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Chapter II

α-Synuclein Metabolism and Aggregation in the Pathogenesis of Parkinson's Disease

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Abstract

α-Synuclein (α-Syn) is a major constituent of Lewy bodies, the pathological intracellular inclusion bodies of Parkinson's disease (PD), dementia with Lewy bodies, and multiple system atrophy. The intracellular aggregates or the accumulation of toxic oligomer of α-Syn is implicated in the pathogenesis of these diseases. Various forms of α-Syn, such as monomer, oligomer, and fibril, are metabolized by the ubiquitin-proteasome system, autophagy-lysosome pathway, chaperone-mediated autophagy, and other proteolytic systems. The impairment of these

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systems leads to the accumulation of α-Syn, resulting in neurodegeneration. In this article, we focus on the current knowledge about the involvement of α-Syn metabolism in PD pathogenesis and discuss proteolytic systems as a therapeutic target for PD.

A. Introduction

The accumulation of misfolded/abnormal proteins, potentially toxic polypeptides, severely damages cells (Goldberg, 2003). Although misfolded proteins are generated by various physical stresses (heat, oxidation, salt, and pH) or changes in the protein properties arising from mutations, their accumulation is generally prevented by proteostasis machineries such as chaperone-mediated refolding and degradation (Figure 1). However, potentially toxic species and aggregates are intracellularly accumulated and induce cell death when proteostasis is severely compromised (e.g., aging and disease) (Hartl et al., 2011).

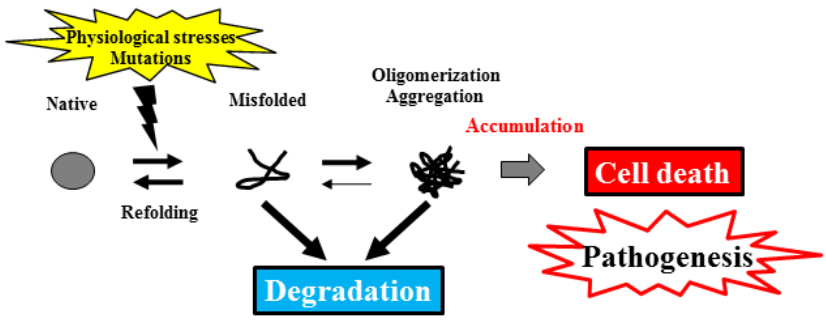

Figure 1. Protein quality control and the pathogenesis of neurodegenerative disease. A subset of cellular proteins misfolds and aggregates in times of physiological stresses (e.g., heat and oxidative conditions) or mutations. These misfolded proteins commonly undergo chaperone-mediated refolding or protein clearance by various proteolytic systems. However, cell death is promoted by the toxicity of misfolded or aggregated proteins when they are accumulated by proteostasis impairment.

The ubiquitin-proteasome system (UPS) and autophagy-lysosome pathway (ALP) are two major cellular mechanisms responsible for the clearance of misfolded and aggregate-prone proteins (Rubinsztein, 2006). Most proteasome substrates are targeted for degradation through the covalent linkage of polyubiquitin chains with a few exceptions (Murakami et al., 1992;

Watanabe & Tanaka, 2011). Ubiquitin conjugation involves ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligase (E3) (Ciechanover, 1994). Poly-ubiquitinated proteins are recognized by the chaperones and the ubiquitin-binding subunits of the regulatory complex of the proteasome and degraded into the catalytic core (Koga et al., 2011). In contrast to UPS, ALP was generally believed to be a non-selective bulk degradation process. However, recent evidence demonstrates that the process also be selective (Kirkin et al., 2009). This selective degradation is mediated by scaffolding adaptor proteins, such as p62/SQSTM1, NBR1, and Alfy (Kraft et al., 2010). UPS and ALP are implicated in several neurodegenerative diseases such as Parkinson's disease (PD) and in polyglutamine disorders including Huntington's disease (HD); their impairment in the brain leads to an accumulation of ubiquitin-positive inclusions (Rubinsztein, 2006).

α-Synuclein (α-Syn) is a natively unfolded protein and a major component of the intraneuronal inclusions called Lewy bodies, a pathological hallmark of PD. Three missense mutations (A30P, E46K, and A53T) and duplication and triplication of the α-Syn locus have been identified in familial PD, suggesting that conformational change and increased intracellular levels in α-Syn are implicated in PD pathogenesis (Forman et al., 2005; Savitt et al., 2006). Recent studies in animal or cultured cell models have disclosed that various proteolytic mechanisms, such as UPS, ALP, chaperone-mediated autophagy (CMA), and other proteases, contribute to α-Syn metabolism (Xilouri et al., 2013). Furthermore, it has been reported that aberrant α-Syn inhibits these proteolytic systems, resulting in further accumulation of α-Syn and neuronal degeneration.

