JCB Journal of Cell Biology

SPOTLIGHT

LC3B is a cofactor for LMX1B-mediated transcription of autophagy genes in dopaminergic neurons

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It is becoming increasingly clear that the Atg8 family of autophagy proteins have roles not only in the cytoplasm, but also in the cell nucleus. In this issue, Jiménez-Moreno et al. (2023. *J. Cell Biol.* https://doi.org/10.1083/jcb.201910133) report that nuclear LC3B binds to the LIM homeodomain transcription factor LMX1B and acts as a cofactor for LMX1B-mediated transcription of autophagy genes, providing stress protection and ensuring survival of midbrain dopaminergic neurons.

Macroautophagy (hereafter autophagy) is an evolutionary conserved lysosomal degradation pathway crucial for maintaining macromolecular and organellar homeostasis important for cell survival and organismal fitness in response to stress (1). Toxic protein aggregates, damaged or surplus organelles, and intracellular pathogens exposed to the cytoplasm are recognized as cargo by autophagy receptors, binding components of the basal autophagy apparatus to nucleate formation of autophagosomes on the cargo, which then fuse with lysosomes. Among about 40 conserved autophagy-related (ATG) proteins orchestrating this multistep process is the Atg8 family of 120 amino acid–long ubiquitin-like proteins that in humans consists of LC3A, -B, -B2, -C, GABARAP, GABARAPL1, and GABARAPL2 (2). Atg8 proteins act as membrane scaffold for other ATG proteins involved in autophagosome formation and fusion with the lysosome, help expand the autophagosome membrane, aid in transport of autophagosomes, and participate in cargo sequestration by bridging autophagy receptors to the autophagosomal membrane in selective autophagy (3). Atg8 proteins also have tasks outside autophagy and roles in the cell nucleus, as exemplified by the autophagic degradation of lamin B1 mediated by nuclear LC3B during oncogene-induced senescence (4). Autophagy receptors and most other proteins that bind directly to Atg8 family proteins do so via a 10–15 amino acid–long LC3 interacting region (LIR) motif with a core sequence W/F/Y-X-X-L/I/V with the aromatic residue docking into a hydrophobic pocket 1 (HP1) and the hydrophobic residue into a hydrophobic pocket 2 (HP2) in the LIR docking site of the Atg8 protein (3).

Autophagy is a pro-longevity mechanism, and autophagy dysfunction is linked to the pathogenesis of major human disorders including cancer and neurodegenerative diseases, like Parkinson's disease (PD; 1, 5). In PD, degeneration of midbrain dopaminergic neurons (mDANs) in the substantia nigra is a characteristic feature. The PD mDANs have compromised autophagy, α-synuclein aggregates, often in strongly ubiquitin-positive Lewy bodies, and mitochondrial defects with increased oxidative stress (1, 5). Mitochondrial dysfunction in PD can be linked to the role of the PD familial genes PTEN-induced putative kinase 1 (*PINK1*) and parkin (*PRKN*) in mediating autophagic degradation (mitophagy) of damaged mitochondria (6). Hence, it is important to determine how autophagy responses are regulated in mDANs, both short term and long term. Short-term responses, like induction of autophagy in response to nutrient or oxidative stress, is regulated by posttranslational modifications of proteins and protein–protein interactions in the cytoplasm. Transcriptional regulation of autophagy genes is important for more long-term changes in autophagy activity, like prolonged starvation.

Transcription factors and transcriptional networks that regulate autophagy in mDANs and possible relevance to PD pathogenesis need to be determined. As shown in a rat model, mDANs can be rescued from α-synuclein toxicity by increasing autophagy through overexpressing transcription factor TFEB, a master regulator of the autophagy–lysosome pathway (ALP; 7). Conditional inactivation of LIM homeodomain transcription factors *LMX1A* and *-B* in the postmitotic dopamine neurons of mice led to loss of mDANs. Abnormally large mDAN axon terminals with strong accumulation of autophagosomes and lysosomes concomitant with reduction of mRNA levels to around 50% for a number of autophagy- and lysosomal-associated genes were observed (8). This was mainly caused by the loss of LMX1B. Overexpression of LMX1B in cultured primary neurons from ventral midbrain increased expression of ALP genes while overexpression of LMX1A did not. Loss- and gain-of-function experiments strongly indicated that LMX1B regulates transcription of autophagy genes in mDANs and that LMX1B is required for maintenance of mDANs (8).

