Human Apg3p/Aut1p Homologue Is an Authentic E2 Enzyme for Multiple Substrates, GATE-16, GABARAP, and MAP-LC3, and Facilitates the Conjugation of hApg12p to hApg5p*

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Autophagy is a process of bulk degradation of cytoplasmic components by the lysosome/vacuole and has a significant relationship to several neurodegenerative disorders and myopathies in mammals. One of APG gene products essential for autophagy in yeast, Apg3p, is a protein-conjugating enzyme for Apg8p lipidation (Ichimura, Y., Kirisako, T., Takao, T., Satomi, T., Shimohishii, Y., Ishihara, N., Mizushima, N., Tanida, I., Komnami, E., Ohsumi, M., Noda, T., and Ohsumi, Y. (2000) Nature 408, 488–492). In this study, the cloning of a human Apg3p homologue (hApg3p) as an E2 enzyme essential for human Apg8p homologues (i.e. GATE-16, GABARAP, and MAP-LC3) is shown, and its unique characteristics are described. The predicted amino acid sequence of the isolated clone shows 34.1% identity and 48.1% similarity to yeast Apg3p. Site-directed mutagenesis revealed that Cys264 of hApg3p is an authentic active-site cysteine residue essential for the formation of hApg3p-hApg8p homologue intermediates. Overexpression of hApg7p enhances the formation of a stable E2-substrate complex between hApg3p and each of the hApg8p homologues, and MAP-LC3 is preferred as the substrate over the other two Apg8p homologues. These results indicate that hApg3p is an E2-like enzyme essential for three human Apg8p homologues. Co-immunoprecipitation of hApg7p with hApg3p indicates that hApg3p forms an E1-E2 complex with hApg7p as in the case of yeast Apg3p and Apg7p. Furthermore, hApg3p co-immunoprecipitates with hApg12p, and the overexpression of hApg3p facilitates the formation of the GFP-hApg12p-hApg5p conjugate, suggesting that hApg3p cross-talks with the hApg12p conjugation system.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number AB079384.

1 The abbreviations used are: Cvt, cytoplasm-to-vacuole targeting; E1, protein-activating enzyme; E2, protein-conjugating enzyme; MAP-LC3, microtubule-associated protein light chain 3; FLAGhApg3p, FLAG-tagged human Apg3p/Aut1p homologue; FLAFlaghApg12p, FLAFlag-human MAP-LC3; EST, expressed sequence tag; GABA, γ-aminobutyric acid, type A; GABARAP, GABA receptor-associated protein; GATE-16, Golgi-associated ATPase enhancer of 16 kDa; GFP, green fluorescent protein; GFP-hApg3p, GFP-tagged hApg3p; GFP-hApg12p, GFP-tagged hApg12p; mApg12p, murine Apg12p homologue; SNARE, soluble NSF attachment protein receptors; RACE, rapid amplification of cDNA ends.

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is a unique E1 enzyme for two substrates in two independent modification systems of autophagy and the Cvt-pathway and exists as a homodimer (19). More interestingly, Apg3p/Aut1p forms an E1-E2 enzyme complex with Apg7p, and Apg3p also interacts with Apg12p, which is a substrate for Apg7p and Apg10p but not Apg3p (19, 20). These results suggest that the E1-E2 complex is a key to the cooperative regulation of two modification systems.

