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Autophagy Shows Its Animal Side

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Most autophagy genes have been discovered in the single-celled yeast *Saccharomyces cerevisiae*, and little is known about autophagy genes that are specific to multicellular animals. In this issue, Tian et al. (2010) now identify four new autophagy genes: one specific to the nematode *Caenorhabditis elegans* and three conserved from worms to mammals.

Autophagy, or "self-eating," is a catabolic process that degrades and recycles cytoplasmic contents. Pioneering studies in the single-celled yeast *Saccharomyces cerevisiae* identified a suite of autophagy (*Atg*) genes required for survival during starvation (Mizushima, 2007). Although many of these genes are functionally conserved from yeast to mammals, autophagy is probably more complex in multicellular animals and most likely requires factors that are absent in yeast. For example, animal tissues maintain homeostasis when nutrients are locally restricted by trading off metabolic and catabolic processes, and this may be one reason that cancer cells with altered metabolism display elevated levels of autophagy (Mathew et al., 2007). However, little is known about autophagy machinery specific to animals. Now in a tour de force study, Tian et al. (2010) identify four previously uncharacterized genes specifically required for autophagy in multicellular animals and establish *Caenorhabditis elegans* as one of the premier genetic models for uncovering new autophagy genes in animals.

During autophagy, cytoplasmic contents, such as proteins and organelles, are engulfed by a double-membrane autophagosome (Figure 1), which then fuses with lysosomes to form autolysosomes. Here hydrolase enzymes degrade the cargo, and the products are subsequently released into the cytosol for reuse (Mizushima, 2007). Besides recycling cytoplasmic material during periods of starvation or stress, autophagy (also called macroautophagy) clears protein aggregates, eliminates pathogens, and influences cell death. Moreover, in many organisms, autophagy defects are associated with decreased life span, neurodegeneration, and tumor progression (Mizushima et al., 2008).

In worms (*C. elegans*), flies, and mammals, autophagy is also important during development (Meléndez and Neufeld, 2008). In *C. elegans*, germ cells contain aggregates of protein and RNA known as P granules, which are absent in somatic cells. A previous study demonstrated that autophagy is required for clearing the aggregate-prone components of P granules from somatic cells in developing *C. elegans* embryos (Zhang et al., 2009), and defects in autophagy lead to the aberrant accumulation of aggregates of P granule proteins in somatic cells.

Now Tian et al. (2010) use the persistence of P granule proteins in somatic cells to find mutant *C. elegans* embryos with defects in autophagy. From the ~160 mutants identified, the authors isolated four new genes, named *epg-2*, *-3*, *-4*, and *-5* (*e*ctopic *P*GL *g*ranules), which do not map to known autophagy genes. The coiled-coil protein, *epg-2*, mediates recognition of cargo (e.g., aggregates of P granule proteins) for delivery to autophagosomes and appears to be specific to nematodes. The other three genes, *epg-3*, *-4*, and *-5*, are also required for starvation-induced autophagy. They are conserved genetically from worms to mammals and appear to lack homologs in yeast.

In addition, the authors isolated numerous new mutations in genes homologous to yeast autophagy genes, which validate and strengthen the results of the study. Not only do these new mutations provide a valuable resource for probing the structure and function of autophagy proteins, but they also establish *C. elegans* as a preeminent system for studying the role and regulation of autophagy in multicellular animals.

