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Effects of Alpha-Synuclein on Cellular Homeostasis

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1. Introduction

1.1 α-synuclein: A "leading act" in the synapse

α-Synuclein is a relatively abundant 140-residue neuronal protein physiologically found in presynaptic neuronal terminals (Abeliovich et al., 2000; Spillantini, Crowther, Jakes, Hasegawa, & Goedert, 1998; Wacker, Zareie, Fong, Sarikaya, & Muchowski, 2004). α-synuclein belongs to a highly conserved family of proteins which includes β- and γ-synucleins. It is an intrinsically unfolded, or natively unfolded, protein, meaning that in its purified form at neutral pH it lacks an ordered secondary or tertiary structure (Trojanowski & Lee, 1998). Three missense point mutations (A53T, A30P and E46K) have been identified in families with autosomal dominant Parkinson's disease (PD) (Figure 1) (Chartier-Harlin et al., 2004; Kruger et al., 1998; Polymeropoulos et al., 1997). In addition, duplications and triplications in the gene encoding for α-synuclein have been shown to cause rare familial forms of PD, suggesting that the levels of the protein are critical in the pathogenesis of the disease (Singleton et al., 2003; Zarranz et al., 2004). Over-expression of mutant α-synuclein in transgenic mice under various promoters presents only certain aspects of PD and some have been shown to lead to neurodegeneration (reviewed in Sulzer, 2010). Knockout mice for α-synuclein do not exhibit severe neuropathological alterations but, at least in the nigrostriatal dopaminergic system, they show an enhancement of response to paired electrical stimuli, suggesting that α-synuclein, normally, negatively controls neurotransmitter release.

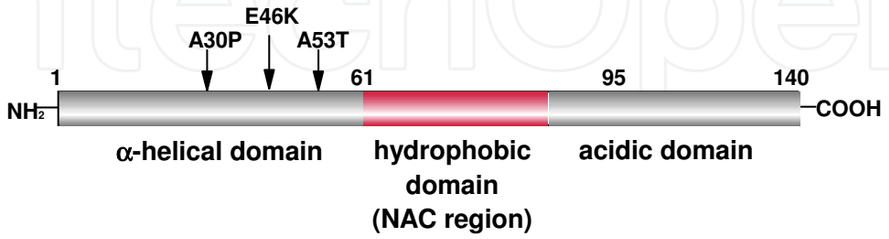

Fig. 1. Schematic representation of α-synuclein showing the basic regions of the protein and the point mutations that have been linked with familial PD.

With respect to neurotransmission, it has been recently reported that excessive α-synuclein induces a series of pathologic changes, including deficits in neurotransmitter release. In a recent study, Nemani et al. (2010) showed that following only modest

increases in α-synuclein, an inhibition of neurotransmitter release was observed in glutamatergic hippocampal pyramidal neurons and mesencephalic dopaminergic neurons (Nemani et al., 2010). This inhibition appeared to be a consequence of the failure to get recycling vesicles clustered near synaptic release sites. In a different study, Scott et al. (2010) demonstrated that α-synuclein can reduce the levels of several critical presynaptic proteins involved in exocytosis and endocytosis (Scott et al., 2010). The group further observed significant reductions in miniEPSC frequency, diminished presynaptic exocytosis and altered vesicle size by EM in α-synuclein-overexpressing neurons.

A role of α-synuclein in the integrity of the SNARE complex was also reported by Darios et al., (2010) who showed that α-synuclein sequesters arachidonic acid and thereby blocks the activation of SNARE protein interactions (Darios et al., 2010). Precisely how increased α-synuclein expression impairs clustering of vesicles near the synapse remains to be elucidated. However, α-synuclein has been found to rescue the disassembly of the SNARE complex and the degeneration of neuritic terminals associated with lack of the presynaptic protein Cystein String Protein-α (CSP- α), by facilitating the assembly of SNARE complexes that mediate vesicle fusion at presynaptic terminals. By directly examining SNARE assembly, Burré et al (2010) showed that this facilitating process depends on synaptic activity and propose that the association of α-synuclein with VAMP may ultimately mediate the process (Burré et al., 2010). Furthermore, the same group generated α-, β-, and γ-synuclein triple knockout mice, which as they aged, made SNARE complexes less, displayed a significant age-dependent decrease in synaptobrevin-2 and perished prematurely. The role of synucleins was fully dispensable in young animals but became essential in aged ones, which suggests that α-synuclein maintains normal synaptic function during aging. Moreover, the authors found that restoring expression of α-synuclein in cultured neurons from the triple knockout mice helped re-establish SNARE complexes, and did so in a dose-dependent way.

Further arguing for a physiological role of synucleins in synaptic transmission, synuclein null mice lacking α-, β-, and γ-synucleins generated by Greten–Harison et al. (2010), exhibited prominent age-dependent changes in synaptic protein composition and axonal structure that led to severe neuronal dysfunction in the central nervous system and neuronal death (Greten-Harrison et al., 2010). Importantly, in the hippocampus, young synuclein null mice exhibited increased basic transmission which could be rescued with both mouse and human α-synuclein transgenes, confirming that synucleins affect basal neurotransmission. In agreement with a role for α-synuclein in synaptic function, Keri et al. (2010) reported impaired learning in people who have two copies of the α-synuclein gene but lack PD-like symptoms (Keri, Moustafa, Myers, Benedek, & Gluck, 2010). Compared to age-matched volunteers whose α-synuclein was normal, the group reported that the gene duplication carriers showed defects in learning. α-Synuclein also binds and inactivates phospholipase D2, and could thus influence synaptic membrane biogenesis through phosphatidic acid metabolism.

Overall, distinct α-synuclein concentration thresholds seem to be permissive for synapse function, raising the question of whether early signs of neurodegenerative pathology reflect a disturbance in synaptic density homeostasis. Targeting synaptic membrane proteins that tend to interact with α-synuclein and affect membrane trafficking under physiological conditions, could present a pool of potential molecular therapeutic targets.

1.2 α-Synuclein toxic species: The search goes on

The feature of α-synuclein that has attracted the most attention is its distinctive propensity to aggregate *in vitro*, through a sequence of conversion from a natively unfolded monomeric form to a fibrillar form; a phenomenon associated with a conformational change from random coil to β-pleeted sheet. Aggregated insoluble α-synuclein is the major constituent of cytoplasmic inclusions termed Lewy bodies and Lewy neurites, which are the pathological hallmarks of inherited and sporadic PD (Spillantini et al., 1998). The presence of Lewy Bodies in the substantia nigra is diagnostic for PD, but α-synuclein pathology is also encountered in other brain regions and may account for the wide range of non-motor symptoms observed (Bate, Gentleman, & Williams, 2010).

