

SPOTLIGHT

Sorting the trash: Micronucleophagy gets selective

Pauline Verlhac  and Fulvio Reggiori 

During micronucleophagy, the nucleolus is targeted by autophagic degradation, but although nucleolar proteins are recycled, ribosomal DNA is spared. Mostofa et al. (2018). *J. Cell Biol.* <https://doi.org/10.1083/jcb.201706164>) reveal that the separation of these two nucleolar components is mediated by the CLIP and cohibin complexes and is vital for cell survival during starvation.

Autophagy is a catabolic process leading to the lysosomal/vacuolar degradation of unwanted cellular components including aggregated proteins, damaged or superfluous organelles, and even pathogens (Galluzzi et al., 2017). This pathway, which is conserved from yeast to mammals, is crucial to maintain cell homeostasis and has numerous physiological implications relevant to human health (Deretic et al., 2013). Mitochondria, peroxisomes, the endoplasmic reticulum, and even portions of the nucleus can be targeted and degraded by autophagy under the orchestrated action of autophagy-related (Atg) proteins (Dikic and Elazar, 2018). Autophagic turnover of nuclear material is termed nucleophagy and has been described in two forms in yeast: macronucleophagy and micronucleophagy. Macronucleophagy involves the sequestration of a portion of the nucleus into autophagosomes and requires the autophagy receptor Atg39 (Mochida et al., 2015). The targeted nuclear material is subsequently delivered and eliminated in the vacuole upon autophagosome fusion with this organelle. During micronucleophagy, a portion of the nucleus is directly engulfed by the vacuole at the nuclear vacuole junction (NVJ), i.e., the NVJ invaginates and pinches off into the vacuolar lumen, leading to a subvacuolar vesicle that is consumed by resident hydrolases (Roberts et al., 2003). Both micro- and macronucleophagy sequester only a portion of the nucleus, indicating a specificity similar to the one observed in other selective types of autophagy such as mitophagy or pexophagy (Galluzzi et al., 2017). In particular, DNA is not degraded by these two processes, but how this is accomplished remained unknown.

Mostofa et al. describe a mechanism allowing the separation of DNA from proteins during micronucleophagy induced by starvation conditions in yeast (Fig. 1). They focused on the nucleolus, which has previously been shown to be targeted by micronucleophagy (Dawaliby and Mayer, 2010; Mochida et al., 2015). They reveal that although nucleolar proteins are brought to the NVJ and delivered into the vacuole for turnover, ribosomal DNA (rDNA) is spared from degradation and kept in the nucleus (Fig. 1). This

mechanism of separation requires the components of the two interacting nuclear complexes, chromosome linkage inner nuclear membrane protein (CLIP) and cohibin, which are composed by Heh1-Nur1 and Csm1-Lrs4, respectively. In cells lacking CLIP or cohibin subunits, rDNA and nucleolar proteins remain associated, and although micronucleophagy is still functional, nucleolar proteins are not degraded. Interestingly, the absence of the CLIP or cohibin complex does not result in the aberrant degradation of rDNA. Thus, the CLIP and cohibin complexes are involved in repositioning of the rDNA away from nucleolar proteins but not in protection of rDNA or degradation of the micronucleophagic cargos per se (Fig. 1). Mostofa et al. (2018) also studied the spatio-temporal dynamics of micronucleophagy during starvation and observed that separation and repositioning of rDNA and nucleolar proteins occurs before the beginning of micronucleophagy. They also observed that these events depend on micronucleophagy as cells defective in this process but not NVJ formation did not show separation of nucleolar proteins and rDNA. Taken together, these findings indicate that separation and repositioning of nucleolar components and subsequent micronucleophagy are coordinated processes. This coordination is similar to the one observed between the fission and Atg machineries during mitophagy and pexophagy (Mao et al., 2013, 2014) and could be essential to avoid rDNA degradation, which would be lethal for the cell. Finally, they found that cells lacking one of the subunits of the CLIP or cohibin complexes have reduced survival during starvation, highlighting that this parting between rDNA and nucleolar proteins could be necessary for cell survival under particular stresses.

