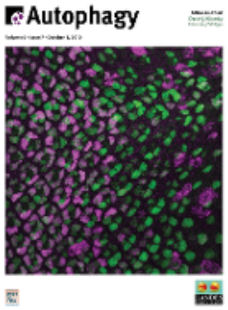

Autophagy

ISSN: 1554-8627 (Print) 1554-8635 (Online) Journal homepage: http://www.tandfonline.com/loi/kaup20

Four metazoan autophagy genes regulate cargo recognition, autophagosome formation and autolysosomal degradation

Ye Tian, Haiyan Ren, Yu Zhao, Qun Lu, Xinxin Huang, Peiguo Yang & Hong Zhang

To cite this article: Ye Tian, Haiyan Ren, Yu Zhao, Qun Lu, Xinxin Huang, Peiguo Yang & Hong Zhang (2010) Four metazoan autophagy genes regulate cargo recognition, autophagosome formation and autolysosomal degradation, Autophagy, 6:7, 984-985, DOI: 10.4161/auto.6.7.13156

To link to this article: http://dx.doi.org/10.4161/auto.6.7.13156

Published online: 01 Oct 2010.

Autophagy 6:7, 984-985; October 1, 2010; © 2010 Landes Bioscience

Four metazoan autophagy genes regulate cargo recognition, autophagosome formation and autolysosomal degradation

Ye Tian, Haiyan Ren, Yu Zhao, Qun Lu, Xinxin Huang, Peiguo Yang and Hong Zhang*
National Institute of Biological Sciences; Beijing. China

The mechanism responsible for induction and maturation of autophagosomes in multicellular organisms is poorly understood. We performed genetic screens in *C. elegans* and identified three essential autophagy genes, *epg-3*, *-4* and *-5*, which have highly conserved homologs in mammals, but are absent in yeast. We also identified a nematode-specific gene, *epg-2*, that is required for degradation of components of the specialized protein aggregates, called PGL granules. *epg-2, -3, -4* and *-5* define discrete genetic steps of the autophagy pathway. We further demonstrated that mammalian homologs of EPG-3, -4 and -5 are essential for starvation-induced autophagy. Our study establishes *C. elegans* as a model to identify components of the basal autophagy pathway specific to higher eukaryotes and to further assemble these genes into genetic pathways.

Key words: autophagy, omegasome, isolation membrane, *VMP1*, *EI24, mEPG5*

Submitted: 07/19/10

Revised: 07/20/10

Accepted: 07/27/10

Previously published online: www.landesbioscience.com/journals/autophagy/article/13156

DOI: 10.4161/auto.6.7.13156

*Correspondence to: Hong Zhang; Email: zhanghong@nibs.ac.cn

Punctum to: Tian Y, Li ZP, Hu WQ, Ren HY, Tian E, Zhao Y, et al. *C. elegans* screen identifies autophagy genes specific to multicellular organisms. Cell 2010; 141:1042–55; PMID: 20550938; DOI: 10.1016/j.cell.2010.04.034.

Autophagy is an evolutionarily conserved catabolic process that delivers portions of the cytosol to the vacuole/lysosomes for degradation. A molecular understanding of autophagy in higher eukaryotes is facilitated by the functional conservation of the yeast Atg proteins. Autophagy in higher eukaryotes, however, involves much more complex membrane dynamics and might require more elaborate molecular machinery. Little is known about essential autophagy components specific to higher eukaryotes.

We showed previously that autophagy is required for degradation of various aggregate-prone proteins during *C. elegans* embryogenesis. The maternally-loaded components of germline-specific P granules, PGL-1 and PGL-3, are removed in somatic cells by autophagy. In autophagy mutants, PGL-1 and PGL-3 accumulate into aggregates in somatic cells, termed PGL granules. Formation and degradation of PGL granules require the coiled-coil domain protein SEPA-1 that colocalizes with PGL granules in autophagy mutants. SEPA-1 itself forms aggregates and is also removed by autophagy, so that SEPA-1 aggregates are present only in early embryos. Large numbers of SEPA-1 aggregates persist in late-stage embryos in autophagy mutants. The *C. elegans* p62 homolog, T12G3.1, is also degraded by autophagy. Weak levels of T12G3.1 are diffusely localized in the cytoplasm in wild-type embryos. Loss of autophagy activity causes accumulation of numerous T12G3.1 aggregates. In autophagy mutants for the two ubiquitin-like conjugation systems and the UNC-51/EPG-1 (Atg1/Atg13) complex, PGL granules and T12G3.1 aggregates are spherical and dispersed in the cytoplasm, and these two types of protein aggregates are separable.

