Paper Title: Human autophagins, a family of cysteine proteinases potentially implicated in cell degradation by autophagy.

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UniProtKB accessions: Q9Y4P1 (Autophagin1, ATG4B_HUMAN); Q8WYN0 (Autophagin2, ATG4A_HUMAN); Q96DT6 (Autophagin 3, ATG4C_HUMAN); Q86TL0 (Autophagin 4, ATG4D_HUMAN)
P53867 (Aut2, ATG4_YEAST); P38182 (Aut7, ATG8_YEAST)

Complementation Studies with Human Autophagins in Autophagy-defective Yeast Strains-- To study the putative implication of the identified proteins in the process of autophagy, we cloned the full-length cDNAs for the four human autophagins in the yeast expression vector pGAD424 (32) under the control of the constitutive ADH1 gene promoter, obtaining four new plasmid constructs pGAD-Autophagin1, pGAD-Autophagin2, pGAD-Autophagin3, and pGAD-Autophagin4. These plasmid constructs were used to transform the *S. cerevisiae* autophagy-defective mutants strains aut2 and aut7, and the properties of the transformed yeasts in terms of restoration of biochemical and morphological markers of autophagy were analyzed.

For this purpose, we first examined the processing of the vacuolar hydrolase aminopeptidase I (API) from its inactive precursor, a process that is defective in autophagy yeast mutants. Thus, wild-type cells and *aut2* mutants carrying the autophagin cDNAs were grown overnight in YPD or selective medium (synthetic medium/Leu$^-$). The cells were collected, lysed in SDS-PAGE sample buffer, and analyzed by Western blot with a polyclonal rabbit antiserum against API. Transformed *aut2* mutant cells lacking the endogenous Apg4/Aut2 activity but carrying the autophagin-1 or the autophagin-3 cDNAs were able to complete the processing of proAPI (Fig. 4*A*). By contrast, the parental *aut2* mutant cells were unable to perform the processing of this marker that signals the integrity of the autophagic process. When the same experiments were performed with autophagin-2 and -4 cDNAs, no obvious processing of proAPI was observed, indicating that these human enzymes do not behave as autophagin-1 and -3 in their ability to restore the autophagy deficiency in *aut2* mutant yeasts (data not shown). To rule out the possibility that the expression of autophagin-1 or -3 in *aut2* cells could complement their autophagy deficiency through a nonspecific effect, *aut7* mutant cells that lack the Apg4/Aut2 substrate and are also deficient in autophagy were transformed with autophagin-1 or -3 cDNA and analyzed as above. As shown in Fig. 4*A*, these transformed yeast cells were unable to complete the proAPI processing, confirming that these human autophagins specifically complement the autophagy deficiency derived from absence of the yeast protease.

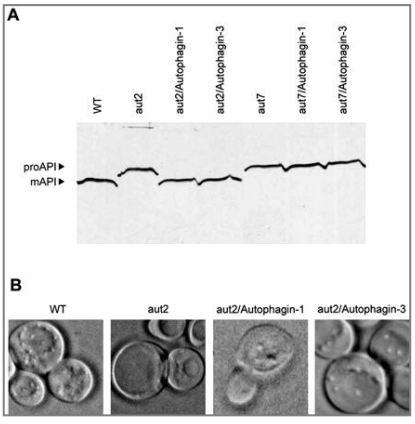

Fig. 4. Complementation studies with human autophagins in autophagy-defective yeast strains. *A*, Western blot analysis of aminopeptidase I (API) in *Aut2* and *Aut7* cells transformed with pGAD-Autophagin1 or pGAD-Autophagin3 or left nontransformed. Indicated cells were lysed as described under "Experimental Procedures," and API processing was analyzed using a rabbit serum anti-API. proAPI and mature aminopeptidase I (*mAPI*) are indicated by *arrowheads*. Wild-type (*WT*) cells were included as a positive control. *B*, *Aut4* yeast mutants were transformed with pGAD-Autophagin1 (*aut2/Autophagin-1*) or pGAD-Autophagin3 (*aut2/Autophagin-3*) or left nontransformed (*aut2*). Micrographs of cells were taken after 4 h of starvation in the presence of 1 mM PMSF to examine the accumulation of autophagic bodies. WT cells were included as a positive control.

Annotation suggestions?