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The significance of α-synuclein (α-syn) phosphorylation to Parkinson disease (PD) pathology has been controversial since the initial identification of this posttranslational modification in intracellular Lewy bodies (1, 2). The modification of serine-129 (S129), which appears to be the most common phosphorylation site on α-syn (3, 4), has had the focus of attention. Although several different kinase families have been linked to modification of S129 in vitro, definitive in vivo evidence that a given kinase family causes the modification has been lacking (5). In addition to the lack of clarity regarding which kinases phosphorylate α-syn in a physiologically relevant context, it has been unclear whether phosphorylation of S129 is protective or harmful to neurons. The most significant problem in this regard has been that genetic manipulation of α-syn at S129—by mutation to either alanine or aspartate—has yielded varying results in cultured cells and different model organisms, including flies, worms, and rodents (5). Because the phosphorylation of S129 appeared to have no consistent effect on α-syn function in the cell, it has been unclear whether the process does anything more than reflect the magnitude of total α-syn levels in diseased neurons. In their article in PNAS, Oueslati et al. (6) now reveal a unique and unexpected effect coupled to S129 phosphorylation.

In many studies of the phospho-specific epitopes of different proteins, it has turned out that knowing the identity of the kinase that phosphorylates a given protein in a physiological context can be just as important as knowing which specific residue is modified. Oueslati et al. (6) show that the phosphorylation of S129 of α-syn is no exception. This is because the kinase of interest, polo-like kinase 2 (PLK2), not only phosphorylates α-syn, but also escorts modified α-syn into the autophagy pathway for degradation. This dual kinase/chaperone activity (Fig. 1) provides key insight into the significance of α-syn phosphorylation, because in the absence of chaperone activity of PLK2, it is not clear that modification of α-syn at S129 has any major functional impact.

The first evidence that α-syn could be phosphorylated at S129 by PLK family members came from a loss-of-function siRNA screen by Elan Pharmaceuticals, which identified both PLK2 and PLK3 as potential mediators of the modification (7). Importantly, this study also demonstrated that small-molecule inhibition and germ-line deletion of mouse *Plk2* lead to decreased S129 phosphorylation in vivo. An ensuing study by Lashuel and colleagues confirmed the finding, and further demonstrated the specificity of PLK2 and PLK3 for α-syn compared with other synuclein isoforms (8). Two critical pieces of information in the PLK2/α-syn connection were missing, however: namely, an understanding of the functional consequences of the interaction and how it affects α-syn pathology.

In addition to its kinase domain, PLK2 also contains a namesake polo-box domain, which functions as a phospho-ser/thr binding motif (9). Oueslati et al. (6) demonstrate that after phosphorylating α-syn at S129, PLK2 binds α-syn via this polo-box domain and escorts it into a degradation pathway, most likely of autophagy/lysosomal origin (Fig. 1). The precise identity of which autophagic pathway is involved is unclear (macroautophagy vs. chaperone-mediated autophagy), but PLK2-mediated α-syn degradation is blocked by both early and late inhibitors of autophagy and does not appear to involve proteosomal activity.

Most importantly, this study by Oueslati et al. (6) also shows that both PLK2 kinase activity and an intact S129 site are required for degradation of excess α-syn in the rat brain. Thus, less α-syn protein accumulated and fewer neurons died when rat brain

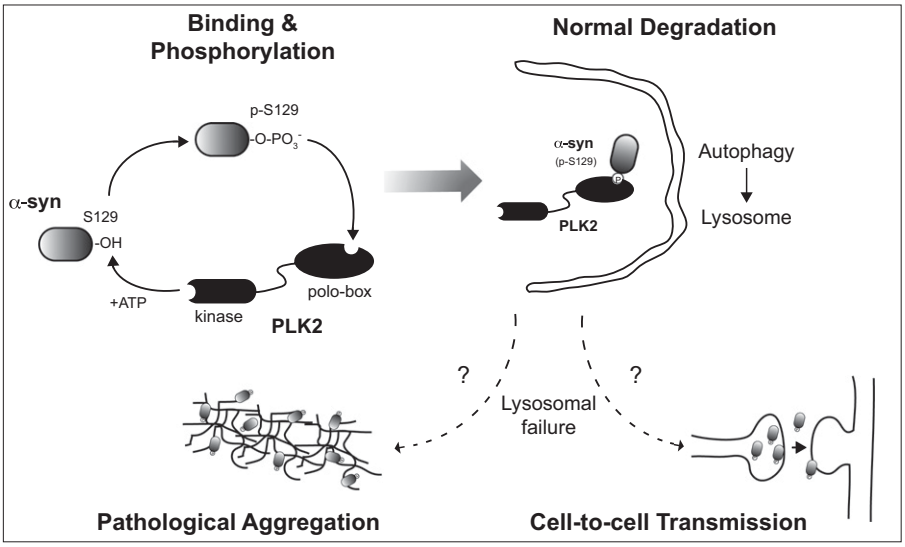

Fig. 1. Dual function of PLK2 in the phosphorylation and degradation of neuronal α-syn. Coordinate expression of α-syn and PLK2 in the same neuronal compartment promotes specific phosphorylation of α-syn at S129 by the PLK2 kinase domain. Upon phosphorylation (p-S129), α-syn is bound by the PLK2 polo-box domain. Formation of this binary complex promotes trafficking of α-syn into the autophagy/lysosomal pathway, which ultimately leads to its degradation. Inhibition of lysosomal function may, however, promote the accumulation of phosphorylated α-syn. Neurons may deal with excess α-syn by storing it in the form of large intracellular aggregates (Lewy bodies) or by releasing into the extracellular space, thus leading to cell-to-cell transmission.

