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Short communication

A POSSIBLE APPROACH TO STUDY AUTOPHAGY IN *DROSOPHILA**

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The process of autophagy, or bulk degradation of cellular proteins and organelles through an autophagosomic-lysosomal pathway constantly functions in all eukaryotic cells. Also a type of physiological cell death exists, which is best characterized with the strenghtening of the autophagic process, but no DNA degradation or caspase activation can be detected, in contrast to apoptosis [2].

Autophagy can be promoted in various ways: addiction of certain drugs (like vinblastine [6]), hormones (like 20-hidroxy-ecdysone [3]) or simply nutrition deprivation [5] leads to the increased amount of proteins degraded by lysosomal enzymes.

The isolation and cloning of yeast autophagy mutants gives an excellent opportunity to examine their putative homologs in *Drosophila melanogaster*. Fourteen genes have been identified in *Saccharomyces cerevisiae* required for autophagy [5], based on several mutant phenotypes, like the sorting problems of vacuolar enzymes such as carboxypeptidase Y or aminopeptidase I, or the less of viability and the inability of degrading cytosolic proteins like fatty acid synthase during starvation. Nine of them *(apg5, apg6, apg7, apg12, aut1, aut2, aut7, aut9, vps4)* appear to have clear homologs in the fly and human genome, using the BLAST tools at http://workbench.sdsc.edu, http://www.ncbi.nlm.nih.gov and http://www.fruitfly.org (BLASTN, TBLASTN services) for sequence similarity searches. The sequence alignment of the yeast, fly and human proteins can be seen in Figure 1.

The high degree of similarity suggests existing homology among these genes, although new and lost functions were identified in some cases [7]. Remarkably, *vps4* exists in two slightly different copies in human, and *aut7* exists in multiple different copies as well (two in *Drosophila* and three in *Homo*), suggesting different roles, or at least different regulation. As expected, fly and human genes are much more similar to each other than to the yeast homolog, promising that *Drosophila* experiments will better contribute to the understanding of the roles of these genes in detail in higher eukaryotes. The precise function of these genes is still unclear, however, molecu-

*Dedicated to Professor János Kovács on the occasion of his 70th birthday.

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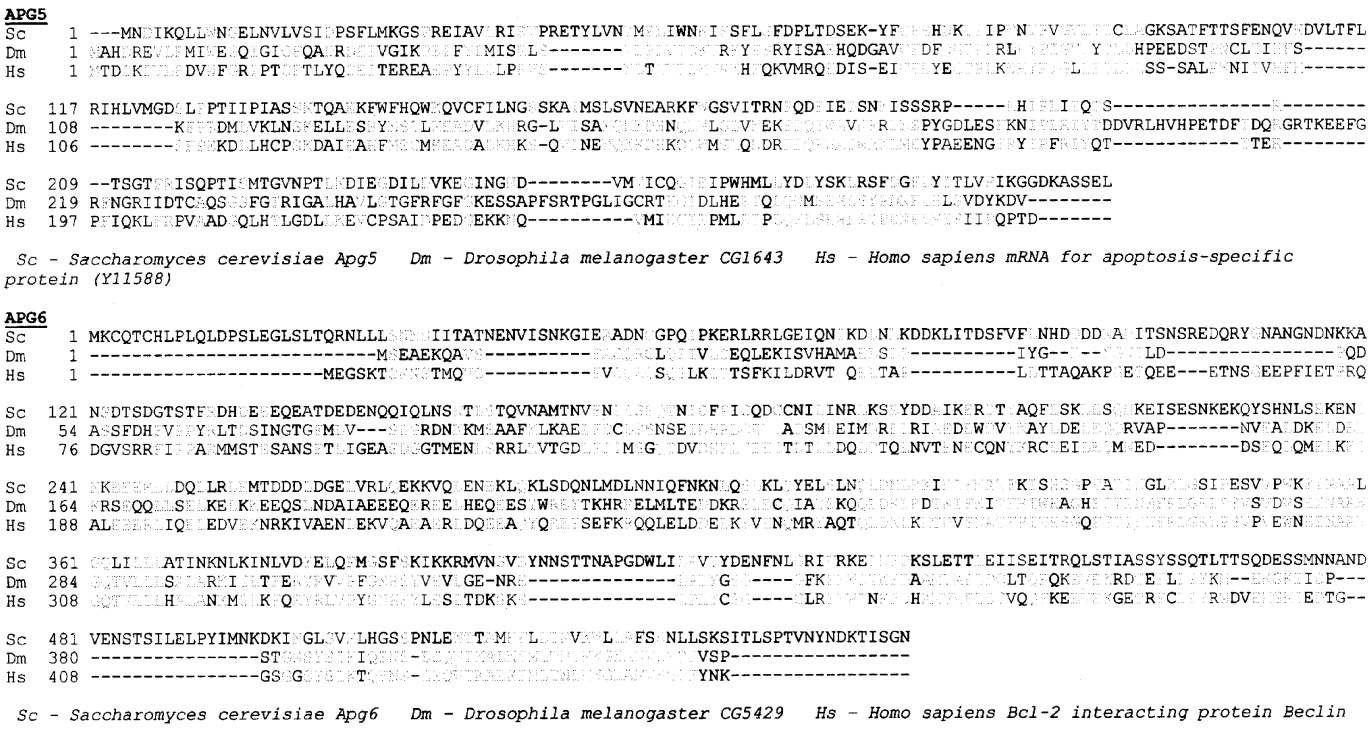
Fig. 1