To identify essential components of the autophagy pathway, we performed genetic screens to isolate mutants with defective degradation of PGL granules or T12G3.1. From ~30,000 genomes screened, ~160 mutants were obtained. In addition to mutations in yeast *ATG* gene homologs, we also isolated four genes, named *epg-2*, *-3*, *-4* and *-5* (*e*ctopic *P*GL *g*ranules), which are specific to higher eukaryotes. *epg-2* is specifically required for degradation of PGL granules, whereas mutations in *epg-3*, *-4* and *-5* cause defects in degradation of PGL granules, T12G3.1 and

other autophagy substrates. *epg-2*, *-3*, *-4* and *-5* mutants exhibit a distinct distribution and organization of PGL granules. In *epg-2* and *epg-5* mutants, PGL granules are spherical and evenly dispersed in the cytoplasm. However, compared to *atg-3* mutants, there are more PGL granules and their size is smaller in *epg-5* mutants. In *epg-3* and *epg-4* mutants, PGL granules form clusters, consisting of several interconnected aggregates. We cloned *epg-2*, *-3*, *-4* and *-5* by transformation rescue. *epg-2* encodes a coiled-coil domain protein whose homologs are only present in nematodes. *epg-3* encodes the homolog of mammalian vacuole membrane protein 1 (VMP1), which is highly expressed in a pancreas with acute pancreatitis. *epg-4* encodes the homolog of EI24/PIG8, a target of the tumor suppressor protein p53. *epg-5* encodes a protein whose human homolog (*mEPG5*) is frequently mutated in breast tumors.

To place these four novel genes in the autophagy pathway, we examined formation and distribution patterns of PGL granules, T12G3.1 aggregates and also autophagic structures labeled by LGG-1/Atg8. In wild-type embryos, the majority of SEPA-1 aggregates are colocalized with LGG-1 puncta. Loss of function of *epg-2* disrupts the association of PGL granules with LGG-1 puncta. EPG-2 itself also forms aggregates that are degraded by autophagy and colocalize with PGL granules in the wild type and in autophagy mutants. EPG-2 may function as a receptor linking PGL granules to the autophagic machinery. EPG-2 is required for removal of SEPA-1, PGL-3 and PGL-1, whereas loss of function of *sepa-1*, *pgl-3* and *pgl-1* has no effect on degradation of EPG-2. SEPA-1 is required for degradation of PGL-1 and PGL-3, whereas removal of SEPA-1 is independent of PGL-1 and PGL-3. Similarly, degradation of PGL-1 depends on PGL-3, but not vice versa. Therefore, components of PGL granules act in a hierarchical order for their removal by autophagy.

Loss of function of *epg-3* and *epg-4* results in accumulation of early autophagic structures. First, in *epg-3* and *epg-4* mutants, LGG-1 puncta are accumulated and are bigger in size and stronger in intensity and the LGG-1 puncta are colocalized with PGL granules, suggesting that PGL granules are enwrapped or surrounded by the LGG-1-labeled autophagic structures. Second, DFCP1-labeled omegasomes, which are transient PtdIns(3)P-enriched ER subdomains and act as a cradle for autophagosome formation, are dramatically accumulated and colocalize with LGG-1 puncta in *epg-3* and *epg-4* mutant embryos. Third, electron microscopy analysis reveals accumulation of phagophores in *epg-3* and *epg-4* mutant animals. Thus, *epg-3* and *epg-4* are essential for progression of omegasomes to autophagosomes. The distinct distribution and organization of PGL granules and LGG-1 puncta in *epg-5* mutants are suppressed by loss of function of *atg-3, atg-13, atg-5, epg-3* and *epg-4*, indicating that *epg-5* functions downstream of these autophagy genes. Taken together, *epg-2*, *-3*, *-4* and *-5* function at discrete genetic steps of the autophagy pathway.

We further demonstrated that mammalian homologs of EPG-3, -4 and -5 are essential for starvation-induced autophagy. Knockdown of *VMP1* extends the duration of omegasomes and consequently leads to accumulation of phagophores. Reduced levels of *EI24* and *mEPG5* result in accumulation of nondegradative autolysosomes, indicating that *EI24* and *mEPG5* function at a late step in the autophagy pathway. Our study provides insights into the mechanisms of cargo-specific recognition, autophagosome formation and autolysosomal degradation in higher eukaryotes and establishes *C. elegans* as a multicellular genetic model to delineate the autophagy pathway.