Although the exact aberrant function of α-synuclein that links it to neurodegeneration is not known, the weight of the evidence suggests that the process of its conversion to toxic oligomers is involved, hence has been the subject of extensive research. Similar to amyloid beta (Aβ), attention has recently shifted from the insoluble amyloid fibrils to the soluble oligomeric intermediates, or protofibrils, in the α-synuclein aggregation process. There is evidence that the soluble oligomeric protofibrils, and not the mature fibrils, are the toxic species. Although the question is still open (Waxman & Giasson, 2009), data obtained in three established model systems for PD, such as primary neurons, C. elegans, and Drosophila, show a strong correlation between α-synuclein oligomers, neuronal toxicity, and behavioral defects (Karpinar et al., 2009), further sustaining a pathogenic role for α-synuclein oligomers in PD. The A30P and A53T mutations of α-synuclein associated with familial forms of PD both promote protofibril formation relative to wild type α-synuclein (J. Li, Uversky, & Fink, 2001; Rochet, Conway, & Lansbury, 2000). The A30P mutation was also shown to delay the formation of amyloid fibrils relative to the wild type protein, suggesting that α-synuclein protofibrils rather than fibrils may be the pathogenic species (Conway et al., 2000). In addition, the presence of soluble protofibrilar species, when compared to frank fibrillar inclusions or monomers, correlates better temporally with death in cellular and *in vivo* models (Danzer et al., 2007; Emmanouilidou, Stefanis, & Vekrellis, 2010; Kayed et al., 2003).

Several α-synuclein post-translational modifications lead to the formation of stable oligomers. These include nitration, oxidation, phosphorylation, and interaction with iron. Oxidative modification of α-synuclein via dopamine adducts may facilitate aggregation (Conway, Rochet, Bieganski, & Lansbury, 2001). Dopamine and its metabolites modulate differently the stability of soluble oligomers and mature fibrils and act as inhibitors of the conversion of protofibrils to fibrils, thus favouring protofibril accumulation (Follmer et al., 2007; Sultzer, Gray, Gunay, Wheatley, & Mahler, 2001). In a recent report Tsika et al. (2010) showed that, despite similarities in basic biochemical properties, α-synuclein oligomeric intermediates obtained from different neural regions demonstrated unexpected divergence in promoting α-synuclein amyloid fibril formation and toxicity (Tsika et al., 2010). This is in agreement with the fact that despite the ubiquitous expression of α-synuclein throughout the CNS, Lewy bodies are found in certain susceptible neuronal subtypes of specific brain nuclei (Braak, Rub, & Del Tredici, 2003).

A possible mechanism by which oligomers could be toxic is through the disruption and permeabilization of cellular membranes (Lashuel et al., 2002; Volles et al., 2001). Oligomeric α-synuclein has been shown to permeabilize negatively charged synthetic phospholipid vesicles (Volles et al., 2001; Zhu, Li, & Fink, 2003). Soluble oligomers are considered to be

cytotoxic and to disrupt cellular membranes, by forming pore-like complexes in the bilayer in a manner similar to bacterial pore forming toxins (Lashuel & Hirling, 2006). The pore formation, inducing disruption of cellular ion homeostasis, may be responsible for the neurotoxic effect (Volles et al., 2001). So far, it has been demonstrated that α-synuclein regulates calcium entry pathways and, consequently, that abnormal α-synuclein levels may promote neuronal damage through disregulation of calcium homeostasis. However, to date, there has been very limited evidence that supports the pore formation by cell-produced α-synuclein oligomers.

Studies aiming to identify the potential toxic species are also based primarily on experiments in which oligomerization of the protein is forced *in vitro* (Danzer et al., 2007; Goldberg & Lansbury, 2000). Recently, a study by the Masliah group in UCSD (2011) using a rat lentiviral system showed that only those mutations of α-synuclein that could cause the formation of soluble oligomers could also confer toxicity to dopaminergic neurons by disturbing the plasma membrane (Winner et al., 2011). Other proposed mechanisms through which these oligomers can confer cell death include alterations in the lysosomal-dependent autophagy pathway (Xilouri, Vogiatzi, Vekrellis, & Stefanis, 2008) and mitochondrial dysfunction (Martin et al., 2006). Evidence on a role of phosphorylation in the oligomerization and neurotoxicity of α-synuclein has also been provided (Cavallarin, Vicario, & Negro 2010). It has been shown that α-synuclein is heavily phosphorylated in Lewy bodies found in patients with synucleinopathies but studies have disagreed about whether this phosphorylation promotes or prevents neurotoxicity (Azeredo da Silveira et al., 2009) because α-synuclein carries a number of potential phosphorylation sites. Phosphorylation of α-synuclein at Ser129 strongly modulates interactions between α-synuclein and synphilin-1 and the formation of inclusions. Soluble α-synuclein oligomeric species are increased by phosphorylation at Ser129 (Chen et al., 2009). Recently, Paleologou et al., (2010) demonstrated that phosphorylation of α-synuclein at Ser87 is elevated in brain extracts from cases of dementia with Lewy bodies (DLB), multiple system atrophy, and from several mouse models of synucleinopathy where most of the modified protein was associated with membranes (Paleologou et al., 2010). The authors also showed that modifications at Ser87 affect both α-synuclein's pathological aggregation and its normal interaction with cell membranes.

Finally, oligomeric α-synuclein can induce microglia activation (Zhang et al., 2005) and cause subsequent neuro-inflammation which aggravates DA neuronal loss (Koprich, Reske-Nielsen, Mithal, & Isacson, 2008).

2. Effects of intracellular α-synuclein on cellular homeostasis

Physiologic function of the cell requires proper and continuous functioning of cellular surveillance mechanisms that identify and clear unwanted adducts in the cell interior. Intracellular buildup of aberrant components disturbs cellular homeostasis ultimately leading to cell death. Chaperones and proteolytic systems are responsible for cellular quality control, and defects in both systems have been involved in PD pathogenesis. Molecular chaperones is a highly conserved class of proteins that are responsible for the proper folding of macromolecules and the refolding of proteins that have become misfolded as a result of cellular stress (Muchowski & Wacker, 2005). As mentioned above, PD is characterized by the accumulation of misfolded α-synuclein in intraneuronal inclusions called Lewy bodies,

and several studies have suggested that failure of chaperone-mediated protection may enhance α-synuclein aggregation and neurotoxicity (Ali, Kitay, & Zhai; Muchowski & Wacker, 2005). Changes in the two major intracellular proteolytic systems, the ubiquitin-proteasome system (UPS) and the lysosome-autophagy system, are widely considered to contribute to the accumulation of aggregated α-synuclein species that in turn affect vital cellular pathways and lead to cell death. Dysfunctions in macroautophagy and chaperone-mediated autophagy, the two main lysosomal degradation systems, have been linked with intracellular α-synuclein accumulation in several studies using both cellular and animal models (Cuervo, Wong, & Martinez-Vicente, 2010; Xilouri & Stefanis, 2011). In this manuscript, we will preferentially focus on the impact of α-synuclein burden on the UPS as a potential pathway to neurodegeneration.

