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Differential roles of the ubiquitin-proteasome-system and the autophagy-lysosomal-pathway in the degradation of α-synuclein in vivo: Implications for Parkinson's disease and other neurodegenerative diseases

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Protein misfolding, aggregation and deposition are common disease mechanisms in many neurodegenerative diseases including Parkinson's disease or Dementia with Lewy bodies. Accumulation of damaged or abnormally modified proteins may lead to perturbed cellular function and eventually to cell death. Thus, neurons rely on elaborated pathways of protein quality control and removal to maintain intracellular protein homeostasis. Molecular chaperones, the ubiquitin-proteasome system (UPS) and the autophagy-lysosomal pathway (ALP) are critical pathways that mediate the refolding or removal of abnormal proteins.

The successive failure of these protein degradation pathways, as a cause or consequence of early pathological alterations in vulnerable neurons at risk, may present a key step in the pathological cascade that leads to spreading neurodegeneration. A growing number of studies in disease models and patients have implicated dysfunction of the UPS and ALP in the pathogenesis of Parkinson's disease and related disorders. Deciphering the exact mechanism by which the different proteolytic systems contribute to the elimination of pathogenic proteins, like α-synuclein, is therefore of paramount importance. A critical question thus remains how α-synuclein is degraded by neurons *in vivo*. To investigate the specific contribution of the UPS and ALP to α-synuclein's degradation in a more biologically and disease relevant experimental paradigm we have developed novel approaches to test our hypothesis in transgenic mouse models. Our study uses α-synuclein transgenic mice, expressing human α-synuclein or α-synuclein-eGFP under the (h)PDGF-β promoter, in combination with *in vivo* pharmacologic and multiphoton imaging strategies to systematically test degradation pathways in the living mouse brain. Using our novel experimental paradigm we demonstrate that the UPS is the

main degradation pathway for α-synuclein under normal conditions *in vivo* while with increased α-synuclein burden the ALP is recruited. We show that levels of α-synuclein at both the somatic and presynaptic compartment *in vivo* are affected by impaired protein clearance through the UPS or the ALP. Moreover, we report alterations of the UPS in α-synuclein transgenic mice and age dependence to the role of the UPS in α-synuclein degradation.

Extending our study to test the degradation of post-translationally modified and potentially pathogenic species of α-synuclein, such as phosphorylated α-synuclein and aggregated species, we find that Serine129 phosphorylated (p-S129) α-synuclein is not only present as monomers but can also be detected as small oligomers that are increased in α-synuclein transgenic and aged mice. We further demonstrate that p-S129 α-synuclein is degraded in an UPS dependent manner *in vivo*. Turning to high-molecular weight human α-synuclein we discovered that proteasome and autophagy impairment leads to a shift towards insoluble species in α-synuclein transgenic mice. Surprisingly when overall levels of α-synuclein are assessed, autophagy inhibition causes a drift towards monomeric species potentially through a dissolution or removal of more compact aggregated species. Following our finding that macroautophagy is recruited to degrade α-synuclein in human α-synuclein transgenic mice, we complemented our study of the turnover of α-synuclein with a set of experiments that evaluates the effects of mTOR-dependent autophagy induction on α-synuclein *in vivo*. Surprisingly, our *in vivo* data show no reduction of α-synuclein after 24 hours of rapamycin treatment and a strong although transient increase in levels of α-synuclein at both presynaptic terminals and the cell body in the living mouse brain, interestingly with different kinetics in both cellular compartments.

Beyond the study of α-synuclein degradation, we provide evidence that the UPS and ALP might be functionally connected such that impairment of one can upregulate the other, a crosstalk that is critically influenced by the level of α-synuclein. In the brain of patients with Dementia with Lewy bodies we found that important ALP signaling molecules are dysregulated. Notably we found increased marker molecules of both chaperone-mediated autophagy (LAMP-2a) and macroautophagy (LC3-II) that strongly co-localized with α-synuclein aggregates, potentially indicating a derangement of autophagic processing.

Collectively, our results provide novel links between the UPS, the ALP and α-synuclein pathology and may have important implications for the evolution and

progression of synucleinopathies as well as for the future approaches of using protein degradation pathways as novel therapeutic targets in Parkinson's disease and related neurodegenerative diseases.