats with mutations that favoured oligomerisation, α-synuclein strongly bound to lipid membranes and formed multimers. Dopamine and its metabolites inhibit the conversion of protofibrils to fibrils and might promote protofibril accumulation.[18] This effect could at least partly explain the selective vulnerability of dopamine neurons to α-synuclein-mediated toxic effects. Research efforts are underway to identify which particular oligomeric species of α-synuclein are toxic.

A major factor that could drive the aggregation and neurotoxic effects of α-synuclein is the total concentration of the protein, as suggested by human genetic multiplication studies.[7] Whether total α-synuclein concentrations are increased in the brains of patients with PD is unclear. One study showed only slightly increased levels of soluble, membrane-bound α-synuclein in the substantia nigra of patients with PD compared with those in controls. This finding was in contrast to the robust increase of α-synuclein levels in vulnerable regions of the brain in patients with multiple system atrophy, where the protein accumulates predominantly within glial cells.[19] Studies that have assessed concentrations of *SNCA* messenger RNA in the brains of patients with PD have been inconclusive, but indirect evidence for the role of the transcriptional regulation of *SNCA* in PD pathogenesis can be derived from specific associations between disease risk and particular polymorphic regions within this gene. Alleles within a Rep1 polymorphic region 10 kB upstream of the *SNCA* promoter that confers risk for sporadic PD[20] have been associated with increased expression of α-synuclein messenger RNA in human and mouse neurons[21,22] and in the temporal neocortex and substantia nigra in human beings.[23] These findings suggest that increased concentrations of *SNCA* messenger RNA constitute a triggering factor for PD pathogenesis.

Mechanisms for *SNCA* transcriptional regulation, therefore, seem likely to be important in PD. Although little work on this regulatory pathway has yet been done, elements within intron 1 of *SNCA* seem to be involved.[24,25] A signal transduction pathway that involves the MAPK 3 and PI3K pathways and converges on the transcription factor ZSCAN21 (Zipro1) could be important.[24,26,27] Additionally, an area containing GATA-1, which is another transcription factor that controls *SNCA* expression[25] that is located a little further down intron 1 and is normally highly methylated, is hypomethylated in the brains of patients with PD.[28] This finding suggests a mechanism through which *SNCA* expression is increased in PD.[28] Post-transcriptional regulation might also be important. MicroRNAs—the hsa-mir-7 family and mmu-mir-153—negatively regulate α-synuclein levels, by the binding of RNA elements at the 3′-UTR of the messenger RNA sequence.[29,30]

Levels of α-synuclein are regulated by a balance of synthesis, degradation, and secretion. The ubiquitin proteasome system and the autophagy-lysosome pathway, (which involves microautophagy, macroautophagy, and chaperone-mediated autophagy) are the two major quality-control systems postmitotic neurons use to maintain intracellular proteostasis.[31] The mechanism of α-synuclein degradation remains unclear. Some reports suggest that monomeric α-synuclein can be degraded by the proteasome.[32] We have found, however, that only a small proportion of soluble-cell-derived intermediate α-synuclein oligomers, not including monomeric α-synuclein, are targeted to the 26S proteasome for degradation.[33] By contrast, total α-synuclein concentrations increase after lysosomal inhibition.[32–36] In particular, wild-type α-synuclein, but not Ala30Pro and Ala53Thr mutant forms, is degraded by the selective process of chaperone-mediated autophagy,[34,35,37] whereas all forms are degraded by macroautophagy (figure 1).[32,35,37]

Inhibition of chaperone-mediated autophagy leads to increased aggregation of high-molecular-weight and detergent-insoluble α-synuclein species in neuronal cells,[35] which suggests that this process has a crucial role in the prevention of oligomerisation or aggregation of α-synuclein. In-vivo support for this idea is provided by a study that showed enhanced chaperone-mediated, autophagy-dependent degradation of α-synuclein in mouse substantia nigra under conditions of stress, such as that induced by an excess of α-synuclein.[38] Furthermore, expression of HSC70 and LAMP-2A, which have regulatory roles in chaperone-mediated authophagy, might be reduced in the brains of patients with PD compared with that in age-matched control brains.[37] This difference would further support the theory that dysfunctional chaperone-mediated autophagy is implicated in PD pathogenesis.

The serine protease neurosin (kallikrein-6) has also been proposed to control α-synuclein degradation. Neurosin is localised within pathological α-synuclein deposits, such as Lewy bodies and glial cytoplasmic inclusions.[39]

Lysosomal involvement and the role of glucocerebrosidase

Lysosomes degrade α-synuclein but lysosomal function also seems to be affected by α-synuclein in a way that leads to neurotoxic effects. The PD-linked Ala53Thr and Ala30Pro mutations and modification of the wild-type protein by dopamine inhibit chaperone-mediated autophagy and, therefore, prevent the degradation of related substrates.[34,37,40,41] The neuronal survival factor MEF2D might be an especially relevant substrate.[42] Inhibition of chaperone-mediated autophagy through aberrant compensatory activation of macroautophagy or by generalised lysosomal dysfunction could lead to neurotoxic effects (figure 1).[40]

