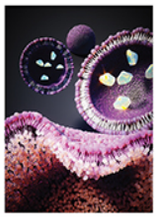
Autophagy



Autophagy

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/kaup20

LAMTOR1 ubiquitination restricts its interaction with the vacuolar-type H^+-ATPase, promotes autophagy and is controlled by USP32

Alexandra Hertel, Stefan Eimer & Anja Bremm

To cite this article: Alexandra Hertel, Stefan Eimer & Anja Bremm (2023) LAMTOR1 ubiquitination restricts its interaction with the vacuolar-type H^+-ATPase, promotes autophagy and is controlled by USP32, Autophagy, 19:8, 2406-2407, DOI: 10.1080/15548627.2023.2184958

To link to this article: https://doi.org/10.1080/15548627.2023.2184958

Published online: 01 Mar 2023.

AUTOPHAGY
2023, VOL. 19, NO. 8, 2406–2407
https://doi.org/10.1080/15548627.2023.2184958

Taylor & Francis
Taylor & Francis Group

AUTOPHAGIC PUNCTUM

LAMTOR1 ubiquitination restricts its interaction with the vacuolar-type H^+-ATPase, promotes autophagy and is controlled by USP32

Alexandra Hertel[a], Stefan Eimer[b], and Anja Bremm [a]

[a]Institute of Biochemistry II, Goethe University Frankfurt - Medical Faculty, University Hospital, Frankfurt am Main, Germany; [b]Institute of Cell Biology and Neuroscience, Goethe University Frankfurt, Frankfurt am Main, Germany

ABSTRACT

Among the various signals governing autophagy, ubiquitination plays a critical role both by controlling the stability of upstream regulators or components of macroautophagy/autophagy pathways and by facilitating the recruitment of cargo to autophagy receptors. As such, modulators of ubiquitin signaling can influence autophagic substrate degradation. Recently, we identified a non-proteolytic ubiquitin signal at the Ragulator complex subunit LAMTOR1 that is reversed by the deubiquitinase USP32. Loss of USP32 promotes ubiquitination within the unstructured N-terminal region of LAMTOR1 and prevents its efficient interaction with the vacuolar-type H^+-ATPase, a prerequisite for full activation of MTORC1 at lysosomes. Consequently, MTORC1 activity is decreased and autophagy is upregulated in USP32 knockout cells. This phenotype is conserved in *Caenorhabditis elegans*. Depletion of USP32 homolog CYK-3 in worms results in LET-363/MTOR inhibition and autophagy induction. Based on our data, we propose an additional control layer of the MTORC1 activation cascade at lysosomes via USP32-regulated LAMTOR1 ubiquitination.

ARTICLE HISTORY
Received 14 February 2023
Revised 20 February 2023
Accepted 22 February 2023

KEYWORDS
ATPase; autophagy; deubiquitinase (DUB); LAMTOR1; ragulator complex; ubiquitin; USP32; V-MTORC1

Autophagy is regulated by ubiquitination at multiple steps. Not only do ubiquitin signals attached to cargo recruit autophagy receptors, also the activity or the turnover of proteins regulating autophagy can be modulated by ubiquitination. In a recent report, we described how a specific ubiquitin signal attached to the Ragulator complex promotes autophagy induction by interfering with the cascade of events at lysosomes leading to MTORC1 activation [1]. This work arose from our long-standing interest in deubiquitinases (DUBs) and the particular goal to understand the physiological function of USP32 (ubiquitin specific peptidase 32). We set out to identify substrates of USP32 using ubiquitin-remnant profiling to ultimately conclude on the cellular processes in which the DUB is involved. Our work was mainly performed in human TERT (telomerase reverse transcriptase)-immortalized primary RPE1 cells, a non-transformed cell line with a stable diploid karyotype. By comparing the ubiquitin-modified proteome of control and *USP32* knockout (KO) RPE1 cells, we detected an accumulation of various ubiquitinated proteins related to the endosomal-lysosomal system in the absence of USP32. Among those potential USP32 substrates are the small GTPase RAB7 and the Ragulator complex subunit LAMTOR1. Increased ubiquitination of RAB7 at lysine residue (K) 191/194 and LAMTOR1 at K20 does not change their expression level. Our data suggested that these are non-proteolytic ubiquitin signals that do not regulate proteasomal degradation.

