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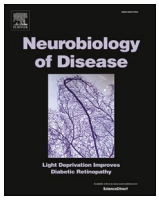

Review

The αSynuclein half-life conundrum

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ABSTRACT

αSynuclein (αSyn) misfolding and aggregation frequently precedes neuronal loss associated with Parkinson's Disease (PD) and other Synucleinopathies. The progressive buildup of pathological αSyn species results from alterations on αSyn gene and protein sequence, increased local concentrations, variations in αSyn interactome and protein network. Therefore, under physiological conditions, it is mandatory to regulate αSyn proteostasis as an equilibrium among synthesis, trafficking, degradation and extracellular release.

In this frame, a crucial parameter is protein half-life. It provides indications of the turnover of a specific protein and depends on mRNA synthesis and translation regulation, subcellular localization, function and clearance by the designated degradative pathways. For αSyn, the molecular mechanisms regulating its proteostasis in neurons have been extensively investigated in various cellular models, either using biochemical or imaging approaches. Nevertheless, a converging estimate of αSyn half-life has not emerged yet.

Here, we discuss the challenges in studying αSyn proteostasis under physiological and pathological conditions, the advantages and disadvantages of the experimental strategies proposed so far, and the relevance of determining αSyn half-life from a translational perspective.

1. Introduction

Impaired regulation of protein homeostasis in neurons is a common theme in neurodegenerative diseases (Ross and Poirier, 2004). With aging, alterations in various steps and components of the quality control pathways lead to the progressive buildup of misfolded and aggregated proteins. Until their accumulation overwhelms the degradation capacity of the challenged neurons, with detrimental neurotoxic outcomes (Kaushik and Cuervo, 2015). In this frame, αSynuclein (αSyn) represents a paradigm of misfolded protein accumulation associated with neurodegeneration. The first observation of aggregated αSyn in neurons emerged from the analysis of brain autoptic samples of patients affected by Parkinson's Disease (PD) and Dementia with Lewy Bodies (DLB) (Spillantini et al., 1997). In the study by Spillantini in 1997, the presence of fibrillar aggregates of αSyn was detected by the immunostaining with a specific antibody against the protein in structures identified as Lewy Bodies (LBs) and Lewy Neurites (LNs). Since then, point mutations on αSyn sequence and gene multiplication have been associated with familial cases of PD. Similarly, the deposition of αSyn amyloid fibrils has been found also in the brains of patients affected by Multiple System Atrophy (MSA), which was then included in the so called Synucleinopathies (Goedert et al., 2017).

Under pathological conditions, several factors can hinder αSyn proteostasis i.e., alterations of *SNCA* gene, failure of protein degradation systems, oxidative stress, toxin exposure, post-translational modifications (PTMs), protein or lipid interactions (Stefanis et al., 2019). In other words, any variation of αSyn sequence, local concentration and interactome, may potentially lead to αSyn accumulation and further aggregation. αSyn monomers may assemble in small oligomers that can serve as nucleating seeds for the evolution into proto-fibrillar structures and finally mature amyloid fibrils that are found in LBs. Otherwise, the small oligomers can follow an off-fibrillar pathway generating aggregates with heterogeneous structure, dimension and stability (Mehra et al., 2019).

An efficient αSyn turnover is therefore essential to maintain the homeostatic regulation of αSyn concentration in neurons. Otherwise, a defective αSyn quality control could dramatically affect its accumulation and pathological aggregation, as it would constantly leave a residual pool of monomeric αSyn prone to alterations and modifications.

From the experimental point of view, several studies attempted to determine αSyn half-life as a read-out of the regulation of its proteostasis

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in neurons, taking advantage of a plethora of cellular models and experimental approaches. Nevertheless, little consensus has been reached, leaving unsolved the conundrum of the actual value of αSyn half-life and its potential as a parameter to monitor αSyn turnover under physio-pathological conditions.

In this perspective, we discuss how the complexity of αSyn proteostasis challenges the determination of a univocal half-life value, the limitations presented by the currently available experimental strategies and whether αSyn basal turnover rate does determine the intrinsic propensity to aggregation.