Multiple sequence alignment (using the CLUSTALW program at http://workbench. sdsc.edu) of the nine yeast autophagy genes and their putative homologs in *Drosophila melanogaster* and *Homo sapiens.* Light grey letters refer to identical, dark grey letters refer to similar amino acid residues

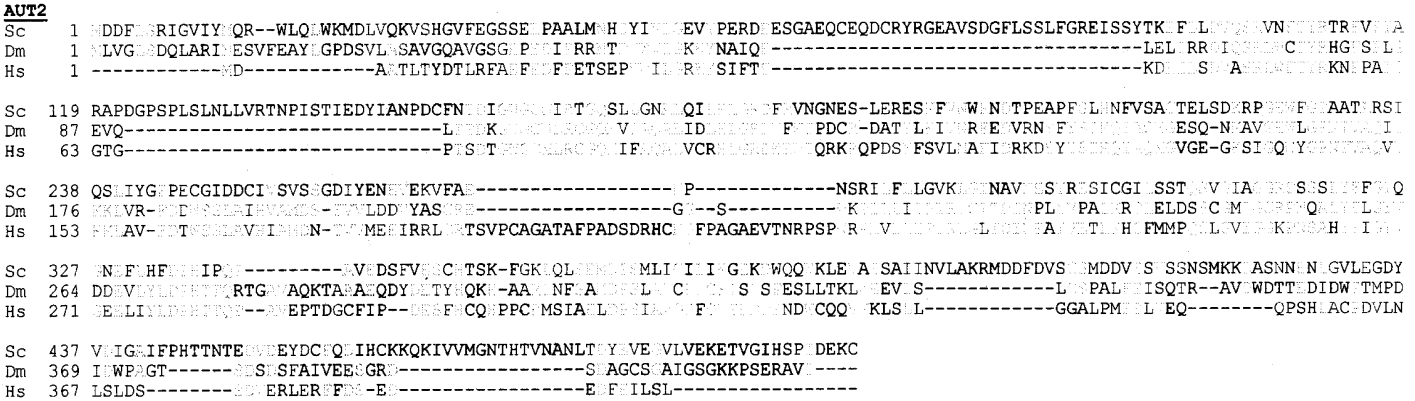


Sc - Saccharomyces cerevisiae Apg7 Dm - Drosophila melanogaster CG5489 alt1 Hs - Homo sapiens E1-like protein
(only the sequences corresponding to the first 626 amino acids of Apg7 are shown)