Very little is known about the mechanisms underlying micronucleophagy, and the study by Mostofa et al. (2018) is pioneering as it provides the crucial groundwork and specific assays for future investigations aimed at understanding this pathway and its relevance for the cell physiology under stress conditions and beyond. By focusing on nucleolar DNA and proteins, they succeeded in answering the lingering question of how rDNA is protected

Department of Cell Biology, University of Groningen, University Medical Center Groningen, Groningen, Netherlands.

Correspondence to Fulvio Reggiori: f.m.reggiori@umcg.nl.

© 2018 Verlhac and Reggiori This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see <http://www.rupress.org/terms/>). After six months it is available under a Creative Commons License (Attribution–Noncommercial–Share Alike 4.0 International license, as described at <https://creativecommons.org/licenses/by-nc-sa/4.0/>).

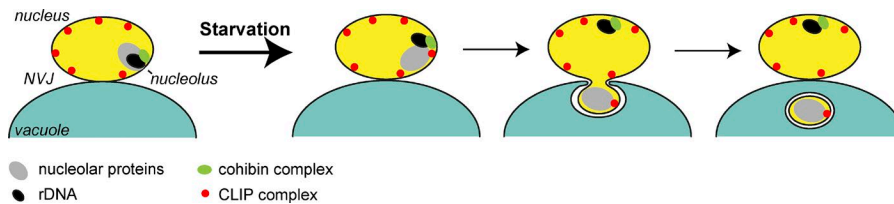


Figure 1. The role of CLIP and cohibin complexes in micronucleophagy. Nucleolar proteins, rDNA, and the cohibin complex are associated at nucleoli under growing conditions, while the CLIP complex is distributed over the entire inner side of the nuclear membrane. TORC1 inactivation triggers the association of nucleolar proteins with part of the CLIP complexes and their subsequent targeting to the NVJ, where they are included into the forming intravacuolar invagination. The pinch-off of this protrusion leads to the vacuolar turnover of both nucleolar proteins and the CLIP complex. In contrast, rDNA and cohibin remain associated and get separated from the nucleolar proteins degradation.

during micronucleophagy. Their work demonstrates that cargo selection and sequestration are key features of nucleophagy as in other forms of selective autophagy. Therefore, this study reinforces the paradigm that autophagic recycling is a meticulous process guarded by fail-safe mechanisms.

It would be of high interest to decipher the precise physiological relevance of this repositioning of rDNA and nucleolar proteins. Although [Mostofa et al. \(2018\)](#) elegantly demonstrated the importance of the CLIP and cohibin complexes during micronucleophagy, they did so using knockout strains. Thus, it cannot be excluded a priori that the relevance of these complexes for cell survival during starvation observed by [Mostofa et al. \(2018\)](#) could be due to one or more other functions of these two complexes. It would therefore be important to generate and use point mutants or possibly ablate known binding partners of the CLIP and cohibin complexes such as Sir2, Cdc14, Net1, Tof2, and rDNA-binding Fob1 to more specifically address this physiological aspect.

Specific mutants will also be key to address some aspects of the molecular mechanism of micronucleophagy. For example, how is DNA directed away and protected from degradation? Although the CLIP-cohibin system is required for rDNA separation, it appears to be unnecessary for its protection from degradation as rDNA is not turned over in the absence of these factors, although micronucleophagy still occurs. This suggests the existence of a different mechanism to avoid delivery of rDNA, and possibly chromosomes in general, to the vacuole during both micro- and macronucleophagy.