2.1 The proteasome: Structure, regulation and proteolytic function

The ubiquitin-proteasome system (UPS) is a major system for intracellular protein degradation, a complex and tightly regulated process (Goldberg et al., 2003). Protein degradation through the UPS pathway consists of two discrete and successive steps. The first step is ubiquitylation, a process through which the small protein ubiquitin is covalently attached to surface-exposed lysine residues of the target protein to be degradated (Glickman & Ciechanover, 2002). Ubiquitin is a highly evolutionarily conserved 76-residue polypeptide that is abundantly found in the cell cytoplasm. Ubiquitylation is accomblished via a three-step cascade mechanism where each step is catalyzed by ubiquitin-activating (E1), ubiquitin-conjugating (E2) and ubiquitin-ligase (E3) enzymes. Once the initial ubiquitin is conjugated, polyubiquitylation of the substrate occurs through the sequential transfer of ubiquitin molecules, thus forming an ubiquitin chain. The minimum signal for UPS-mediated proteolytic degradation is a chain of four ubiquitin moieties (Gallastegui & Groll; Glickman & Ciechanover, 2002; Layfield et al., 2001). In the second step of the degradation process, this poly-ubiquitin chain is recognized by the proteolytic core engine of the UPS, the 26S proteasome complex, which in turn degrades the substrate protein into defined oligopeptides with release of free and reusable ubiquitin. The removal of the ubiquitin tag is mediated by ubiquitin recycling enzymes (Ardley & Robinson, 2005; Glickman & Ciechanover, 2002).

The 26S proteasome is a 2-2.5 MDa proteolytic enzyme that is highly conserved among eukaryotes (Besche, Haas, Gygi, & Goldberg, 2009). It comprises two multimeric protein complexes, the 20S core particle, where actual proteolysis occurs, and two 19S regulatory particles, that regulate the function of the 20S (Benaroudj, Zwickl, Seemuller, Baumeister, & Goldberg, 2003; Crews, 2003; Goldberg et al., 2003). Each one of the two regulatory units attach to the outer surface of the core particle. The 20S core complex is a ~700 kDa barrel-shaped structure made up of four stacked hetero-heptameric rings: two identical outer rings consisting of α-type subunits and two identical inner rings consisting of β-type subunits. Each of the β-rings possesses three different catalytic activities for cleavage after specific amino acids. The 19S regulatory particle, also known as the PA700 particle, caps one or both ends of the 20S cylinder and is itself composed of two subcomplexes, the lid and the base, that have distinct regulatory functions. The base complex is thought to mediate the ATP-dependent unfolding of protein substrates and their translocation to the 20S chamber through a narrow gated pore channel. This gate is formed by the interlacing N-termini of the α subunits thereby preventing nonspecific degradation of cellular proteins. The lid complex

is responsible for the initial recognition, binding and de-ubiquitination of the ubiquitin-tagged polypeptides (Benaroudj et al., 2003; Besche et al., 2009; Glickman & Ciechanover, 2002).

Perhaps the most intriguing question is how the proteasome achieves its high specificity and selectivity. Collective data suggest that two distinct groups of proteins, the E3 ligases and the proteasome ancillary proteins, determine the proteasome specificity through control of the substrate recognition process (Glickman & Ciechanover, 2002). As already mentioned, E3s catalyze substrate recognition prior to their ubiquitination. Such recognition can be accomplished by direct binding of substrates to the appropriate E3 enzyme via their NH_2-terminal residue (the N-end rule pathway). However, in most cases indirect pathways facilitate protein recognition. Alternative modes of recognition include post-translational modifications or allosteric activation of the E3 enzyme, phosphorylation of the substrate and/or E3s, or changes in the conformation state of the substrate protein. Ancillary proteins, such as chaperone proteins or transcription factors, also facilitate substrate recognition through a variety of mechanisms (Glickman & Ciechanover, 2002).

Proteasome regulation is tightly controlled by the 19S complex. The base subcomplex of the 19S particle consists of six different ATPase subunits (Rpts1-6) that promote gate opening upon ATP binding (Demartino & Gillette, 2007). This process also requires binding of the C-terminal tails of Rpt1, Rpt3 and Rpt5 to the 20S through a specific conserved sequence motif (HbYX motif). Interestingly, binding of the C-terminus of Rpt2 acts as a potent repressor of substrate accessibility into the 20S particle. The ATPases Rpt4 and Rpt6 are not involved in gate opening; rather they are thought to regulate the ordered assembly of the 19S complex via binding of their C-terminus tail with external chaperone proteins. Finally, the UPS system can also be regulated at the level of proteasomal activity either by enhancing the catalytic activities or by altering the specificity of the cleavage sites (Gallastegui & Groll, 2010). Alternatively, regulator non-ATPase complexes, such as the PA28 activator, can interact with the one end of the 20S enhancing the overall proteolytic activity of the proteasome, possibly by removing the occlusion at the chamber pore. Since these activators do not bind to polyubiquitin chains, their assembly into the 19S/20S complex results in ubiquitin-independent proteolysis (Demartino & Gillette, 2007).

2.2 The impact of protein aggregation on the UPS

Neurodegenerative diseases like Parkinson's disease, Alzheimer's disease, Huntington's disease and others share a common neuropathological characteristic which is the aggregation of abnormal proteins that fold improperly and impair neuronal function. Accumulation of aberrant proteins could be achieved by several mechanisms including mutations, overproduction or impairment of their clearance. Clearly, such accumulations are indicative of a malfunction of the process of protein turnover since they are not found in healthy individuals. Proteolysis is an important cellular process which involves the tightly regulated removal of unwanted proteins. The ubiquitin-proteasome system (UPS) eliminates mutated or abnormally folded proteins by degradation to prevent their accumulation in the cell and the subsequent formation of inclusion bodies. Most likely, dysfunction of the UPS contributes to the neuropathogenesis of various types of conformational diseases such as the ones mentioned above. It is plausible that failure of protein degradation from the UPS can lead to imbalance in protein homeostasis that could in turn promote the toxic accumulation of proteins which is detrimental for neuronal viability (Sherman & Goldberg, 2001). It is

interesting that in postmortem studies proteasomal activity was significantly reduced in the brain of PD patients (McNaught, Perl, Brownell, & Olanow, 2004).

A rather important question still under investigation is why protein quality control mechanisms, such as the UPS, fail to keep cells free from misfolded proteins. Recent evidence clearly suggests that there is a reciprocal relationship between protein aggregation and the UPS, meaning that one can influence the other. The selective accumulation of oligomeric-prone proteins could be explained by the inability of the UPS to cope with these proteins once their levels have been increased as a consequence of cellular stress. In an elegant study, Bence, et al. reported that protein aggregation directly impaired the function of the UPS (Bence, Sampat, & Kopito, 2001). They demonstrated that expression of two aggregation-prone proteins, the DF508 mutant of cystic fibrosis conductance regulator (CFTR) and an N-terminal fragment of Huntingtin with an expanded polyglutamine repeat (Q103), in Human Embruonic Kidney (HEK) cells was associated with accumulation of the artificial fluorescent proteasome substrate GFPu. Moreover, the authors reported that fluorescence was proportional to the inclusion size, indicating that larger inclusions were associated with more intense proteasome dysfunction. Transient expression of two unrelated aggregation-prone proteins, a huntingtin fragment containing a pathogenic polyglutamine repeat and a folding mutant of cystic fibrosis transmembrane conductance regulator, caused nearly complete inhibition of the UPS.