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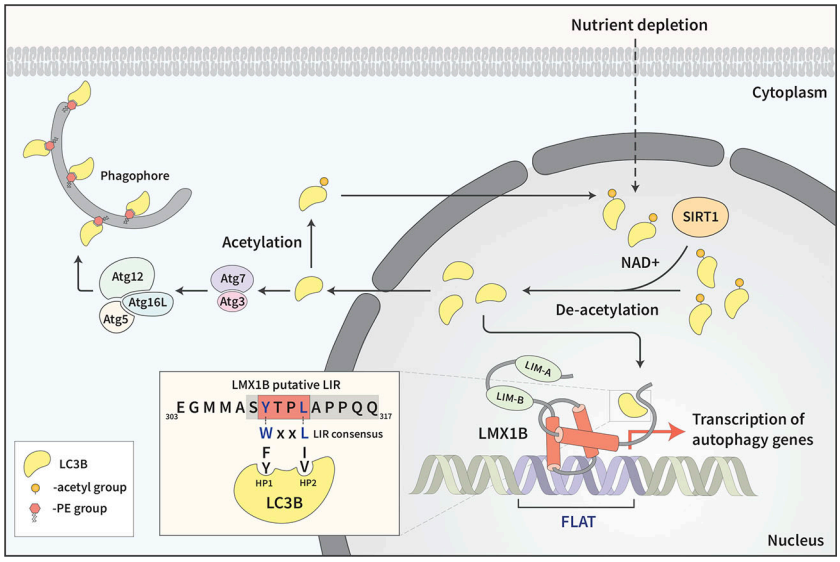

Figure 1. **LMX1B interacts with de-acetylated nuclear LC3B via a LIR-like sequence to stimulate transcription of autophagy genes in mDANs.** LMX1B binds to FLAT elements in promoters of autophagy genes. Nuclear LC3B, de-acetylated on K49 and K51, binds to the LIR-like sequence of LMX1B in a non-canonical manner to enhance transcription of autophagy genes. Nutrient depletion is known to induce SIRT1-mediated de-acetylation of LC3B (10). The de-acetylated LC3B exported out of the nucleus will be conjugated to phosphatidyl ethanolamine (PE) and incorporated in the membranes of the forming autophagosomes (phagophore).

In this issue of *JCB*, Jiménez-Moreno et al. (9) used siRNA and shRNA knockdown and CRISPR/CAS9-mediated knockout of *LMX1B* in cells combined with rescue experiments with WT and mutants to confirm that LMX1B is indeed a transcriptional regulator of a number of autophagy genes. To address the knowledge gap on the role of LMX1B in regulating autophagy in mDANs, the authors first used bioinformatic analyses to identify LMX1B binding sites, called FLAT elements, in the promoters of autophagy genes. Subsequently, they verified promoter occupancy by LMX1B and positive regulation of mRNA levels of genes encoding proteins involved in initiation (ULK1) and expansion (ATG7, ATG3, ATG16L1) of the forming autophagosome, autophagy receptors (OPTN and NDP52, not p62/SQSTM1), *PINK1* (which is involved in mitophagy), and TFEB (important for ALP). Very interestingly, the authors found that LMX1B interacts directly with human Atg8 proteins and that LC3B acts as a nuclear cofactor to stimulate transcription of autophagy genes (Fig. 1). All this was demonstrated in HEK293T cells and in in vitro induced pluripotent stem cell–derived human mDANs. However, direct binding of LMX1B to LC3B was not directly demonstrated in mDANs, only in HEK293T cells.