2. The complex regulation of αSynuclein proteostasis in neurons

αSyn is a small protein of 140 amino acids, encoded by the *SNCA* single gene of seven exons, located in chromosome 4q22.1 (Shibasaki et al., 1995). It was independently described by several research groups in the late 1980's in studies on rat brain cDNA libraries and working on the electric organ of *Torpedo Californica* (Rosen et al., 2020). It was then identified by Maroteaux in 1988 as a neuron-specific protein localized in the synapses and the nucleus (Maroteaux et al., 1988). Since the discovery of αSyn role in PD pathology more than two decades ago, the molecular mechanisms regulating αSyn proteostasis in neurons have been extensively investigated, revealing a quite intricate scenario which results from the simultaneous interplay among several cellular processes, here summarized in Fig. 1 and briefly described.

αSyn expression. αSyn is highly expressed in the neurons of the central nervous system, particularly in the hippocampus, basal ganglia, striatum, olfactory bulb and cerebellum (Kim and Lee, 2008). αSyn expression starts in the late embryonic stages in vertebrates (Burré, 2015), under the regulation of transcriptional factors i.e., nerve growth factor (NGF), Nurr-1 (nuclear receptor related-1 protein) and others, as well as methylation and other epigenetic mechanisms (Kim and Lee, 2008; Surguchov, 2023). Altogether, αSyn accounts for 0.5–1% of the total soluble brain proteins (Iwai et al., 1995) and it is highly concentrated (~40 μM) in pre-synaptic terminals of neurons (Wilhelm et al., 2014).

αSyn neuronal distribution. αSyn subcellular localization spans among cell body, neurites and it progressively accumulates in the pre-synaptic terminals in the latter stages of neuron development (Kim and Lee, 2008), where it is thought to exert its physiological function. αSyn axonal transport to the distal synaptic boutons is reported to occur by both fast and slow components, mainly in association to vesicular membranes rather than axon skeleton proteins (Jensen et al., 1999). According to Utton et al., αSyn traffics in both anterograde and retrograde directions on intact microtubules, as αSyn was shown to interact with both kinesin 1- and dynein-containing complexes (Butkevich et al., 2016; Utton et al., 2005).

αSyn at synapse. αSyn has a high structural plasticity, being in constant equilibrium among diverse conformers. It is considered a natively unfolded protein, when referring to the soluble monomer in the cytoplasm (Breydo et al., 2012). Instead, the N-terminus domain can acquire a helical folded state, which is able to both mediate the association of αSyn with curved negatively charged membranes like synaptic vesicles and modulating their curvature (Fusco et al., 2018; Middleton and Rhoades, 2010). Although the physiological function of αSyn has not been fully untangled yet, the localization on synaptic vesicle membranes underscores its possible role in the regulation of synaptic vesicles dynamics and exocytotic events (Burré, 2015; Logan et al., 2017). In addition, it has been shown to associate to mitochondrial and endosomal structures, contributing to organelle trafficking, dynamics and quality control (Teixeira et al., 2021; Thorne and Tumbarello, 2022). αSyn interacts with several proteins involved in vesicle dynamics, among which synaptobrevin-2 and other proteins of the SNARE complex, Rab3a and synapsin-III (Burré, 2015; Zaltieri et al., 2015). Many other αSyn