Furthermore, an aspect that will probably attract further research is the identification of the pathway that conveys TORC1 signaling into the nucleus to communicate both the repositioning of rDNA and the targeting to degradation of nucleolar proteins. TORC1 is a cytosolic complex that allows cells to adapt to environmental changes through an integrated orchestration of anabolic and catabolic pathways including micronucleophagy. Identifying the signaling axis that regulates micronucleophagy in the nucleus will help to understand intracellular communication and the potential crosstalk of micronucleophagy with other processes and possibly cell death, a crosstalk suggested by the survival defect of cells lacking nucleophagy. An interesting candidate signaling axis could be the newly identified Nemi1/Spo7-Pah1 cascade, which has been implicated in micro- and macronucleophagy induction notably through positioning of

Atg39 and Nvj1, a SNARE-mediated NVJ establishment ([Rahman et al., 2018](#)). Other lingering questions include determining whether a similar rDNA-sorting mechanism takes place during macronucleophagy and whether macronucleophagic cargoes are different from those of micronucleophagy.

The findings from [Mostofa et al. \(2018\)](#) also provide fascinating new perspectives for the study of the precise physiological role(s) of micronucleophagy. First, the observation that micronucleophagy still occurs while the repositioning of rDNA and nucleolar proteins is impaired suggests that other nuclear components might be degraded through this pathway. Identifying such cargoes would be crucial to better understand the cellular implications of an eventual defect in micronucleophagy. Moreover, [Mostofa et al. \(2018\)](#) observed a reduced survival under starvation in cells where the repositioning is impaired but nucleophagy is still functional, in line with previous observations that nucleophagy is necessary for cell survival under stress. Nevertheless, the fact that impairing the repositioning alone is enough to induce a survival defect also suggests that either degradation of nucleolar proteins is capital or other cargoes need to be degraded for cells to survive starvation.

Intriguingly, the segregation mechanism described by [Mostofa et al. \(2018\)](#) shares some conceptual aspects with what was shown for mitophagy in yeast, where specific proteins are protected from degradation by forming intramitochondrial aggregates while others are preferentially turned over via segregation into the subdomains of the mitochondria targeted by mitophagy ([Abeliovich et al., 2013](#)). A similar phenomenon might be taking place during micro- and possibly macronucleophagy, where specific nuclear proteins are preferentially degraded and thus concentrated at the site of nucleophagy in a CLIP-cohibin system-dependent manner, whereas others could be protected by another system through, for example, a retention and/or exclusion mechanism. If some proteins are protected from micronucleophagy, it would be interesting to demonstrate whether the mechanism is similar to the one protecting rDNA. Conversely, it would be interesting to explore whether mitochondrial DNA is protected during mitophagy.

Finally, although autophagy is highly conserved among eukaryotes, the same cannot currently be said for nucleophagy. Nucleophagy has rarely been observed in mammalian cells and exclusively in pathological contexts ([Park et al., 2009](#)). The findings of [Mostofa et al. \(2018\)](#) provide the framework that could

help elucidating whether micronucleophagy occurs in mammalian cells by studying for example the mammalian counterpart(s) of the CLIP and cohibin complexes. As Mostofa et al. (2018) stated in their study, it is also possible that the mechanisms of nucleophagy differ greatly between yeast and mammals. The answer could lie in the fact that key mammalian ATG components such as LC3 proteins, the homologues of yeast Atg8, are also present in the nucleus, but a role has not been assigned to this pool yet. Defects in selective types of autophagy like mitophagy, reticulophagy, and aggrephagy have been associated to severe human pathologies, and therefore, the modulation of these specific processes rather than the one of bulk autophagy has great therapeutic potential (Rubinsztein et al., 2012). It is thus crucial to determine the molecular mechanism and physiological functions of micronucleophagy in mammalian cells, and more in general, those of nucleophagy, as this could be relevant for human health.

Acknowledgments

We thank Dr. Mario Mauthe for his critical reading of the manuscript.

F. Reggiori is supported by ZonMW VICI (016.130.606), the Netherlands Organization for Scientific Research ALW Open Program (ALWOP.310), and the European Commission's Marie Skłodowska-Curie Cofund (713660) and Marie Skłodowska-Curie Innovative Training Networks (765912) grants.

The authors declare no competing financial interests.