Because of the central role of ubiquitin-dependent proteolysis in regulating fundamental cellular events such as cell division and apoptosis, these data suggest a potential mechanism linking protein aggregation to cellular disregulation and cell death. The exact species of the aggregate-prone proteins responsible for the dysfunction of the proteasome are unknown. For example, we have not found evidence for accumulation of total α-synuclein after proteasomal inhibition following either pharmacologic treatment or overexpression of the protein, in the cellular systems that we have used (Emmanouilidou, Stefanis et al., 2010; Rideout, Larsen, Sulzer, & Stefanis, 2001; Rideout & Stefanis, 2002). However, α-synuclein does seem to be turned over by the proteasome in other experimental settings (Tofaris, Razzaq, Ghetti, Lilley, & Spillantini, 2003; Webb, Ravikumar, Atkins, Skepper, & Rubinsztein, 2003). Similarly, studies suggest that expanded polyglutamine regions are by themselves intrinsically resistant to degradation by purified proteasomes (Venkatraman, Wetzel, Tanaka, Nukina, & Goldberg, 2004), although not all studies agree to that (Michalik & Van Broeckhoven, 2004).

Although these results suggest that inclusions are the primary deleterious species causing proteasomal dysfunction, it is also possible that other species of the aggregation process, rather than the inclusions themselves, are responsible for inhibition especially if proteasome inhibition itself accelerates inclusion formation. In this respect, *in vitro* studies show that α-synuclein, especially the oligomeric-aggregated conformation, can directly inhibit proteasomal function (Lindersson et al., 2004). These data suggest that the inhibition of proteasomal function observed in the cellular systems with mutant α-synuclein overexpression, are likely due to soluble oligomeric forms of the protein. Clearly, well-formed inclusions are unlikely to interact with the proteasome as substrates. They may, however, sequester chaperones and proteasomes that are recruited during the increased effort of the cell to degrade the misfolded proteins. This may eventually lead to the depletion of the proteasome and of other UPS components from their usual site of action and subsequent UPS dysfunction. Consistent with this idea, Jana NR., et al. 2001

showed that expression of N-terminal Huntingtin with expanded polyglutamine repeats in cells and in transgenic animals led to the incorporation of the 20S proteasome in inclusions (Jana, Zemskov, Wang, & Nukina, 2001). Although the UPS appears as a very attractive option for therapeutic intervention in neurodegenerative diseases, the confirmation of the identity of the toxic proteins species involved remain unresolved. Importantly, as yet there are no strong *in vivo* data linking proteasome inhibition to cellular toxicity.

2.3 α-synuclein impairs UPS function

Several lines of evidence suggest that dysfunction of protein degradation through the UPS may be involved in PD-related neurodegeneration (Lang-Rollin, Rideout, & Stefanis, 2003). *In vivo,* data from studies using the *gad* mouse model directly support this statement. The *gad* mouse lacks expression of murine UCH-L1, a deubiquitinating E3 ligase that is highly abundant in neuronal cells. UCH-L1 has been found in Lewy inclusions which characterize the PD pathology (Lowe, McDermott, Landon, Mayer, & Wilkinson, 1990). These mice display neuronal degeneration with progressive accumulation of ubiquitin-positive inclusions into sensory and motor neurons (Saigoh et al., 1999). The two components of the UPS, UCH-L1 and parkin, are genetically implicated with familial PD (Kitada et al., 1998). Additionally, α-synuclein and DJ-1, two key proteins in PD pathogenesis, have been shown to be degraded by the UPS (Ardley & Robinson, 2005; Miller & Wilson, 2003; Snyder et al., 2003).

A great number of studies propose a link between α-synuclein and the UPS system, although it remains controversial whether the proteasome is responsible for the degradation of this protein (Bennett et al., 1999; Rideout et al., 2001). *In vitro* work has demonstrated that the expression of mutant or wild type α-synuclein is sufficient to cause proteasomal inhibition in neuronal cell culture systems (Petrucelli et al., 2002; Smith et al., 2005; Snyder et al., 2003; Stefanis, Larsen, Rideout, Sulzer, & Greene, 2001; Tanaka et al., 2001). In accordance with these data, α-synuclein can directly bind to Rpt5 *in vitro*, a subunit of the 19S regulatory particle (Ghee, Melki, Michot, & Mallet, 2005). In most of the cases, aggregated or oligomeric α-synuclein had a stronger effect on proteasome function compared with the monomeric form of the protein (Lindersson et al., 2004; Snyder et al., 2003). However, Martin-Clemente *et al.* failed to show UPS inhibition in PC12 cells overexpressing mutant α-synuclein or in α-synuclein transgenic mice (Martin-Clemente et al., 2004).

Given the complexity of the mechanism through which α-synuclein affects proteasome function, we have addressed, in a cellular context, the identity of α-synuclein species that are implicated in UPS dysfunction (Emmanouilidou, Stefanis et al., 2010). We have shown that stable overexpression of wild type or A53T mutant α-synuclein in PC12 cells significantly reduces all proteasome activities (chymotrypsin-like, trypsin-like and caspase-like activities). In this study, the assessment of proteasome activity was performed in functional 26S proteasomes isolated by size exclusion chromatography (SEC) from cell extracts rather than analysing crude cell lysates. This method provides increased accuracy in the measurement of enzymatic activity since it precludes any interference from other common proteinases present in the cell lysate (Rodgers & Dean, 2003). The observed proteasome inhibition was not due to decreased levels of proteasome subunits or abnormal assembly of the complex. Separation of cytosolic proteins by SEC showed that a small

amount of α-synuclein (corresponding only to 0.5% of the total α-synuclein contained in the lysate) co-eluted in the 26S proteasome-containing fractions. Ultrafiltration experiments verified the presence of α-synuclein in these high MW fractions. This co-elution was shown to be specific for α-synuclein and not an artifact of mere protein overexpression or the result of producing high levels of an aggregation-prone protein in the cell model used (Emmanouilidou, Stefanis et al., 2010).

Non-denaturing gel electrophoresis revealed that the α-synuclein co-eluting with the 26S proteasome was oligomeric in nature, migrating between 150 and 450 kDa (Emmanouilidou, Stefanis et al., 2010). Importantly, these species were detected in the cortex homogenates of homozygous transgenic mice which express the human A53T α-synuclein under the control of the prion promoter (Giasson et al., 2002). Further proving the oligomeric state of these specific species, treatment of the cell lysates with Congo Red (CR), a compound known to disrupt preformed oligomeric/aggregated forms of various proteins (Carter & Chou, 1998), significantly reduced α-synuclein burden in the 26S proteasome fractions. In doing so, CR treatment restored the proteasome activity without interfering with the assembly of the 26S complex. In another approach, treatment with the heat shock protein inducer, geldanamycin, resulted in the reduction of α-synuclein species from the proteasome fractions. Again, removal of the proteasome-associated oligomeric α-synuclein led to restoration of proteasome activity (Emmanouilidou, Stefanis et al., 2010). Application of selective proteasomal and lysosomal inhibitors further demonstrated that these specific α-synuclein oligomers are indeed degraded by the proteasome but not the lysosome. Overall, these data indicated that specific oligomeric α-synuclein species of intermediate size are targeted to, and impair the 26S proteasome possibly through a functional interaction.