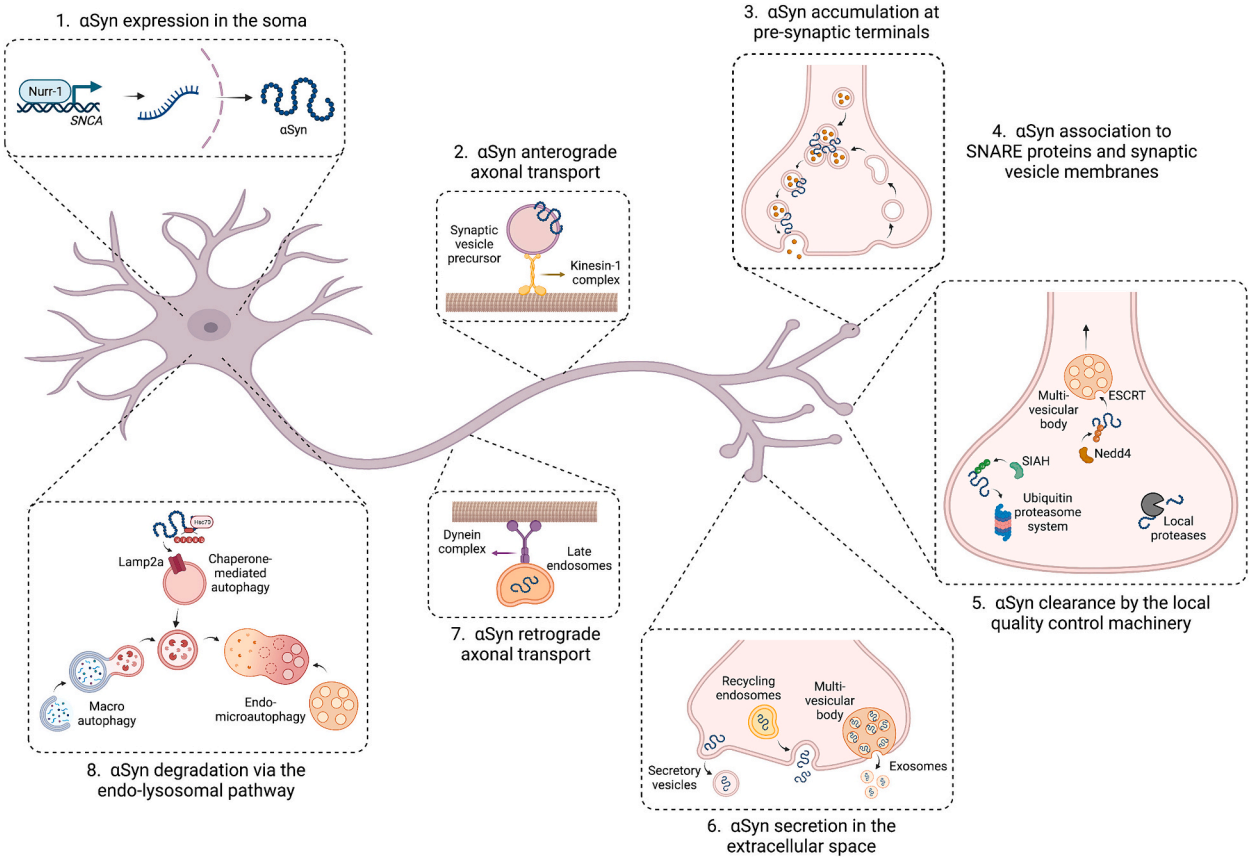

Fig. 1. Schematics of αSyn proteostasis in neurons. *Generated with BioRender.com*

interactors have been described, overall modulating αSyn proteostasis in terms of folding and trafficking, modification, secretion and degradation (Hernandez et al., 2020).

αSyn clearance and secretion. Different proteolytic pathways are involved in αSyn clearance, both intracellular and extracellular (Stefanis et al., 2019). In the periphery, a fraction of αSyn is likely to be degraded by the local quality control machinery. Aside cytosolic proteases i.e., calpain and neurosin, intracellular monomeric αSyn is primary degraded by the proteasome by multi-mono-ubiquitination by the E3-ubiquitin ligase SIAH (Rott et al., 2017), by poly-ubiquitination mediated by K48-linkages or through ubiquitin-independent mechanisms. Alternatively, monomeric αSyn can be recognized by the chaperone Hsc70 on its chaperone mediated autophagy (CMA)-recognition motif $_{95}$VKKDQ$_{99}$, that vehicles αSyn to the LAMP2a receptor on the lysosomal membrane[17]. Furthermore, Tofaris and colleagues identified the E3 ubiquitin ligase Nedd4 as the most relevant for αSyn ubiquitination, promoting αSyn degradation by the endosomal-lysosomal pathway (Tofaris et al., 2011). Nedd4 recognizes the C-terminus of αSyn that contains the required proline-rich motif and synthesized K63-linkages on lysines-21 and -96 of αSyn. This process enhances the degradation of membrane-bound αSyn by the lysosomal pathway, through the recruitment of the endosomal-sorting complex required for transport (ESCRT) that mediates the loading of ubiquitinated proteins into endosomes, which are retrogradely transported to the soma to fuse with proteolytic lysosomes. More recently, additional lysines i.e., K45, K58, K60 have been identified as critical ubiquitination sites to target the protein to autophagic degradation (Zenko et al., 2023). Although small soluble oligomers can be digested by the ubiquitin-proteasome system (Emmanouilidou et al., 2010), large oligomeric forms are likely to be degraded by the autophagic routes (Pantazopoulou et al., 2021). Lysosomal cathepsins B, C and L together contribute to the degradation of αSyn within the lysosomes, however their efficiency was found to decrease towards αSyn fibrils (McGlinchey and Lee, 2015).

