Role of selective autophagy in cellular remodeling
“Self-eating” into shape

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The Atg1 Ser/Thr kinase, although now a well-established regulator of autophagy, was first identified genetically in C. elegans as a requirement for axonal elongation. However, possible connections between Atg1 functions in cellular morphogenesis and in autophagy were previously unaddressed. In the recent paper highlighted in this punctum, we reconciled these dual roles for Atg1, demonstrating a requirement for p62-mediated selective autophagy in the dynamic regulation of cell shape, in both fly and mammalian macrophages, with effects on immune cell functions. This work further strengthens the emerging importance of autophagy as a post-translational regulatory mechanism in diverse cell signaling contexts, including the cortical remodeling and function of immune cells.

Immune cells undergo environmentally responsive cell shape changes for surveillance functions that involve cell spreading and formation of cell protrusions. A central theme in the remodeling of cell shape is reorganization of cell structural components through the modulated balance between assembly and disassembly of the cytoskeleton and focal adhesions. Our identification of Atg1 in an RNAi screen for cellular morphogenesis, coupled with previously described requirements in neuronal morphogenesis and autophagy, led us to investigate a novel role for the membrane-mediated process of autophagy in the regulation of immune cell shape. Drosophila macrophages, called hemocytes, dissected out of larvae display a spreading response, starting as round cells that flatten while extending and retracting filopodial-like, filamentous (F)-actin protrusions. In contrast, hemocytes from Atg1 mutant larvae attached but did not flatten or extend F-actin protrusions. We found that addition of the autophagy inhibitor, 3-methyladenine (3MA), to wild-type hemocytes or hemocyte-targeted knockdown of other genes required for autophagy (atg4, atg6, atg7, atg8a and atg9) mimicked the Atg1 knockdown phenotype. Importantly, hemocyte recruitment to larval wound sites was impaired in Atg1 mutants, indicating the significance of an autophagy role in hemocyte cortical remodeling to immune cell function. This demonstrates that autophagy is cell-autonomously required for hemocyte shape changes critical for functions in Drosophila.

Autophagy is best understood as a nonselective, catabolic, pro-survival or pro-death mechanism. Therefore, we addressed whether a role for basal (uninduced) autophagy in cellular remodeling was required independent of these more familiar functions. We found that Atg1 mutant larvae had normal hemocyte survival, proliferation, differentiation and adhesion in the absence of basal autophagy. Neither was the defect in cellular remodeling associated with decreased energy production. Finally, addition of 3MA to spread hemocytes caused them to round up, whereas washing away the 3MA allowed the hemocytes to spread, pointing to a continuous role for autophagy in the initiation and maintenance of hemocyte spreading. Altogether, these studies support the idea that basal autophagy plays a specific, immediate and continuous role in cortical remodeling.
How does autophagy specifically regulate cell spreading and protrusion extension? Cell spreading involves coordinated events in cytoskeletal remodeling, both for removal of inhibitory cortical tension and the generation of dynamic protrusive forces. We found that the hemocyte cortex was dynamic with membrane ruffling both in control and Atg1 mutant conditions. This suggests that autophagy is unessential for protrusion initiation, but appears to regulate protrusion extension that occurs with cell flattening. The requirement for autophagy was also specific for an integrin-mediated cell spreading, demonstrating that the general structural machinery required for cell flattening is independent of autophagy. We found that expression of a constitutively active form of the Rho1 GTPase, a known regulator of the F-actin cytoskeleton, drives abnormally long cell protrusions in spread hemocytes. However, hemocytes disrupted for autophagy blocked these Rho1-induced protrusions, indicating that autophagy is needed to mediate a Rho1 pathway leading to protrusion extension. Interestingly, the adapter protein, p62, which targets ubiquitinated cargoes to autophagic destruction, was similarly required for cell spreading and protrusion formation. From all of this, we conclude that selective autophagy is required continuously, possibly through p62-mediated degradation of a regulator of the Rh1 pathway, for dynamic F-actin-based protrusions.

Importantly, we established that a requirement for autophagy in cell shape changes is conserved in mammalian macrophages. Changes in cell shape are induced with macrophage activation, and are required for proper immune functions. Basal autophagy was required for the characteristic cell shape changes elicited in response to different stimuli in mouse cell lines and primary macrophages. We thus uncovered a previously unknown role for autophagy in macrophage cell shape changes, with relevance to their proper immune functions.

It is curious how autophagy, leading to lysosomal degradation, provides a continuous regulatory mechanism for the cyclical processes of cell spreading and protrusion formation. One possible explanation is that cells, especially environmentally responsive and migratory cells, must be primed to assemble or disassemble sub-structures that enable a switch between morphological responses, e.g., cell spreading or rounding up. In this respect, selective autophagy could target for degradation a factor that promotes the cortical F-actin ring in rounded cells, and thereby permits extension of F-actin bundles in cell protrusions. Another untested example of this switch is the rounding up and subsequent respreading of cells for mitosis. Our work does not rule out the interesting possibility that sequestration, and possible re-release, of specific substrates from autophagosomes, but not their degradation in lysosomes is the impetus for autophagy-dependent cell shape changes. This is an intriguing consideration given the rapid cycles of Rho GTPase hydrolysis and cytoskeletal assembly-disassembly involved in cortical remodeling.

The implication of a selective, basal form of autophagy in cell remodeling, versus the bulk cytoplasmic turnover seen in stress-induced autophagy, adds more support to the emerging theme of autophagy as a key regulator of signaling pathways, possibly as a rheostat that controls pathway flux. Since p62 can bind and target specific ubiquitinated proteins for degradation by autophagy, drawing analogies to regulation of signaling by proteomic degradation, p62 is an attractive candidate to pursue in aiding the identification of relevant autophagy substrates. Discovery of the molecular mechanisms underlying regulation of cell remodeling by autophagy will add to our understanding of the numerous—and often, surprising—roles played by autophagy.

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