Increased accumulation of autophagic vacuoles and alterations in macroautophagic markers, which are indicative of excessive macroautophagy induction or failure of proper vacuole fusion with lysosomes, have been seen in various cellular and animal models of synucleinopathies.[43–46] Increases in macroautophagy in such models lead to decreases in α-synuclein load and improvements in neuronal survival and function.[32,47] Conversely, in our own work, inhibition of macroautophagy protected against neurotoxic effects mediated by α-synuclein, which indicates that induction of macroautophagy can be harmful rather than protective.[40] Moreover, in another study, overexpression of α-synuclein impaired an early stage of autophagic vacuole formation, through an interaction with Rab1a.[48] These conflicting results and differential effects could be attributed to timing. For instance, macroautophagy might be beneficial as a clearing mechanism early on in the pathogenic process of PD, but later, when more general neuronal dysfunction is present, it could contribute to neuronal death. Although the details of these processes remain to be untangled, a harmful cycle forms, in which aberrant α-synuclein affects the lysosome, degradation of α-synuclein becomes diminished, and further lysosomal damage occurs (figure 1). Whether accumulation of α-synuclein precedes the impairment of autophagic pathways or vice versa remains unclear.

A lysosomal enzyme of particular interest is glucocerebrosidase. Loss-of-function mutations in *GBA* (which encodes glucocerebrosidase) that are normally associated with Gaucher's disease are linked to an increased risk of classic Lewy-body-associated PD, and related synucleinopathies.[49] In support of a link between *GBA* mutations and synucleinopathies through a toxic gain of function, overexpression of such mutants but not wild-type *GBA* promoted α-synuclein accumulation in cell culture and in mouse models without alteration of glucocerebrosidase activity; by constrast, pharmacological inhibition of glucocerebrosidase had no effect on α-synuclein levels.[50] Another study, however, showed that pharmacological inhibition of glucocerebrosidase led to the accumulation of α-synuclein in cultured neuronal cells and in rat nigral cell bodies and astroglia.[51] Consistent with the loss-of-function connection, an extensive histological analysis of several mouse models of Gaucher's disease showed α-synuclein oligomerisation and aggregation in various brain regions only when glucocerebrosidase activity was substantially downregulated over long periods of time.[52]

A major breakthrough in the understanding of a relation between glucocerebrosidase, lysosomal function, and α-synuclein has been provided by three reports. In the first, glucocerebrosidase encoded by wild-type *GBA* and *GBA* carrying the Asn370Ser mutation, which is related to Gaucher's disease, interacted physically and selectively with α-synuclein in vitro, in human tissue, and in neuronal cultures under lysosomal solution conditions.

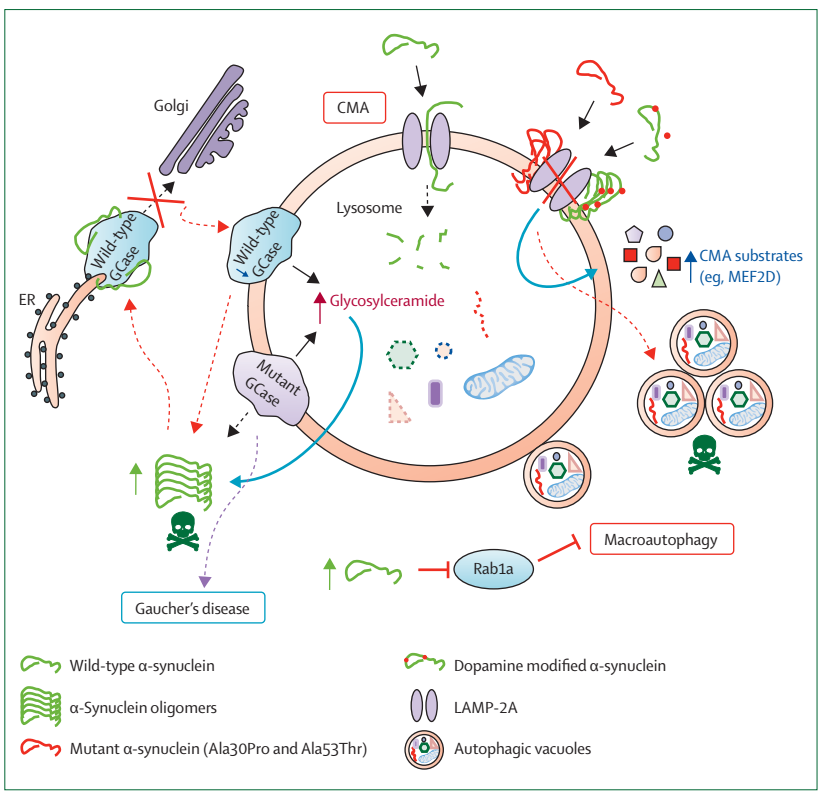

***Figure 1*: Lysosomal effects of α-synuclein**
Wild-type α-synuclein is degraded by CMA upon binding to the CMA-specific receptor LAMP-2A. In patients carrying the Ala30Pro and Ala53Thr mutations of *SNCA*, which are linked to Parkinson's disease, or where α-synuclein is modified by dopamine, binding is stronger and α-synuclein is not internalised, which leads to inhibition of degradation of α-synuclein and other CMA substrates. This dysfunction can lead to macroautophagy alterations and accumulation of autophagic vacuoles, which might result in neuronal death. Some studies have shown that increased concentrations of α-synuclein are associated with impaired formation of autophagic vacuoles through interaction with Rab1a, which leads to suppression of macroautophagy. In patients with Gaucher's disease and in carriers of *GBA* mutations with synucleinopathies, activity of GCase is lowered, which can lead to lysosomal dysfunction and consequent increases in α-synuclein accumulation. At the same time glycosylceramide accumulates, which increases oligomerisation of α-synuclein. Raised concentrations of oligomerised α-synuclein might inhibit ER–Golgi trafficking of wild-type GCase, which leads to reduced GCase activity and, therefore, reduced degradation of α-synuclein. This cycle can cause neurotoxic effects. CMA=chaperone-mediated autophagy. ER=endoplasmic reticulum. GCase=glucocerebrosidase.