αSyn clearance from neurons also includes the secretion to the extracellular space through various proposed mechanisms: by secretory vesicles; by loading into early endosomes with direct secretion to the extracellular space once the recycling endosome fuses with the plasma membrane; by incorporation of αSyn into intraluminal vesicles of the multi-vesicular bodies (MVBs) to be then released as exosomes (Danzer et al., 2012; da Fonseca et al., 2015). SUMOylation on lysine 96 and lysine 102 is thought to participate into the sorting of αSyn into extracellular vesicles (Kunadt et al., 2015).

Together with ubiquitination and SUMOylation, other post-translational modifications can modulate and eventually alter αSyn turnover. For instance, both phosphorylation at serine 129 and dopamine modification at the C-terminus have been reported to prevent αSyn sorting into lysosomes via CMA (Martinez-Vicente et al., 2008). DOPAL covalent modification of αSyn lysines was shown to dramatically affect αSyn subcellular localization and clearance, promoting the overload of misfolded and oligomeric αSyn in the *endo*-lysosomal pathway and exosomal secretion (Masato et al., 2023).

3. Measuring αSynuclein half-life in cells

The complexity of the regulatory mechanisms of αSyn proteostasis accounts for the challenges that can be faced in uniquely determining its half-life value by conventional experimental approaches.

The time dependence of the concentration of a protein in the cellular environment is commonly described by the following differential equation(Alvarez-Castelao et al., 2012):

$$Protein\ (t) = \frac{dP}{dt} = \vartheta_{synthesis}[mRNA\ (t)\,] - \vartheta_{degradation}[P\ (t)\,]$$

where the protein levels depend on both the de novo protein synthesis, which is defined by the copy number of mRNA available; the rate of translation per mRNA molecule ($\vartheta_{\ synthesis}$); as well as the protein degradation rate ($\vartheta_{\ degradation}$). Nonetheless, for protein like αSyn, $\vartheta_{\ degradation}$ needs to consider the many pathways involved (proteases and proteasome digestion, autophagic routes, secretion) with their respective degradation velocities.

When measuring protein levels and protein half-life, different experimental strategies can be applied(Alvarez-Castelao et al., 2012; Hinkson and Elias, 2011): a) steady-state or kinetic studies; b) biochemical or imaging techniques; c) genetic interference of protein expression i.e., tet-ON/OFF systems; d) pharmacological modulation of either protein synthesis (i.e., blocking de novo protein synthesis with cycloheximide) or degradation (i.e., inhibition of proteasome by MG132, inhibition of autophagy by chloroquine, bafilomycin A, etc); e) protein-oriented approaches by specifically detecting a protein of interest via immunolabeling or unbiased approaches coupled with mass spectrometry and proteomics analysis such as stable isotope labeling by amino acids in cell culture (SILAC).

In the case of αSyn, we searched in the literature for publications where an estimate of αSyn half-life has been determined by using combinations of the following keywords for our research: αSynuclein, half-life, turn-over, proteostasis, degradation/clearance. The data were then listed in Table 1 and visualized by the graph presented in Fig. 2, where the different half-lives identified are plotted in relation to the technique and the cellular model used in the experiment.