In this article, we highlight recent insights into three themes: (B) aggregation and toxicity of α-Syn, (C) α-Syn metabolism mediated by UPS, ALP, CMA, and other proteolytic systems, and (D) PD therapeutic strategies targeted to α-Syn metabolism.

B. Structural Change in α-Synuclein (α-Syn)

α-Syn is a protein of 140 amino acids that consists of three regions: (i) the N-terminal amphipathic region (residues 1–60); (ii) the hydrophobic self-aggregating sequence known as NAC (non-Aβ component, residues 61–95); and (iii) the acidic C-terminal region (residues 96–140). Secondary structure

analysis demonstrates that α-Syn is an intrinsically disordered protein under physiological conditions (Orcellet & Fernandez, 2011). A marked increase in the levels of truncated, full-length, oligomeric forms, and aggregates of α-Syn is observed in the substantia nigra (SN) of the sporadic PD brain (McNaught & Olanow, 2006). Moreover, three missense mutations (A30P, E46K, and A53T) and duplication and triplication of the α-Syn locus have been identified in familial PD, suggesting that conformational change and increased intracellular levels in α-Syn are implicated in PD pathogenesis (Forman et al., 2005; Savitt et al., 2006). α-Syn aggregation involves a multi-step nucleated polymerization process via the formation of dimmer, oligomer, protofibril, and fibril (Figure 2). Although an α-Syn fibrillar form was initially suspected as the main cause of neurodegeneration, recent evidence of potential neurotoxic quaternary structure intermediates that precede fibril formation, referred to as oligomer and protofibril, and the finding that several pathogenic mutations promote these formations suggest that the oligomer and protofibril rather than the fibrils are the pathogenic species (Volles & Lansbury, 2003; Lansbury & Lashuel, 2006). α-Syn oligomer enriched in a cross-β structure is 4 nm in height and 20 nm wide, and its molecular weight is estimated in the range of 140–800 kDa (Lundvig et al., 2005). In cell culture models, α-Syn oligomer is associated with enhanced toxicity as measured by the release of adenylate kinase from damaged cells, adenosine triphosphate levels, or caspase 3/7 activity (Kalia et al., 2013). A similar result is obtained in animal models. A transgenic fly bearing an α-Syn mutant with increased propensity to form soluble oligomers shows loss of dopaminergic neurons and motor deficits (Karpinar et al., 2009). These studies indicate that the accumulation of α-Syn toxic species is involved in PD pathogenesis, and that the prevention of this accumulation is important in PD treatment.

C. α-Syn Metabolism

C-i. Ubiquitin-Proteasome System (UPS) and Sumoylation

The UPS commonly functions in the degradation of damaged or unnecessary proteins, which are tagged with a polyubiquitin chain by ubiquitin ligases (Murata et al., 2009). The 26S proteasome is an unusually large, multi-subunit proteolytic complex, consisting of the 20S proteasome, a central catalytic machine, and 19S regulatory particles (Murata et al., 2009). The fact that Lewy bodies contain UPS-associated proteins such as ubiquitin,

proteasomal subunits, and ubiquitin C-terminal hydrolase (UCH-L1) has predicted the involvement of α-Syn and a dysfunctional UPS in PD pathogenesis (Lowe et al., 1990; Kwak et al., 1991; Ii et al., 1997). α-Syn has been found to undergo ubiquitination by some E3 ubiquitin ligases such as UCH-L1, parkin, ubiquitin-protein ligase E3A (UBE3A/E6-AP), and seven in absentia homolog (SIAH). Mutations in UCH-L1 and parkin genes are also known to be responsible for familial forms of PD (Kitada et al., 1998; Leroy et al., 1998). Nonaka et al. showed that α-Syn fibril is less ubiquitinated than the soluble form and that the major ubiquitination sites of α-Syn fibril are different from those of the soluble form (Nonaka et al., 2005). In addition, other studies revealed that phosphorylation of α-Syn at Ser^{129} was required for its ubiquitination (Hasegawa et al., 2002). Despite these facts, it remains unclear whether ubiquitination is indispensable for proteasomal degradation of α-Syn. Although many studies have indicated ubiquitin-dependent proteasomal degradation of α-Syn, there is some evidence showing that α-Syn is degraded by a proteasome in a ubiquitin-independent manner. For example, different reports show that 20S and 26S proteasomes can degrade non-ubiquitinated α-Syn *in vitro* (Tofaris et al., 2001; Liu et al., 2003). Moreover, part of α-Syn purified from Lewy bodies is modified by monoubiquitination, which can serve important functions in many biological processes such as the regulation of gene transcription, protein trafficking, and DNA repair (Hasegawa et al., 2002; Tofaris et al., 2003). This evidence speculates about the involvement of α-Syn monoubiquitination in a signal for vesicular compartments trafficking because α-Syn is localized to a synaptic vesicle (Kahle et al., 2000). Similarly to ubiquitin, the small ubiquitin-like modifier (SUMO) proteins are also covalently conjugated to α-Syn (Pountney et al., 2005; Dorval & Fraser, 2006). SUMO acceptor sites in α-Syn are mapped at Lys^{96} and Lys^{102}. Intriguingly, an α-Syn mutant of sumoylation sites shows increased propensity for aggregation and cytotoxicity in a cell-based assay and increased cytotoxicity in dopaminergic neurons of the SN *in vivo*, suggesting that sumoylation of α-Syn promotes protein solubility (Krumova et al., 2011).