Mutant glucocerebrosidase displayed reduced affinity for α-synuclein compared with the wild-type protein.[53]

The second study showed that downregulation of glucocerebrosidase activity led to decreased lysosomal protein degradation and consequent α-synuclein accumulation and aggregation-dependent neurotoxic effects in various cellular models, including human neurons derived from induced pluripotent stem cells originating from Gaucher's disease fibroblasts.[54] Furthermore, accumulation of glucosylceramide, which occurs in Gaucher's disease owing to glucocerebrosidase dysfunction, stabilised oligomeric intermediates of α-synuclein, which further increased the pathogenic effects.[54] In accordance with reports of disruption of endoplasmic reticulum–Golgi protein trafficking by excess α-synuclein,[55] overexpression of α-synuclein inhibited the intracellular trafficking of wild-type

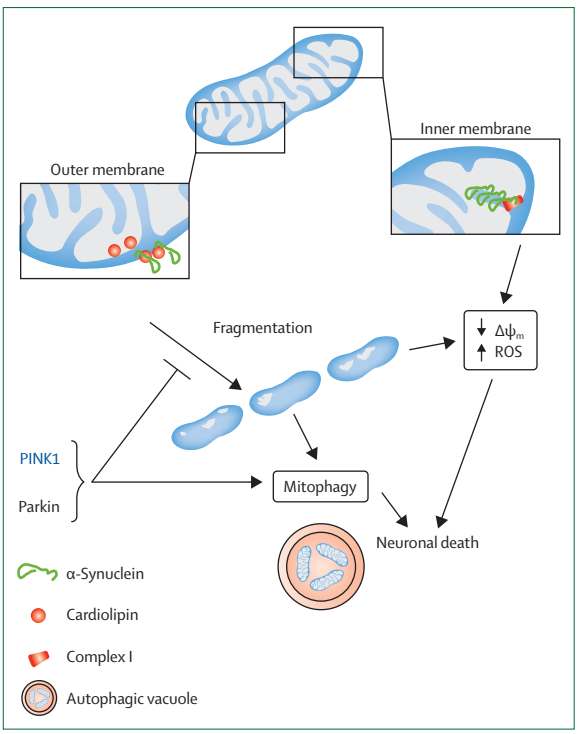

***Figure 2*: Effects of α-synuclein on mitochondria**
Accumulation of monomeric or oligomeric α-synuclein occurs in the outer mitochondrial membrane, where it interacts strongly with cardiolipin. Overexpression of α-synuclein induces mitochondrial fragmentation, which leads to increased removal of mitochondria through mitophagy. α-Synuclein can also accumulate within the inner mitochondrial membrane and interact directly with and inhibit the activity of complex I of the respiratory chain. Wild-type forms of Parkin and PINK1 can inhibit α-synuclein-induced mitochondrial fragmentation, but stimulate mitophagy. Mitochondrial fragmentation and inhibition of complex I activity can lower mitochondrial membrane potential, which leads to increased production of ROS and neuronal death. $\Delta\psi_m$=membrane potential. ROS=reactive oxygen species.

glucocerebrosidase, which led to a decline in the enzyme's lysosomal activity and, therefore, a pathogenic cycle of α-synuclein accumulation and glucocerebrosidase dysfunction (figure 1).[54]

The third study showed that overexpression of wild-type glucocerebrosidase in a mouse model of Gaucher's disease reversed α-synuclein accumulation and the related histopathological and behavioural alterations.[56] Whether mutant glucocerebrosidase leads to an increased risk of PD through gain or loss of function, or both, is not completely clear. Restoration of glucocerebrosidase activity and general lysosomal function might, however, be a therapeutic strategy, at least in patients with PD who have *GBA* mutations.

Mitochondrial involvement and recessive parkinsonism

Mitochondrial alterations are well recognised in PD. Data that suggest a relation between the pathobiology of α-synuclein and damage to this organelle are, therefore, of interest. Endogenous α-synuclein is detected within rodent brain mitochondria, especially in the outer mitochondrial membrane.[57–59] Some studies, one of which was in patients with PD,[60] have shown α-synuclein accumulation within the inner mitochondrial membrane, dependent on the N-terminal membrane-binding domain.[59–61] In other studies, however, only outer-membrane binding of overexpressed α-synuclein has been reported.[62]

Numerous studies have shown that overexpression of α-synuclein impairs mitochondrial complex I[59,60,63–66] or complex IV[61] activity. In one study, Ala53Thr α-synuclein was more potent than wild-type α-synuclein in this regard; furthermore, α-synuclein interacted directly with complex I to exert this effect (figure 2).[60]