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neurons were cotransduced in vivo with viral vectors for both human α-syn and PLK2 than when the brains were injected with α-syn vectors alone. A vector encoding kinase-dead PLK2, however, was ineffective at reducing α-syn levels. Furthermore, when the neurons were transduced with a viral vector encoding S129A–α-syn (which cannot undergo phosphorylation), cotransduction of PLK2 did not affect α-syn protein levels and neuronal loss. Previous studies have shown that when other putative S129 kinases from the G protein-coupled receptor kinase family are coexpressed with α-syn, the α-syn protein levels and neuronal survival are not changed, despite the levels of phosphorylated S129–α-syn going up (10).

It is notable that PLK2, unlike other PLK family members, appears to be enriched in postsynaptic dendrites in the brain, and is transcriptionally up-regulated in response to synaptic hyperactivity (11, 12). Within this context, PLK2 activity is required for homeostatic synaptic scaling in hyperstimulated neurons and results in decreased dendritic spine density and AMPA receptor down-regulation (13, 14). Although this may at first appear irrelevant to α-syn, which is mostly located at presynaptic terminals in neurons, it should be noted that increased synaptic activity promotes α-syn secretion from neurons, and that phosphorylated α-syn is enriched in the secreted fraction of some cell types (15, 16). Given the suggestion that α-syn secretion is a key step in a prion-like cell-to-cell transfer of α-syn pathology and thereby contributes to progression of PD symptoms (17), the findings by Oueslati et al. (6) suggest that PLK2 might influence transmission of misfolded α-syn variants.

Interestingly, the autophagy/lysosomal pathway by which PLK2 facilitates α-syn degradation has already been implicated in the pathogenesis of PD (18, 19). Regardless of which distinct autophagic mechanism is used for α-syn clearance, disruption of lysosomal function—either by genetic changes or toxic insults—would be expected to result in accumulation of excess phosphorylated α-syn (Fig. 1). Because phosphorylated α-syn is enriched in Lewy bodies in PD patients, it is possible that these inclusions form in response to lysosomal failure in the context of high synaptic activity. Under these circumstances, α-syn would be expected to undergo higher rates of cell-to-cell transfer and become phosphorylated by the increased levels of PLK2 in postsynaptic neurons. In the absence of proper lysosomal function, however, α-syn/PLK2 complexes would fail to become degraded, and could conceivably trigger α-syn aggregation or further release into the extracellular space (Fig. 1) (16). Application of the recently described α-syn transmission model of PD to *Plk2*$^{-/-}$ mice, which are viable into adulthood, would provide insight into whether this hypothesis is valid (20).

Despite the findings of Oueslati et al. (6) providing new exciting directions for future PD research, the immediate therapeutic implications of the results are less clear. The authors' results suggest clearly that increased PLK2 activity would be beneficial, although this effect would be challenging to achieve with small molecules. Preventing α-syn dephosphorylation by targeting the pS129 phosphatase is similarly unlikely to provide benefit, because phosphorylation of α-syn is not sufficient for protection in the absence of increased PLK2 chaperone activity (Fig. 1). In this context it would appear that gene therapy aimed at PLK2 gene overexpression or modulation of its breakdown using miRNA technology—with all of the challenges this would entail—are currently the most likely means by which one could increase PLK2 levels in the brain.

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However, one then has to ask the question whether chronically increased PLK2 activity in neurons is likely to be a good thing. Viral vector-mediated overexpression of PLK2 in the rat model used by Oueslati et al. (6) did not cause toxicity in the timeframe of their study, but this does not exclude detrimental effects in a chronic setting. Brains from patients with Alzheimer's disease and Lewy body disease have been shown to contain increased levels of PLK2 (8). This finding is possibly because of the well-described loss of calcium homeostasis in diseased neurons, which mimics synaptic hyperactivity and would promote PLK2 up-regulation (13). Increased calcium levels in diseased neurons are also linked to synaptic dysfunction and reduced spine density—two effects associated with PLK2 activation (21). Therefore, excessive PLK2 activity for prolonged periods might be harmful to neurons, despite it promoting α-syn degradation. Future studies will hopefully address this paradox and provide a clear answer to whether PLK2 is a friend or foe in the battle against neurodegenerative disease.

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