What this interaction involves is still unclear (Figure 2). One possibility could be the direct binding of α-synuclein oligomers to the active sites of the 20S β-subunits. However, this mechanism would require translocation of the oligomers into the catalytic cylinder through a narrow open-gated channel (Pickart & Cohen, 2004). Alternatively, α-synuclein oligomers may interfere with processes controlled by the 19S complex. Our data (Emmanouilidou, Stefanis et al., 2010) show that proteasome assembly is not affected by the presence of such species. 19S inhibition may involve a physical interaction between α-synuclein and a 19S subunit (Ghee et al., 2005). It is possible that α-synuclein oligomers are targeted to the 26S proteasome by means of their aberrant conformation. In line with this notion, α-synuclein present in the proteasome-enriched fractions lacks ubiquitylation, the targeting signal for degradation via the UPS system (Emmanouilidou, Stefanis et al.). As reported previously, misfolded polypeptides are recognized by the 26S proteasome by the help of certain molecular chaperones (Benaroudj et al., 2003; Glickman & Ciechanover, 2002). In this context, the bulky α-synuclein oligomers may prevent 19S-mediated protein unfolding and translocation due to steric hindrance preventing further interactions of other substrates with the proteasome. Such an idea has also been suggested in the case of oligomeric PrP (Kristiansen et al., 2007). Recently, Machiya Y et al. (2010) showed that Ser-129-phosphorylated α-synuclein is targeted to the proteasome pathway in an ubiquitin-independent manner, in addition to undergoing dephosphorylation (Machiya et al., 2010). Thus, the proteasome pathway may also have a role in the biogenesis of Ser-129-phosphorylated α-synuclein-rich LBs.

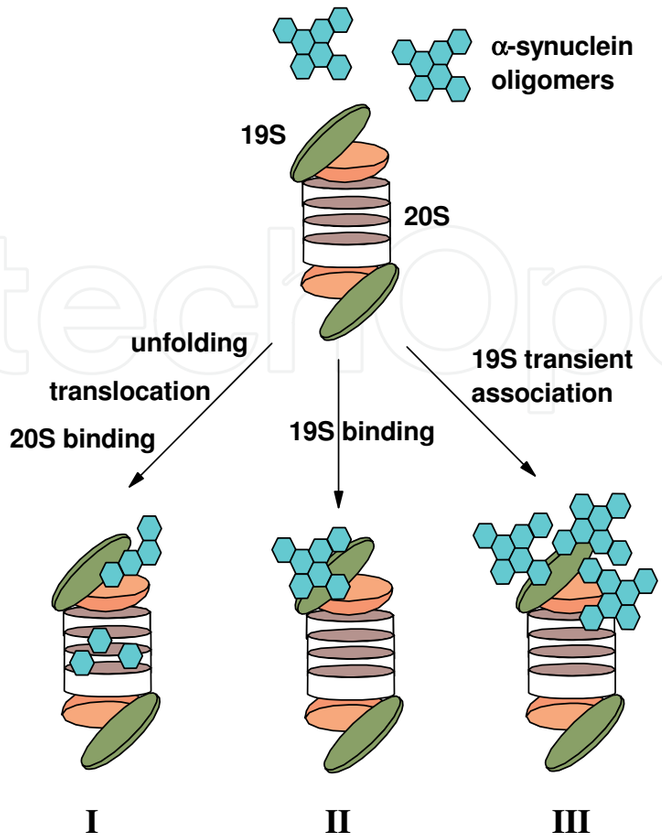

Fig. 2. Schematic illustration of the possible interaction between α-synuclein and the 26S proteasome. α-synuclein can gain access into the interior of the 20S champer and bind to one or more of the catalytic β-subunits. Such changes would interfere with the active site(s) thus decreasing the overall proteolytic activity of the proteasome (I). Alternatively, oligomeric α-synuclein can directly bind to a subunit of the 19S complex thereby inhibiting substrate recognition or gate opening (II). Finally, α-synuclein oligomers can transiently interact with the 19S particle and its function possibly by preventing 19S subunits from obtaining the appropriate conformation. This "clogging" of the 19S cap would result in the cytoplasmic accumulation of other proteins due to their deficient degradation (III).

3. Effects of extracellular α-synuclein on cellular homeostasis

3.1 α-synuclein is detected in biological fluids

Since α-synuclein lacks a signal peptide targeting the protein for ER-mediated exocytosis, it was considered to be primarily localized in the cytoplasm where it would exert its pathogenic effects. However, a number of studies suggest that α-synuclein can be secreted in the medium of cultured cells and is detectable in human biological fluids such as CSF and plasma of PD patients and controls. The first studies to demonstrate detection of α-synuclein in the CSF and plasma utilized biochemical techniques such as immunoprecipitation and western blotting in a small number of human samples (Borghi et al., 2000; El-Agnaf et al., 2003). However, these initial studies failed to show a significant difference in the levels of α-synuclein between PD and healthy subjects.

In an attempt to assess the applicability of α-synuclein concentration in biological fluids as a biomarker for PD, α-synuclein was measured by specific ELISAs that provide higher sensitivity and accuracy. Some of these studies (Mollenhauer et al., 2008; Tokuda et al., 2006), but not all (Ohrfelt et al., 2009), reported significant differences in the levels of α-synuclein in PD and control samples. Since there is substantial evidence indicating that α-synuclein aggregation is central in PD pathogenesis, some other studies focus on the quantification of α-synuclein oligomers in CSF (Tokuda et al., 2010) or plasma (El-Agnaf et al., 2006) using oligomer-specific ELISAs. Overall, there is great variability in the amount of α-synuclein quantified in either blood plasma or CSF. Two basic reasons could account for this discrepancy. First, the ELISA system employed for the measurement of α-synuclein varies between groups in terms of both the antibodies and the detection method used. This results in differences in the specificity and the sensitivity of the measurement. Second, each group does not follow similar protocols for sample collection and processing. Protein integrity and erythrocyte contamination are important parameters related to sample acquisition and processing and should be carefully monitored to assure valid assessment of α-synuclein in biological fluids. While future work is required to establish a correlation between disease and α-synuclein levels in biological fluids, α-synuclein remains an appealing protein to be used as a diagnostic marker for PD.

3.2 Mechanism of α-synuclein release

Numerous studies employing a variety of cell systems reveal a dynamic network of molecular communication between cells, involving secretion. Deciphering the components of this network as well as their biological significance represents a major challenge in the field of neurodegeneration in particular.

In this respect, α-synuclein has been shown to be released from neuronal cells in culture independently of the expression method used; stable overexpression (El-Agnaf et al., 2003), inducible overexpression (Emmanouilidou, Melachroinou et al., 2010), transient transfection (Sung et al., 2005) or viral-mediated expression (H. J. Lee, Patel, & Lee, 2005). The presence of α-synuclein in the conditioned media (CM) of the α-synuclein-expressing cells reflects physiologic secretion of the protein and not an artifact of membrane leakage, since other abundant cytoplasmic proteins are not detected in the CM. The secretion of α-synuclein has been reported to be insensitive to brefeldin A (H. J. Lee et al., 2005), suggesting that it is secreted via an ER/Golgi-independent pathway. In accordance with a vesicular mechanism of secretion, a portion of intracellular α-synuclein has been found in the lumen of vesicles from rat brain homogenates, rat embryonic cortical neurons and human neuroblastoma cells (H. J. Lee et al., 2005). Electron microscopy and density gradient ultracentrifugation suggested that the vesicles containing α-synuclein have morphologies and sedimentation properties similar to the dense core vesicles (H. J. Lee et al., 2005), but their exact identities remain unknown.