Expression of α-synuclein in cultured cells[45] or in transgenic mice leads to swelling[61] or damage to the mitochondria, resulting in distorted membranes or cristae,[59,63] and fragmentation has been seen after expression of α-synuclein in mammalian cells or in *Caenorhabditis elegans*.[59,62] These structural changes occur with even low overexpression of α-synuclein, and in the virtual absence of structural defects in other intracellular organelles.[59] The harmful effects arise independently of the mechanisms known to control mitochondrial fusion and fission, and seem to be exerted directly on mitochondrial membranes, for which α-synuclein might have special affinity because of their rich cardiolipin content.[59,62] Whether mitochondrial fragmentation represents an increase in fission or an inhibition of fusion is controversial. What is important, however, is the connection with mitochondrial dynamics, which seem to play a crucial part in the pathogenesis of recessive parkinsonism owing to loss-of-function mutations in Parkin or PINK1. Overexpression in cultured cells of wild-type Parkin or PINK1, but not of disease-related mutant forms, prevented mitochondrial fragmentation induced by α-synuclein, which suggests that all such PD-related genes might affect a common pathway (figure 2).[62]

No data clearly indicate which α-synuclein species cause these mitochondrial effects. The inhibitory effect of α-synuclein on membrane fusion might represent an intrinsic property of the monomeric protein, because mitochondrial fragmentation was reduced in the absence of α-synuclein.[62] By contrast, the effects of individual species in an in-vitro assay with artificial membranes suggest that small oligomers are the cause.[59]

It has been proposed that a direct effect of α-synuclein on mitochondrial fragmentation sets off a sequence of events that is followed by the loss of mitochondrial transmembrane potential and neuronal death.[59] Thus, mitochondrial fragmentation might represent a useful therapeutic target. This sequence of events, however, would not account for the direct effects of α-synuclein on complex I mentioned above (figure 2).[60] Additionally, the effects on mitochondria may lead to release of reactive oxigen species, which may in turn lead to secondary

induction of α-synuclein levels, oligomerisation, and aggregation and, therefore, create a vicious amplification cycle.[67]

The effects of α-synuclein on mitochondria could be related to those reported on other intracellular constituents. Similar structural effects to those induced by α-synuclein on mitochondrial membranes could be important to the interaction with vesicles (at the level of the presynaptic terminal), the lysosomal membrane, or the endoplasmic reticulum–Golgi apparatus. A study showed that aberrant α-synuclein expression in cortical neurons induced excessive macroautophagy but also, more specifically, was associated with excessive autophagy of mitochondria, termed mitophagy, which led to mitochondrial depletion. When mitophagy was inhibited, either through general suppression of macroautophagy or through specific mitophagy suppression by depletion of Parkin, the harmful effects of α-synuclein on survival were partly reversed (figure 2).[68] In another study, transgenic overexpression of α-synuclein led to in-vivo induction of mitophagy in the substantia nigra.[66] These effects in the cellular model are in accordance with observations in double transgenic mice, where the absence of Parkin actually ameliorated neuropathological and behavioural deficits of α-synuclein overexpression.[69] These data suggest that mitophagy, which is viewed as dysfunctional in autosomal recessive parkinsonism, might assume a death-mediator role when excessively activated in the context of synucleinopathies. These opposing effects suggest caution regarding the lumping of all PD-related genetic defects in a similar linear biochemical pathway.

α-Synuclein, LRRK2, tau, and cytoskeletal effects

Genome-wide association studies have identified strong associations with PD for *SNCA*, *MAPT* (which encodes microtubule-associated protein tau), and *LRRK2*.[8] The identification of these genes in such analyses does not necessarily mean that their protein products interact synergistically or otherwise to facilitate the pathogenesis of PD. Functional links might, however, exist between the proteins that affect the cytoskeleton. Oligomerisation of α-synuclein could destabilise cytoskeletal units, which in turn might accelerate the formation of α-synuclein oligomers and further cytoskeletal disruption and result in the neuritic degeneration observed in synucleinopathies (figure 3). For example, aggregated α-synuclein applied extracellularly decreased tubulin polymerisation, even in the absence of a direct interaction.[70] Alternatively, the region of α-synuclein between residues 60 and 100 has been suggested to directly interact with tubulin and to inhibit microtubule assembly in cultured cells.[65] Similarly, α-synuclein oligomers selectively recruit tubulin, but not actin, in degenerating neurites.[71] Conversely, in yeast the inhibition of microtubule assembly can trigger aggregation of α-synuclein,[72] although other studies have found that enhancement of tubulin oligomerisation also promotes

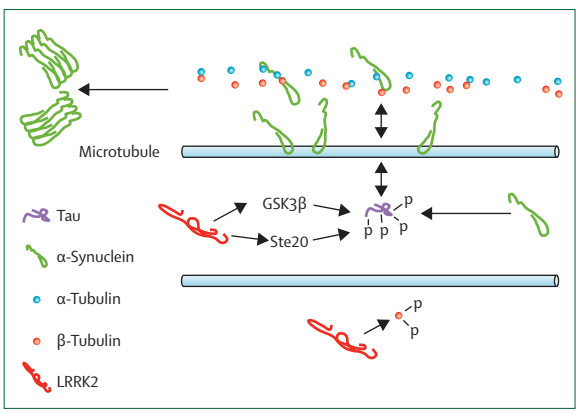

***Figure 3*: Effects of α-synuclein on the cytoskeleton**
α-Synuclein enhances the phosphorylation of the microtubule-stabilising protein, tau, possibly via GSK3β or other kinases. Reduced interaction of tau with the microtubule network alters its stability. Similarly, α-synuclein can directly affect the microtubule network by binding with fully polymerised microtubules or the component monomers, α-tubulin and β-tubulin. Some studies show that accumulation of α-synuclein inhibits microtubule polymerisation, but others show stimulation of polymerisation. Reciprocal interaction between depolymerised microtubules and α-synuclein that triggers oligomerisation of α-synuclein and leads to a pathological state has been proposed. Similarly to α-synuclein, LRRK2 can affect microtubule stability through increased phosphorylation of tau, through GSK3β or Ste20 kinases, or directly by increased phosphorylation of β-tubulin.