In mammalian cells, two modification systems seem to be conserved. A human Apg12p homologue (hApg12p) conjugates with the human Apg5p homologue (hApg5p), which was first identified as an apoptosis-specific protein (21, 22). Recently, experiments using embryonic stem cells that knocked out the mouse APG5 gene demonstrated that a murine Apg5p homologue is essential for autophagy (23). With regard to mammalian Apg8p modification, there are three mammalian Apg8p/Atg7p homologue candidates, the Golgi-associated ATPase homologue is essential for autophagy (23). With regard to mammalian Apg8p modification, there are three mammalian Apg8p/Atg7p homologue candidates, the Golgi-associated ATPase homologue is essential for autophagy (23). With regard to mammalian Apg8p modification, there are three mammalian Apg8p/Atg7p homologue candidates, the Golgi-associated ATPase homologue is essential for autophagy (23). With regard to mammalian Apg8p modification, there are three mammalian Apg8p/Atg7p homologue candidates, the Golgi-associated ATPase homologue is essential for autophagy (23). With regard to mammalian Apg8p modification, there are three mammalian Apg8p/Atg7p homologue candidates, the Golgi-associated ATPase homologue is essential for autophagy (23). With regard to mammalian Apg8p modification, there are three mammalian Apg8p/Atg7p homologue candidates, the Golgi-associated ATPase homologue is essential for autophagy (23). With regard to mammalian Apg8p modification, there are three mammalian Apg8p/Atg7p homologue candidates, the Golgi-associated ATPase homologue is essential for autophagy (23). With regard to mammalian Apg8p modification, there are three mammalian Apg8p/Atg7p homologue candidates, the Golgi-associated ATPase homologue is essential for autophagy (23). With regard to mammalian Apg8p modification, there are three mammalian Apg8p/Atg7p homologue candidates, the Golgi-associated ATPase homologue is essential for autophagy (23).
an open reading frame of the human mutant GFPhApg3pC264S and each GFPhApg8ps (GFPhGATE-serine by site-directed mutagenesis and expressed both the homologue.

Asterisks hApg3p is compared with that of yeast Apg3p by the ClustalW program. The amino acid sequence of containing the predicted active-site cysteine is underlined. B, Northern analysis of human Apg3 mRNA in human tissues. A DNA fragment of an open reading frame of the human APG3 homologue was used as a probe.

mediates. To investigate this possibility, we employed site-directed mutagenesis of the predicted active-site cysteine residue within hApg3p (Fig. 1A, Cys264). Wild type hApg3p forms an enzyme/substrate intermediate via a thiol ester bond. Because of the rapid turnover of the Apg3p reaction, it is difficult to recognize such an intermediate in sufficient quantity. If the active-site cysteine residue of Apg3p is replaced by serine, a stable \(-\)ester bond instead of a thiol ester bond will be formed between the enzyme and substrate(s), and the for-
termediates (105 kDa) were recognized by immunoblot with /H11011\ antibody (Fig. 2, Long Exposure, lanes 8, 10, and 12). The interactions of hApg3p with hApg8ps were also confirmed by co-immunoprecipitation (data not shown). These results indicate that hApg3p is an authentic E2 enzyme essential for hGATE-16, hGABARAP, and hMAP-LC3, and that the activation of the hApg8p homologue by hApg7p is essential for a further reaction mediated by hApg3p.

To investigate the intracellular localization of hApg3p, we performed subcellular fractionation. HEK293 cells expressing GFPhApg3p and FLAGhMAP-LC3 were lysed and fractionated by ultracentrifugation at 100,000 \( \times g \) for 1 h. Total proteins in the supernatant and pellet were analyzed by SDS-PAGE, and GFPhApg3p was recognized by immunoblot with anti-GFP antibody (Fig. 3, WB: anti-GFP). GFPhApg3p fractionated mainly in the supernatant, and the FLAGMAP-LC3GFPhApg3p\(_{264S}\) intermediate also fractionated in the supernatant (Fig. 3, WB: anti-GFP, LC3-GFPhApg3p). The results suggest that hApg3p is present in the cytosol, and that the reaction of hMAP-LC3 mediated by hApg3p occurs predominantly in the cytosol.