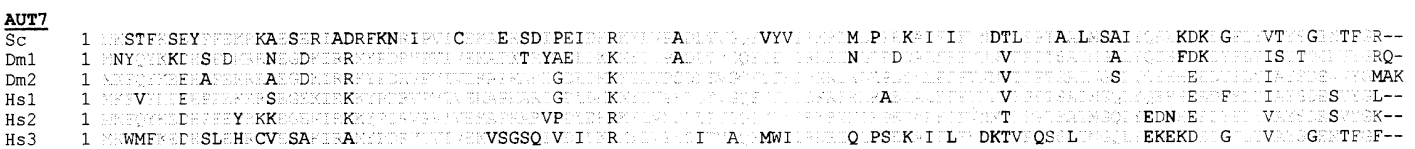
Sc - Saccharomyces cerevisiae Apg12 Dm - Drosophila melanogaster CG10861 Hs - Homo sapiens cDNA clone AW085439

Sc - Saccharomyces cerevisiae Aut1 Dm - Drosophila melanogaster CG6877 Hs - Homo sapiens cDNA clone AL137515

Fig. 1 (cont.)



Sc - Saccharomyces cerevisiae Aut2 Dm - Drosophila melanogaster CG4428 Hs - Homo sapiens cDNA clone AL080168 (KIAA0943)



Sc - Saccharomyces cerevisiae Aut7 Dm1 - Drosophila melanogaster CG12334 Dm2 - Drosophila melanogaster CG1534 alt1
Hs1 - Homo sapiens GABA-receptor associated protein Hs2 - Homo sapiens cDNA clone AA476809 Hs3 - Homo sapiens Gef-2 protein
(the last two amino acids [I,N] of Dm2 are not shown)

Sc - Saccharomyces cerevisiae Aut9 Dm - Drosophila melanogaster CG3615 Hs - Homo sapiens clone AC011234
(only partial sequences are shown, as indicated by the numbering)

Sc - Saccharomyces cerevisiae Vps4 Dm - Drosophila melanogaster CG6842 Hs1 - Homo sapiens Vps4 Hs2 - Homo sapiens End13

lar and genetic studies are under way on yeast cells and cell lines of various organisms.

Holometabolic insects are promising model organisms, as massive autophagy-type programmed cell death occurs in several organs during metamorphosis [1, 8], inevitable for the formation of adult organs. The known complete genome sequence and the broad variety of molecular and genetic tools make *Drosophila* a good choice to study autophagy. There are P-element mutants for two of the genes in Fig. 1.: *l(3)00096* for *CG5429* and *EP(X)0362* for *CG1534*, making it possible to remobilize the P-element and gain null allele of the gene of interest. In the other cases, "knock-out", or in other words, hypomorphic mutants can be generated by the RNAi (RNA interference) method [9], thus silencing the gene corresponding to the injected dsRNA (double-stranded RNA). During the RNAi reaction, both strands of the dsRNA are processed to RNA segments 21–23 nucleotides in length. Processing of the dsRNA to the small RNA fragments does not require the targeted mRNA. The mRNA is cleaved only within the region of identity with the dsRNA. The improved version of this method is the EIR (expressed inverted repeat) technique [4], when *in vivo* transcription of an inverted repeat transgene might also produce a dsRNA "hairpin" that is capable of triggering post-transcriptional gene silencing (PTGS). *In vivo* dsRNA formation can be promoted simply by keeping the stock at 29 °C, although it is not known yet which step is temperature-dependent: either dsRNA formation, or the enzymes participating in RNAi, or the Gal4-UAS system.

Drosophila experiments will hopefully help us better understand the molecular biology of autophagy.

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