From the graph, it emerges that a wide range of half-lives have been measured in the different publications, spanning from a few hours to days. While in stable cell lines the majority of αSyn half-lives are shorter than one day, especially in neuroblastoma-derived cell lines like SH-SY5Y and BE(2)-M17, a relatively high variability has been observed in primary neuronal cultures. One explanation is that immortalized cell lines will continue to grow and divide, thus diluting their proteome due to the increase in cell volume and subsequent cell division(Alvarez-Castelao et al., 2012). Hence, the second part of the equation should be corrected for the growth rate or volume increase factor per unit time (V) (Alvarez-Castelao et al., 2012), overall resulting in a faster protein decay:

$$Protein\ (t) = \frac{dP}{dt} = \vartheta_{synthesis}[mRNA\ (t)\,] - \left(\vartheta_{degradation} + \boldsymbol{V}\right)[P\ (t)\,]$$

On the contrary, primary neurons represents a more relevant physiological model to study αSyn turnover. They are post-mitotic aging cells in constant adaptation to balance new protein synthesis and clearance of old and misfolded proteins, to maintain a healthy and functional cellular milieu. When mature, they display the typical polarization and compartmentalization, with the specific local machinery for protein synthesis, folding, transport and degradation. In this regard, the equation for αSyn should include an additional parameter accounting for the anterograde and retrograde axonal transport, which is likely to affect its turnover rate while the protein is trafficked from the soma to the synapses and back. In addition, it should be noted that the local αSyn half-life at the synapse might be different from the cell body, which would be interesting to assess especially under pathological conditions, when one district could be more affected than the other. This can be potentially achieved by live cell imaging techniques; however, these strategies usually rely on the overexpression of fluorescent protein tags with caveats that will be discussed in the next paragraph. Moreover, to dissect the local degradation rate, the neuronal compartment should be isolated by blocking the inward and outward axonal transport using chemicals like Nocodazole which inhibits microtubule polymerization (Masato et al., 2023; Utton et al., 2005). However, this is likely to heavily impact the regulation and functionality of the local protein quality control, thus altering an accurate estimate of the physiological protein half-life. On the other hand, biochemical approaches like western blot or proteomics are usually performed without subcellular fractionation, thus preventing the differential half-life determination in the soma versus the peripheral synapses. In addition, other variables should be taken into

Table 1
List of half-lives of αSyn (wild-type sequence) determined in previous publications in various cellular models, using alternative experimental approaches.