In a recent study, treatment of MES cells in culture with aggregated recombinant α-synuclein results in the internalization of the protein which is subsequently released in the extracellular space by rab11a/HSP90-mediated exocytosis (Liu et al., 2009). The mechanism of exocytosis was found to be temperature-sensitive and time-dependent. Part of this internalized protein is also degraded through the lysosomal-endosomal pathway (Liu et al., 2009). Indeed, we recently demonstrated that a non-classical secretory pathway is involved in the physiological and constitutive release of α-synuclein in the extracellular space

(Emmanouilidou, Melachroinou et al., 2010). In this study, α-synuclein was exported in a calcium-dependent manner in association with externalized membrane vesicles that involved in the endocytic pathway (Figure 3).

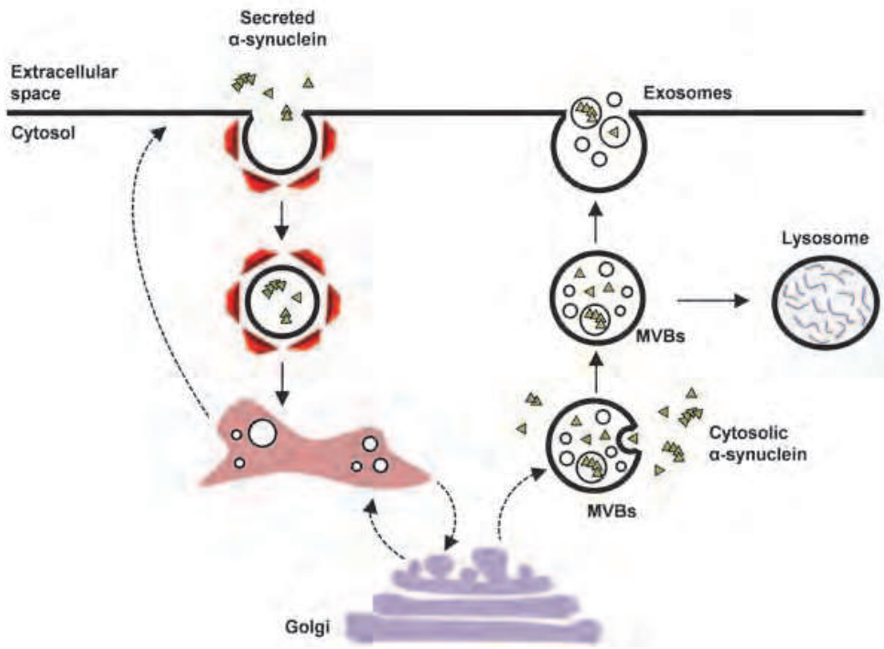

Fig. 3. α-Synuclein transportation through the endocytic pathway. Along with membrane proteins, secreted α-synuclein can enter the cell via endocytosis of clathrin-coated vesicles which fuse with early endosomes. In early endosomes, protein material is either recycled back to the plasma membrane or sorted to MVBs. Cytoplasmic α-synuclein can also enter MVBs at this point via inward budding of the limiting membrane of these vesicles. For protein degradation, MVBs fuse with lysosomes. Alternatively, MVBs can fuse with the plasma membrane releasing their content in the extracellular space as exosomes.

In the first step of the endocytic pathway (Figure 3) internalized proteins via clathrin-coated vesicles are delivered to early endosomes. Proteins are then either recycled back to the plasma membrane or accumulate in multivesicular endosomes, commonly called multivesicular bodies (MVBs). Proteins destined for degradation are sorted into small (40-100 nm in diameter) intraluminal vesicles (ILVs) that are generated by inward budding from the limiting membrane of MVBs (Fevrier & Raposo, 2004; Keller, Sanderson, Stoeck, & Altevogt, 2006). Degradation of the vesicle-associated proteins and lipids is achieved upon fusion of the MVBs with lysosomes. This process allows the cell to remove certain transmembrane proteins and excessive membranes. Alternatively, MVBs can fuse with the plasma membrane releasing ILVs in the extracellular environment as exosomes (Fevrier & Raposo, 2004; Keller et al., 2006).

Exosomal protein content includes cytosolic proteins, heat shock proteins, tetraspanins and transmembrane proteins; proteins originating from mitochondria, ER or nucleus are excluded (Thery, Zitvogel, & Amigorena, 2002). Exosomes share common characteristics, most important of which is that they are delimited in a cholesterol-rich lipid bi-layer containing cytosolic compounds. Most secreted exosomes contain lipid rafts, a characteristic which

signifies selectivity of protein sequestration (Vella, Sharples, Nisbet, Cappai, & Hill, 2008). Interestingly, this bi-layer also carries transmembrane cell adhesion molecules such as integrins, which enable the dynamic communication of the cytoskeleton with the extracellular matrix (ECM) and neighboring cells. The origin of exosomes led to the suggestion that this mechanism was an alternative to autophagic degradation, another means of "discarding" unwanted cytosolic material. Recently, it was found that under certain conditions, exosomes can be biologically active entities, important for intercellular communication (Valadi et al., 2007) and key players in significant biological processes. They are secreted by most cells that have been examined so far including primary neurons (Lachenal et al., 2010). Furthermore, exosomal release by neurons was shown to be dependent on synaptic activity. It is suggested that exosomes could be a mechanism of releasing proteins in the extracellular space in order to be proteolytically processed. Alternatively, exosomes can mediate cell-to-cell communication since they can attach and fuse with membranes of neighbouring target cells transferring exosomal molecules from one cell to another (Thery et al., 2002; Vella et al., 2008). The exosomal pathway thus seems to represent a well-designed mechanism for local and systemic inter-neuronal transfer of information (Smalheiser, 2007).

Several groups have reported that exosomes contain pathological proteins. Biochemical studies from L. Rajendran et al. in 2006 reported that Aβ peptides were indeed found on vesicles positive for specific markers of exosomal identity (Rajendran et al., 2006). This suggested that toxic species of processed Amyloid beta Precursor Protein (APP) are also excreted via exosomes. Most importantly, neuritic plaques are co-localized with exosomal markers, indicating that exosomes are able to act at a distance from their source of generation like amyloidogenic fragments of the APP (Rajendran et al., 2006). Similarly, Fevrier and Raposo demonstrated association of prion protein with exosomes (Fevrier & Raposo, 2004). In this sense, exosomes are the central component of a theory that is starting to gain scientific traction over the past few years. The "Trojan horse" hypothesis is an appealing hypothesis according to which, toxic protein contents of a cell are packed into exosomes, shipped extracellularly and are subsequently received by neighboring cells in the context of cell-to-cell communication (Ghidoni, Benussi, & Binetti, 2008). Upon membrane fusion, exosomal cargo is released and causes spread of disease. Up-regulation of exosome secretion is correlated with conditions that promote protein misfolding and impair proteolysis (Alvarez-Erviti et al.; Eldh et al.; Jang et al.), hence, increase cytosolic cargo of a particular protein. In our study, α-synuclein was shown to be associated with both the exosomal membrane and lumen. Importantly, not only monomeric α-synuclein but also oligomeric forms of the protein were found in our exosomal preparations (Emmanouilidou, Melachroinou et al., 2010), further suggesting that exosomes can indeed carry "potentially toxic" cargo. The finding that α-synuclein can be partly externalized via the exosomal pathway provides a common mechanism for the delivery of a potentially cytotoxic protein in the extracellular space (Figure 3).

