Dimeric Coiled-coil Structure of Saccharomyces cerevisiae Atg16 and Its Functional Significance in Autophagy

Yuko Fujioka, Nobuo N. Noda, Hitoshi Nakatogawa, Yoshinori Ohsumi, and Fuyuhiko Inagaki

From the Department of Structural Biology, Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, the Integrated Research Institute, Tokyo Institute of Technology, Yokohama 226-8503, and the Precursory Research for Embryonic Science and Technology, Japan Science and Technology Agency, Saitama 332-0012, Japan

Atg16 interacts with the Atg12-Atg5 protein conjugate through its N-terminal domain and self-assembles through its coiled-coil domain (CCD). Formation of the Atg12-Atg5-Atg16 complex is essential for autophagy, the bulk degradation process conserved among most eukaryotes. Here, we report the crystal structures of full-length Saccharomyces cerevisiae Atg16 at 2.8 Å resolution and its CCD at 2.5 Å resolution. The CCD and full-length Atg16 each exhibit an extended α-helix, 90 and 130 Å, respectively, and form a parallel coiled-coil dimer in the crystals. Although the apparent molecular weight of Atg16 observed by gel-filtration chromatography suggests that Atg16 is tetrameric, an analytical ultracentrifugation study showed Atg16 as a dimer in solution, consistent with the crystal structure. Evolutionary conserved surface residues clustered at the C-terminal half of Atg16 CCD were shown to be crucial for autophagy. These results will give a structural basis for understanding the molecular functions and significance of Atg16 in autophagy.

Autophagy is a starvation-induced response that mediates the bulk degradation of cytoplasmic components in the lysosome/vacuole (1, 2). In higher eukaryotes, autophagy plays a crucial role in fundamental biological processes such as intracellular clearance, differentiation, development, programmed cell death, and antigen representation, and autophagy dysfunction is associated with severe diseases such as neurodegenerative disorders and cancers (3). In autophagy, a double-membrane structure called an autophagosome sequesters a portion of the cytoplasm and fuses with the lysosome/vacuole to deliver its inner contents into the organelle lumen. The autophagosome-formation step requires at least 18 Atg proteins (4), among which eight constitute two ubiquitin-like conjugation systems, the Atg12 and Atg8 systems (5). In the Atg12 system, Atg12 is covalently linked to Atg5 by sequential reactions catalyzed by Atg7 and Atg3 (another E2-like enzyme) (6, 7). The Atg5 noncovalently interacts with the N-terminal domain of Atg16, but Atg12-Atg5 conjugation is not required for this process (8). Because Atg16 self-assembles through the predicted coiled-coil motif at the C-terminal region (8), Atg12-Atg5-Atg16 is considered to behave like a large protein complex and has been estimated by gel-filtration chromatography to be 350 kDa (9, 10). Multimerization of Atg12-Atg5 conjugates mediated by the coiled-coil region of Atg16 is essential for the autophagosome formation (9).

The Atg12 and Atg8 systems are well conserved from yeasts to mammals, although the sequence similarity of Atg16 with a mammalian Atg16 counterpart, Atg16L, is low. Similarly with yeast Atg16, mammalian Atg16L was shown to form a large protein complex with mammalian Atg12-Atg5 conjugates (11). Furthermore, the mammalian Atg12-Atg5-Atg16L complex localizes to the convex surface of autophagic isolation membranes (11), suggesting its pivotal role in autophagosome formation. Recently, genome-wide association studies have linked human Atg16L to the pathogenesis of Crohn disease, a chronic inflammatory disorder of the bowels (12), attracting significant attention to this protein.

Despite the importance of the Atg12-Atg5-Atg16 complex, the functional role of this complex during autophagy has remained unclear. However, recent studies have shown that the complex functions as an E3-like enzyme in the Atg8 system (10, 13, 14). In the Atg8 system, Atg8 is conjugated to phosphatidyethanolamine by sequential reactions catalyzed by Atg7 and Atg3 (another E2-like enzyme) (15), in which the Atg12-Atg5 conjugate has been shown to promote the transfer of Atg8 from Atg3 to phosphatidyethanolamine by direct interaction with both Atg3 and phosphatidyethanolamine (10). Furthermore, the mammalian Atg12-Atg5-Atg16L complex has been shown to specify the lipidation site of LC3, a mammalian Atg8 orthologue, through interaction with Atg3 and some unidentified factor(s) on the membrane (14).

