PARKINSON’S DISEASE

PD is the second most common neurodegenerative disorder, after Alzheimer’s disease, and is classically characterized by symptoms such as resting tremor, postural instability, bradykinesia and muscular rigidity [1]. Pathologically, PD is defined by a progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta, and the presence of intracellular inclusions, known as Lewy Bodies, in surviving neurons [1]. Despite its initial classification as a motor disorder, PD is currently perceived as a whole-brain pathology since it affects multiple brain areas and presents a broad variety of symptoms in addition to the typical motor symptoms mentioned above. According to Braak’s staging hypothesis, the pathological process is thought to initiate in either the lower brainstem or in the olfactory bulb. Then, as the disease evolves, Lewy body pathology occurs in other brain regions and circuits, including the cerebral cortex, leading to non-motor symptoms such as depression, cognitive decline and hallucination episodes [2-5]. The vast majority of PD cases are sporadic and only approximately 5-10% have been linked to genetic factors. Thus far, more than 20 genes have been associated with PD, and this number tends to increase as novel and more powerful studies are conducted [6]. The proteins encoded by these genes play a wide range of cellular roles but the precise functions of some of them are still not fully understood.

Understanding the interplay between different PD genes and, consequently, unravelling the molecular mechanisms underlying PD will bring new hope for the development of novel diagnostic and therapeutic tools.

ALPHA-SYNUCLEIN

Asyn is, by far, the most extensively studied protein in the...
context of PD. Nevertheless, our understanding of its physiological function is still not entirely established. Three functional domains can be defined in the primary sequence of asyn (Fig. 1A). While the protein was initially thought to be natively unfolded and monomeric, recent studies proposed it may also adopt a tetrameric structure [7, 8]. Although these studies are controversial, they brought new perspectives into the process of asyn misfolding and aggregation, thought to be central in the context of PD [6]. In fact, aggregated asyn is the main component of Lewy Bodies [9]. Thus far, 6 point mutations have been associated with familial cases of PD. Interestingly, all 6 mutations are located in the N-terminal region of the protein [10-15]. In addition, multiplications of the SNCA gene, encoding for asyn, have also been associated with familial forms of PD [16, 17]. Furthermore, polymorphisms in the SNCA gene are known to increase the risk of PD [18-21].

The normal cellular function of asyn is still unclear, but distinct roles have been proposed, ranging from transcriptional regulation [22-27], mitochondrial homeostasis [28], vesicle trafficking [29], and neurotransmitter release [30-33]. Despite all these putative functions, mice lacking asyn reveal only minor physiological alterations [34] suggesting the existence of compensatory cellular mechanisms that can overcome the absence of the protein.

**Fig. 1.** Schematic representation of asyn and ATP13A2. (A) Asyn can be divided in three domains according to its amino acid composition: an N-terminal amphipathic domain where all 6 PD-associated point mutations are located; a central region known as non-amyloid component (NAC) domain, responsible for the amyloidogenic properties of the protein; and the C-terminal region which is highly acidic. (B) ATP13A2 is an 1180 aa protein with 10 transmembrane domains and four functional domains: catalytic phosphorylation (P1), nucleotide binding (P2 and N) and actuator domain (A).

ATP13A2

ATP13A2 is a transmembrane protein of 1180 amino acids (aa) localized in the lysosomes and late endosomes [35] that belongs to a family with 5 members (ATP13A1-5) - the P5 type pump ATPases. ATP13A2 has 10 predicted transmembrane domains and three functional domains (Fig. 1B) [36]. The protein is highly expressed in the brain, especially in the substantia nigra pars compacta and is upregulated in the dopaminergic neurons of this region in the brains of PD patients [35, 37]. Mutations in ATP13A2 have been associated with different diseases including Kufor-Rakeb syndrome, PD [35, 38-46], and Neuroid Ceroid Lipofuscinosis (NCL) [47-49]. Interestingly, ATP13A2 knockout mice display characteristics of both NCL and PD, such as hippocampal accumulation of asyn, sensorimotor deficits and lipofuscinosis [50], suggesting that the phenotypes in these may not be solely gene dependent.

Mitochondrial impairment was observed in fibroblasts from patients carrying ATP13A2 mutations. This impairment was associated with reduced ATP production and increased maximum respiration capacity, due to an impairment of mitochondrial degradation that resulted in their accumulation. These phenotypes could be partially rescued upon ATP13A2 overexpression [51]. The process of autophagic mitochondria degradation, known as mitophagy, is a crucial quality control mechanism to ensure the proper function of the organelle [52] and has been associated to PD [53]. Interestingly, ATP13A2 has been directly linked to mitophagy in several studies [51, 54, 55] but, so far, little is known about the mechanisms involved. In addition to a role in mitophagy, ATP13A2 has been connected with protein autophagy [50, 56, 57] and metal/cation homeostasis [37, 55, 57-66]. These are also central cellular processes that have been associated with asyn biology, as discussed below.

