

# The Clp chaperone-protease is a central regulator of chloroplast proteostasis; the search for substrate selection and regulation

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**ABSTRACT** Different proteases and peptidases are present within chloroplasts and non-photosynthetic plastids to process precursor proteins and to degrade cleaved chloroplast transit peptides and damaged, misfolded, or otherwise unwanted proteins. Collectively, these proteases and peptidases form a proteolysis network, with complementary activities and hierarchies, and build-in redundancies [1]. The challenge is to determine the contributions of each peptidase (system) to this post-translational network. This will require an understanding of substrate recognition mechanisms, degrons, substrate and product size limitations, as well as the capacity and degradation kinetics of each protease. Extra-plastidial degradation pathways complement these intra-chloroplast proteases. Following a conceptual overview of this intra-plastid protease network, I will provide an update on our search for substrates, substrate selection and regulation of the essential and abundant chloroplast Clp chaperone-protease system and its adaptors in Arabidopsis [2]. This search includes loss-of-function analysis, in vivo trapping, protein half-life measurements, N-terminomics, in vitro interactome analysis, combined with protein mass spectrometry. I will also highlight our recent proposal that UVR protein motifs play a regulatory role in substrate selection and Clp activity [3].

## REFERENCES

1. van Wijk, K.J., Intra-chloroplast proteases: A holistic network view of chloroplast proteolysis. *Plant Cell*, 2024. 36(9): p. 3116-3130.
2. Bouchnak, I. and K.J. van Wijk, Structure, function, and substrates of Clp AAA+ protease systems in cyanobacteria, plastids, and apicoplasts: A comparative analysis. *J Biol Chem*, 2021. 296: p. 100338.
3. Annis, M.Y., C.M. Ravenburg, and K.J. van Wijk, Uvr motifs regulate the chloroplast Clp chaperone-protease system. *Trends Plant Sci*, 2024.

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