We have been carrying out comprehensive structural studies of Atg proteins (16). Thus far, the crystal structures of plant Atg12 (17) and yeast Atg5 complexed with the N-terminal domain of Atg16 (18) have been determined. Intriguingly, these studies showed that both Atg12 and Atg5 are ubiquitin-fold proteins. However, the structure of the predicted coiled-coil motif (residues 58–123) of Atg16, essential for the formation of the multimeric Atg12-Atg5-Atg16 complex (9), has not been elucidated. Here, we report the crystal structure of the coiled-coil domain (CCD) 

2 The abbreviations used are: CCD, coiled-coil domain; ALP, alkaline phosphatase.

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residues. Both structures showed that Atg16 is a parallel dimeric coiled-coil protein, and the dimeric state was confirmed in solution by analytical ultracentrifugation. Further, structure-based mutational analyses suggested that the conserved surface residues of Atg16 in CCD are critical for autophagy.

EXPERIMENTAL PROCEDURES

Expression and Purification—Expression and purification of Saccharomyces cerevisiae Atg16<sup>CCD</sup> as well as the construction of the expression vector of Atg16<sup>CCD</sup> were described elsewhere (19). The expression vectors of glutathione S-transferase-fused Atg16 and its mutants were constructed as follows. The full-length <i>S. cerevisiae</i> ATG16 gene was amplified by PCR and inserted into pGEX-6P-1 (GE Healthcare). Mutations leading to the indicated amino acid substitutions were introduced by PCR-mediated site-directed mutagenesis. All of the constructs were sequenced to confirm the identities and were expressed in <i>Escherichia coli</i> strain BL21 (DE3) cells. After cell lysis, glutathione S-transferase-fused Atg16 and its mutants were purified by affinity chromatography using a glutathione-Sepharose 4B column (GE Healthcare), followed by excision of glutathione S-transferase from the proteins with PreScission protease (GE Healthcare). The proteins were again applied to a glutathione-Sepharose 4B column to remove the excised glutathione S-transferase. Further purification was performed using a Superdex200 gel-filteration column (GE Healthcare) eluted with 20 mM Tris-HCl, pH 8.5, and 500 mM NaCl.

To express the Atg5-Atg16 complex, the full-length of <i>S. cerevisiae</i> Atg5 was inserted into a pHT1 vector and the full-length of <i>S. cerevisiae</i> Atg16 was inserted into a PET-11a vector (Novagen). The Atg16 was coexpressed with N-terminally hexahistidine-tagged Atg5 in <i>E. coli</i> strain BL21 (DE3). After cell lysis, Atg5 was purified by affinity chromatography using a nickel-nitrilotriacetic acid column (Qiagen). Throughout the purification steps, Atg16 comigrated with Atg5, indicating that Atg16 forms a stable complex with Atg5. After affinity chromatography, the protein complexes were purified on a Superdex200 column (GE Healthcare) eluted with 20 mM Tris-HCl, pH 8.5, and 150 mM NaCl. Further purification was performed using a HiTrap DEAE FF column (GE Healthcare) equilibrated with 20 mM Tris-HCl, pH 8.5, and the purified product was eluted with a 0–300 mM NaCl gradient in the same buffer. Final purification was carried out on a HiPrep desalting column (GE Healthcare) eluted with 20 mM Tris-HCl, pH 8.5, and 500 mM NaCl.