in-vitro fibrillisation of α-synuclein.[73] Consistent with the latter notion, in a transgenic mouse model of multiple system atrophy, in which α-synuclein was overexpressed in oligodendrocytes, microtubule depolymerisation lessened α-synuclein-related neuropathological effects.[74]

Evidence suggests that tau hyperphosphorylation is indirectly mediated by α-synuclein (figure 3).[75–77] Jensen and colleagues[75] showed that an interaction between α-synuclein and soluble, but not microtubule-bound, tau led to tau phosphorylation, which is required for its binding to microtubules. Tubulin blocked the binding of tau to α-synuclein, which suggests a regulatory step in the maturation of the microtubule network.[75] What remains to be clarified, however, is whether phosphorylated tau binds tubulin more readily than non-phosphorylated tau or whether this modification leads to the dissociation of tau and tubulin or microtubule networks. The specific residue of tau that undergoes phosphorylation might dictate its tubulin-binding properties.

A growing number of reports indicate a functional association between the cytoskeleton and another dominantly inherited PD gene, leucine-rich repeat kinase 2 (*LRRK2*). The gene product, which has kinase activity, has a direct link with the cytoskeleton, as seen by the phosphorylation by LRRK2 of the substrates moesin[78] and β-tubulin.[79] An indirect link also exists via kinase signalling cascades involving Ste20 kinase family members as potential phosphorylation substrates of LRRK2, or the identification of PKC-ζ as an upstream kinase that phosphorylates LRRK2.[80] Together with the multiple reports of abnormal accumulation of

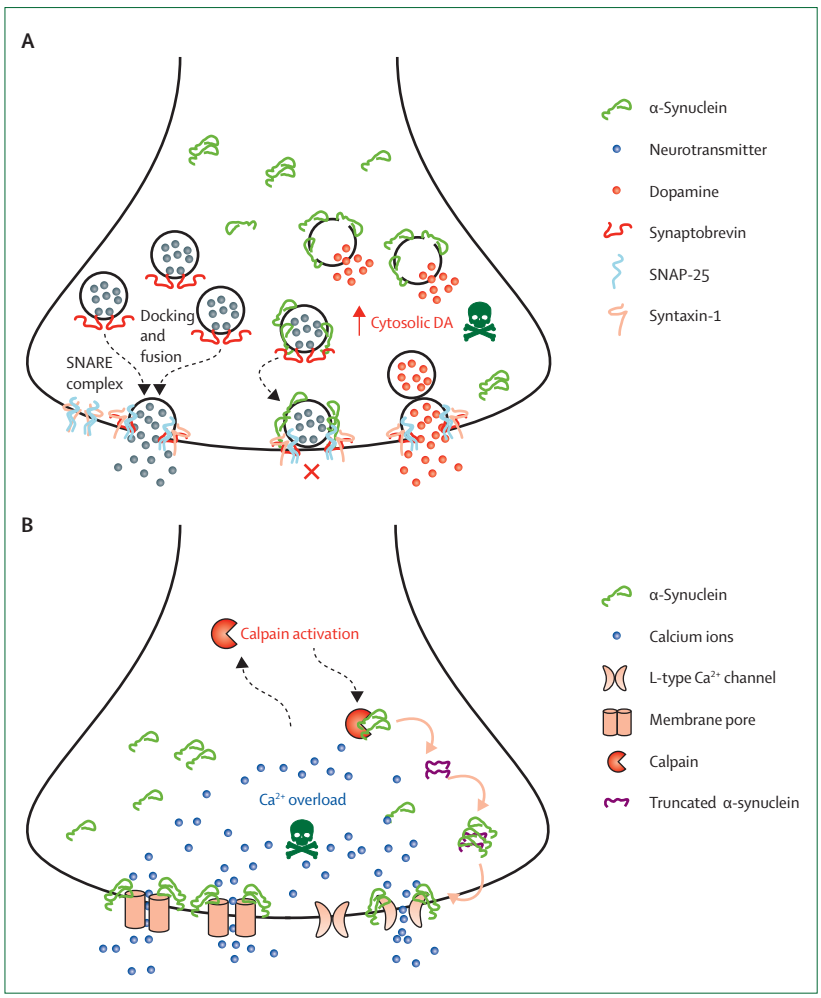

***Figure 4*: Effects of α-synuclein on synapse integrity and calcium homoeostasis**
(A) Release of neurotransmitters from nerve terminals requires SNARE-complex assembly and disassembly. Accumulation of α-synuclein causes assembly defects, reducing the synaptic vesicle pool and adversely affecting neurotransmitter release. α-Synuclein also attacks synaptic vesicles within the nerve terminal, which leads to leakage of neurotransmitter in the cytosol; high cytosolic concentrations of DA can be harmful to the cell. (B) Oligomeric species of α-synuclein are thought to promote the formation of ion-permeable pores in the plasma membrane, through which Ca^{2+} can enter the cytosol. The increased concentrations of Ca^{2+} might affect the opening of L-type voltage-gated calcium ($Ca_v1.3$) channels and lead to intracellular Ca^{2+} overload. Increased cytosolic concentrations of Ca^{2+} can trigger a harmful cascade, including calpain activation, which might end in neuronal death. Additionally, calpain-mediated cleavage of α-synuclein can generate aggregation-prone species with C-terminal truncation that can increase Ca^{2+} entry through L-type Ca^{2+} channels. DA=dopamine.

phosphorylated tau in the brains of animals expressing *LRRK2*,[81,82] a strong functional link is suggested between the wild-type gene and tau dynamics under physiological conditions, and between *LRRK2* mutations and tau under pathological conditions (figure 3).

