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Small differences make a big impact: Structural insights into the differential function of the 2 Atg8 homologs in *C. elegans*

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ABSTRACT

The 2 *C. elegans* homologs of Atg8, LGG-1 and LGG-2, show differential function in the degradation of protein aggregates during embryogenesis. LGG-1 is essential for the degradation of various protein aggregates, while LGG-2 has cargo-specific and developmental stage-specific roles. LGG-1 and LGG-2 differentially interact with autophagy substrates and ATG proteins. LGG-1 and LGG-2 possess 2 hydrophobic pockets, the W-site and the L-site, which recognize the LIR motif in Atg8-binding proteins. The plasticity of the W-site and the size and shape of the L-site differ between LGG-1 and LGG-2, thus determining their preferences for distinct LIR motifs. The N-terminal tails of LGG-1 and LGG-2 adopt unique closed and open conformations, respectively, which may result in distinct membrane tethering and fusion activities. LGG-1 and LGG-2 have different affinities for ATG-7 and ATG-3, and lipidation of LGG-2 is regulated by levels of lipidated LGG-1. Taken together, the structural differences between LGG-1 and LGG-2 provide insights into their differential functions in the aggrephagy pathway.

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Macroautophagy (hereafter called autophagy) delivers cytoplasmic contents in a double-membrane autophagosome to the vacuole/lysosomes for degradation. In yeast, a series of evolutionarily conserved Atg proteins function at distinct steps of autophagosome formation. The autophagic machinery is more complex in higher eukaryotes, which have multiple homologs of the same yeast *ATG* genes and also possess metazoan-specific autophagy genes such as the *EPG* genes. The ubiquitin-like protein Atg8, which is conjugated to phosphatidylethanolamine (PE), acts at multiple steps of autophagosome formation by directly interacting with other Atg proteins, including the Atg1/ULK1 complex and the Atg12–Atg5-Atg16 complex. Atg8–PE also contains membrane-tethering activity, which may participate in phagophore expansion. Autophagy can selectively recognize and remove damaged organelles and protein aggregates. In selective autophagy, Atg8–PE interacts with autophagy receptor proteins (e.g. SQSTM1/p62 and NBR1), which recognize different cargoes for degradation. Atg8/LC3 (the mammalian Atg8 homolog) interacts with proteins via the [W/F/Y]xx[I/L/V] motif, known as the LC3-interacting region (LIR). The aromatic and hydrophobic [I/L/V] residues in the LIR motif are recognized by 2 hydrophobic binding pockets termed the W-site and L-site, respectively, in Atg8 family members. Mammals have at least 7 Atg8 homologs, belonging to the LC3 and GABARAP subfamilies. The differential function of distinct Atg8 homologs during animal development is poorly understood.

We investigated structure/function relationships of the 2 *C. elegans* Atg8 homologs, LGG-1 and LGG-2, in the degradation of protein aggregates, a process known as aggrephagy. The overall structure of LGG-1 and LGG-2 resembles a typical Atg8-family fold, with evident differences at the N- and C-terminal tails. The C-terminal tails of Atg8 family members, which interact with distinct enzymes such as Atg4 and Atg7, are flexible and divergent. The N terminus of LGG-1 and GABARAP family proteins shows a rigid closed conformation (C-form), resulting from hydrophobic interactions between residues in the N-terminal tail and the ubiquitin fold. The N-terminus of LGG-2, the LC3 family and Atg8 adopts the open form (O-form), which is detached from the ubiquitin fold.

Despite their structural similarity, LGG-1 and LGG-2 function nonredundantly in autophagic degradation of PGL granules during embryogenesis. However, LGG-1 and LGG-2 have distinct roles in the degradation of the SQSTM1 homolog SQST-1. LGG-1 is essential for removal of SQST-1 and its scaffold protein EPG-7, which mediates degradation of SQST-1 and is itself degraded by autophagy. LGG-2 is differentially required for SQST-1 degradation during development; it is essential during early embryogenesis, is needed less at the comma stage and onwards, and is dispensable at the L2 larval stage and onwards. LGG-2 also acts in a cargo-specific manner, being dispensable for degradation of EPG-7 at the 200-cell stage onwards.

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LGG-1 and LGG-2 differentially interact with autophagy substrates and Atg proteins. Both bind to the autophagy substrates SQST-1, SEPA-1 and AIN-1, but only LGG-1 binds to EPG-7. LGG-1 binds to components of the UNC-51-ATG-13/EPG-1 complex, while LGG-2 binds to LGG-3 and ATG-16.1/ATG-16.2. These differential interactions may contribute to hierarchical recruitment of Atg proteins in the aggrephagy pathway. Both LGG-1 and LGG-2 possess the W-site and the L-site. However, LGG-1 binds to "W," "F" and "Y" type LIRs, while LGG-2 prefers Phe as the aromatic residue. In the LGG-1 W-site, a key Phe residue is juxtaposed with a Gly residue, which permits rotation of the Phe benzene ring and enhances the plasticity of the site. In LGG-2, the conformation of the equivalent Phe is severely restricted by juxtaposition with a bulky Val residue. Thus, the W-site in LGG-1 has greater plasticity and accommodates more divergent aromatic residues than the LGG-2 W-site. The L-sites in LGG-1 and LGG-2 also differ in size and shape. The differences in the hydrophobic pockets and surrounding regions in LGG-1 and LGG-2 determine their binding specificity for interacting proteins.

Lipidation of LGG-1 and LGG-2 is mediated by 2 enzymes, ATG-7 and ATG-3. LGG-1 is synthesized as a precursor and then cleaved by the cysteine proteases ATG-4.1 and ATG-4.2 to expose the conjugated glycine. The C-terminal conjugated glycine in LGG-2, however, is directly exposed, and only a small portion of LGG-2 is PE-conjugated. LGG-1 and LGG-2 exhibit different affinities for ATG-7 and ATG-3. During embryogenesis, the number of LGG-2 puncta depends on the level of LGG-1 lipidation. Excess lipidated LGG-1, or a lipidation-incompetent mutant LGG-1 with the conjugated glycine replaced by alanine, inhibits LGG-2 puncta formation. Modulating the lipidation of one Atg8 homolog by another provides an additional mechanism for regulating their differential function in the pathway.

Lipidated LGG-1 mediates membrane tethering and fusion at a physiologically relevant PE concentration (20%) in an in vitro membrane-mixing assay, while LGG-2 only contains weak tethering activity. The distinct membrane tethering and/or fusion activity appears to result from their unique N-terminal closed and open conformations. Interestingly, LGG-2, LC3 and Atg8, which share an open conformation at the N terminus, all control autophagosome size.

These structural analyses help us to understand the differential function of LGG-1 and LGG-2 in autophagy in *C. elegans* and of GABARAP and LC3 subfamily members in mammals. Moreover, it has been largely assumed that autophagy gene products are equally employed for removal of different substrates. Our results demonstrate that during multicellular organism development an autophagy protein can be differentially used to accommodate the characteristics of distinct developmental stages and types of cargo.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.