Diffraction Data Collection—Crystallization of native and L103M mutant of Atg16<sup>CCD</sup> were performed as described previously (19). Crystallization of full-length Atg16 D101A/E102A mutant was performed by the sitting-drop vapor diffusion method at 293 K. Drops of 10 mg ml<sup>-1</sup> Atg16 D101A/E102A in 20 mM Tris buffer, pH 8.5, and 500 mM NaCl were mixed with equal amounts of reservoir solution consisting of 0.5 M CaCl<sub>2</sub>, 100 mM acetic acid buffer, pH 4.6, and equilibrated against 100 μl of the same reservoir solution by vapor diffusion. Crystals, 0.50 × 0.25 × 0.25 mm, were obtained within a week. Diffraction data of native Atg16<sup>CCD</sup>, selenomethionine-labeled Atg16<sup>CCD</sup> L103M mutant as well as full-length Atg16 D101A/E102A mutant were collected using a Rayonix MX-225 charge-coupled device detector at the SPring-8 beamline BL41XU. Because diffractions in the lower resolution shell were not good for native Atg16<sup>CCD</sup>, the diffraction data were merged with those collected using a RAXIS-VII imaging plate detector and an in-house CuKα x-ray generator (Rigaku). All diffraction data were indexed, integrated, and scaled with HKL2000 (20).

Structure Determination—The initial phasing was performed by the single-wavelength anomalous dispersion method using the peak data of the selenomethionine-labeled Atg16<sup>CCD</sup> L103M crystal. After identifying six selenium sites and calculating the initial phases using the SOLVE program (21), density modification and automated model building were performed using the RESOLVE program (22). Further model building was performed manually using the COOT program (23), and crystallographic refinement was performed using the Crystallography and NMR system software (24). The structure of full-length Atg16 D101E102A mutant was determined by the molecular replacement method using CNS software, for which the Atg16<sup>CCD</sup> structure was used as a search model.

In Vivo Assays—The significance of the conserved amino acids in Atg16<sup>CCD</sup> in vivo was examined as follows. Point mutations were introduced by PCR-based site-directed mutagenesis using pRS316- or pRS426-based plasmids containing the ATG16-myc gene as templates. Successful introduction of the point mutations was confirmed by sequencing. These plasmids were introduced into Δatg16:LEU2 cells on a SEY6210 background (MATα leu2 ura3 his3 trp1 lys2 suc2), and SEY6210 Δatg16::LEU2 cells expressing Atg16 mutants were cultured in synthetic defined medium + casamino acids + AdeTrp medium to logarithmic phase and then transferred to synthetic defined (-N) medium. After culture for 4.5 h in synthetic defined (-N) medium, cells were collected. The lysates were prepared by breaking cells with glass beads in 250 mM Tris-HCl, pH 9.0, 10 mM MgSO<sub>4</sub>, 10 μM ZnSO<sub>4</sub>, and 1 mM phenylmethanesulfonyl fluoride. Using the lysates, alkaline phosphatase (ALP) assay was performed in the manner described in previous studies (25, 26). For Western blotting, the lysates were resuspended in SDS-PAGE sample buffer and were separated by urea-SDS-PAGE (15), followed by immunoblotting using anti-aminopeptidase I (anti-Ape1) or anti-Atg16 antibodies. Signals were detected using an ECL system (GE Healthcare) with a LAS4000 bioimaging analyzer (Fujifilm).

Analytical Ultracentrifugation—Sedimentation equilibrium studies were conducted in a Beckman Optima XL-A equipped with absorbance optics. Concentrations of the loaded Atg16 were 0.45, 0.75, and 1.50 mg ml<sup>-1</sup>, and for the Atg5-Atg16 complex sample 0.13, 0.21, 0.42 mg ml<sup>-1</sup>, in 20 mM Tris-HCl at pH 8.5 and 500 mM NaCl, 110 μl of the protein samples and 130 μl of the reference buffer were loaded in a six-channel centerpiece. The Atg16 was centrifuged at rotor speeds of 12,000, 16,000, and 20,000 rpm at 293 K, and the Atg5-Atg16 complex at rotor speeds of 6,000, 8,000, and 10,000 rpm, also at 293 K. The absorbance profiles were acquired at a wavelength of 280 nm. After 22 h of centrifugation, the absorbance profiles were compared at 2-h intervals to ensure that the sedimentation equilibrium was reached. Data analysis was carried out using the Beckman Optima XL-A/AXL-I software, version 4.1 (Beckman Coulter, Inc.) based on the Origin software (Microcal, Inc.).
RESULTS

Structure of Atg16<sup>CCD</sup>—Atg16 is comprised of the N-terminal Atg5-binding domain and the coiled-coil domain (Fig. 1A). In the case of mammalian Atg16L1, the WD40 domain is attached to the C terminus of the coiled-coil domain. <i>S. cerevisiae</i> Atg16 has been suggested to contain a coiled-coil motif at the C-terminal region (residues 58–123) (9). Various lengths of the Atg16 construct containing this motif were applied to crystallization and a crystal for the fragment (residues 50–123) was obtained. This construct will be referred to as Atg16<sup>CCD</sup> hereafter. The crystal structure of Atg16<sup>CCD</sup> was refined against 2.5 Å data to an R-factor of 0.272 and a free R-factor of 0.299 (Table 1).