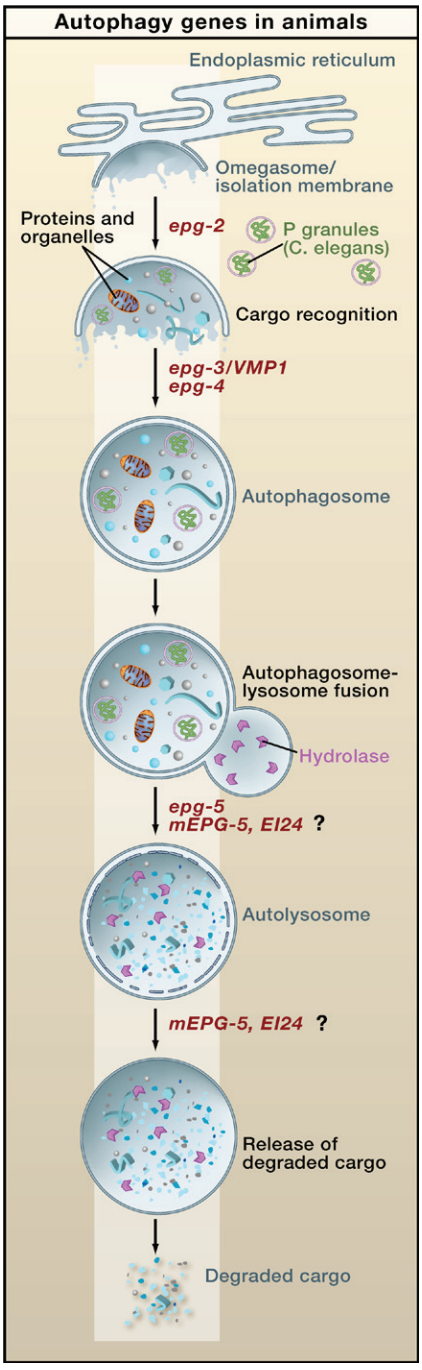

Figure 1. Genes Required for Autophagy in Animals

Autophagy degrades and recycles cytoplasmic contents. For example, during the development of *Caenorhabditis elegans* embryos, autophagy clears from somatic cells aggregates of proteins and RNA molecules known as P granules. Upon autophagy induction, isolation membranes nucleate at structures derived from the endoplasmic reticulum (ER) called omegasomes. As an isolation membrane expands, it engulfs P granules and then closes up to produce an autophagosome. Lysosomes fuse with the outer membrane of an autophagosome to form an autolysosome, where hydrolase enzymes degrade the inner membrane and cargo. Degraded cargo is then released into the cytosol for reuse. Using a genetic screen, Tian et al. (2010) now uncover four new genes in *C. elegans* (*epg-2*, *-3*, *-4*, and *-5*), which are required specifically for autophagy in animals. Whereas *epg-2* is required for recognizing cargo, mutations in *epg-3* and *epg-4* lead to the accumulation of isolation membranes and omegasomes. Embryos with mutations in *epg-5* accumulate autolysosomes that fail to degrade cargo. Strikingly, mammalian homologs of *epg-3*,*-4*, and *-5* are also required for autophagy in cell cultures. *VMP1* functions at an early step of autophagosome formation, whereas *mEPG-5* and *EI24* act at later stages.

Tian and colleagues found that EPG-3 is similar in sequence and function to the human vacuolar membrane protein 1, VMP1. Expression of human *VMP1* remarkably rescues the P granule degradation defect in worm embryos with mutations in *epg-3*, and VMP1 is required for autophagy during starvation. In yeast, autophagosomes form at a specific cellular location called the pre-autophagosomal structure. Mammalian cells do not contain a clearly defined pre-autophagosomal structure. Instead, autophagosomes form from cup-shaped membrane fragments, called isolation membranes, which nucleate at multiple sites in the cytosol, including endoplasmic reticulum (ER)-derived structures termed omegasomes (Figure 1) (Axe et al., 2008). Defects in *epg-3* or *VMP1* lead to the accumulation of omegasomes in *C. elegans* embryos or rat kidney cells, respectively. Thus, although the exact roles of EPG-3 and VMP1 remain unknown, both proteins must function at an early step in autophagy. Perhaps EPG-3 and VMP1 facilitate the elongation of the isolation membrane or the closure of the double-membrane vesicle during autophagosome assembly (Figure 1).

Isolation membranes and omegasomes also accumulate in embryos with mutations in *epg-4*. Consequently, EPG-4 probably also functions in an early step of autophagosome formation. EPG-4 localizes to the ER, suggesting that it helps to convert ER membranes to autophagic membranes. In contrast, reducing the expression of *EI24*, the mammalian homolog of *epg-4*, does not affect omegasome formation in mammalian cells but instead results in the accumulation of autolysosomes that fail to degrade their contents. This suggests that *EI24* functions later in the autophagy pathway than *epg-4*. The apparent phenotypic differences between defects in *epg-4* and *EI24* may be due to inefficient silencing of *EI24*, or these homologs may have diverged functionally during evolution.