Although the necessity of α-Syn ubiquitination for its degradation remains obscure, treatment with a proteasome inhibitor has led to neurodegeneration and the formation of ubiquitin- and α-Syn-positive inclusions in cultured cells or primary neurons (Bennett et al., 1999; McLean et al., 2001; Rideout et al., 2004). In addition, a progressive model of PD in rats can be generated by systemic administration of PSI, a proteasome inhibitor. Postmortem studies of this rat model show neuronal loss in the SN, as well as in the locus ceruleus, nucleus basalis of Meynert, and dorsal motor nucleus of the vagus (McNaught

et al., 2004). Bedford et al. established a conditional knockout mouse of proteasomal subunit PSMC1 (Rpt2), an ATPase of the 19S regulatory complex (Bedford et al., 2008). This mouse displays 26S proteasome depletion in targeted neurons (the SN or forebrain), in which the 20S proteasome is not affected. Remarkably, α-Syn- and ubiquitin-positive inclusions developed in the brain regions with impaired 26S proteasome function. In the forebrain-restricted knockout model, progressive neurodegeneration and learning deficits were observed, while in the knockout restricted to SN neurons, striking neurodegeneration and inclusion body formation occurred (Bedford et al., 2008).

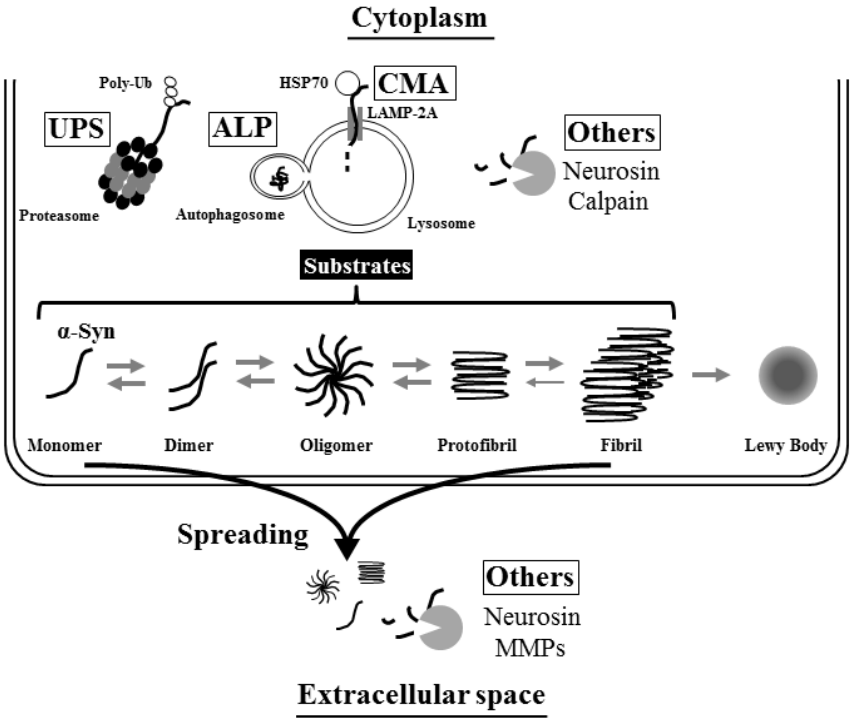

Figure 2. Structural properties and clearance mechanism of α-Syn molecular species. α-Syn undergoes structural changes in the formation process of Lewy bodies. These intermediates (dimers, oligomers, protofibrils, and fibrils) are characterized by their structures. In particular, oligomeric species take on distinct forms such as rings or stellate spheres. Various α-Syn molecular species including α-Syn monomers are degraded by UPS, ALP, CMA, and other proteases both intra- and extra-cellularly.

