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Commentary

Controlling proteolysis of Clp peptidase: a possible target for combating mitochondrial diseases

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ABSTRACT

Some mechanisms of cellular stress, aging, and apoptosis are related to proteolysis. With respect to ClpP, little is known about the mechanical manner in which the substrate is hydrolyzed in and released from the degradation chamber. Furthermore, what would be the real influence of ClpP in mammalian UPR^{mt}?

1. Proteolytic equilibrium and cellular dynamics

Knowing the structures and functions of intracellular peptidases is fundamental for understanding protein processing (Silva, 2017). Some mechanisms of cellular stress, aging, and apoptosis are related to proteolysis (Vahidi et al., 2018). Because of this, the equilibrium of cellular activity depends greatly on proteolytic complexes and their perfect functioning in the cell.

In this article, I would like to share my interpretation and deepen the discussion on the importance of these studies, as well as discuss the catalytic specificity information of the multimeric ClpP. We are addressing a deeply important topic for cellular biochemistry and that, in particular for ClpP, still lacks some important clarifications on its mechanistic performance in mitochondrial proteolysis.

ClpP is a highly conserved multimeric serine peptidase (Vahidi et al., 2018) with crucial roles in bacterial and mitochondrial proteostasis. X-ray crystallography has shown that ClpP consist of a pair of stacked homoheptameric rings (Stahl et al., 2018). It requires association with unfoldases ATPases (Vahidi et al., 2018) for the processing of intracellular proteins.

Indeed, it is well known that m-AAA peptidases are fundamental for cellular homeostasis. In this sense, Patron et al. (2018) described a variety of cellular dysfunctions associated with the imbalance in the activity of various peptidases (Table 1). Sica and Kroemer (2018) also discussed the function of IMMP2L suppressing cellular senescence. Cellular senescence contributes to organismal aging and tumor suppression.

Imbalances in proteolysis, either by reduction or an increase of proteolysis, cause negative effects on the cell. Thus, in understanding the causes of this perturbation in the cell it is vitally important to look for therapeutic routes or to serve as biomarkers (for example, overexpressed peptidases in cancer). For this reason, many investigations have searched for alternative ways to control the cellular proteolysis. Research on these proteolytic complexes contributes greatly to the better understanding of these enzymes and pathologies.

Recently, Vahidi et al. (2018) demonstrated a novel way to control the activity of the Clp peptidase from *Staphylococcus aureus* (SaClpP). They did so by means of an N-terminal conformational switch rather than using the active site as a target for suppression of catalysis. In that same year, Stahl et al. (2018) described a selective activation of human ClpP. The authors reported a molecule (D9) capable of acting as a potent and selective activator of human ClpP.

Living organisms naturally have proteolysis activation/deactivation mechanisms. One of the ways of limiting broad proteolysis is through substrate specificity. In the case of ClpP, the specificity study was recently discussed. Gersch et al. (2016) evaluated the specificity of ClpP from *E. coli, S. aureus*, and human mitochondria, and proposed that the barrel-shaped architecture of ClpP favors proteolysis even when the enzyme is anchored to amino acid sequences for which it does not exhibit high preference. In the physiological proteolysis scenario, the high molar concentration of substrate inside the degradation chamber could enable increased proteolysis.

This indicates that the selectivity of the ClpP enzyme is in fact dependent on the substrates that have access to the degradation chamber, via ATP-dependent unfolding and control by the N-terminal domain of the protomer.

2. Current challenges and future directions

With respect to ClpP, little is known about the mechanical manner

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Table 1
Some pathologies associated with mitochondrial peptidases.

Enzyme	Catalytic type	Diseases associated with proteolysis imbalance	References
LONP1	Serine peptidase	CODAS syndrome	Patron et al. (2018)
ClpP	Serine peptidase	Perrault syndrome	Patron et al. (2018)
		ClpP is overexpressed in human cancer cells	Vahidi et al. (2018); Seo et al. (2016)
HTRA2	Serine peptidase	Parkinson's disease	Gulsuner et al. (2014)
IMMP2L	Serine peptidase	Tourette syndrome, Alzheimer's disease and autism	Zhang et al. (2017)
PMPCB	Metallopeptidase	Friedreich ataxia	Patron et al. (2018)
YME1L1	Metallopeptidase	Optic atrophy	Patron et al. (2018)
AFG3L2	Metallopeptidase	Spinocerebellar ataxia type 28 (SCA28)	Patron et al. (2018)
OMA1	Metallopeptidase	Heart failure	Wai et al. (2015)
		Neurodegenerative pathologies	Korwitz et al. (2016)