Of the several disease-associated mutations identified in ATP13A2, only a few were investigated in detail thus far. In cells, ATP13A2 mutants exhibited loss of protein function, subcellular mislocalization in the endoplasmatic reticulum, increased cellular toxicity, and shorter protein half-life [67]. In a comprehensive study of the effects of ATP13A2 missense mutations associated with early-onset parkinsonism, several novel phenotypes were identified, including disruption of the protein vesicular localization, impairment of ATPase activity and of neurite outgrowth [68].

**THE INTERPLAY BETWEEN ATP13A2 AND ALPHA-SYNUCLEIN**

Most of the existing knowledge about the function of ATP13A2

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has arisen from studies focusing on the interaction between this protein and asyn. Thus, in the sections below, we focus on our current understanding of the interplay between these two proteins in order to provide a framework for the challenges that lie ahead in this area of research.

**METAL HOMEOSTASIS**

Despite the lysosomal localization of ATP13A2, the first connection to asyn was not due to a putative role in protein degradation, but rather due to a role in metal homeostasis. ATP13A2 was shown to exert a protective effect in manganese (Mn⁺)-mediated asyn toxicity, in both yeast and SH-SY5Y cells [60]. This connection with Mn⁺ homeostasis was further explored in yeast and resulted in the identification of several genes being part of the network [62]. In addition, some ATP13A2 mutants were unable to rescue Mn⁺ induced toxicity in mammalian cell culture [64]. Interestingly, two ATP13A2 polymorphisms enhance Mn⁺ neurotoxic effect in patients [63].

Since Mn⁺ has long been associated with asyn oligomerization and aggregation [69, 70], and with Parkinsonism itself [71], this metal was seen as an interesting culprit in the asyn:ATP13A2 interaction. However, a recent contradictory report concluded that lower levels of ATP13A2 did not affect Mn⁺ sensitivity in SH-SY5Y cells [58].

In addition to Mn⁺, ATP13A2 was shown to be protective against niquel-(Ni²⁺), cadmium-(Cd²⁺) and selenium-(Se²⁻) induced toxicity in yeast and in mammalian cell culture [65, 66] but little is known about the role of these metals in the context of asyn toxicity and in PD.

ATP13A2 was also associated with zinc (Zn²⁺) homeostasis in a study showing that mitochondrial impairment, due to high levels of Zn²⁺, could be rescued by overexpression of ATP13A2 [55] (Fig. 2A).

It is unlikely that ATP13A2 is able to directly mediate the clearance of all these different metals. Thus, seems more plausible that ATP13A2 might act as a general, intermediary player. Since both metals and asyn are thought to have a great impact in mitochondria [28, 72-75], it is possible that the protective effect of ATP13A2 could be related to mitochondrial function as it was also found to be upregulated under oxidative stress conditions [76]. Nevertheless, additional studies are required to test this hypothesis.

**AUTOPHAGY**

Autophagy-mediated protein degradation is an important component of the protein quality control system in the cell that is mobilized upon the accumulation of misfolded, damaged, or unnecessary proteins. In PD, autophagy has assumed the central stage due to its involvement in the clearance of misfolded and aggregated asyn. Nevertheless, whether the interplay between autophagy and asyn is beneficial or deleterious to the cell is still controversial [77-85].

The finding that ATP13A2 is also present at the lysosomal membrane has further underscored the relevance of this proteolytic compartment in the context of PD. Strikingly, autophagy seems to directly connect asyn and ATP13A2 since knockdown [56] or knockout [50] of the latter resulted in impaired degradation of asyn (Fig. 2B).

In medaka fish, knockdown of ATP13A2 had a direct effect in the activity of the lysosomal aspartase cathepsin D [86], albeit no conclusive results were obtained regarding the intracellular content of asyn. In zebrafish, knockout of ATP13A2 led to embryonic lethality [87].

In other model systems, ATP13A2 deficiency also resulted in autophagy impairment, with alterations of lysosomal pH and in the levels of hydrolases, in the failure in autophagosome clearance, and in decreased proteolytic processing [56, 57, 59].

Besides its classical lysosomal localization, a recent report noted that ATP13A2 can be also found in multivesicular bodies (MVB) [58], which can have an important role in autophagy. Interestingly, this study reported exocytosis as the final outcome of MVB instead of autophagy. MVB are late endosomes that play a role in several intracellular trafficking mechanisms, including autophagy [88], but also in the clearance of exosomes [89]. Furthermore, considering that mitochondria can be degraded by autophagy (mitophagy) [90], it would be important to understand how asyn and ATP13A2 might affect this process (Fig. 2B). Previously, it was shown that accumulation of asyn at the mitochondria can enhance mitophagy [91, 92], so one possibility is that this process is mediated by ATP13A2 [58].