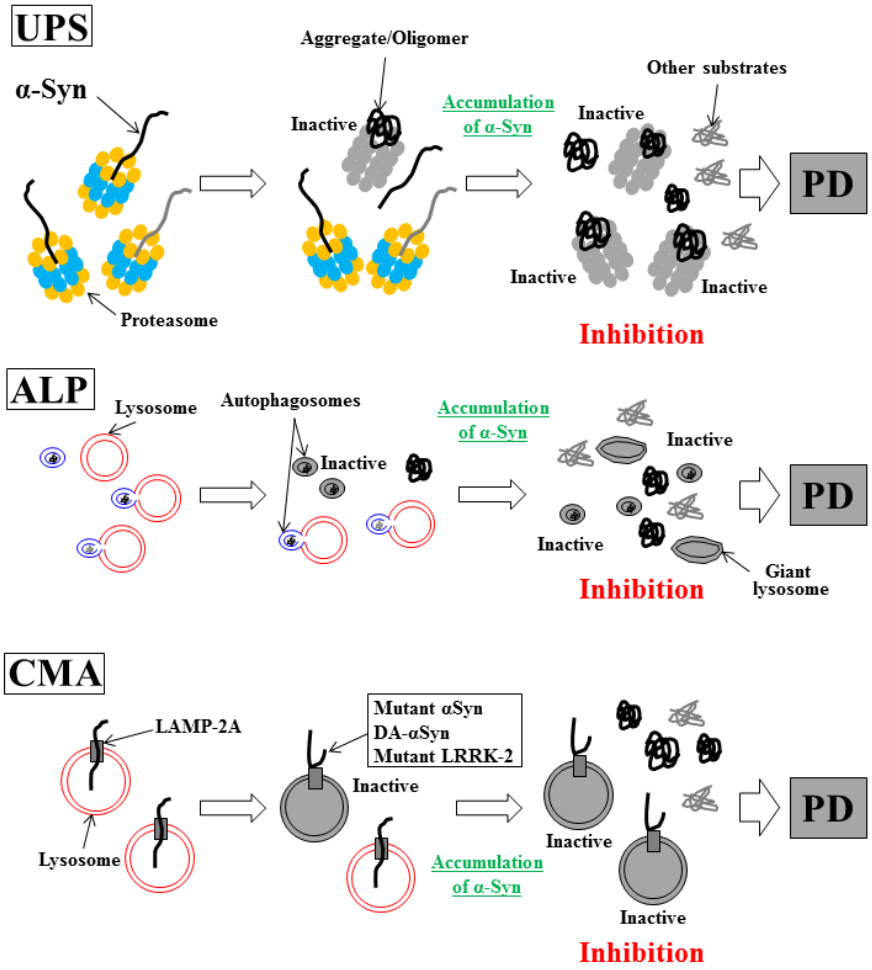

Figure 3. The models of proteostasis impairment by α-Syn aberrant forms in the pathogenesis of PD. α-Syn aberrant forms, such as oligomers, fibrils, PD mutants, and dopamine-modified forms (DA-α-Syn), inhibit several proteolytic systems. In the case of UPS, α-Syn fibril and Lewy bodies resist full entry and cause steric occlusion of the proteasome cavity, resulting in UPS dysfunction. This dysfunction leads to a further increase in α-Syn aberrant forms and neurodegeneration. Similarly, ALP is also affected by α-Syn fibrils. Although α-Syn fibrils are sequestrated into autophagosomes, they cause accumulation of immature autophagosomes. Consequently, overall macroautophagy is impaired by reducing autophagosome clearance, which may accelerate cell death. In the case of CMA, α-Syn PD mutants and DA-α-Syn inhibit its activity. In addition, it is also severely impaired by mutant proteins of another PD–associated protein, LRRK2.

What kinds of α-Syn forms are targets for proteasomal degradation? Soluble aggregation intermediates of α-Syn such as monomer, dimmer, and oligomer would be potential target substrates for proteasome because they can readily enter the 20S proteasome (Figure 2). In contrast, insoluble fibril and Lewy body may resist full entry and cause steric occlusion of the proteasome cavity. Indeed, aggregated α-Syn strongly inhibits proteasome activity *in vitro* (Snyder et al., 2003; Lindersson et al., 2004). This result suggests that attenuation of UPS activity causes the accumulation of toxic oligomers and aggregates of α-Syn, which synergistically impairs UPS proteolytic function, resulting in neurodegeneration (Figure 3).