Mutations in *epg-5* lead to the persistent colocalization of P granule aggregates with protein markers known to associate with autophagosomes (Figure 1). Thus, although protein aggregates are near the autophagic machinery in these mutant embryos, the aggregates are probably not properly degraded. Mutations in other autophagy genes (i.e., *atg-3*, *atg-13*, or *atg-5*) suppressed the *epg-5* phenotype, and these epistasis analyses suggest that *epg-5* acts downstream of genes that regulate autophagosome formation.

As with *EI24*, silencing the *epg-5* mammalian homolog *mEPG-5* led to the persistence of autolysosomes that fail to degrade their contents. Transmission electron microscopy images revealed significant differences in the ultrastructures of the autolysomes present in embryos with reduced levels of *EI24* and *mEPG-5*. Thus, future studies are needed to determine if these genes function in different steps in the degradation of autophagosome cargo.

In this landmark study, Tian et al. define discrete steps in the autophagy pathway that are specific to multicellular animals. Defects in autophagy are associated with numerous pathological conditions, including aging, neurodegeneration, and cancer (Mizushima et al., 2008). Therefore, it is interesting that the mammalian homologs of EPG-3, -4, and -5 are all associated with either human diseases or models of human disease. VMP1 is highly expressed in the pancreas of rats with acute pancreatitis (Dusetti et al., 2002), and it will be interesting to determine if VMP1 specifically functions in autophagy in the pancreas. *EI24* expression is activated by tumor suppressor p53 and by etoposide, a chemotherapy drug that activates p53 (Gu et al., 2000). It could be that EI24 functions in a p53-independent or -dependent process. Notably, *mEPG-5* is altered in human

breast tumors (Sjöblom et al., 2006). Therefore, EI24 and mEPG5 may specifically regulate autophagy in cancer cells. The identification of these new genes by Tian et al. (2010) highlights the importance of autophagy in human diseases and may lead to exciting new discoveries about the role of autophagy in cancer and other disorders.

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ATM Creates a Veil of Transcriptional Silence

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The ATM kinase orchestrates diverse responses to DNA damage. By simultaneously monitoring transcription and DNA-damage responses in single cells, Shanbhag et al. (2010) now uncover a role of ATM in preventing transcription near DNA double-strand breaks.

The chromatin domains that flank DNA double-strand breaks (DSBs) harbor a plethora of posttranslational protein modifications. Although these modifications decorate megabase-size regions and are generally thought to promote DNA repair and cell survival, the functional roles of many remain to be determined, among them monoubiquitinated histone 2A (uH2A). Stemming from previous studies that implicate uH2A in transcriptional silencing (Weake and Workman, 2008), Greenberg and colleagues now examine whether uH2A may also exert similar gene silencing activities near sites of DNA damage (Shanbhag et al., 2010).

To do this, the authors borrow a previously described transcriptional reporter (Janicki et al., 2004) and re-engineer it so that a defined DSB can be generated at a stretch of sequence adjacent to the transcription unit. By employing fluorescence-based designs, the system makes it possible to simultaneously observe, both qualitatively and quantitatively, nascent transcription, protein production, as well as DNA-damage responses—all at the single-cell level.

Introduction of DSBs not only disrupts the physical integrity of interphase chromatin but is thought to interrupt numerous processes that take place at this dynamic structure. Whereas DSBs appear to inhibit DNA replication by preventing global origin firing and slowing the progression of local replication forks, it is not known whether and how these DNA lesions modulate local transcription. Now using this experimental setup, Shanbhag et al. (2010) address this question by measuring transcriptional activities adjacent to the engineered DSB site. They find that transcriptional activities at the chromosomally integrated reporter are largely repressed when a DSB is introduced. What's more interesting is that this DSB-associated gene silencing response is only effective on regions of chromatin proximal to the lesion and does not affect transcription at distal sites.

The authors call this phenomenon DNA *d*ouble-strand break-*i*nduced silencing in c*is* (DISC), and they uncover a strict requirement for the ataxia telangiectasia mutated (ATM) kinase in mediating DISC. Notably, DISC coincides with two hallmarks of transcriptional repression: stalling of RNA polymerase II (indicated by hypophosphorylation) and impaired chromatin decondensation, pointing to the notion that DISC affects through