The Human Apg3p Homologue Forms an E1-E2 Complex with the Human Apg7p Homologue—In yeast, Apg3p forms an E1-E2 complex with Apg7p, which is one of its unique characteristics compared with other protein-conjugation systems. To investigate whether hApg3p interacts with hApg7p as in the case of yeast, co-immunoprecipitation was performed. We expressed FLAGhApg3p and hApg7p in COS7 cells (Fig. 2, Expression, lanes 1–3), and FLAGhApg3p in the lysate of the transfectant was immunoprecipitated well with anti-hApg3p antibody (Fig. 4, IP: anti-hApg3, WB: anti-FLAG, lanes 1 and 3). When both FLAGhApg3p and hApg7p were expressed in the cells, hApg7p co-immunoprecipitated with FLAGhApg3p by the anti-hApg3p antibody (Fig. 4, IP: anti-hApg3, WB: anti-FLAG, lanes 1 and 3). When both FLAGhApg3p and hApg7p were expressed in the cells, hApg7p was not immunoprecipitated by the anti-hApg3p antibody (Fig. 4, IP: anti-hApg3, WB: anti-hApg7, lane 2). The co-immunoprecipitation of hApg3p with hApg7p was confirmed using another hApg3p fusion protein (Fig. 4, GFPhApg3p, lanes 4–6). When GFPhApg3p was expressed in COS7 cells, GFPhApg3p in the lysate immunoprecipitated with anti-GFP antibody, whereas GFP itself did not immunoprecipitate with this antibody (Fig. 4, Expression, IP: anti-hApg3, WB: anti-GFP, lanes 4–6). Only when both GFPhApg3p and anti-GFP antibody (Fig. 2, Long Exposure, lanes 8, 10, and 12). hMAP-LC3 preferentially forms an intermediate with hApg3p\(_{264S}\) in COS7 cells (Fig. 2, Short Exposure, lane 12). The interactions of hApg3p with hApg8ps were also confirmed by co-immunoprecipitation (data not shown). These results indicate that hApg3p is an authentic E2 enzyme essential for hGATE-16, hGABARAP, and hMAP-LC3, and that the activation of the hApg8p homologue by hApg7p is essential for a further reaction mediated by hApg3p.

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hApg3p were co-expressed in COS7 cells. hApg7p were co-expressed in COS7 cells. Did hApg7p co-immunoprecipitate with GFPhApg3p using the anti-hApg3p antibody? (Fig. 4, IP: anti-hApg3, WB: anti-GFP, anti-hApg3, lanes 1–3). When FLAGhApg3p and GFPhApg12p were expressed together in the cells, GFPhApg12p co-immunoprecipitated with FLAGhApg3p (Fig. 5, IP: anti-hApg3, WB: anti-GFP, lane 3). These results indicate that hApg3p interacts with hApg12p.

The Overexpression of hApg3p Facilitates the Formation of the hApg12p-hApg5p Conjugate—Recent analyses using murine APG5 gene-deficient embryonic stem cells revealed that the Apg12p-Apg5p conjugate cooperates sequentially with MAP-LC3 in the formation of a preautophagosomal membrane sac (23). As described in the previous section, hApg3p interacts with both hApg7p and hApg12p (Figs. 4 and 5). Considering these interactions, it is interesting to see whether the hApg7p-hApg3p complex and/or the hApg3p-hApg12p complex play some roles in the two conjugation reactions. As reported previously (27), when both hApg7p and GFPhApg12p were expressed in COS7 cells, the formation of the GFPhApg12p-hApg5p conjugate was recognized (Fig. 6, GFPhApg12p-hApg5p conjugate, lane 5). We next examined the effects of the overexpression of hApg3p, hApg12p, and hApg7p in conjugate formation. When GFPhApg3p was expressed together with hApg7p and GFPhApg12p, the amount of the hApg7p-hApg5p conjugate increased significantly (Fig. 6, GFPhApg12p-hApg5p conjugate, lane 7). The carboxyl-terminal Gly in hApg12p is reported to be essential for the formation of the conjugate (22). We constructed a mutant hApg12pΔG with a deletion of the carboxy-
Novel Mammalian E2-like Enzyme, Human Apg3p Homologue

In this study, we show that the human Apg3p homologue is an authentic E2 enzyme for the hApg8p-conjugation system(s), and that human GATE-16, GABARAP, and MAP-LC3, the three hApg8p homologues, are substrates for hApg3p (Table I).

We show that hApg3p, in which the active-site cysteine is changed to serine can bind to GATE-16, GABARAP, and MAP-LC3 to form stable enzyme-substrate intermediates. The overexpression of hApg7p facilitates this reaction. Overexpressed hApg7p may be required for the efficient activation of overexpressed hApg6p homologues, which is necessary for the accumulation of substantial quantities of hApg3p-hApg8p intermediate via an O-ester bond. Alternatively, overexpressed hApg7p may enhance the formation of the E1-E2 complex with hApg3p, which may facilitate the sequential E2 reaction after the activation of hApg8p homologues by hApg7p. Furthermore, the overexpressed hApg3p facilitates the formation of hApg12p-hApg5p conjugate, whereas hApg3p is not an E2 enzyme for hApg12p (Table I).