A study in *Drosophila* confirmed the link between *LRRK2* and the cytoskeleton and implicated an additional kinase. *Drosophila* expressing the *LRRK2* Gly2019Ser mutant form showed substantially increased phosphorylation of tau by the kinase Sgg (ninein in human beings).[81] These data are consistent with neuropathological evidence that tau is hyperphosphorylated in PD[83] and in particular in *LRRK2*-associated PD.[84] The net effects of aberrant phosphorylation of tau are the destabilisation of the microtubule network, accumulation of this protein, frequently within aggregated tangles, and neurite retraction. In these respects, the aberrant effects on tau function are features of both α-synuclein and LRRK2-mediated neurodegeneration. By contrast, the direct phosphorylation of β-tubulin by LRRK2 seems to stimulate microtubule formation.[79] The apparent opposing effects on cytoskeletal dynamics should be viewed as part of the overall picture of disruption of the cytoskeleton network.

Direct evidence of a functional interaction between α-synuclein and LRRK2 was provided by double transgenic models, in which mice conditionally expressed the Ala53Thr mutation in *SNCA* and forms of *LRRK2*, or the Ala53Thr mutation in *SNCA* on an *LRRK2*-deficient background.[85] In each assessment of α-synuclein-induced neuropathology the phenotype was worsened by overexpression of LRRK2, whether wild-type or the Gly2019Ser mutant. Conversely, neuropathological changes induced by the Ala53Thr mutation were less severe in mice null for *LRRK2*. This finding suggests a synergistic interaction between LRRK2 and α-synuclein that affects neuronal survival and that might involve cytoskeletal elements and tau.

Effects on neurotransmitter release and calcium homoeostasis

Data largely derived from α-synuclein knockout mice suggest that α-synuclein normally mediates negative control of neurotransmitter release and has a possible role in assembly of SNARE family complexes.[3–6] Whether these apparently physiological functions at the presynaptic terminal contribute to the pathogenic effects of α-synuclein has been investigated.

Iwai and colleagues[86] showed abnormal localisation of the protein in presynaptic terminals. The researchers suggested that this accumulation leads to the synaptic dysfunction associated with amyloid plaques. In the brains of patients with dementia with Lewy bodies, α-synuclein aggregates were located in presynaptic terminals and resulted in severe synaptic pathology, leading to almost complete loss of dendritic spines at the postsynaptic area.[87] More recent findings have broadly supported these observations, although details and suggested mechanisms have differed.

After only slight increases in α-synuclein, neurotransmitter release was inhibited in glutamatergic hippocampal pyramidal and mesencephalic dopaminergic neurons, potentially by a reduction in the pool of readily releasable synaptic vesicles.[88] In another study, overexpression of α-synuclein in cultured neurons was associated with low concentrations of several critical presynaptic proteins involved in exocytosis and endocytosis[89] and substantial reductions in the frequency of excitatory postsynaptic currents, diminished exocytosis, and altered vesicular size. A different perspective was

provided by a study in another model, in which α-synuclein with C-terminal truncation was transgenically expressed in mouse nigral dopaminergic neurons. Functional deficits were related to severe reductions in dopamine release, which were in turn probably due to redistribution, but not loss, of presynaptic proteins (figure 4).[90]

A mechanism through which α-synuclein may ensure the integrity of SNARE complexes was provided by the finding that α-synuclein sequesters arachidonic acid and thereby blocks the activation of SNARE-protein interactions.[91] In another study, however, overexpression of α-synuclein had no effect on synaptic efficacy.[3] Despite the differences in the proposed mechanisms, the studies overall suggest that impaired SNARE-complex function and synaptic transmission owing to even slightly raised α-synuclein expression lead to behavioural consequences, although the processes that link these outcomes and neurodegeneration remain unclear. Whether soluble oligomeric species participate in such effects also needs to be determined. Of note is that the absence of endogenous monomeric α-synuclein leads to neuritic degeneration in elderly mice;[92] the effect was increased in elderly mice deficient in α, β, and γ forms of synuclein, and led to motor deficits, axonal and synaptic structural alterations, and early death.[3,93] Despite the lowered frequency of SNARE-complex assembly and structural changes in synapses, effects on synaptic transmission were not obvious in the triple knockout mice, perhaps because the age of the mice differed at electrophysiological assessment.[3] In conjunction, knockout and transgenic studies have shown that a lack of expression or overexpression of α-synuclein can lead to age-associated synaptic degeneration (figure 4). A loss of functional α-synuclein might arise in patients with PD owing to sequestration within aggregated structures.