C-ii. Autophagy-Lysosome Pathway (ALP)

Macroautophagy (hereafter called autophagy) is a highly conserved process in eukaryotes. The cytoplasm, including excess or aberrant organelles, is sequestered into double-membrane vesicles (termed an autophagosome). This autophagosome is delivered to the lysosome, for breakdown and recycling of the resulting macromolecules (Yorimitsu & Klionsky, 2005). In yeast, the isolation of autophagy-defective mutants has enabled the study of autophagy at the molecular level (Takeshige et al., 1992; Tsukada & Ohsumi, 1993), and 31 autophagy-related genes (Atgs) are identified; among them, at least 18 genes (Atg1–10, Atg12–14, Atg16–18, Atg29, and Atg31) are essential for normal autophagosome formation (Mizushima, 2007). The sequestration of substrates into autophagosomes is initiated by the formation of the phagophore (also called the isolation membrane). Various substrates are selectively/non-selectively engulfed and completely sequestrated by the elongating phagophore, and then they are delivered to the lysosome for degradation. Recent studies reveal that adaptor proteins (cargo receptors), such as p62/SQSTM1, NBR1, NDP52, and optineurin, are required for selective autophagosomal degradation (Isakson et al., 2013). Adaptor proteins are able to interact directly with both substrates and an Atg8/LC3 family member involved in autophagosome formation, resulting in a selective sequestration of substrates.

In vitro and *in vivo* studies have demonstrated that ALP is also involved in α-Syn metabolism. Autophagy inhibition with bafilomycin A1 (Baf A1) or 3-methyladenine led to an obvious increase of α-Syn levels in PC12 cells expressing α-Syn A53T mutant (Webb et al., 2003). In accordance with *in vitro* data, the accumulation of α-Syn is observed in the brain of α-Syn-EGFP

transgenic mice treated with Baf A1 (Ebrahimi-Fakhari et al., 2011). Conversely, clearance of both α-Syn wild-type (WT) and mutants in cultured cells is enhanced by rapamycin treatment, which activates autophagy (Webb et al., 2003). However, it is not yet clear what kinds of α-Syn forms are the preferred substrates for ALP (Figure 2). What is known is that the exogenously introduced α-Syn fibrils can be a target for ALP. As shown in Figure 4, α-Syn fibrils are subsequently associated with p62 and LC3, which are then engulfed and digested by lysosomes when its fibrils are introduced into HEK293 cells with a transfection reagent (Watanabe et al., 2012). Conversely, direct evidence for ALP-mediated clearance of a non-ubiquitinated α-Syn monomer or oligomer has not been reported yet. However, the above-mentioned study that the α-Syn level is elevated in Baf A1-treated mice raises the possibility of these non-ubiquitinated forms as a potential target for ALP (Webb et al., 2003).

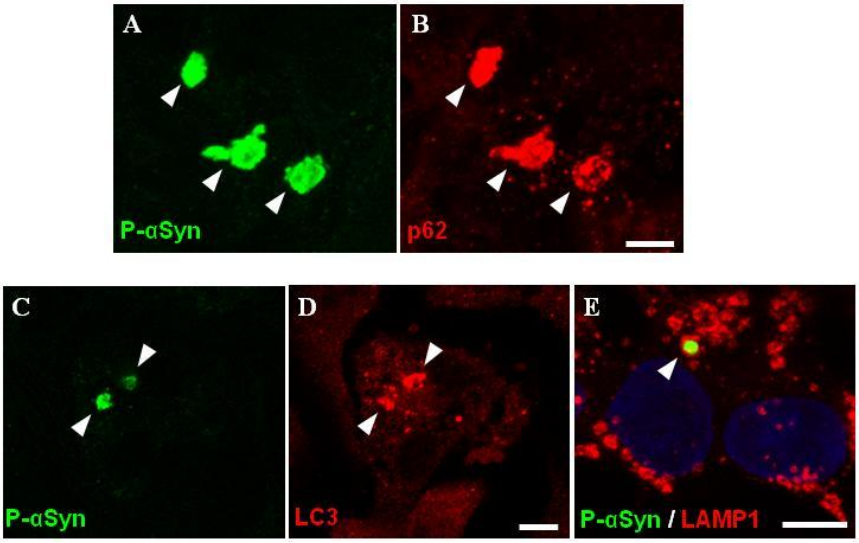

Figure 4. Clearance of α-Syn fibrils by ALP. To examine colocalization of p62 (A and B), LC3 (C and D), or LAMP-1 (E) with α-Syn fibrils, α-Syn fibrils were exogenously supplied to cultured cells, followed by immunocytochemical analysis (Watanabe et al., 2012). Phosphorylated α-Syn-positive fibrils (P-αSyn) are merged to the autophagic adaptor protein p62, the autophagosome marker LC3, or the lysosome marker LAMP-1 (arrowheads). Blue, DAPI. Scale bar, 10 μm.