One Atg16<sup>CCD</sup> molecule is comprised of a single /H9251/H9252-helix. About 10 N-terminal and 5 C-terminal residues did not show any defined electron density and were omitted from the model. The asymmetric unit of the crystal contains six Atg16<sup>CCD</sup> molecules (A–F) (supplemental Fig. S1). The molecules A and B, C and D, and E and F...
interact as pairs, all in a similar manner. As a result, these three pairs of molecules form similar dimer structures with root mean square differences of 0.6–0.9 Å for the Cα atoms. These three dimers further interact with each other in the crystal in various fashions (supplemental Fig. S1). Therefore, Atg16\(^{CCD}\) was considered to form a homodimer as the basic unit in the crystal. The dimer structure of Atg16\(^{CCD}\) is a highly twisted, parallel oriented, two-stranded coiled-coil that is 20–25 Å wide and 90 Å long with an approximate 2-fold symmetry (Fig. 2A).

**Structure of Full-length Atg16**—Although crystallization of full-length Atg16 was not possible, crystallization was possible when introducing mutations at exposed acidic residues (substitution of Asp\(^{101}\) and Glu\(^{102}\) with alanine), and the structure at 2.8 Å resolution was established (this construct will be referred to as Atg16\(^{F}\) hereafter) (Table 1). The asymmetric unit contains two Atg16\(^{F}\) molecules (supplemental Fig. S2, A and B). Residues 55–142 of molecule A and residues 58–133 of molecule B show defined electron densities, whereas other regions, including the whole N-terminal domain, are disordered and omitted from the model. Each molecule is composed of a single α-helix, and they form a parallel coiled-coil dimer similar to the Atg16\(^{CCD}\) structure but longer, as long as 110 Å (Fig. 2B). Residues 55–60 and 134–142 of molecule A (yellow in Fig. 2B) lack binding partners but have an α-helical structure, resulting in the 130 Å length. The other intermolecular interactions observed in the crystal, some of which seem to be achieved by point mutations, are completely different from those observed in the Atg16\(^{CCD}\) crystal (supplemental Fig. S2). The dimer structures of Atg16\(^{CCD}\) can be superimposed on that of the same region of Atg16\(^{F}\) with root mean square differences of 1.6–2.4 Å for Cα atoms. This somewhat large structural difference may be due to differences in the crystal packing and/or distinct lengths of the protein
Structure of Atg16

although point mutations could also affect the conformation of Atg16^F. Whatever the case, it is certain that Atg16 has a parallel, dimeric coiled-coil structure, but the 2-fold symmetry axis observed in Atg16^{CCD} is somewhat distorted in Atg16^F. The dimer structure is stabilized by extensive hydrophobic interactions by a number of hydrophobic side chains faced at the dimer interface. In contrast, there are 14 acidic and 15 basic side chains that distribute evenly on the dimer surface (Fig. 2C).

Residues Constructing the Coiled-coil Dimer Interface—Coiled-coil motifs commonly share a seven-amino acid sequence, the 3–4-heptad repeat, with the positions denoted by the letters a to g (27) (Fig. 1B). The first (α) and fourth (δ) positions are predominantly occupied by hydrophobic residues. Previous studies have demonstrated a crucial role for the interior packing of the side chains at positions α and δ to enable the establishment of the parallel coiled-coil conformation. For example, the presence of β-branched residues (Val, Ile, and Thr) in position α and non-β-branched residues in δ promotes dimer formation, while the reverse arrangement favors tetramers (27). In the case of Atg16^{CCD}, β-branched residues are abundant in position α but not in position δ (Fig. 1B); therefore, the rule suggests the sequence of Atg16^{CCD} as favorable for homodimer formation, consistent with the dimer structure in the crystal.