Reference	αSyn half-life	Cellular model	Experimental approach
(Bennett et al., 1999)	1.84 ± 0.16 h	SH-SY5Y cells overexpressing 6XHis-tag-αSyn	Pulse-chase radiolabeling with [^{35}S] methionine coupled to immunoprecipitation
(Cuervo et al., 2004)	16.8 ± 2 h	Primary rat midbrain neurons - endogenous αSyn	Pulse-chase radiolabeling with [^{35}S] methionine coupled to immunoprecipitation
	16.1 ± 2.4 h	PC12 cells - endogenous αSyn	
	19.7 ± 2.1 h	PC12 stable cell line overexpressing hαSyn	
(Li et al., 2004)	~ 26-50 h	Primary mouse cortical neurons (DIV 7-14) – endogenous αSyn	Pulse-chase radiolabeling with [^{35}S] methionine coupled to immunoprecipitation
	~ 115-160 h	Primary mouse cortical neurons (DIV 35-42) – endogenous αSyn	
	13.0 ± 2.2 h	Undifferentiated SH-SY5Y stable cell line expressing hαSyn	
	52.3 ± 3.3 h	Differentiated SH-SY5Y stable cell line expressing hαSyn	
(Vogiatzi et al., 2008)	> 36 h	Tet-Off stable inducible PC12 and SH-SY5Y cell lines overexpressing αSyn	Doxycycline treatment and western blot analysis
	~ 8 h	Tet-Off stable inducible PC12 and SH-SY5Y cell lines overexpressing αSyn	Pulse-chase radiolabeling with [^{35}S] methionine coupled to immunoprecipitation
(Mazzulli et al., 2011)	~ 10 h	Tet-Off stable inducible H4 cell line overexpressing αSyn	Doxycycline treatment and western blot analysis
(Rott et al., 2011)	~ 15 h	SH-SY5Y cells overexpressing HA-αSyn	Pulse-chase radiolabeling with [^{35}S] methionine coupled to immunoprecipitation
(Tofaris et al., 2011)	> 8 h	SH-SY5Y cells - endogenous αSyn	Treatment with 20 μg/ml cycloheximide and western blot analysis
(Cohen et al., 2013)	NA	Primary rat cortical neurons	SILAC coupled to LC/MS/MS
(Sugeno et al., 2014)	~ 24 h	SH-SY5Y cells - endogenous αSyn	Treatment with 50 μg/ml cycloheximide and western blot analysis
(Fishbein et al., 2014)	145 h	Primary mouse cortical neurons (DIV 10): - endogenous mouse αSyn	Pulse-chase radiolabeling with [^{35}S] methionine coupled to immunoprecipitation
	61 h	- transgenic (non-overexpressing) hαSyn	
(Alexopoulou et al., 2016)	~ 7 h	HEK293 cells - endogenous αSyn	Treatment with 20 μM cycloheximide and western blot analysis
(Rott et al., 2017)	~ 5 h	HEK293 cells overexpressing HA-αSyn	Treatment with 50 μM cycloheximide and western blot analysis
(Vicente Miranda et al., 2017)	~ 8 h	H4 cells co-expressing SynT and Synph I	Treatment with 100 μM cycloheximide and western blot analysis
(Dörrbaum et al., 2018)	10.81 d	Primary mouse hippocampal cultures (DIV 18-19): - Mixed culture	SILAC coupled to LC/MS/MS
	7.98 d	- Neuron-enriched culture	
	4.29 d	- Glia-enriched culture	
(Mathieson et al., 2018)	~ 7 d	Human monocytes	SILAC coupled to LC/MS/MS
	~ 34 h	Primary mouse neurons	
(Pinho et al., 2018)	~ 12 h	H4 cells overexpressing hαSyn	Treatment with 100 μM cycloheximide and western blot analysis
(Ho et al., 2020)	~ 48 h	MEF cells overexpressing PAmCherry-SNCA-NE	Photoactivation of fluorescent protein and flow cytometry
(Sampognaro et al., 2023)	~ 3 h	Differentiated SH-SY5Y stable cell line expressing inducible Flag-hαSyn	Doxycycline treatment and western blot analysis
(Masato et al., 2023)	2.6 ± 0.4 h	Primary rat cortical neurons expressing hαSyn-TimeSTAMP-YFP-miniSOG: - cell body	Pulse-chase coupled to live cell confocal imaging in the presence of 5 μg/ml Nocodazole
	6.3 ± 0.2 h	- synapses	
	18.8 h	BE(2)-M17 cell overexpressing hαSyn-HaloTag	Treatment with 50 μM cycloheximide and western blot analysis
(Zenko et al., 2023)	~ 8 h	HEK293 cells overexpressing αSyn-VC	Treatment with 35 μM cycloheximide and western blot analysis
	~ 6 h	Primary mouse neurons - endogenous αSyn	

consideration, i.e., the maturation or age of the neurons in cultures, whether they reached a good level of branching of the neurite web and whether they developed synaptic contacts (usually, not earlier than 12–14 days-in-vitro). Also, the type of neurons and the region of the brain they were dissected from can heavily influence they morphology, level of branching as well as proteome profile.

Interestingly, whilst endogenous αSyn presents high variability of turnover rates across the different publications, overexpressed αSyn appears to have a relatively short half-life, 0.6 days on average. On one side, the overexpression of tagged proteins usually comes in hand for efficient protein immunoprecipitation following radiolabeling or Halo-tag labeling of the protein of interest, as well as for imaging approaches with fluorescent tags. However, both transient transfection and viral transduction usually results in expression levels much higher than physiological. This might have an impact on the whole cellular proteome, a potential feedback inhibition of protein synthesis, and impaired functionality of degrative pathways. Moreover, when introducing a tag to a protein, either under overexpressing condition or by knock-in, it could be misleading to assume this wouldn't affect the proteostasis of the tagged protein (Alvarez-Castelao et al., 2012). Whether it is a small peptide or an entire protein like GFP, the tag likely affects the protein structure, potential post-translational modification, the interactome, localization and function. Also, introducing the tag at the N-terminus or at the C-terminus can change the fate of the protein if it is subjected to truncation, like in the case of αSyn.