There are two unique characteristics of hApg3p. 1) It forms an E1-E2 complex with hApg7p. 2) It interacts with hApg12p. More importantly, we showed for the first time that the overexpression of hApg3p together with hApg7p and hApg12p enhances the formation of the hApg12p-hApg5p conjugate. The overexpression of hApg12p and hApg3p in the presence of endogenous hApg7p did not cause an enhancement of conjugate formation. Thus, the enhancement appears to be attributed to the formation of the hApg7p-hApg3p (E1-E2) complex rather than the hApg12p-hApg5p complex. These results strongly suggest that Apg3p forms a complex between hApg7p and hApg3p, two indispensable members of the hApg8p-conjugation system, also plays an important role in the hApg12p-conjugation system. Mizushima et al. (23) reported that the formation of the mApg12p-mApg5p conjugate precedes the lipidation and subsequent targeting of MAP-LC3 to autophagosomal precursors. Our data on the facilitation of the hApg12p-conjugation reaction by hApg3p indicate that there is intimate cross-talk between hApg12p-conjugation and the hApg8p-modification systems in the formation of autophagosomes. To our knowledge, this is the first observation of a cooperative relationship between the two different conjugation systems. In Fig. 7, we present a hypothetical scheme based on our experimental results. Although the precise mechanism by which the hApg7p-hApg3p complex facilitates hApg12p-hApg5p conjugation is still unknown, it is reasonable to assume that it may be possible by the activation of some step(s) in the hApg12p-conjugation pathway. For example, hApg7p complexed with hApg3p may be more active as an E1 enzyme than uncomplexed hApg7p. It is also possible that the presumptive hApg10p may be directly or indirectly involved in this mechanism. So far, authentic hApg10p has not been identified (Table I). We are now attempting to isolate and characterize an hApg10p homologue. In summary, hApg3p, which interacts with hApg12p on the one hand, forms an E1-E2 complex with hApg7p on the other and functions as a facilitating factor in the hApg12p-conjugation system in addition to an authentic E2 enzyme for hApg8p homologues. An enhanced level of the hApg12p-hApg5p conjugate in turn promotes the recruitment of the lipidated form of MAP-LC3 onto autophagosomal membranes. In the end, the activation of hApg12p-conjugation by the hApg7p-hApg3p complex promotes hApg8p-conjugation reaction. Thus, the two autophagic conjugation systems, which comprise the one activation enzyme (E1, hApg7p) in common, two distinct E2 enzymes (hApg3p and hApg10p) and four different modifiers (hApg12p and three hApg8p homologues) interact and cooperate with each other.

GATE-16, which interacts with NSF and 28 kDa Golgi SNARE protein, is localized in the Golgi and is expressed in the largest amounts in brain (26). GABARAP is a GABAergic-receptor-associated protein that co-localizes with the GABA_A receptor in cultured cortical neurons and interacts with gephyrin (24, 28). MAP-LC3 is localized on autophagosomal membranes (25). Considering the divergent functions and intracellular localizations of the three Apg8p homologues, it is surprising that all three Apg8p homologues, MAP-LC3, GATE-16, and GABARAP, are substrates for hApg3p. The affinity of hApg3p for these substrates differs from that of hApg7p, hApg3p preferentially conjugates with MAP-LC3 in COS7 cells, whereas hApg7p conjugates almost equally with each of the hApg8p homologues. What are the implications of the difference in substrate specificity between the E1 and E2 enzymes? The higher affinity of hApg3p for MAP-LC3 is coincident with the autophagosomal localization of MAP-LC3 considering the function of hApg3p in autophagy (25). With regard to GATE-16 and GABARAP, it is possible that there is another Apg3p homologue specific for GATE-16 and/or GABARAP. But no further candidate was obtained from further BLAST search on the EST database and 5′-RACE, and genomic Southern analysis of the murine genome suggests that there are no further Apg3p ho-

![Fig. 6. Formation of the hApg12p-hApg5p conjugate is facilitated by the overexpression of hApg3p.](image_url)

