



## Ribophagy – the novel degradation system of the ribosome

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#### Summary

Recently biochemists have discovered a new pathway by which the cell selectively degrades ribosomes. The pathway is called ribophagy. Two proteins were identified as crucial for the selective degradation of ribosomes by autophagy: ubiquitin-specific protease 3 (Ubp3) and Ubp3-associated cofactor, Bre5. This fact strengthens the connections between the autophagy and proteasome pathways of protein degradations.

#### Key words:

ribophagy, autophagy, proteasome, ubiquitin, ribosome, 60S, yeast.

### 1. Introduction

Autophagy is a catabolic process involving the degradation of a cell's own components through the lysosomal machinery. It is a specific and tightly-regulated process, normal during cell growth, development, and homeostasis, helping to maintain a balance between the synthesis, degradation, and subsequent recycling of cellular products. It also plays a role during nutrient starvation, leading to the breakdown of non-vital components and the release of nutrients, ensuring that vital processes can continue. Autophagy also plays a role in the destruction of some bacteria within the cells. It has also been proposed that autophagy resulting in the total destruction of the cell is one of several

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types of programmed cell death; yet, no conclusive evidence exists for such a process (1). Autophagy is usually divided into three types: macroautophagy, microautophagy and chaperone-mediated autophagy. Majority of cellular proteins is ultimately degraded either by macroautophagy or the ubiquitin-proteasome pathway and there are some indirect functional connections between these two separate processes (2,3). By contrast to the degradation of cytoplasmatic components by macroautophagy, ubiquitin modification selectively targets protein substrates for destruction by the proteasome (4), but it has been also shown that monoubiquitination of membrane proteins can trigger their endocytosis and nonautophagic degradation in the vacuole (5,6). Macroautophagy involves *de novo* formation of membrane sealing on itself (autophagic vacuole) to engulf cytosolic components (proteins and/or whole organelles), which are degraded after its fusion with the lysosome; whereas microautophagy is the direct invagination of materials into the lysosome. Specific types of autophagy include: (i) chaperone-mediated autophagy – degradation of specific cytosolic proteins, marked with a specific peptide sequence, (ii) pexophagy – degradation of peroxisomes, (iii) mitophagy – degradation of mitochondria and (iv) xenophagy – degradation of intracellular bacteria and viruses. Recently Matthias Peter's group from the Institute of Biochemistry in Zurich has discovered a new metabolic pathway identified in starving yeast cells to dispose of ribosomes very quickly and selectively. The cells do this by using a novel autophagy process, which the biochemists have named "ribophagy". This mechanism was described recently in "Nature Cell Biology" (7).

## 2. The discovery of the novel ribosomal autophagy

Under nutrient-rich conditions, large amounts of ribosomal subunits are assembled, which raises the possibility for the need to remove excess ribosomes in response to changing environmental conditions. To examine whether ribosomes are degraded by autophagy following starvation, Kraft et al. tagged proteins of the large (RPL25) and small (RPS2) ribosomal subunits with green fluorescent protein (GFP) and observed the accumulation of GFP in the vacuole. They concluded that this relocalization required components of the autophagy machinery but was independent of the selective cytoplasm-to-vacuole (CTV) autophagic pathway.

What was also noticed, is that the tested ribosomal proteins had increased turnover kinetics compared with two other cytoplasmic proteins, taken as controls: low-abundance protein Hog1p and high-abundance protein Fba1p (7). This fact suggested that ribosome degradation involves a new selective autophagy pathway, which the authors termed "ribophagy". Both ribosomal and cytoplasmic protein accumulation in the vacuole was abolished in mutant cells that lacked the basal autophagy machinery; the authors concluded that ribosome degradation relies on both selective and non-selective autophagy processes.

To identify genes that are specifically required for the ribophagy, Kraft et al. used starvation-sensitive mutants that were screened for defects in their ability to translocate the ribosome to the vacuole upon nutrient deprivation (7). They provided the first identification of two proteins exclusively involved in the selective degradation of ribosomes by autophagy: Ubp3 (ubiquitin-specific protease 3), enzyme already known to be involved in the deubiquitination of damaged or misfolded proteins (8), and Ubp3-associated cofactor, Bre5. What was noticed is the fact, that mutants lacking *ubp3* (*ubp3Δ*) and *bre5* (*bre5Δ*) died upon nitrogen starvation (possibly because of their inability to digest the ribosomes) and *ubp3Δ* revealed the reduction of polysomes. The observed polysome profiles in mutant cells revealed that the ribophagy affects the entire 60S subunit, however not the 40S. This fact suggests differential degradation of small and large subunits.