Finally, when the interference of protein synthesis is applied, both pharmacologically by cycloheximide treatment and genetically by tet-ON/OFF systems, it results in shorter αSyn half-life. Once again, these

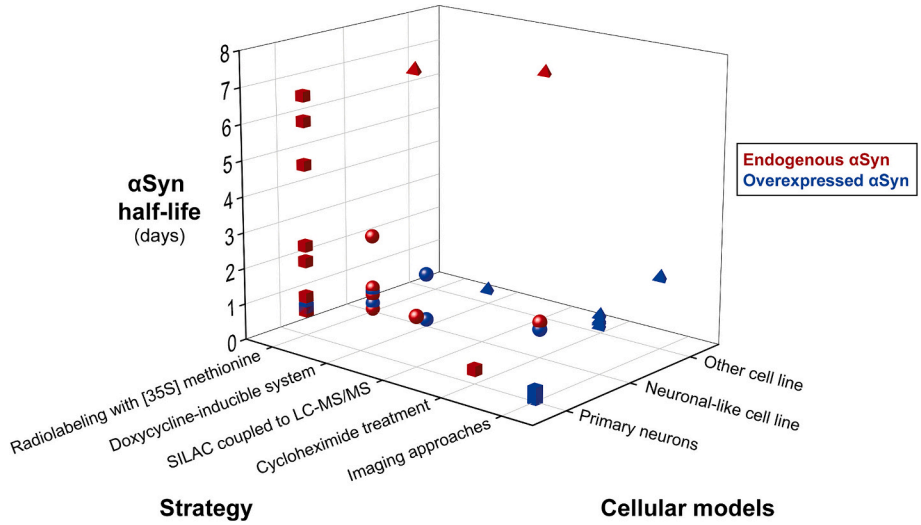

Fig. 2. The graph displays the αSyn half-lives (z-axis; expressed in days) measured in previous publications. Each data point is shown in relation to the experimental approach applied (x-axis) and the cellular model used (y-axis; cubes: primary neurons, spheres: neuronal-like cell line, tetrahedrons: other cell line), either focusing on the endogenous αSyn (red symbols) or by overexpressing tagged αSyn (blue symbols). Each data can be traced back to the relevant publication based on the information reported in Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

data should be carefully considered in the light of any alterations triggered by treatments with compounds, or genetic disruption caused by the insertion of the tetracycline responsive promoter cassette.

4. αSynuclein half-life: still under debate

The study of the regulation of αSyn proteostasis in neurons, and the different experimental strategies and models that could be employed to address this topic generate several questions and points of discussion.

First of all, the data presented in Table 1/Fig. 2 only refer to the estimate of wild-type αSyn turn-over in cells under physiological conditions, however these values are likely to change when studying different isoforms of αSyn. For example, high damage score mutations like G51D and A53T, which generate different αSyn conformers (Moretti et al., 2020) and promote aggregation, significantly prolonged αSyn half-life of more than twice the turnover of the wildtype isoform (or the low damage score mutations A30P and E46K) when measured in a dox-inducible system in SH-SY5Y cells overexpressing Flag-tagged αSyn (Sampognaro et al., 2023). On the contrary, mouse primary neurons expressing the human sequence of αSyn (on a null endogenous αSyn background) didn't show a significant difference in half-life between the wild-type isoform and the pathogenic mutant A53T assessed by radiolabeling coupled to immunoprecipitation (Fishbein et al., 2014). However, in the same experimental setup, the endogenous mouse αSyn displayed a half-life value more than double the human proteins. Interestingly, whilst the mouse and human sequences share 95% identity, the mouse αSyn naturally harbors the A53T substitution, thus suggesting that various factors other than a simple mutation contribute to defining protein turnover.