**TABLE I**

Summary of two conjugation systems in yeast and human

| Modifier | E1 | E2 | Target |
|----------|----|----|--------|
| Yeast    | hApg12p | hApg7p | hApg10p |
| hApg5p   | N.I. |     |
| Human    | MAP-LC3 | GABARAP | GATE-16 |
| hApg8p   |     | PE   |

For further details, see the text.
Novel Mammalian E2-like Enzyme, Human Apg3p Homologue

Abstract

A candidate for a mammalian E2-like enzyme, human Apg3p homologue, was purified from COS7 cells and its properties were analyzed. The purified enzyme, hApg3p, is a monomer of 21 kDa containing 33% alanine. It is a homologue of yeast Apg3p, which is a member of the HECT family of E3 ubiquitin ligases. In vivo experiments revealed that hApg3p is expressed ubiquitously. In vitro experiments demonstrated that hApg3p is able to conjugate hApg7p with a ubiquitin-like protein MAP-LC3 and hApg5p conjugates with hApg7p. These results suggest that hApg3p is a novel mammalian E2-like enzyme and that the hApg3p-hApg7p conjugate is involved in the formation of the autophagosome.

Keywords: Autophagy, E2-like enzyme, Human Apg3p homologue

1. Introduction

Autophagy is a highly conserved cellular process that involves the degradation of cytoplasmic components by lysosomes. In mammals, autophagy is initiated by the formation of an autophagosome, which is a double-membrane structure that sequesters cytoplasmic components and delivers them to lysosomes for degradation. The formation of the autophagosome is a complex process that involves the recruitment of various protein complexes to form the pre-autophagosomal membrane (PAM) and the formation of the autophagosome itself.

2. Results

2.1. Purification of hApg3p

hApg3p was purified from COS7 cells using a combination of immunoprecipitation and chromatography. The purified enzyme was a monomer of 21 kDa containing 33% alanine. Its properties were analyzed, and it was shown to be a homologue of yeast Apg3p, which is a member of the HECT family of E3 ubiquitin ligases.

2.2. Conjugation of hApg7p with hApg3p

In vivo experiments revealed that hApg3p is expressed ubiquitously. In vitro experiments demonstrated that hApg3p is able to conjugate hApg7p with a ubiquitin-like protein MAP-LC3 and hApg5p conjugates with hApg7p. These results suggest that hApg3p is a novel mammalian E2-like enzyme and that the hApg3p-hApg7p conjugate is involved in the formation of the autophagosome.

3. Discussion

The results presented in this study suggest that hApg3p is a novel mammalian E2-like enzyme that plays a role in the formation of the autophagosome. The conjugation of hApg7p with hApg3p and hApg5p conjugation with hApg7p suggest that hApg3p is involved in the regulation of the autophagic process.

References

[1] K. Tanida, T. Ueno, and E. Kominami, unpublished results.
[2] Tanida, I., Mizushima, N., Kiyooka, M., Ohsumi, M., Ueno, T., Ohsumi, Y., and Kominami, E. (2000) Mol. Biol. Cell 11, 1367–1379.
[3] Shibata, T., Mizushima, N., Yamasaki, M., Ohsumi, M., Ueno, T., Ohsumi, Y., and Kominami, E. (1999) J. Biol. Chem. 274, 32866–32873.

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Fig. 7. Working hypothesis of the facilitation of the hApg12p-conjugation system by the hApg7p-hApg3p (E1-E2) complex in autophagy. In the hApg12p-conjugation system, hApg12p is activated by hApg7p (E1 enzyme), transferred to hApg10p (hypothetical E2 enzyme), and finally conjugated to hApg5p. The hApg12p-hApg5p conjugate plays an indispensable role in the formation of the autophagosome precursor. The second modification system is essential for the next step in the formation of the autophagosome. MAP-LC3 is activated by the same E1 enzyme, transferred to hApg3p, and finally conjugated to phosphatidylethanolamine. The modification of MAP-LC3 is essential for the formation of the preautophagosomal membrane. Therefore, the cup-shaped preautophagosomal is closed to form the autophagosome. The hApg7p-hApg3p (E1-E2) complex facilitates the formation of the hApg12p-hApg5p conjugate to promote the cooperation of the hApg12p-conjugation system with the MAP-LC3-modification system for the formation of the autophagosome.
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