**BRIDGING METAL HOMEOSTASIS AND AUTOPHAGY**

The complex interaction between ATP13A2 and asyn has been studied primarily from one of two perspectives: metal dyshomeostasis or autophagy impairment. However, one possibility is that both pathways are connected in the biology of the two proteins. In fact, two recent studies investigated the interaction between asyn and ATP13A2 by looking at both metal homeostasis and autophagy regulation, and proposed a chain of deleterious events starting upon Zn²⁺ dyshomeostasis. The first study reported that alterations in Zn²⁺ intracellular levels and cellular sub-localization could promote lysosomal dysfunction and
Fig. 2. Putative intracellular pathways connecting asyn and ATP13A2. (A) ATP13A2 may be responsible for metal clearance via the lysosome (left side). A failure in this process, caused by mutations or reduced activity of ATP13A2, would lead to the toxic accumulation of metals in the cytoplasm (right side). Furthermore in disease conditions, asyn may increase the intracellular levels of metals, exacerbating cytotoxic effects [55, 60, 62, 64]. (B) Protein and mitochondria degradation by autophagy and mitophagy, respectively, may be critically regulated by ATP13A2 (left side). Upon deficient ATP13A2 activity, accumulation of defective mitochondria or proteins (such as asyn) would contribute to cytotoxicity and disease (right side) [50, 54, 56, 86]. (C1) ATP13A2 may also impact on intracellular Zn\(^{2+}\) homeostasis. Under normal conditions, ATP13A2 may mediate Zn\(^{2+}\) transport across the lysosomal membrane, a process that is thought to influence lysosomal degradation of asyn. On the other hand, under pathological conditions, impaired Zn\(^{2+}\) clearance, caused by defective ATP13A2 activity at the lysosome, can trigger cytoplasmic accumulation and aggregation of asyn [59]. (C2) ATP13A2 may also play a role at the level of multivesicular bodies (MVBs). Thus, functional ATP13A2 might mediate the entrance of Zn\(^{2+}\) into MVB. MVBs may later fuse with autophagosomes containing asyn and be targeted to exocytosis [58].
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Interestingly, this phenotype was exacerbated in ATP13A2 knock-down cells and in fibroblasts from patients carrying ATP13A2 mutations, and could be rescued upon ATP13A2 overexpression (Fig. 2C1) [59]. On the other hand, a separate study placed MVBs in the center of the interplay between asyn and ATP13A2. The authors found that MVBs are targeted to exocytosis, instead of autophagy, and constitute the main pathway underlying the decrease of asyn intracellular levels [58]. In this perspective, ATP13A2 was shown to modulate Zn\(^{2+}\) levels which, in turn, may influence the biogenesis of exosomes (Fig. 2C2).

Ultimately, these two studies suggest that ATP13A2 can impact on asyn levels. Moreover, considering the converging players and organelles involved in autophagy and exocytosis pathways, one can hypothesize that the fate of asyn, as well as other accumulated proteins, can be determined by ATP13A2 levels, behaviour and its interactors at the membrane level of the MVB.

In a recent study investigating possible ATP13A2 interactors, histone deacetylase (HDAC) 6 was identified as an attractive candidate [93]. HDAC6 is a cytoplasmic HDAC that has been linked to (i) asyn clearance in a cell model [94], (ii) to its accumulation in Drosophila [95] and (iii) is present in Lewy Bodies from PD patients [96, 97]. Interestingly, HDAC6 has also been linked to mitophagy in PD [98]. Furthermore, this Zn\(^{2+}\) binding enzyme has been associated with key steps in the autophagy process, including aggresome formation and delivery to lysosomes [96], fusion between autophagosomes and the lysosomes, and was also found associated with MVBs [99]. Since a direct interaction between ATP13A2 and asyn is still to be proven, one can speculate that the effect of ATP13A2 on asyn clearance and in the protection against asyn-induced toxicity might be, at least partially, mediated by HDAC6 (Fig. 3).

Interestingly, the same study also identified HSPA8 (also known as HSC70 and HSP73) as an interactor of ATP13A2 [93, 100]. HSPA8 is an essential player in chaperone mediated autophagy, a process that was found to be involved in the clearance of soluble asyn [84, 101]. Thus, the interaction between ATP13A2 and HSPA8 might also play a role in the degradation of asyn.

**CONCLUDING REMARKS**

Currently, asyn and ATP13A2 are thought to be members of the same intracellular network, with the latter having a direct impact on the fate of asyn in the cell. Nevertheless, the precise biological function of ATP13A2 and whether it plays a direct or indirect role in the processing of asyn is still unknown. Although two main interacting networks have been proposed separately, recent studies tend to converge in a more appealing and comprehensive hypothesis that comprises a single process that includes both alterations in the levels of metals and in autophagy.

It will also be important to determine the effects of familial mutations in both ATP13A2 and in asyn on the interaction between the two proteins, and whether the occurrence of ATP13A2 in the lysosome can rescue deleterious effects of mutant asyn. In this context, since most studies focused on the effect of ATP13A2 knockdown on asyn, it will be important to assess the impact of asyn knockdown on ATP13A2. On the other hand, it seems likely that the function of ATP13A2 in the cell goes beyond its effects on asyn, suggesting that we need a broader understanding of its
role in both metal homeostasis and autophagy in order to better understand the biological function of ATP13A2 and, consequently, how it causes disease. With this knowledge at hand, it might then be possible to design novel strategies for therapeutic intervention in PD and other disorders associated with asyn and ATP13A2 dysfunction.

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