FUNCTIONAL ANALYSIS OF NEUROPROTECTIVE GENES USING *CAENORHABDITIS ELEGANS* AND MAMMALIAN MODELS OF PARKINSON'S DISEASE

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Abstract:
Dysfunction of the protein degradation machinery has emerged as a leading cellular defect associated with age-onset proteotoxicity in neurodegenerative diseases, including Parkinson's disease (PD). While the etiology of PD is not fully understood, the protein α-synuclein (α-syn) is thought to play a central role in the pathology associated with this disease, in that accumulation of α-syn is observed in both familial and sporadic forms of PD and mutations or overexpression of α-syn result in enhanced dopaminergic (DA) neurodegeneration. Without an effective therapeutic treatment for the progression of PD, we set out to identify and validate potential genetic and chemical modifiers of key pathological features of PD, including α-syn accumulation and DA neurodegeneration using *Caenorhabditis elegans in vivo* studies and mammalian cell culture *in vitro* assays. Using these approaches, we validated the lysosomal trafficking protein, VPS41, as a potential neuroprotective candidate, in that overexpression of VPS41 suppressed both α-syn and chemical neurotoxicity in *C. elegans* and mammalian cell culture, as well as suppressed α-syn accumulation in mammalian cells. Through a structure/function analysis, we identified the minimal domains required for the protective function of human VPS41. Using worms engineered to enable DA-neuron specific RNAi, we showed that post-Golgi trafficking of AP-3 vesicles to the lysosome affected α-syn-induced neurodegeneration, indicating that VPS41 may be mechanistically eliciting protection via Golgi to lysosome trafficking. Furthermore, we functionally analyzed two VPS41 single nucleotide polymorphisms (SNPs) that naturally occur in human populations and found that both SNPs prevent VPS41 from suppressing α-syn accumulation and neurotoxicity in mammalian cells and *C. elegans*, respectively. These SNP data may represent additional genetic susceptibility factors for PD onset or progression. Additionally, using our *C. elegans* model of α-syn-induced DA neurodegeneration, we analyzed other genetic and chemicals for potential neuroprotective capacity. In this regard, the protein 14-3-3*θ* and the small molecule bafilomycin, originally identified in mammalian experiments, were functionally validated in *C. elegans*. Taken together, these studies support the predictive nature of *C. elegans* in validating potential modifiers of α-syn neurotoxicity, and highlight the importance of lysosomal function in maintaining α-syn homeostasis and its implications for suppressing the pathology associated with PD.