References

- Abeliovich, H., M. Zarei, K.T.G. Rigbolt, R.J. Youle, and J. Dengjel. 2013. Involvement of mitochondrial dynamics in the segregation of mitochondrial matrix proteins during stationary phase mitophagy. *Nat. Commun.* 4:2789. <https://doi.org/10.1038/ncomms3789>
- Dawaliby, R., and A. Mayer. 2010. Microautophagy of the nucleus coincides with a vacuolar diffusion barrier at nuclear-vacuolar junctions. *Mol. Biol. Cell.* 21:4173–4183. <https://doi.org/10.1091/mbc.e09-09-0782>
- Deretic, V., T. Saitoh, and S. Akira. 2013. Autophagy in infection, inflammation and immunity. *Nat. Rev. Immunol.* 13:722–737. <https://doi.org/10.1038/nri3532>
- Dikic, I., and Z. Elazar. 2018. Mechanism and medical implications of mammalian autophagy. *Nat. Rev. Mol. Cell Biol.* 19:349–364. <https://doi.org/10.1038/s41580-018-0003-4>
- Galluzzi, L., E.H. Baehrecke, A. Ballabio, P. Boya, J.M. Bravo-San Pedro, F. Cecconi, A.M. Choi, C.T. Chu, P. Codogno, M.I. Colombo, et al. 2017. Molecular definitions of autophagy and related processes. *EMBO J.* 36:1811–1836. <https://doi.org/10.15252/emboj.201796697>
- Mao, K., K. Wang, X. Liu, and D.J. Klionsky. 2013. The scaffold protein Atg11 recruits fission machinery to drive selective mitochondria degradation by autophagy. *Dev. Cell.* 26:9–18. <https://doi.org/10.1016/j.devcel.2013.05.024>
- Mao, K., X. Liu, Y. Feng, and D.J. Klionsky. 2014. The progression of peroxisomal degradation through autophagy requires peroxisomal division. *Autophagy.* 10:652–661. <https://doi.org/10.4161/auto.27852>
- Mochida, K., Y. Oikawa, Y. Kimura, H. Kirisako, H. Hirano, Y. Ohsumi, and H. Nakatogawa. 2015. Receptor-mediated selective autophagy degrades the endoplasmic reticulum and the nucleus. *Nature.* 522:359–362. <https://doi.org/10.1038/nature14506>
- Mostofa, G.M., M.A. Rahman, N. Koike, A.M.S.T. Yeasmin, N. Islam, T.M. Waliullah, S. Hosoyamada, M. Shimobayashi, T. Kobayashi, M.N. Hall, et al. 2018. CLIP and cohibin separate rDNA from nucleolar proteins destined for degradation by nucleophagy. *J. Cell Biol.* <https://doi.org/10.1083/jcb.201706164>
- Park, Y.E., Y.K. Hayashi, G. Bonne, T. Arimura, S. Noguchi, I. Nonaka, and I. Nishino. 2009. Autophagic degradation of nuclear components in mammalian cells. *Autophagy.* 5:795–804. <https://doi.org/10.4161/auto.8901>
- Rahman, M.A., M.G. Mostofa, and T. Ushimaru. 2018. The Nem1/Spo7-Pah1/lipin axis is required for autophagy induction after TORC1 inactivation. *FEBS J.* 285:1840–1860. <https://doi.org/10.1111/febs.14448>
- Roberts, P., S. Moshitch-Moshkovitz, E. Kvam, E. O'Toole, M. Winey, and D.S. Goldfarb. 2003. Piecemeal microautophagy of nucleus in *Saccharomyces cerevisiae*. *Mol. Biol. Cell.* 14:129–141. <https://doi.org/10.1091/mbc.e02-08-0483>
- Rubinsztein, D.C., P. Codogno, and B. Levine. 2012. Autophagy modulation as a potential therapeutic target for diverse diseases. *Nat. Rev. Drug Discov.* 11:709–730. <https://doi.org/10.1038/nrd3802>