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ATG8 proteins are co-factors for human dopaminergic neuronal transcriptional control: implications for neuronal resilience in Parkinson disease

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ABSTRACT

Parkinson disease (PD) is caused by the loss of ventral midbrain dopaminergic neurons (mDANs) in the substantia nigra pars compacta (SNpc). These cells are especially vulnerable to stress but can be protected by autophagy enhancement strategies in vitro and in vivo. In our recent study, we focused on the LIM (Lin11, Isl−1, and Mec−3)-domain homeobox transcription factors LMX1A (LIM homeobox transcription factor 1 alpha) and LMX1B (LIM homeobox transcription factor 1 beta), crucial drivers of mDAN differentiation with roles in autophagy gene expression for stress protection in the developed brain. Using human induced pluripotent stem cell (hiPSC)-derived mDANs and transformed human cell lines, we found that these autophagy gene transcription factors are themselves regulated by autophagy-mediated turnover. LMX1B possesses a non-canonical LC3-interacting region (LIR) in its C-terminus through which it interacts with ATG8 family members. The LMX1B LIR-like domain enables binding to ATG8 proteins in the nucleus, where ATG8 proteins act as co-factors for robust transcription of LMX1B target genes. Thus, we propose a novel role for ATG8 proteins as autophagy gene transcriptional co-factors for mDAN stress protection in PD.

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Main

Parkinson disease (PD) is the second most-common neurodegenerative disease thought to affect more than 10 million people worldwide with enormous socioeconomic impact. Progressive collapse of the dopamine circuitry causes the motor and cognitive dysfunctions characteristic of PD. Thus, interventions to protect midbrain dopaminergic neurons (mDANs) of the substantia nigra (SNpc) via cell autonomous stress protection pathways represent viable strategies to slow or prevent the disease. (Macro)autophagy augmentation protects against mDAN loss following α-synuclein toxification in mice, providing compelling evidence for its beneficial effects in PD models, and further highlighting the need for better knowledge of mDAN autophagy regulatory control in humans.

The LIM (Lin11, Isl−1, and Mec−3)-domain homeobox transcription factors LMX1A (LIM homeobox transcription factor 1 alpha) and LMX1B (LIM homeobox transcription factor 1 beta) encode related, highly conserved transcription factors with developmental roles in the brain and elsewhere. E.g., mutations in *LMX1B* cause the human disease nail-patella syndrome, which affects the skeletal systems, the kidneys, and the eyes. LMX1A and LMX1B play crucial, partially redundant roles during mDAN specification and maturation, through downstream control of dopaminergic neuronal genes (e.g., *NURR1* [nuclear receptor related 1], *PITX3* [pituitary homeobox 3] and *TH* [tyrosine hydroxylase]). Tellingly, conditional simultaneous ablation of *Lmx1a* and *Lmx1b* in the mature mouse brain is associated with reduced autophagy-related gene expression, autolysosomal disruption, and mDAN loss, accompanied by accumulation of α-synuclein-rich Lewy body-type deposits. These observations highlight *LMX1A* and *LMX1B* as possible routes to mDAN functional resilience augmentation in PD. For this reason, we set out to confirm their autophagy transcription factor roles in human iPSC-derived mDAN cultures with the aim of better understanding their molecular control and contribution to human mDAN functional maintenance [1]. We validated their influence on autophagy control in human mDANs and provided the first evidence for a novel layer of reciprocal regulatory control linking these transcription factors with the core macroautophagy machinery.

Our initial data suggested a possible role for autophagy in LMX1A and LMX1B turnover, as this was found to be Bafilomycin A1-sensitive and elevated during amino acid/growth factor starvation. Furthermore, LMX1B accumulated in the nucleus during acute starvation – from where it would be poised to increase autophagy-related gene transcription – but this was reversed during prolonged starvation, with LMX1B subsequently colocalizing with cytoplasmic LC3B-positive puncta (Figure 1). Using a variety of pull-down approaches, we determined that both LMX1A and LMX1B can bind to all ATG8 family members, and that binding is blocked when using LC3-interacting region (LIR)-binding domain mutant LC3B (F52A; L53A) as the bait. Consistently, binding also required a non-canonical LIR-like domain located on the C-terminal side of the homeodomain of LMX1B.