Interestingly, it has recently been reported that the Ubp3/Bre5 complex is also able to interact with Atg19 protein and to modulate its ubiquitination (2). Atg19p is one of the components that are uniquely employed in other specific autophagy pathway, CVT (cytoplasm-to-vacuole). Atg19p binds specifically to cargo proteins Ape1p and Ams1p and mediates the interaction of CTV complex with components required for formation of the double membrane that encloses the CTV vesicle. Atg19p is shut in the Cvt vesicle along with the cargo proteins, and when the vesicle fuses with the vacuolar membrane and the inner membrane is degraded, Atg19p is released into the lumen and destroyed (2). From these findings, it can be hypothesized that Ubp3/Bre5 regulates different forms of selective autophagy during starvation (through ribophagy) and under non-starvation conditions (throughout the CVT pathway) (9).

### 3. Ribophagy – the mechanism of action

The mechanism of the ribophagy is not fully understood yet. The signalling mechanism controlling the elimination of 40S is still unknown. What is clear is that this subunit is also selectively degraded. In case of the large subunit, Kraft suggested that by analogy with endocytosis, in which monoubiquitination serves as an internalization signal, it is possible that deubiquitination of one or more proteins of the 60S is required to trigger its uptake by autophagy vacuoles (Fig. 1). Previous studies identified few ribosomal proteins to be ubiquitinated (10,11), but no function for this process was seen until now. Kraft suggested that these ubiquitin modifications may prevent the degradation of mature ribosomes by ribophagy (7). Upon starvation, deubiquitination by the Ubp3p/Bre5p could be the signal for the engulfment of ribosomal subunits by autophagic membranes, followed by their degradation in the autophagy vacuole (the equivalent of the mammalian lysosome) into its individual components (Fig. 1a). Alternatively, ubiquitination of some of the ribosomal proteins could be the signal for the engulfment of 60S and deubiquitination by the Ubp3p/Bre5p would take place inside the vacuole (Fig. 1b).

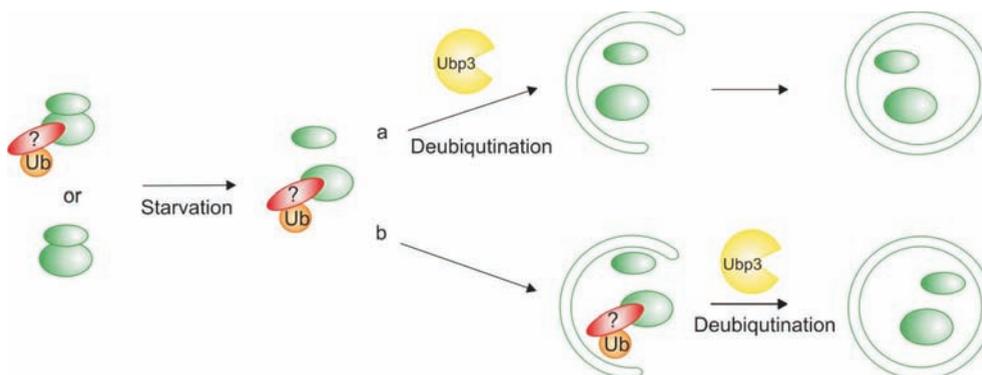


Fig. 1. The proposed mechanism of ribophagy (7).

#### 4. More implications from ribophagy

The work performed by Kraft et al. is strong evidence for a direct connection between the selective autophagy and the ubiquitin-proteasome pathway (7). Misfolded or damaged proteins are recognized intracellularly by protein quality mechanisms like chaperones and the ubiquitin-proteasome system, which aim at restoration of protein function and protein removal, respectively. A number of studies have outlined the functional significance of the ubiquitin-proteasome system as well as autophagy for the heart and, as of recently, for the vascular system (12). Abnormal abundance of ubiquitin is also a diagnostic characteristic of Huntington's disease (13,14) and many other neurodegenerative disorders (15,16) including Alzheimer's and Parkinson's diseases (17,18). Thus it has been suggested that dysfunction in ubiquitin metabolism may contribute to the pathogenesis of these diseases. Modification of proteins with polyubiquitin chains regulates many essential cellular processes including protein degradation, cell cycle, transcription, DNA repair and membrane trafficking (19), therefore disrupted ubiquitin signalling is likely to have broad consequences for neuronal function and survival. That is why a direct connection between proteasome and autophagy pathways, revealed by Kraft et al. (7) is of high importance in better understanding of protein-damage related diseases.

#### 5. Conclusions

The discovery of the fact, that ribosomes are selectively eliminated by autophagy is important for at least three reasons (9): (i) it is the first evidence of a new selective form of autophagy; (ii) ribosomes are very stable, and it is important to understand whether ribophagy plays a role in adjusting the number and quality of ribosomes in new environmental conditions; (iii) ribophagy involves deubiquitination enzyme

that strengthen the cross-talk between the ubiquitin-dependent processes and autophagy. According to Kraft, the cross-talk between autophagy and proteasome activity is noteworthy. In recent years scientists have discovered that both processes play an important role in many diseases. Therefore ribophagy is another piece in the jigsaw towards a better understanding of diseases such as Alzheimer's or Parkinson's.

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