Developmental Cell
Previews

The Autophagy Connection

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DOI 10.1016/j.devcel.2010.07.005

For a process intimately connected to an immense range of physiological processes, the molecular understanding of macroautophagy remains far from complete. Recent large-scale studies, including those of Behrends et al. in *Nature* and Lipinski et al. in *Developmental Cell*, are now providing new insight into the machinery of autophagy regulation.

Macroautophagy (hereafter referred to as autophagy) is usually a catabolic process in which portions of the cytoplasm are sequestered within cytosolic double-membrane vesicles called autophagosomes and subsequently delivered to the lysosome to allow degradation and recycling of the cargo (Mizushima et al., 2008). Autophagy has attracted increasing research interest because it is associated with a wide array of physiological processes that range from embryogenesis to life span extension, whereas its dysfunction may play a role in numerous diseases (Figure 1). The autophagic pathway is complex, and 33 proteins have already been identified in fungi that are primarily devoted to various steps, in particular those of cargo recognition and autophagosome formation. Although the majority of these proteins have been identified and characterized in yeasts, new high-throughput global analysis studies, such as that of Behrends et al. (2010), recently published in *Nature*, and Lipinski et al. (2010), recently published in *Developmental Cell*, are providing large amounts of information on proteins associated with autophagy in higher eukaryotes.

Although autophagy was initially observed in mammalian cells, mutants defective in this process, and subsequently the complementing genes and gene products, were first identified in yeasts (Klionsky et al., 2003). Many, but not all, of these proteins have clear homologs in higher eukaryotes (Klionsky et al., 2010); in general, the exceptions are seen with autophagy-related (Atg) proteins, such as Atg11, Atg30, or Atg32, that are involved in selective types of autophagy. Conversely, there are some components, such as Atg101, that are only found in higher eukaryotes (Mercer et al., 2009). In addition, many organisms have multiple isoforms of the Atg proteins relative to a single version in yeast. Some of the autophagy proteins also function in complexes that, in mammals, may contain additional components. Despite these variations in the proteins involved during autophagy among different model organisms, the processes of autophagosome formation and autophagy regulation remain too poorly understood to know whether there are substantial differences between organisms in the overall process. Nonetheless, it would not be surprising if mammals displayed a greater complexity, at least in the regulatory control of autophagy. In part, this may represent development- or tissue-specific regulation not relevant to yeasts. Some aspects of autophagic regulation—such as the cues for organelle degradation—may also not be fully conserved in mammals, possibly reflecting the differing metabolic regulation as yeasts tend to carry out fermentation rather than respiration. Moreover, autophagy in yeasts is primarily a starvation response. In contrast, autophagy in higher eukaryotes is involved in a greater diversity of functions, including roles in diseases such as cancer and neurodegeneration that have little or no relevance to yeast.

Screens to identify regulatory and structural components of autophagy have been carried out in several model systems, and this has definite advantages. For example, the facility of the yeast system for molecular genetic studies makes it possible to conduct synthetic gene arrays encompassing the entire genome (Costanzo et al., 2010). Indeed, large-scale screens in yeast, such as those for mutants that are defective in selective mitochondria (Kanki et al., 2009; Okamoto et al., 2009) or peroxisome (Manjithaya et al., 2010) degradation, are still uncovering new autophagy components. However, most studies of fungal autophagy have focused on its role as a starvation response triggered by changes in nutrient conditions, which may not be a common occurrence in mammalian cells under physiological conditions. Thus, both Behrends et al. (2010) and Lipinski et al. (2010) took advantage of recent advances in molecular genetic methodologies that can be applied to higher eukaryotes to examine in human cells the regulation of basal autophagy, which occurs constitutively. Beyond providing a foundation for an extended understanding of the complex regulatory network and mechanism of autophagy in mammals, this approach also has the potential advantage of identifying starvation response-independent autophagy components that may not have been uncovered in the yeast screens.