Undoubtedly, deciphering networks of intercellular communication is a fascinating field of research. Understanding the physiological mechanisms of exchanging information between cells will allow the identification of new, effective therapeutic targets for late-onset neurodegenerative diseases, including Parkinson's disease. The dynamic nature of neuron-to-neuron interactions leads us to the thought that more enlightening answers are to come from the field of synaptic plasticity and function. So far, data interpretation in most studies focuses on cell-autonomous effects and networks. Perhaps, data interpretation should be

realized under the scope of a three-dimensional neuronal interface in order to uncover the moving forces underlying cell content alterations and communication at a systemic level. The exact role and contribution of exosomes in this dynamic interplay remains to be elucidated.

3.3 Pathologic neuronal interplay mediated by α-synuclein?

Recent studies by Desplats et al. demonstrated that neurons overexpressing α-synuclein can transmit the protein to neural precursor cells in tissue culture and in transgenic animals (Desplats et al., 2009). Interestingly, the precursors were shown to readily uptake and propagate α-synuclein oligomers leading to cellular dysfunction as well as to inflammatory responses. Therapeutic strategies directed at reducing the formation and propagation of α-synuclein oligomers might be critical in developing new treatments for PD and DLB. Among them, considerable effort has been devoted in the last few years to promoting the clearance. This can be achieved by increasing lysosomal activity (autophagy) or degradation with immunotherapy or by pharmacologically blocking α-synuclein aggregation with small organic molecules.

Host-to-graft propagation of α-synuclein pathology has recently been demonstrated with the discovery that fetal dopaminergic neurons (derived from multiple, genetically unrelated donors) that had been implanted into PD patients 11–14 years earlier developed Lewy body pathology immunopositive for α-synuclein and thioflavin-S (Kordower, Chu, Hauser, Freeman, & Olanow, 2008; J. Y. Li et al., 2008). It is possible that these inclusions were formed as a result of the "disease environment" of the PD brain. One plausible explanation is that oligomeric α-synuclein was transmitted from the already affected host neurons to healthy implanted fetal neurons, and induced endogenous α-synuclein to misfold. Such an infective process mechanism is supported by the Desplats et al., data and could be an explanation of the step-wise progression of the disease pathology and the involvement of specific neural pathways as suggested by the Braak staging of PD progression (Braak, Del Tredici et al., 2003). Importantly, Patric Brundin and his group recently demonstrated that this process may be indeed involved in the spread of aggregated synuclein in a manner similar to that suggested for prion diseases (J. Y. Li et al., 2008). The group showed *in vivo* and *in vitro* that α-synuclein not only can transfer from one cell to another, but also that the transferred protein can seed aggregation of α-synuclein in recipient cells. Alternatively, the source of "seeding" might be microparticles, like exosomes containing α-synuclein, which following uptake by healthy "acceptors" accelerate aggregation of endogenous α-synuclein.

Collectively, recent data provide good evidence to speculate that α-synuclein exhibits prion-like behaviour. For example, oligomers from both misfolded prion and α-synuclein can "instruct" the misfolding of the normal proteins (Ferreon, Gambin, Lemke, & Deniz, 2009). Therefore it is possible, that α-synuclein is a prion itself that in a misfolded oligomeric conformation can be transmitted to neighbouring healthy neurons, thus extending the disease process. However, the cause of such an infectious spread has to be more multifactorial. The parkinsonian milieu that causes α-synuclein accumulation and extension of pathology is not yet known and could be the result, of a combination of factors. For example, aging, oxidative stress, and inflammation, may contribute to altered metabolism of α-synuclein, resulting in the pathogenesis of sporadic PD. Furthermore, continuous accumulation of misfolded proteins ,which is a common pathological phenomenon in various neurodegenerative disorders, compromises the ability of the cell's proteolytic

systems. Impairement of lysosomal and proteasomal protein degradation increases the burden of uncleared, unwanted proteins thus promoting their further accumulation and the development of a self-propagating cycle that eventually leads to cell death. Lysosomal function has been reported to decrease in PD patients (Alvarez-Erviti et al.; Chu, Dodiya, Aebischer, Olanow, & Kordower, 2009) and α-synuclein has been shown to be degraded by the lysosome specific mechanism of chaperone mediated autophagy (Cuervo, Stefanis, Fredenburg, Lansbury, & Sulzer, 2004; Xilouri et al., 2008).

Interestingly, Alvarez-Erviti et al. (2010), recently demonstrated that lysosomal inhibition in cells dramatically increased the intracellular and secreted pools of α-synuclein (Alvarez-Erviti et al.). The group further demonstrated a neuron-to-neuron exchange of cytosolic content via exosomes. It could be that under conditions which promote the intracellular accumulation of misfolded proteins, such as lysosomal and proteasomal dysfunction, the homeostatic mechanisms favor the secretion of aggregated forms of α-synuclein. Although the evidence for extracellular α-synuclein internalization in Emmanouilidou (2010) and Alvarez-Erviti (2010) studies are slightly debatable, there are strong indications at both that exosomes are an important mediator of intercellular communication. Exosome exchange between neurons might also represent a way for propagating pathological alterations throughout the brain during neurodegenerative diseases (Aguzzi & Rajendran, 2009; Smalheiser, 2007).

A demonstration that exosomes allow exchange of proteinaceaous or genetic material within the nervous system would provide an an explanation of how pathologies like Alzheimer's Creuzfeld Jacob or Parkinson's diseases, which begin in discrete regions spread overtime to connected regions of the central nervous system. This idea proposes that drugs directed toward reducing the formation and/or facilitating the clearance of misfolded α-synuclein, in order to arrest or reverse the self-propagation process, might represent novel therapeutic interventions for the treatment of PD. In addition, understanding how the neuropathology spreads throughout the nervous system in Parkinson's disease, will open up avenues for new treatments.

3.4 Effects of extracellular α-synuclein on cellular homeostasis

There are several studies addressing the role of extracellular α-synuclein especially in the context of PD pathology. The first indications that high levels of extracellular α-synuclein can impact cell viability came from studies using the recombinant protein. Exogenous addition of recombinant α-synuclein to the cultured medium of neuronal cells significantly decreased the viability of the recipient cells. Cell death was linearly correlated with the concentration of exogenous α-synuclein and was amplified when the applied protein also contained soluble oligomers (Albani et al., 2004; Du et al., 2003; Sung et al., 2001; Zhang et al., 2005). Application of recombinant monomeric or aggregated α-synuclein also revealed that this protein can be readily be uptaken by neuronal cells or even neural stem cells in culture (Ahn, Kim, Kang, Ryu, & Kim, 2006; Desplats et al., 2009; H. J. Lee et al., 2008; Luk et al., 2009; Sung et al., 2001). It has been suggested that the mechanism for α-synuclein internalization involves receptor-mediated endocytosis of the protein (Desplats et al., 2009; H. J. Lee et al., 2008; Sung et al., 2001). It has been proposed that this mechanism specifically mediates the uptake of oligomeric and fibrillar α-synuclein whereas monomeric α-synuclein enters cells via simple diffusion across the plasma membrane. Following internalization, extracellular α-synuclein was shown to move through the endosomal compartment and

finally, to be degraded by lysosomes (H. J. Lee et al., 2008). However, these results were obtained by using very high concentrations of recombinant α-synuclein and cationic liposomes to assist the uptake.