It was recently demonstrated that ALP activity is impaired by Lewy body-like α-Syn inclusions. When fibrillar α-Syn is exogenously introduced into α-Syn-expressing cells, Lewy body-like inclusions are intracellularly formed (Luk et al., 2009; Volpicelli-Daley et al., 2011). As shown in Figure 3, the autophagy analysis using this culture model reveals that Lewy body-like α-Syn inclusions cause accumulation of immature autophagosomes and morphological changes in lysosomes, resulting in autophagy impairment (Tanik et al., 2013). Its severe impairment is implicated in PD pathogenesis.

C-iii. Chaperone-Mediated Autophagy (CMA)

CMA is an intracellular catabolic pathway that mediates the selective degradation of cytosolic proteins in lysosomes (Arias & Cuervo, 2011). CMA substrates possess a KFERQ-like motif that is recognized by a molecular chaperone, the heat-shock cognate protein of 70 kDa (Hsc70) (Chiang et al., 1989). Biochemical analysis shows that approximately 30% of cytosolic proteins contain this motif (Chiang & Dice, 1988). Hsc70-bound substrates are delivered to the surface of lysosomes, where they bind to a CMA receptor, the lysosome-associated membrane protein type-2A (LAMP-2A) (Cuervo & Dice, 1996). After unfolding, the substrate proteins are translocated into the lysosomal lumen in an ATP-dependent manner and finally degraded by lysosomal proteases (Arias & Cuervo, 2011).

Cuervo et al. demonstrated that α-Syn was a substrate for CMA (Cuervo et al., 2004). A potential CMA recognition motif exists in the α-Syn sequence ($_{95}$VKKDQ$_{99}$). In fact, the substitution of this motif with alanine ($_{95}$VKKAA$_{99}$) reduces the association of α-Syn with lysosomes (Cuervo et al., 2004). Interestingly, two pathogenic mutants of α-Syn (A53T and A30P) can bind to LAMP-2A but are poorly translocated into lysosomes, resulting in the disturbance of the uptake and degradation of other CMA substrates (Cuervo et al., 2004). Moreover, *in vitro* lysosomal uptake analysis reveals that dopamine-modified α-Syn (DA-α-Syn) is poorly translocated inside lysosomes and blocks degradation of other substrates by the CMA pathway, even though the binding of DA-α-Syn to lysosomes was significantly higher than that of unmodified α-Syn (Martinez-Vicente et al., 2008). This observation suggests that reduction in CMA by DA-α-Syn may contribute to selective degeneration of dopaminergic neurons in PD. Recently, it has been shown that another PD-associated protein, leucine-rich repeat kinase 2 (LRRK2), also undergoes CMA proteolysis (Orenstein et al., 2013). As is the case in α-Syn, LRRK2 PD

mutants (G2019S, D1994A, and R1441C) cause a reduction in CMA activity (Orenstein et al., 2013). Furthermore, it is noteworthy that α-Syn markedly colocalizes with LAMP-2A and accumulates in neurons differentiated from induced pluripotent stem cells (iPS) of PD patients with a LRRK2-G2019S mutation (Orenstein et al., 2013). These findings predict that an α-Syn/LRRK2 self-perpetuating inhibitory effect on CMA would underlie toxicity in PD by compromising the degradation of α-Syn (Figure 3).

C-iv. Other Proteolytic Systems

Besides proteasome and autophagy, α-Syn is known to undergo proteolysis by various proteases. Intracellularly, calpain-1 and neurosin can cleave α-Syn at specific sites. Two major forms, calpain-1 and -2, are a Ca^{2+}-activated cytosolic cysteine protease and regulated by the specific endogenous inhibitor calpastatin (Wendt et al., 2004). They are involved in many processes such as skeletal remodeling, cell proliferation, differentiation, apoptosis, platelet activation, and membrane fusion (Govindarajan et al., 2008). Calpain activation seems to be implicated in PD pathogenesis. Increased expression of calpain-2 is observed in the SN and locus coeruleus of patients with PD (Mouatt-Prigent et al., 1996). Biochemical analysis demonstrated that calpains cleave α-Syn including mutant or fibril forms at multiple sites (Mishizen-Eberz et al., 2003; Mishizen-Eberz et al., 2005; Dufty et al., 2007). Interestingly, cleavage sites of α-Syn by calpain-1 are different among α-Syn forms. Calpain-1 cleaves WT α-Syn predominantly after amino acid 57 and within the NAC region (Figure 5). In contrast, it cleaves fibrillized α-Syn primarily in the region of amino acid 122 to generate fragments like those that increase susceptibility to dopamine toxicity and oxidative stress (Figure 5). Further, while calpain-1 cleaves WT α-Syn after amino acid 57, this does not occur in mutant A53T α-Syn (Figure 5). The difference in cleavage sites suggests that calpain-1-mediated cleavage of native WT α-Syn could protect against fibrillization by cleaving within the NAC region, altering the structure of this region, whereas cleavage of fibrillized α-Syn in the C-terminal region could further enhance the fibrillization process (Mishizen-Eberz et al., 2003; Mishizen-Eberz et al., 2005).