Dimer Formation of Atg16 in Solution—Gel-filtration chromatography study has suggested that Atg16 forms a ~350 kDa complex with Atg12-Atg5 conjugates due to Atg16 oligomerization, and therefore Atg16 was proposed to form a tetramer (9). As suggested by supplemental Fig. S3, it is likely that the recombinant Atg16^{CCD} and full-length Atg16 behaved as a 4–5-mer in gel-filtration chromatography, not affected by the concentration of NaCl. To verify the stoichiometry in solution, we employed analytical ultracentrifugation, which gives a molecular weight independent of the molecular shape. Sedimentation velocity ultracentrifugation experiments indicated that a portion of both Atg16 and the Atg5-Atg16 complex tend to aggregate in the presence of 150 mM NaCl, whereas they were highly homogeneous in the presence of 500 mM NaCl (data not shown), suggesting that high concentrations of NaCl may stabilize Atg16 and the Atg5-Atg16 complex. Therefore, sedimentation equilibrium ultracentrifugation experiments were performed in the presence of 500 mM NaCl. The sedimentation equilibrium data for Atg16 and the Atg5-Atg16 complex are shown in Fig. 3, in which the equilibrated concentration of proteins (absorbance at 280 nm) is plotted against the distance from the center of centrifuge. At equilibrium, sedimentation of proteins is balanced by diffusion opposing the concentration gradients. Because the average molecular mass of the proteins determines the balance, it can be calculated from the distribution pattern of proteins at equilibrium. The average molecular mass of Atg16 was estimated to be 31 kDa, which is 1.8 times the calculated molecular mass of the Atg16 monomer (17 kDa). The average molecular mass of the Atg5-Atg16 complex was estimated to be 108 kDa, which is twice the calculated molecular mass of the Atg5-Atg16 heterodimer (54 kDa). These results suggest that Atg16 exists as a dimer in solution, and the Atg5-Atg16 complex exists as a 2:2 heterotetramer, consistent with the dimer structure of Atg16 observed in the crystals.
Conserved residues on the surface of Atg16CCD are required for autophagy. A, autophagic activity was estimated using an ALP assay (see under “Experimental Procedures”). White and gray bars indicate ALP activities at 0 and 4.5 h after starvation, respectively. B, shown is the monitoring of Ape1 maturation under growing (upper) or starvation (lower) conditions using a CEN plasmid pRS316 as an expression vector for Atg16. The lysates were separated by urea-SDS-PAGE and subjected to immunoblotting with anti-Ape1 antibody. C, shown are experiments using a 2-μm plasmid pRS426 as an expression vector (Vec.) for Atg16 (left, growing; right, starvation). Upper panels, expression level of Atg16 mutants. The lysates were separated by SDS-PAGE and subjected to immunoblotting with anti-Ape1 antibody. Lower panels, a monitoring Ape1 maturation is shown. The lysates were separated by urea-SDS-PAGE and subjected to immunoblotting with anti-Ape1 antibody. WT, wild-type.

mid, pRS316. The autophagic activity was estimated by an ALP assay (25). The ALP assay monitors the transport of ALP from the cytoplasm into the vacuole via autophagy by measuring the activity of the alkaline phosphatase that is artificially expressed in the cytosol as a proform and transported into the vacuole via autophagy, where it is processed into the mature form. As shown in Fig. 5A, Δatg16 cells expressing Atg16(60–118) or Atg16(119–150) showed significantly reduced autophagic activity, whereas those expressing Atg16(119–150) showed autophagic activity comparable with that expressing wild-type Atg16. The Δatg16 cells expressing Atg16(60–118) or ΔAtg16(119–150) showed no autophagic activity. Next, the activity of Atg16 mutants was assayed by monitoring the maturation of the proform of Ape1 (aminopeptidase I), which is transported into the vacuole via the cytoplasm-to-vacuole targeting pathway under nutrition-rich conditions and by autophagy under starvation conditions, following this procedure, the protein is processed into a mature form within the vacuole. The Ape1 maturation in Δatg16 cells was restored in cells expressing wild-type Atg16, Atg16(60–118), or Atg16(119–150) in both growing and starvation conditions (Fig. 5B, left panels). In contrast, there was only a weak restoration of Ape1 maturation in cells expressing Atg16(60–118) or Atg16(119–150) and no restoration was observed for cells expressing ΔCCD mutants irrespective of the nutrient condition. The results were closely correlated with those of the ALP assay. We then monitored the Ape1 maturation in Δatg16 cells expressing a single mutant of Atg16 (D101A, E102A, I104A, I108A, or V112A) to clarify which residue is especially important for autophagy. All these residues other than Val112 are partially important for autophagic activity (Fig. 5B, right), suggesting that they collectively contribute to the function of Atg16 in autophagy.