Importantly, recent data using cell-secreted α-synuclein have verified its impact on neuronal survival. Application of conditioned medium containing cell-secreted α-synuclein to neuronal cells induced cell death to the recipient cells (Emmanouilidou, Melachroinou et al., 2010). This toxic effect was concentration-dependent and was conferred synergistically by both oligomeric and monomeric α-synuclein species present in the conditioned medium. In this study, however, there was evidence of very low, if any, α-synuclein uptake by neuronal cells (Emmanouilidou, Melachroinou et al., 2010). Similarly, apoptotic death of neurons, both *in vitro* and *in vivo*, was observed upon their exposure to cell-derived extracellular α-synuclein (Desplats et al., 2009). Secreted α-synuclein, that was readily endocytosed by neurons, was transmitted from one cell to another thereby supporting the idea of a mechanism of pathological propagation in PD (Desplats et al., 2009). Cell-to-cell transfer of α-synuclein was also demonstrated using co-culture systems (Hansen et al., 2010). In fact, this transfer did not require cell contact and was independent of the aggregation state of the protein. Fluorescently-labeled recombinant α-synuclein was uptaken by neuronal cells *in vitro* and *in vivo* via an endocytic mechanism. Altogether, these data demonstrated that endocytosed extracellular α-synuclein can be internalized by recipient cells, interact with the pool of intracellular α-synuclein and seed aggregation (Hansen et al., 2010).

An alternative mechanism of neurodegeneration induced by extracellular α-synuclein may involve the initiation of neuroinflammatory responses. Microglia are resident immune cells that are sensitive to even minor disturbances in the homeostasis of the central nervous system (Soulet & Rivest, 2008). Activation of microglia results in a change in cell morphology (from a ramified to amoeboid shape) accompanied by alterations in surface receptor expression, production of reactive oxygen species (ROS) and release of chemokines and cytokines (Kim & Joh, 2006; Soulet & Rivest, 2008). There is increasing evidence suggesting that extracellularly added recombinant α-synuclein can trigger microglia activation which induces the production of various cytokines, such as IL1β and IL6, and inflammation-related enzymes (Su et al., 2008; Zhang et al., 2005). In fact, microglia activation has been shown to be one of the mechanisms by which α-synuclein induces dopaminergic neurodegeneration, rather than being an epiphenomenon following cell death (Zhang et al., 2007). Further dissection of the pathway of microglia activation, suggested that α-synuclein potentially binds to Mac-1 receptors which subsequently activate PHOX, a ROS-generating enzyme, to produce $O_2^{\bullet}$ ultimately leading to neurotoxicity. Importantly, microglia activation did not require internalization/phagocytosis of α-synuclein by microglial cells (Zhang et al., 2007).To this end, microglial prostaglandin E_2 receptor subtype 2 (EP2) plays a critical role in α-synuclein-induced neurotoxicity partly by decreasing PHOX activation (Jin et al., 2007). Cell-produced α-synuclein also resulted in the activation of primary microglia, leading to the induction of inflammatory signaling pathways (E. J. Lee et al., 2010). It was suggested that α-synuclein-induced microglia activation involves the secretion of MMPs which in turn activate PAR-1 receptor (E. J. Lee et al.). Alternatively, recent data indicate that cell-released α-synuclein can also be internalized by astrocytes thereby producing inflammatory responses both *in vitro* and *in vivo* (E. J. Lee et al., 2010).

4. Conclusion

α-Synuclein is genetically linked to PD. Maintenance of intracellular steady-state concentration of α-synuclein is considered to be a key challenge for neuronal homeostasis and total levels of the protein have been directly linked with PD pathogenesis. Importantly, Genome-Wide association Studies (GWAS) have provided a strong genetic link between α-synuclein and sporadic PD, and clearly point to α-synuclein as being one of the very few genetic loci consistently associated with disease progression. The physiological and aberrant functions of α-synuclein are still under investigation. However, cytoplasmic soluble oligomers/protofibrils of the protein appear to be one of the primary "suspects" in the pathogenesis of PD. Therefore, prevention of α-synuclein aggregation and intervention in the mechanisms of abnormal protein turnover appears to be a highly promising therapeutic target for the treatment of PD as well as other synucleinopathies.

From a therapeutic standpoint, it follows that enhancement of α-synuclein clearance via proteasomal or lysosomal degradation may represent a valid therapeutic intervention for PD. New evidence, suggests that α-synuclein is also physiologically secreted, and as such, it can exert as yet unknown paracrine effects in the brain. Still, the presence and exact levels of α-synuclein in the interstitial fluid in the brain remain to be clarified. Recent clinical observations have suggested that secreted α-synuclein may aggravate PD pathology via a mechanism that underlies cell-to-cell propagation of the protein. It is possible that a dynamic equilibrium between intracellular and extracellular α-synuclein exists, ensuring normal function of neuronal cells. In this respect, dysfunctions in the mechanism(s) regulating extracellular α-synuclein levels, such as mechanisms of secretion or extracellular clearance, may affect neuronal survival. Increases in extracellular α-synuclein may trigger the formation of toxic oligomers in neighbouring neurons and in the extracellular space, and result in inflammatory glia activation, utterly leading to a vicious cycle of neurodegeneration. Along these lines, compounds which block other signalling pathways - switched on as a consequence of microglial activation which may ultimately lead to neuronal death- might also represent new targets for therapeutic intervention. Under this scope, manipulation of regulatory mechanisms that alleviate the extracellular α-synuclein "burden" represents a potential target for the development of novel treatment strategies for PD. It is obvious that α-synuclein can affect neuronal cell homeostasis in numerous ways and at multiple levels. The intrinsic complexity of the neuronal interface may suggest that its actions be considered within the context of non cell-autonomous models and thus be interpreted by taking into account that the nature of communication between brain cells is indeed very dynamic.

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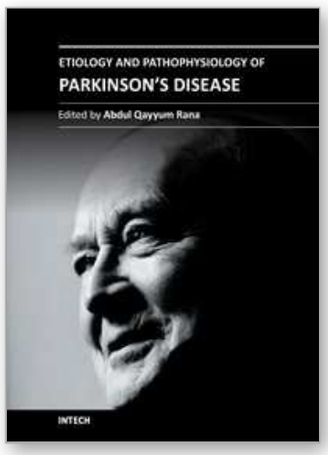

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This book about Parkinsonâ€™s disease provides a detailed account of etiology and pathophysiology of Parkinsonâ€™s disease, a complicated neurological condition. Environmental and genetic factors involved in the causation of Parkinsonâ€™s disease have been discussed in detail. This book can be used by basic scientists as well as researchers. Neuroscience fellows and life science readers can also obtain sufficient information. Beside genetic factors, other pathophysiological aspects of Parkinsonâ€™s disease have been discussed in detail. Up to date information about the changes in various neurotransmitters, inflammatory responses, oxidative pathways and biomarkers has been described at length. Each section has been written by one or more faculty members of well known academic institutions. Thus, this book brings forth both clinical and basic science aspects of Parkinsonâ€™s disease.

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