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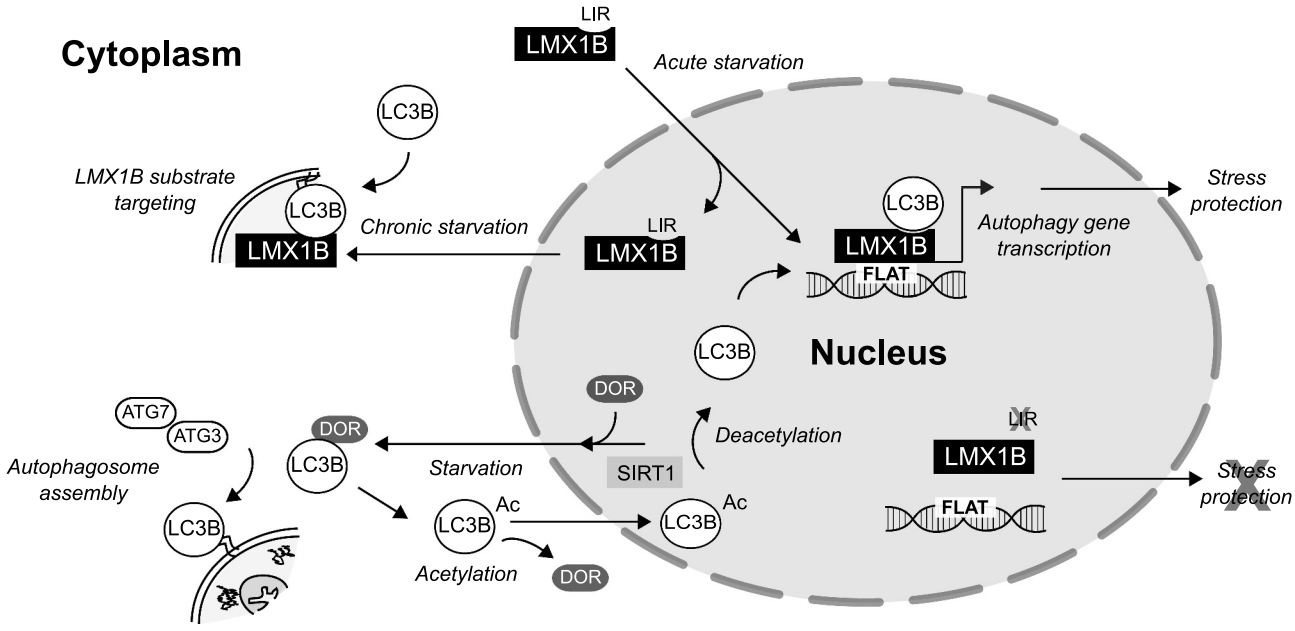

Figure 1. Schematic showing the relationships between human ATG8 proteins (exemplified by LC3B) and LMX1B. Binding to LC3B via a non-canonical LIR-like domain (depicted here as "LIR") stimulates transcription of autophagy-related genes for enhanced stress protection. Ablation of the LMX1B non-canonical LIR abolishes this protective influence. LMX1B and LC3B exhibit parallel nuclear-cytoplasmic trafficking itineraries, with their encounters in these distinct compartments resulting in different outcomes. LC3B trafficking is regulated by acetylation, while LMX1B trafficking between nucleus and cytoplasm responds to the duration and/or intensity of nutrient starvation.

Unexpectedly, ablation of the proposed LIR-like domain in LMX1B did not alter its turnover kinetics, arguing for alternative, non-degradative roles. Based on the interactions between LMX1B and ATG8 proteins being consistently observed in the nucleus, we reasoned that human ATG8 proteins might act as LMX1B co-factors for enhanced autophagy and associated gene transcription. Indeed, our detailed analysis showed that ATG8 binding-deficient LMX1B failed to trigger autophagy gene transcription to the same extent as wild-type LMX1B, with consistent, parallel impacts on autophagy flux restoration and cell stress protection in LMX1B knockdown/rescue experiments in HEK293T cells and iPSC mDANs.

Previous studies have described the acetylation-dependent cycling of LC3 between nucleus and cytoplasm, with SIRT1 (sirtuin 1)-mediated deacetylation and TP53INP2 (tumor protein p53 inducible nuclear protein 2), commonly known as DOR (diabetes and obesity-regulated nuclear factor)-dependent nuclear-cytoplasmic shuttling, which establish LC3 lipidation competency (Figure 1). Using acetylation-mimicking (K49Q/K51Q) and acetylation-resistant (K49R/K51R) GFP-LC3B variants, we found that acetylation prevents LC3B binding to LMX1B. We therefore suggest that LMX1B and LC3B observe parallel transport itineraries, with LMX1B entering the nucleus during early starvation where it binds LC3B as a co-factor for enhanced autophagy-related gene transcription (Figure 1). Under sustained nutrient stress, the gradual depletion of nuclear ATG8 proteins would curtail LMX1B-mediated autophagy augmentation, with mDANs consequently entering a phase of reduced stress resilience and increased cell death (Figure 1). This biphasic protection/cell death response is predicted to shift further toward cell death with LMX1B subsequently exiting the nucleus to be degraded by autophagy (Figure 1).

Our findings suggest exciting, new roles for ATG8 protein family members as transcriptional co-factors, and we recognize the likelihood that other transcription factors are regulated through ATG8 protein binding. Future studies are therefore needed to identify further ATG8-regulated transcription factors and to decipher the underlying mechanistic function of ATG8 binding in transcriptional control (e.g., transcription factor structural changes and DNA binding affinity; recruitment of additional regulatory components; associated post-translational modifications), and the possible influence of specific ATG8 family members on transcriptional target specificity. The broader influence of this regulatory interface on development, cell and tissue maintenance, and disease progression will need to be addressed using appropriate models in future targeted studies.

List of abbreviations

ATG8	autophagy-related protein 8
hiPSC	human induced pluripotent stem cell
LC3	microtubule-associated protein 1A/1B-light chain 3
LIM	Lin11, Isl–1, and Mec–3
LIR	LC3-interacting region
LMX1A	LIM homeobox transcription factor 1 alpha

LMX1B LIM homeobox transcription factor beta
PD Parkinson disease

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Reference

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