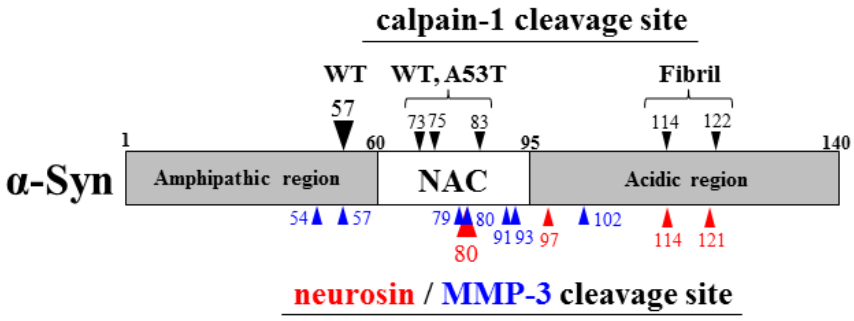

Figure 5. α-Syn cleavage sites by calpain-1, neurosin, and MMP-3 proteases. α-Syn has an N-terminal amphipathic region (1–60 amino acids), a non-amyloid-β component (NAC) domain (61–95 amino acids), and an acidic region containing several phosphorylation sites (96–140 amino acids). Its cleavage sites by each proteases are indicated by arrowheads (calpain-1; black-filled arrowheads, neurosin; red-filled arrowheads, MMP-3; blue-filled arrowheads) and numbers of amino acids. α-Syn WT-, A53T-, or fibril-specific cleavage sites by calpain-1 are also indicated at the top.

Serine protease neurosin is also involved in proteostasis of α-Syn. Neurosin belongs to the tissue kallikrein gene family and is also referred to as kallikrein 6/KLK6 (Watanabe & Tanaka, 2012). The degradation of α-Syn by this protease occurs intra- and extra-cellularly. Iwata et al. found that intracellular α-Syn is degraded by neurosin released from mitochondria into the cytosol under stressed conditions (Iwata et al., 2003). Conversely, when cultured cells expressing neurosin are incubated in a culture medium containing α-Syn, this degradation occurs in the extracellular space (Tatebe et al., 2010). Recent studies have demonstrated that α-Syn is secreted into the extracellular space via exocytosis and may move from neuron to neuron to propagate the α-Syn pathology (Lee et al., 2005; Desplats et al., 2009; Luk et al., 2012). Potential cleavage sites within α-Syn by neurosin were identified by a cleavage experiment *in vitro* (Kasai et al., 2008). Mass spectrometric analysis demonstrated that the major cleavage site is localized between Lys^{80} and Thr^{81} within the NAC region (Figure 5). In addition, the PD-associated mutant A30P and phosphorylated α-Syn are more resistant to degradation by neurosin (Kasai et al., 2008). This cleavage shows a protective effect against synucleinopathies including PD and dementia with Lewy bodies (DLB). When the neurosin gene is delivered to WT α-Syn transgenic (Tg) mice using a lentivirus vector, hippocampal neuronal loss of these mice is rescued (Spencer et al., 2013), suggesting that neurosin digestion of α-Syn reduces the generation of α-Syn toxic forms.

Furthermore, α-Syn is extracellularly cleaved by various matrix metalloproteinases (MMPs), such as MMP-1, -2, -3, and -9 (Sung et al., 2005; Levin et al., 2009). Sung et al. determined cleavage sites of *in vitro*-digested α-Syn with MMP-3 using MALDI-TOF mass spectrometry (Sung et al., 2005). As α-Syn is gradually broken down by MMP-3 from its C-terminal end, it is likely that MMP-3 would have an effect on the aggregation of α-Syn. In fact, protein aggregation of intact α-Syn is induced in the presence of the MMP-3 digested α-Syn fragments, resulting in increased cytotoxicity (Sung et al., 2005).

