Structural Analyses of ATG9 and Hsp21 by Cryo-EM

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Macroautophagy, henceforth called autophagy, is a major bulk degradation pathway responsible for the removal of unwanted or harmful materials from cells, including protein aggregates, damaged organelles and invading pathogens. This process is critical to cell homeostasis and is highly conserved throughout the eukaryotes. Autophagy proceeds by the *de novo* formation of a cup-shaped membrane, the phagophore, that expands and engulfs cytoplasmic cargo. The expanding membrane eventually seals to generate a double-membrane compartment termed the autophagosome, which then fuses with the lysosome or the vacuole where the sequestered cargo is degraded and recycled. Of the core autophagy machinery components, Atg9 is the integral membrane protein proposed to act as an organizing center and, at the same time, provide an essential source of lipids/membranes for phagophore nucleation. However, the molecular events and mechanisms underlying phagophore nucleation remain poorly understood. Here we use single-particle cryo-EM to elucidate structures of Atg9. Our work provides insights into the Atg9 architecture and testable hypotheses for the molecular mechanism of autophagy progression regulated by Atg9.

Environmental stresses often lead to protein unfolding and the formation of cytotoxic aggregates that compromise cell survival. In the cell, proper folding of proteins is constantly monitored and maintained by the protein quality control system. One essential component of protein quality control is molecular chaperones that stabilize non-native protein conformations and facilitate the refolding of proteins. The small heat shock proteins (sHsps) are ubiquitous and diverse family of molecular chaperones present in all living organisms. While they bind to and sequester misfolding proteins to prevent their aggregation, they themselves do not promote substrate refolding, distinguishing them from other major chaperone families. How do sHsps recognize a wide variety of unfolding substrate proteins? Currently, little is known about the structural organization of the sHsp-substrate complexes due to their inherent heterogeneity and lack of high-resolution information. Our group is interested in the chloroplastic Hsp21, a member of the organelle-localized sHsps unique to plants. In preliminary

analysis we identified a natural substrate of Hsp21, which catalyzes the synthesis of precursors to metabolites that function in photosynthesis, growth regulation and plant-environment interaction. To obtain insights into the regulation and determinants of substrate binding and specificity of Hsp21, we use cryo-EM combined to study the Hsp21 structure and its interaction with substrate. Our work improves our understanding of the molecular mechanism of protein aggregation suppression by sHsps. Because overexpression of Hsp21 in transgenic plants enhances tolerance towards heat stress, the outcome of this research will offer potential applications for agricultural biotechnology to develop stress-resistant crops.