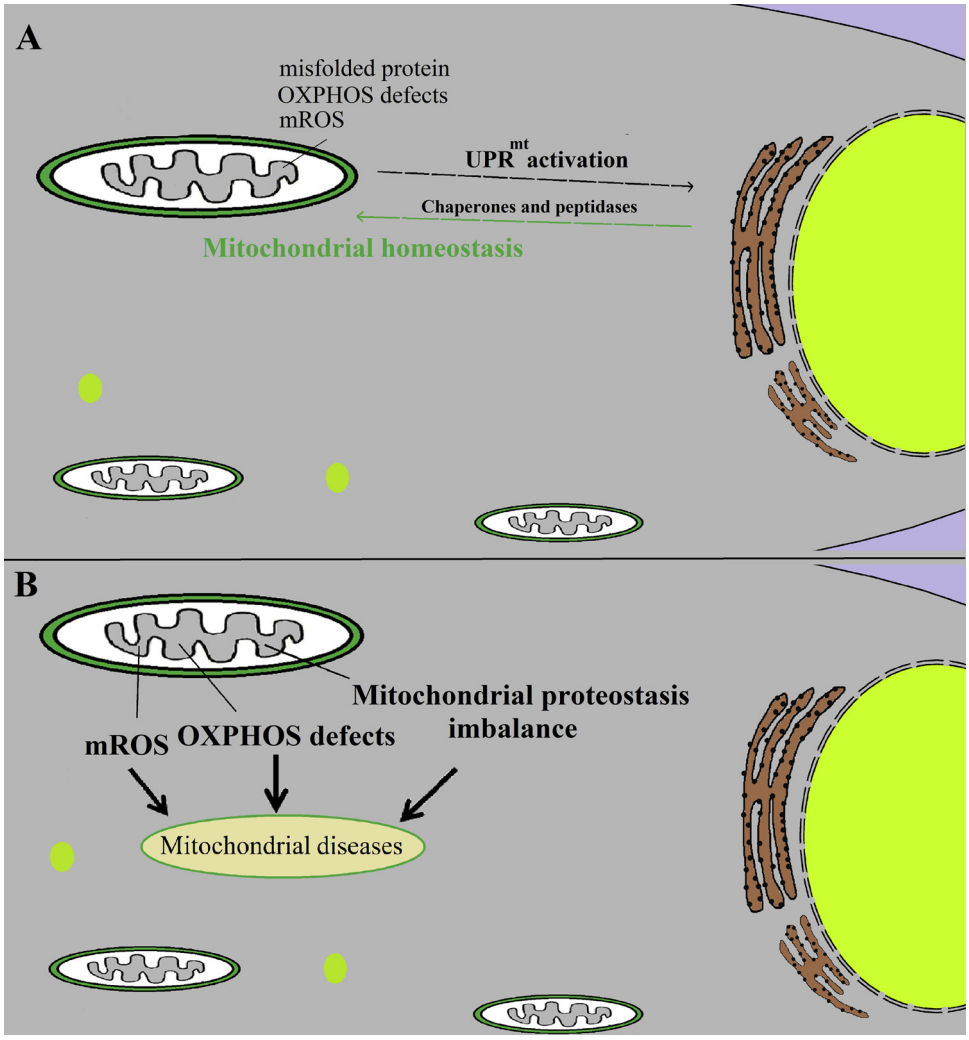

Fig. 1. Eukaryotic cell, (A) Proper functioning of peptidases and chaperones, and UPR^{mt} activation: nuclear transcription of mitochondrial chaperones and peptidases. (B) Deficiencies in multimeric peptidases functioning.

in which the substrate is hydrolyzed in and released from the degradation chamber. Details on substrate processing still require further investigation.

Another important aspect to be considered is the influence of ClpP on mitochondrial unfolded protein response (UPR^{mt}). UPR is a signaling event to respond to the mitochondrial stress caused by unfolded protein and other perturbations (Fig. 1), including Oxidative phosphorylation (OXPHOS) defects, and excessive ROS (Lin and Haynes, 2016; Tian et al., 2016; Shpilka and Haynes, 2018; Vahidi et al., 2018). In *Caenorhabditis elegans* the involvement of ClpP has been fundamental for UPR^{mt}. However, little is known about the influence of ClpP in mammalian UPR^{mt}.

Recently, Seiferling et al. (2016) demonstrated that, in mammals, loss of ClpP did not affect activation of UPR^{mt} and surprisingly alleviates mitochondrial cardiomyopathy. This causes uncertainties about ClpP as a possible target for UPR^{mt} intervention. There is still much to be discovered about mitochondrial ClpP. Further studies of this enzyme complex will provide a better understanding of its role in protein and peptide processing within the mitochondria.

Although there are still uncertainties regarding ClpP in human UPR^{mt}, many pathologies demonstrate close association with multimeric enzymes, including human malignancy (Patron et al., 2018)

(Table 1). In this sense, by reflecting the work conducted by Vahidi et al. (2018), controlling proteolysis could possibly be important for combating some pathologies. The Clp peptidase is a key component in the degradation of intracellular proteins and therefore assumes an important role in cellular dynamics. Its overexpression has already been verified in pathologies such as acute myeloid leukemia, among others (Seo et al., 2016).

In view of these results, it is very important to highlight the advances that these researches have achieved. By analyzing the works of Vahidi et al. (2018) and Stahl et al. (2018), we observed the positive and negative modulation of ClpP activity. Vahidi et al. (2018) have provided us with an alternative way to control the activity of this enzyme complex. Alternatively, Stahl et al. (2018) offers an important tool to analyze mechanistic features of human ClpP. This study opens up an opportunity to provide further clarifications on its mechanistic performance in mitochondrial proteolysis. In fact, knowing the structures and functions of intracellular peptidases is fundamental for understanding protein processing. This finding is a significant contribution to this area of research.

For this perspective article, I have proposed a brief discussion on this topic in cellular biochemistry, interpreting recent investigations on mitochondrial ClpP, in order that it serve as an avenue for new researches that will support new discoveries in this field.

Conflict of interest

The author declares no competing financial interest.

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