Besides investigating the cellular consequences of RAB7 ubiquitination, we mainly focused on the impact of the USP32-controlled ubiquitin signal attached to K20 of LAMTOR1. In our study, we combined CRISPR-Cas9 technology with RNA interference (RNAi) using a state-of-the-art siRNA pool for USP32 depletion to exclude off-target effects. Given that RAB7 has been linked to both autophagosome and autolysosome maturation and that LAMTOR1, as part of the Ragulator complex, is involved in MTORC1 activation, we hypothesized that hyperubiquitination of those proteins and a resulting change in functionality, could affect autophagy induction. We combined the complementary approaches of monitoring lipidation of the autophagy marker LC3 by western blotting and quantification of autophagosomes by immunofluorescence confocal microscopy with flow cytometry-based autophagy flux analysis using a well-established fluorescent probe. Loss of USP32 indeed increases autophagy induction in both RPE1 and U2OS cells.

Next, we tackled the question of how ubiquitination of LAMTOR1 elevates autophagic substrate degradation. Because K20 is close to LAMTOR1′s N-terminal myristoylated glycine residue that anchors the Ragulator complex to lysosomes, we hypothesized that a mono- or poly-ubiquitin signal attached to K20 could interfere with the positioning of LAMTOR1 and the entire Ragulator complex at the lysosomal membrane and thereby alter its ability to drive the MTORC1 activation cascade. We co-stained LAMTOR1 and the lysosomal marker LAMP2 for confocal microscopy and quantified colocalization of both proteins in control and *USP32* KO cells. A decreased Pearson's coefficient in cells depleted of USP32 indicates that less LAMTOR1 localizes to lysosomes. Interestingly, this observation was made both under full growth conditions and upon amino acid

CONTACT Anja Bremm bremm@em.unifrankfurt.de Institute of Biochemistry II, Goethe University Frankfurt - Medical Faculty, University Hospital, Theodor-Stern-Kai 7, Frankfurt am Main 60590, Germany

starvation, indicating an amino acid-independent effect. To further understand the consequences of LAMTOR1 ubiquitination at K20, a residue that lies within its unstructured, N-terminal region, we determined the interactome of LAMTOR1 by mass spectrometry. LAMTOR1 is hyperubiquitinated in *USP32* KO cells and our data showed that accumulation of this ubiquitin signal impairs binding of LAMTOR1 to the vacuolar-type H^+-ATPase (V-ATPase), another node in the network of interacting proteins at the lysosomal surface executing the multi-step process of MTORC1 activation. The lysosomal V-ATPase senses amino acid levels and stimulates the guanine nucleotide exchange factor (GEF) activity of Ragulator, which scaffolds heterodimeric RRAG GTPases and activates them. Activated RRAGs have increased affinity toward MTORC1 and recruit the kinase complex to the lysosomal surface, the site of its activation by the GTP-binding protein RHEB. Now, we have established that ubiquitination of LAMTOR1 at K20 interferes with this cascade. Complementary interactome studies of LAMTOR1 in *USP32* KO cells expressing either wild-type or catalytic inactive exogenous USP32 further support our model that LAMTOR1 needs to be deubiquitinated at K20 to efficiently interact with the V-ATPase. In accordance with those findings, less MTOR kinase localizes to lysosomes in *USP32* KO cells. Further, we observe decreased phosphorylation of a specific, autophagy-related subset of MTORC1 substrates in the absence of USP32 and elevated translocation of TFEB (transcription factor EB) into the nucleus. Our data indicate a reduction of MTORC1 activity upon loss of USP32 and the accompanying increase in LAMTOR1 ubiquitination. Finally, we tested if the role of USP32 in autophagy regulation is evolutionarily conserved. The nematode *Caenorhabditis elegans* expresses the USP32 homolog CYK-3. We used RNAi to deplete CYK-3 in worms expressing the ubiquitin-like modifier GFP:LGG-1, a homolog of human GABARAP, and detect an increase of GFP-LGG-1 puncta in the dermis of one-day-adult worms as compared to control animals. Further microscopy-based experiments confirm that CYK-3 knockdown promotes autophagy and results in impaired LET-363/TORC1 signaling in *C. elegans* as shown by nuclear accumulation of the TFEB homolog HLH-30-GFP. In conclusion, we identify a ubiquitin-dependent mechanism that restricts the engagement of LAMTOR1 and V-ATPase, an interaction required for full MTORC1 activation at lysosomes. We demonstrate that USP32 terminates this ubiquitin signal and thereby ultimately controls autophagy induction.

Acknowledgments

We would like to thank the other co-authors who contributed to the original manuscript: Ludovico Martins Alves, Henrik Dutz, Georg Tascher, Florian Bonn, Manuel Kaulich, Ivan Dikic and Florian Steinberg.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Project ID 323732846 and Project ID 25913077-SFB 1177.

ORCID

Anja Bremm http://orcid.org/0000-0003-1386-0926

Reference

[1] Hertel A, Alves LM, Dutz H, et al. USP32-regulated LAMTOR1 ubiquitination impacts mTORC1 activation and autophagy induction. Cell Rep. 2022;41(10):111653.