A prevailing hypothesis suggests that oligomeric species of α-synuclein can promote the formation of ion-permeable pores on membranes and alter cellular homoeostasis.[94,95] Increased calcium (Ca^{2+}) influx is thought to be the main resulting toxic effect,[96–100] and could be further augmented via glutamate AMPA receptors (figure 4).[101] Catecholaminergic neurons might be especially vulnerable to oscillations in Ca^{2+} concentrations because L-type voltage-gated calcium ($Ca_v1.3$) channels help to maintain their spontaneous pacemaker activity.[102] Although neurons might compensate for high cytosolic concentrations of free Ca^{2+} through a functional sarcoendoplasmic reticulum pump, this system is thought to break down in conditions of energy depletion or oxidative stress, which might be caused by mitochondrial dysfunction in PD.[103,104]

The same pore-forming mechanism is thought to enable oligomeric α-synuclein to attack synaptic vesicles, which leads to leakage of neurotransmitter into the cytosol; for dopamine, high cytosolic concentrations could lead to oxidative stress, intracellular interactions between Ca^{2+}, dopamine, and α-synuclein, and the triggering of a neurodegenerative cascade.[105] Another contributing factor could be calcium-mediated activation of calpains, which might lead to C-terminal truncation and oligomerisation of α-synuclein,[106] further Ca^{2+} influx, and neurotoxic effects (figure 4).

α-Synuclein secretion and disease propagation

α-Synuclein has no endoplasmic reticulum signal peptide and was thought at first to be an exclusively intracellular protein. This notion was challenged when α-synuclein was detected in biological fluids, such as blood plasma and CSF.[107,108] Furthermore, α-synuclein could be secreted in the culture medium of neuronal cells independent of whether stable overexpression,[108] inducible overexpression,[109] transient transfection,[110,111] or viral-mediated expression[112] was used.

The mechanism of α-synuclein release has not been fully elucidated, but data point towards a non-classic, secretory pathway that involves vesicle trafficking but is independent of the endoplasmic reticulum–Golgi apparatus. Lee and colleagues[112] detected intracellular α-synuclein in the lumen of vesicular structures with properties similar to those of dense core vesicles. We used an inducible neuroblastoma cell line that expresses α-synuclein and found that soluble oligomeric and monomeric α-synuclein species were released in association with externalised membrane vesicles.[109] The protein composition, morphology, and size of these vesicles were characteristic of exosomes, which are endosome-derived vesicles secreted by a multitude of cell types after fusion of multivesicular bodies with the plasma membrane (figure 5). Release depended on intracellular Ca^{2+} concentration and the endocytic pathway, which is consistent with exosome-mediated α-synuclein secretion.

Exosomes enable the transfer of membrane and cytosolic components from donor cells to the extracellular matrix and recipient cells by various mechanisms, such as endocytosis, receptor–ligand binding, or fusion with the plasma membrane (figure 5).[113] As such, these small vesicles could serve as a system to facilitate cell-to-cell communication, rather than merely providing a way of disposing of unwanted proteins. In PD, exosomes might enable accumulation of α-synuclein to spread. This theory is supported by the discovery that fetal mesencephalic grafts transplanted into the striatum of patients with PD develop α-synuclein-positive and ubiquitin-positive Lewy bodies more than a decade after transplantation.[114,115] This finding suggests host-to-graft transmission of pathology, although other interpretations are possible. Transmission of α-synuclein oligomers between neurons and from neurons to non-neuronal cells has been shown in vitro.[111] The intercellular transmission of α-synuclein from host to graft has also been seen in vivo in transgenic mice.[116,117] Endocytosis-governed neuronal uptake of α-synuclein might be a prerequisite step for such a mechanism.[117,118]