Jiménez-Moreno et al. (9) identified a putative LIR motif in LMX1B located C-terminal to the homeodomain (Fig. 1). Mutation of the core amino acids binding to HP1 and HP2 to alanine, in this case Y and L, usually abolish binding. This did not occur in this case. However, the deletion, Δ308-317, encompassing the core LIR and some flanking residues, did abolish binding. This LIR lacks the acidic residues flanking the core LIR that usually are present to ensure strong binding and contains a proline that is often inhibitory for binding by canonical LIRs (3). Hence, it will be interesting to solve the structure of the complex between LC3B and the LMX1B LIR to reveal how this non-canonical binding occurs. LMX1B Δ308-317 could neither effectively stimulate expression from FLAT element reporters, nor rescue the expression of autophagy genes in mDANs (or HEK293T) upon knockdown of endogenous LMX1B, nor protect against rotenone-induced cell death in mDANs with LMX1B shRNA knockdown. Another case of nuclear Atg8 acting as a cofactor to regulate autophagy genes is reported in *Drosophila* where Sequoia, a repressor of autophagy gene transcription, binds in a LIR-dependent manner to nuclear Atg8a, thereby repressing autophagy genes (11). Hence, Atg8–transcription factor interactions can have different outcomes. The complexity involved is suggested by the observation that *Drosophila* mutants lacking Atg8a show reduced expression of autophagy genes.

Nuclear LC3B is deacetylated at K49 and K51 by SIRT1 to enable its redistribution to the cytoplasm to mediate starvation-induced autophagy (10; Fig. 1). Upon 2 h starvation, the cytoplasmic fraction of LMX1B decreased and the nuclear fraction increased, likely in order to meet the demand of increased autophagy gene expression upon starvation. Consistently, LMX1B bound to deacetylated nuclear LC3B. Sequoia also preferentially bound to deacetylated Atg8a (11). Hence, Atg8 binding in the nucleus is regulated by acetylation, and nucleo-cytoplasmic shuttling of these transcription factors and Atg8s is likely also regulated to coordinate starvation stress with transcriptional regulation of autophagy genes.

In future experiments it would be interesting to see if co-occupancy of LMX1B and LC3B on target gene promoters can be verified. How does LC3B act to enhance transcription? Is it an adaptor binding to established transcriptional cofactors? The authors show that siRNA knockdown of GABARAPL1 results in reduced LMX1B-mediated reporter gene expression, suggesting that other Atg8 proteins may also act as nuclear cofactors.

The study by Jiménez-Moreno et al. (9) represents a very important contribution to our understanding of transcriptional regulation of autophagy genes with particular relevance to PD. Several layers of interconnectivity between LMX1B and LC3B in the regulation of autophagy are revealed. This study also highlights that upregulation of autophagy by modulating the activity of transcription factors like LMX1B and TFEB needs exploration for possibility of drug development to combat or slow progression of PD and other neurodegenerative diseases.

Acknowledgments

We thank Trond Lamark for critical reading of the manuscript.

We acknowledge the support of the Research Council of Norway (grant 249884), the Norwegian Cancer Society (grant

190214) and UiT The Arctic University of Norway (grant eph 2021/929).

References

1. Klionsky, D.J., et al. 2021. *EMBO J.* https://doi.org/10.15252/embj.2021108863
2. Yamamoto, H., et al. 2023. *Nat. Rev. Genet.* https://doi.org/10.1038/s41576-022-00562-w
3. Johansen, T., and T. Lamark. 2020. *J. Mol. Biol.* https://doi.org/10.1016/j.jmb.2019.07.016
4. Dou, Z., et al. 2015. *Nature.* https://doi.org/10.1038/nature15548
5. Karabiyik, C., et al. 2017. *Essays Biochem.* https://doi.org/10.1042/EBC20170023
6. Malpartida, A.B., et al. 2021. *Trends Biochem. Sci.* https://doi.org/10.1016/j.tibs.2020.11.007
7. Decressac, M., et al. 2013. *Proc. Natl. Acad. Sci. USA.* https://doi.org/10.1073/pnas.1305623110
8. Laguna, A., et al. 2015. *Nat. Neurosci.* https://doi.org/10.1038/nn.4004
9. Jiménez-Moreno, N., et al. 2023. *J. Cell Biol.* https://doi.org/10.1083/jcb.201910133
10. Huang, R., et al. 2015. *Mol. Cell.* https://doi.org/10.1016/j.molcel.2014.12.013
11. Jacomin, A.C., et al. 2020. *Cell Rep.* https://doi.org/10.1016/j.celrep.2020.107695