**Drosophila** Golgi membrane protein Ema promotes autophagosomal growth and function

Sungsu Kim, Sarah A. Naylor, and Aaron DiAntonio

Department of Developmental Biology, Washington University in St. Louis, St. Louis, MO 63110

**AUTHOR SUMMARY**

Autophagy is a self-digestion process in eukaryotic cells. During this process, organelles and other components in the cell's cytosol are engulfed in double-membrane structures called “autophagosomes” and are trafficked to degradative lysosomes, thereby recycling cellular resources. Autophagy is essential for normal development and homeostasis at both the cellular and organism level and is implicated in many human diseases, including cancers and neurodegenerative disorders. Although the biogenesis of autophagosomes and interactions between autophagy and other cellular membrane-trafficking pathways have been explored in detail, the molecular mechanisms governing the growth and ultimate size of autophagosomes are not well described. Here, we used *Drosophila* fat body cells to demonstrate that the *Drosophila* Golgi membrane protein Ema promotes the normal growth of autophagosomes and is required for the efficient degradation of autophagic substrates (Fig. P1).

Our previous finding that Ema protein promotes the maturation of endosomes, membrane-bound compartments that carry material to various destinations within the cell, led us to hypothesize that the *ema* gene might be required for autophagosomal maturation (1). To test this hypothesis, we chose *Drosophila* fat body cells, in which autophagosomes often are >10 μm in diameter, much larger than their submicron counterparts in mammalian cells and yeast. We labeled autophagosomes with fluorescent Atg8a protein, labeled lysosomes with LysoTracker (Invitrogen), a fluorescent dye that stains acidic lysosomes, and performed live confocal imaging.

Contrary to our initial hypothesis, autophagosomes in the *ema*-mutant fat body cells matured effectively into acidic lysosomes. However, autophagosomes were dramatically smaller in the mutants than in wild-type cells. Electron microscopic analysis verified the formation of abnormally small but mature autophagosomes in the *ema*-mutant fat body cells. Mosaic clonal analysis and genetic rescue experiments, methods of determining the effects of specific genetic changes, demonstrated that *ema* functions within fat body cells to generate autophagosomes of normal size. Analysis of the time course of autophagosomal development following starvation, which stimulates autophagosome formation, demonstrated that autophagosomal growth was impaired at an early stage of autophagy in the *ema* mutant. Hence, *ema* controlled the growth of autophagosomes and their ultimate size.

How does Ema promote autophagosomal growth? An autophagosomal growth defect could result from impaired autophagosomal fusion with endosomes or lysosomes. This hypothesis is attractive, because we previously demonstrated in endocytic Garland cells that *ema* promotes endosomal trafficking (1). Here, however, we demonstrated that the endocytic tracer Avidin-Cy3 and the lysosomal protein Lamp1-GFP were readily incorporated into autophagosomes in both wild-type and *ema*-mutant fat body cells, demonstrating that fusion persists in the mutant. Furthermore, mature autolysosomes were much smaller in the mutant than in wild type. This finding is not consistent with the hypothesis that the autophagosomal growth defect is caused by a failure to fuse with endosomes or lysosomes. Instead, it suggests that Ema has a function in promoting autophagosomal growth that is distinct from its role in endosomal maturation.

To investigate this possibility, we assessed the localization of the Ema protein. In contrast to Garland cells, where we previously demonstrated that Ema is primarily an endosomal protein (1), we found that Ema localized to the Golgi in fat body cells, as well as in salivary gland, muscle, and epithelial cells. The Golgi is a major trafficking organelle in the cell. Upon starvation, Ema protein localized to autophagosomal membranes, consistent with the trafficking of Ema from the Golgi to autophagosomes upon induction of autophagy. Furthermore, we demonstrated that the *Drosophila* Golgi protein Lva localized to autophagosomes upon starvation and that this localization to autophagosomes required *ema*. These findings suggest that *ema* may promote autophagosomal growth by recruiting Golgi-to-autophagosomal membrane traffic.

Is *ema* also required for autophagosome function? The ubiquitin-binding protein p62 recruits ubiquitinated substrates to autophagosomes and is subject to autophagic turnover. Ubiquitination is a common form of marking cellular proteins, particularly those that are intended for degradation. Mitochondria also undergo autophagic degradation, termed “mitophagy.” We...
found that the *Drosophila* p62 protein accumulated in starved *ema*-mutant fat body cells. In addition, mito-GFP–labeled fragmented mitochondria accumulated in the autophagosomes of the *ema*-mutant fat body cells but were rarely detectable in wild-type autophagosomes. These results demonstrate that p62 turnover and mitophagy are impaired in the *ema* mutant. Although the *ema* mutant’s autophagosomes can mature, fuse with lysosomes, and become acidic autolysosomes, functionally defective endosomes and lysosomes could contribute to this defective autophagic degradation. These findings demonstrate that Ema is required not only for autophagosomal growth but also for autophagic function.

In this study we identified a role for the *Drosophila* Golgi membrane protein Ema in autophagy and found an aspect of autophagosome morphogenesis that controls the growth and ultimate size of autophagosomes. Furthermore, Ema is the *Drosophila* ortholog of human Clec16A, a candidate autoimmune-susceptibility locus (2, 3). Expression of hClec16A can rescue the *ema* mutant’s autophagosome growth defect, suggesting that this gene family has a conserved role in regulating autophagosome morphogenesis. Finally, our results support the seminal hypothesis of Locke and Collins (4) that the Golgi complex contributes to autophagosome membranes. Future studies will explore the molecular mechanism of the Golgi-to-autophagosome relationship.