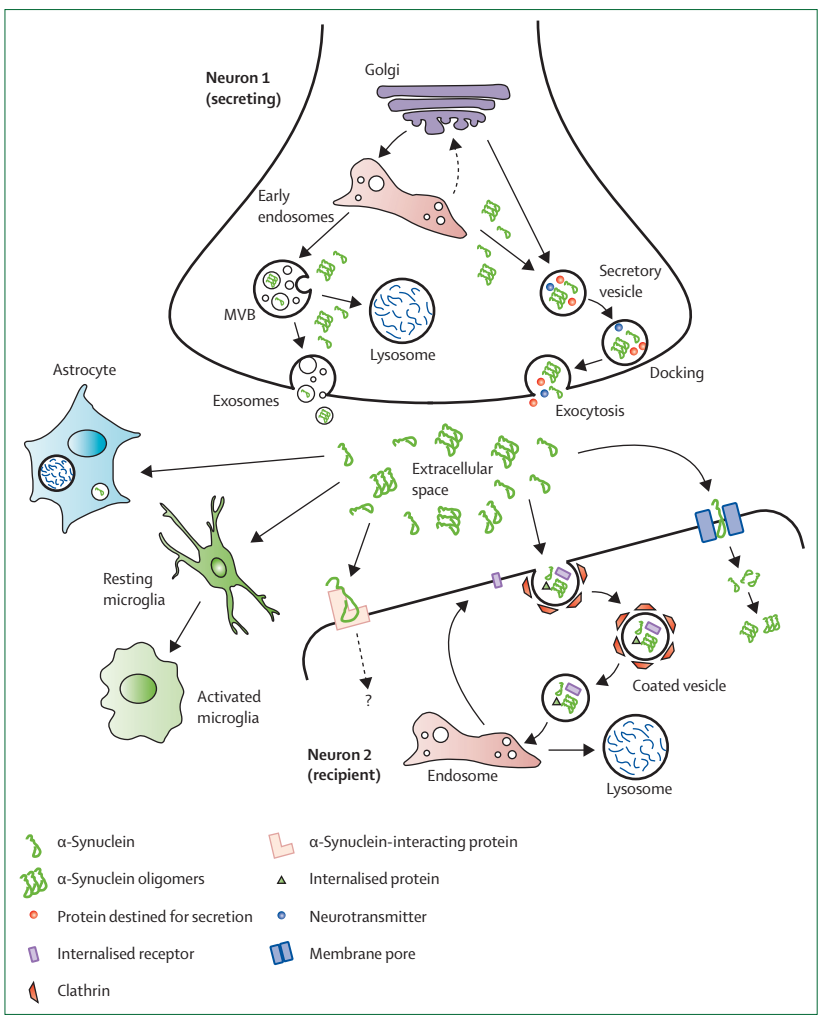

***Figure 5*: α-Synuclein secretion and paracrine actions**
α-Synuclein is secreted through non-classic endocytic or exocytic pathways. In early endosomes, protein material is either recycled back to the plasma membrane or sorted to MVBs. Cytoplasmic α-synuclein species can enter MVBs at this point, via inward budding of the limiting membrane of these vesicles. Subsequently, MVBs fuse with lysosomes for degradation or with the plasma membrane to release their content in the extracellular space as exosomes. Alternatively, α-synuclein species can be incorporated into secretory vesicles and be released via exocytosis. Once in the extracellular space, α-synuclein can affect the homoeostasis of recipient neurons by association with as yet unknown membrane proteins or receptors, endocytosis of clathrin-coated pits, or formation of pores on the recipient plasma membrane. In parallel, α-synuclein triggers neuroinflammatory responses via microglia activation. Finally, α-synuclein can be cleared through endocytosis by astrocytes. MVB=multivesicular body.

Overall, this evidence adds validity to the hypothesis that cell-to-cell transmission and perhaps permissive templating of α-synuclein contribute to the spread of pathological accumulation of α-synuclein in PD, as suggested by Braak staging.[11] Whether neurons can take up α-synuclein, however, continues to be debated. Cell-secreted α-synuclein that is readily taken up by cycling neuroblastoma cells is not internalised by neurons.[109] Luk and colleagues[119] had to use cationic liposomes to facilitate the delivery of exogenously added α-synuclein into the cytoplasm of recipient cells. These data imply that mechanisms other than endocytosis mediate the effects of extracellular α-synuclein on recipient cells, for instance an interaction at the level of the plasma membrane of the recipient neuron, perhaps via a controlled type of diffusion or specialised binding.[109,118] The possibility that this process is species specific, cell-type dependent, or can be modulated by specific protein modifications might account for the discrepancies seen across studies. In one study, intragastric administration of the complex I inhibitor rotenone led to enteric α-synuclein pathology that gradually spread to the CNS, including dopaminergic neurons, and caused delayed neurotoxic effects. This outcome suggests that mitochondrial dysfunction triggers aberrant α-synuclein conformations, and that once these are initiated they could propagate independently of the initial insult and cause neurotoxic effects. This finding thus bridges together the mitochondrial dysfunction and α-synuclein propagation theories.[120]

Secreted forms of α-synuclein might be biologically important because of the potential for causing paracrine effects on neighbouring cells. Secreted α-synuclein lessens the viability of recipient neuronal cells in cell-culture models, in a concentration-dependent fashion,[109,110] and this effect is largely mediated by oligomeric species.[109,111] Extracellular α-synuclein could also trigger a neuroinflammatory response through glial activation. The activation of microglia via binding to integrin α-M receptors and without the need for internalisation of α-synuclein has been proposed.[121,122] By contrast, astrocytes internalise α-synuclein via endocytosis, probably in an attempt to clear potentially toxic conformations of the protein.[118,123] Excessive uptake of α-synuclein could, however, lead to astroglial inflammatory responses and might account for astrocyte pathology (figure 5).

The finding that soluble α-synuclein can be measured in vivo in the brain parenchyma of mice and human beings,[124] strengthens the notion that this protein could have paracrine effects that exacerbate PD pathology. Maintenance of extracellular α-synuclein at physiological concentrations might be crucial for homoeostasis in the nervous system. As such, the mechanisms governing either the release of α-synuclein into or its withdrawal from the extracellular environment must be tightly regulated; disturbance of such mechanisms might trigger or contribute to the progression of neurodegeneration.

Search strategy and selection criteria

We searched PubMed for original reports and reviews published in peer-reviewed journals, with the search terms "alpha-synuclein", "neurodegeneration", "Parkinson's disease", "lysosomes", "synapse", and "secretion". Although we set no parameters for years of publication, most of the original research we used to support this Review was published from January, 1993, to August, 2011; we also selected the most recent reviews from leaders in the subject.

Conclusions

Aberrant expression of α-synuclein can lead to multiple intracellular and potentially extracellular pathogenetic effects. The mechanisms involved, especially feed-forward amplification loops, might be amenable to therapeutic interventions, although, in view of the range of toxic effects of α-synuclein, the inhibition of neurodegeneration is likely to be incomplete. The discovery of a common denominator for the harmful effects that could be effectively targeted would be most useful. Additionally, the identification of biomarkers for the earliest steps of α-synuclein deregulation in synucleinopathies would help in the development of effective treatments.

Contributors

All authors contributed equally to the manuscript. MX, EE, and HJR prepared and edited the figures. All authors determined the structure of the review and assembled the different sections. LS wrote the final draft and made corrections.

Conflicts of interest

We declare that we have no conflicts of interest.

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