How do variations in αSyn turn-over affect its propensity to pathology? A long half-life might indicate a relatively stable protein but also higher probability of modifications, misfolding and association with non-canonical interactors. On the other hand, a fast turnover might result in sustained protein availability which leads to fast aggregation when the system is impaired. In both scenarios, there would be consequences on the integrity and homeostasis of pre-synaptic terminals and neuronal projections, which constitute the most vulnerable compartments to proteotoxicity and degeneration (Cajigas et al., 2010; Uchihara, 2017). Of note, the challenge of determining αSyn half-life increases exponentially under pathological conditions when increased rate of synthesis of the *SNCA* gene (i.e., due to gene multiplication), impaired axonal transport in both directions, protein aggregation and defective clearance might all happen at the same time. In addition, pathological αSyn is not only a target of quality control pathways but it becomes one of the main impinging factors for their functionality.

Then, is it really feasible to determine αSyn half-life? All the techniques and cellular models used so far present both advantages and caveats, and the results obtained should be weighed in the light of the specific aim of the work reported in each publication. In other words, each measurement is strictly depends on the basal conditions of the specific setup applied and the relative differences, rather than the absolute numbers, should be considered when testing potential therapeutic strategies in pre-clinical cellular models of αSyn pathology.

5. Conclusions

We here collected several publications where αSyn half-life has been quantified, however it emerges that a unique value is yet to be defined as it strongly depends on the cellular models and the experimental strategy used in each specific study. Hence, the use such values as readout of αSyn proteostasis in neurons needs to be carefully revised especially when comparing physio-pathological conditions.

Moreover, further focus should be addressed to investigate αSyn turn-over at organism level. In this regards, metabolic labeling and LC-MS/MS represents a feasible strategy to study in vivo protein turnover (Shi et al., 2023). For instance, Price and colleagues performed organism-wide isotopic labeling (SILAC) and determined protein turn-over rates in mouse brain, liver and blood (Price et al., 2010). Of note, the authors suggested that the regulation of protein turn-over varies upon the specific tissue, organelle and molecular complex where the protein is expressed, thus resulting in significant variations of half-life values among the three groups. Here, αSyn half-life was estimated about 33 days in the brain and 12 days in the blood (Price et al., 2010).

On the same line, using specific tracers to study αSyn turn-over in the brain or measuring variations of αSyn levels in the blood of patients would be relevant from a translational point of view. Although it could be argued that these analysis would represent indirect quantifications of αSyn accumulation under pathological conditions rather than defective clearance. Nevertheless, αSyn is a neuronal protein that can be secreted in the extracellular space, building up in the cerebrospinal fluid and blood in Synucleinopathies. At the same time, high levels of αSyn are present in the red blood cells, thus confounding its measurement in the

blood stream. As a consequence, a complete understanding of αSyn proteostasis at systemic level is still out of reach with the currently available technologies.

It is currently being discussed whether busting protein degradative pathways constitutes an effective, sensible and feasible therapeutic approach for neurodegenerative diseases, with higher chances of positive outcomes than immunotherapies. As recently reviewed by Engelender and co-authors, gaps and controversies emerge when considering treating proteinopathies with enhancers of protein clearance, targeting either the UPS or the autophagic pathway (Engelender et al., 2022). Among them, whether the protein aggregation is an actual cause or effect of neurodegeneration; the specificity of any pharmacological modulation and the impact on ubiquitous processes; whether the augmented protein turnover would affect the pool of soluble functional monomers rather than pathological aggregates; when it would be the proper time to intervene and how much this approach could be generalized to all patients.

Nevertheless, a crucial aim is still to regulate, sooner rather than later, αSyn neuronal proteostasis in patients affected by PD and Synucleinopathies. At the same time, it is mandatory to identify a reliable biomarker of αSyn levels in the central nervous system. Whether to use the absolute value of αSyn half-life as a readout of a successful strategy remains still debatable and should be further explored.

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CRediT authorship contribution statement

Anna Masato: Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Luigi Bubacco:** Writing – review & editing, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no conflicts of interest.

Data availability

The data showed in this manuscript derive from published research articles.

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