The report by Behrends et al. (2010) uses a proteomic approach to focus on identifying interaction partners associated with autophagy. The authors started with a subset of human proteins that were previously linked with autophagy or vesicle trafficking. Tagged versions of these proteins expressed in HEK293T cells were affinity isolated, and coimmunoprecipitated proteins were identified by mass spectrometry. This analysis identified 409 "high-confidence candidate interaction proteins" that comprise 751 interactions, forming an "autophagy interaction network." One caveat of this

approach is that it is inherently limited to identifying interactors of only known components. Nonetheless, a consideration of putative interactors of the Atg8 protein family demonstrates the potential utility of this approach. Atg8 is the classic autophagy marker because it is the only protein in higher eukaryotes that clearly remains associated with the completed autophagosome. The posttranslational conjugation of Atg8 to phosphatidylethanolamine (PE) is critical for phagophore expansion during autophagosome formation (Figure 1). Despite its central role in this process, the function of Atg8 is not clearly defined. Similarly, the function(s) of the various Atg8 homologs in mammals is not known, nor is it known why multiple isoforms of this protein are needed. Thus, the identification by Behrends et al. (2010) of 67 potential interaction partners for human ATG8, most of which were previously unknown, provides a substantial number of leads that may yield further insight into the function of these proteins.

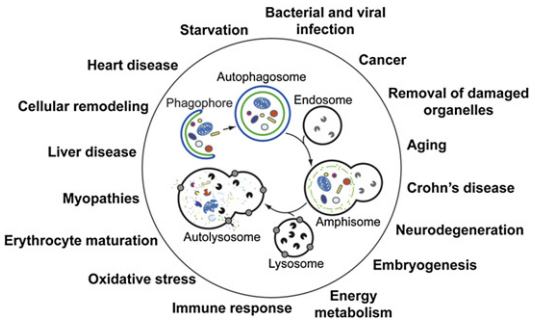

Figure 1. Macroautophagy Is Extensively Involved in Cellular Homeostasis

The morphological features of macroautophagy are illustrated schematically. The initial sequestering compartment, the phagophore, expands into the double-membrane autophagosome. Fusion with an endosome generates the single-membrane amphisome, which subsequently fuses with a lysosome. The degraded cytoplasm is released back into the cytosol through permeases. Some of the physiological connections between macroautophagy and human health and disease are indicated by the surrounding terms.

Using a very different approach, the study by Lipinski et al. (2010) relies on a siRNA screen to identify genes that regulate autophagy. Focusing on basal autophagy, they uncovered regulatory components that are involved in stress other than that of nutrient limitation. The authors transfected human neuroblastoma cells expressing the GFP-LC3 autophagy reporter with siRNA pools and screened for changes in GFP-LC3 puncta or in the level of LC3-II (the human Atg8–PE homolog). One caveat concerns the use of neuroblastoma H4 cells, which are derived from a tumor and may thus have regulatory pathways that are altered from that in normal cells. Furthermore, the function of autophagy in the neuroendocrine system is not fully understood. Of course these very facts can also be viewed as compelling justifications for studies that could have ultimate therapeutic applications. The particular impact of this analysis is that it identified a group of receptor-mediated signaling pathways, responding in part to growth factors and other cytokines, that regulate autophagy through the activity of phosphatidylinositol 3-kinase rather than mTOR, the best-characterized factor that regulates autophagy (primarily as a nitrogen sensor). Cytokines play important roles in extracellular signal transduction pathways, but they are not present in fungi. Thus, this screen provides a striking example of components that would not be readily, or ever, identified through studies that rely solely on unicellular organisms. Furthermore, autophagy is a mechanism of cellular homeostasis that must respond to varying nutritional and environmental cues, but little is actually known about the signal transduction pathways that modulate this process. This study provides a wealth of new information that connects autophagy with extracellular signaling, setting the stage for an increased understanding of this coordination.

Clearly, the challenge for the future in following up either of these reports is to fully characterize the interaction networks. However, as increasing studies implicate autophagy in ever more aspects of cellular and organismal physiology, and, in the case of these recent studies, tremendously expand the number of proteins that impinge on autophagy, we may soon reach a time when we will face the need for a new screen—one for components that are *not* involved in some way with autophagy.

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