Taken together, some forms of α-Syn fragment cleaved by various proteases may be implicated in the pathogenesis of α-Synucleinopathies including PD, but others may contribute to a protective effect against their diseases. To achieve a clinical application in targeting proteases, it is very important to characterize individual proteolytic fragments of α-Syn.

D. Potential Targets for Parkinson's Disease (PD) Treatment

As described above, α-Syn proteostasis has been noted as a novel therapeutic target for PD. In particular, the enhancement of lysosomal function including ALP and CMA would be a promising approach. Indeed, many research groups have performed drug screening for neurodegenerative diseases such as HD and PD, as a target for these clearance systems. Rapamycin, an immunosuppressive drug, is well known to activate ALP by inhibition of the mammalian target of rapamycin (mTOR). In cultured cells, ALP activation by rapamycin increased clearance of α-Syn (Webb et al., 2003). Similarly, the same group found that trehalose, a disaccharide, also induces ALP activity via an mTOR-independent pathway, followed by accelerated α-Syn clearance (Sarkar et al., 2007a). These studies suggest that autophagy inducers would be a new approach in the treatment of PD. Novel autophagy inducers have already been identified using yeast and cultured cells. From 50,729 compounds screened, Sarkar et al. found three kinds of small molecules that activate ALP either independently or downstream of the target of rapamycin. These compounds effectively enhance the clearance of ALP substrates such as A53T α-Syn and mutant huntingtin in cultured cells (Sarkar et al., 2007b). Besides autophagy induction by these compounds, transcription factor EB (TFEB) may also be a target for therapy because TFEB overexpression

stimulates autophagy and has an effect on neuroprotection via the clearance of α-Syn oligomers (Decressac et al., 2013). Similarly, CMA inducers are also identified, and the glucose-6-phosphate dehydrogenase inhibitor, 6-aminonicotinamide, and geldanamycin (an Hsp90 inhibitor) have the ability to activate CMA (Finn et al., 2005). However, whether α-Syn clearance is accelerated by these CMA-activating drugs remains to be verified

Alternatively, the direct enhancement of lysosomal function would be an effective PD treatment. Recently, other lines of evidence have also implicated the Gaucher disease (GD)-associated lysosomal enzyme glucocerebrosidase (GCase) in the pathogenesis of PD and related synucleinopathies. Indeed, mutations in GBA1, the GCase encoding gene, are known as a genetic risk factor for PD (Sidransky et al., 2009). In addition, down-regulation of GCase activity compromised lysosomal degradation and evoked α-Syn accumulation followed by neuronal toxicity in human iPS neurons derived from GD fibroblasts (Mazzulli et al., 2011). Interestingly, overexpression of α-Syn inhibits the intracellular trafficking of GCase, leading to its decreased lysosomal activity. Because of this inhibition, a pathogenic amplification loop of α-Syn accumulation and GCase dysfunction occurs, resulting in PD pathogenesis (Mazzulli et al., 2011). Thus, specific treatments that promote targeting of GCase to lysosomes are expected to diminish the formation of toxic α-Syn oligomers and break the pathogenic cycle of α-Syn aggregation and toxicity in PD.

Furthermore, the reduction of extracellular α-Syn levels is also useful in therapy for PD. As described above, the infection of a lentiviral vector expressing neurosin in the brain of α-Syn transgenic mice reduces the accumulation of α-Syn and rescues neurodegenerative alteration of its transgenic mice (Spencer et al., 2013). This may provide the way for the development of novel gene therapies for PD. Conversely, immunotherapy is also a very promising approach for the clearance of extracellular and plasma membrane-bound α-Syn. When the antibody against α-Syn is injected into the brain of α-Syn transgenic mice, antibody-assisted clearance of α-Syn occurs mainly in microglia through the Fcγ receptor (Bae et al., 2012). Another study shows that the original monoclonal antibody against the C-terminus of α-Syn is able to cross into the central nervous system and binds to cells that display α-Syn accumulation, and the antibody-antigen complex is then endocytosed and degraded by ALP (Masliah et al., 2011).

E. Perspective

We described here that various proteolytic systems are involved in α-Syn clearance, which are targets for drug development in PD and other synucleinopathies. For the establishment of the specific exclusion of pathogenic α-Syn forms, however, it is essential to identify the recognition mechanism of pathogenic α-Syn for individual proteolytic systems. Furthermore, a declining proteostasis accompanies aging and initiates an irreversible process of toxic protein aggregation and cell death (Douglas & Dillin, 2010). This implies that the reduction of α-Syn metabolism with aging contributes to PD pathogenesis. Therefore, an elucidation of the reduction mechanism of proteostasis with aging is also an important issue.

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