In above experiments, the expression of wild-type and mutant Atg16 proteins was not clearly detected by Western blot analysis (data not shown). We then expressed wild-type and mutant Atg16 proteins using a 2-μm plasmid pRS426 as an expression vector. The expression level of all Atg16 mutants other than Atg16(60–150) was comparable with that of wild-type Atg16 under both nutrient-rich and starvation conditions (Fig. 5C, upper panels). The Ape1 maturation was then monitored in these cells (Fig. 5C, lower panels), which showed close correlation with the results obtained using pRS316 plasmids (Fig. 5B). These results suggest that the autophagic defects observed in Δatg16 cells expressing Atg16 mutants are not due to their low expression levels and that Atg16CCD and its conserved surface residues are crucial for autophagy.

DISCUSSION

Atg16 Is a Dimeric Coiled-coil Protein—The previous analyses using gel-filtration chromatography showed that Atg12-Atg5:Atg16 forms a ~350 kDa complex, suggesting that Atg16 may self-assemble into a tetramer and that it tethers four Atg12-Atg5 conjugates. However, the present structural analyses of both CCD and the full-length of Atg16 showed that Atg16 forms a parallel coiled-coil homodimer in the crystals, and furthermore, the analytical ultracentrifugation experiments showed that Atg16 and the Atg5-Atg16 complex exist as a dimer...
and a 2:2 heterotetramer, respectively, in solution. The differences between the results of the gel-filtration chromatography and analytical ultracentrifugation studies may be due to the rod-like structure of Atg16 as gel-filtration chromatography often overestimates the molecular mass of nonglobular, extended proteins (28). Sequence alignment of Atg16/Atg16L homologues suggests that the feature favored by coiled-coil dimers, i.e. \( \beta \)-branched residues (enclosed with a green square in Fig. 1A) are abundant at position \( a \) but not at position \( d \), is conserved among most Atg16 homologues, indicating that the dimeric coiled-coil is a conserved feature among the Atg16 homologues. Therefore, mammalian Atg16L, which was reported to form a very large ~800 kDa complex with mammalian Atg12-Atg5 conjugates by gel-filtration chromatography studies (11), also may have a dimeric coiled-coil structure. Mammalian Atg16L has a WD repeat domain and other insertions that are absent in yeast Atg16, and structural studies of Atg16L are required to establish the structural similarities and differences between Atg16 and Atg16L.

The linker region of Atg16, the flexible C-terminal tail of Atg12 as well as the side chain of Atg5 K149, may allow the flexible arrangement of three moieties (Atg12, Atg5-Atg16(22–46), and Atg16\textsubscript{CCD}). Because Atg16\textsubscript{CCD} is a parallel coiled-coil dimer, two Atg5 molecules are positioned at the same edge of the long rod-like structure of Atg16\textsubscript{CCD}. Although each component has a well known fold (ubiquitin fold and coiled-coil), the overall architecture of the Atg12-Atg5-Atg16 complex, containing six ubiquitin folds at one edge of the elongated coiled-coil dimer, is unique. In mammals, the Atg12-Atg5-Atg16L complex was reported to localize to the convex surface of isolation membranes and to detach immediately before or after the completion of autophagosome formation (11, 29). This localization pattern is similar to that of coat proteins involved in various membrane trafficking events, which could lead to a hypothesis that the Atg12-Atg5-Atg16 complex could also function as a coat-like protein specific to autophagosome formation. However, the Atg12-Atg5-Atg16 complex possesses neither \( \beta \)-propeller nor \( \alpha \)-solennoid motifs, both commonly observed in clath-
rin and coat protein complex II cages (30). Furthermore, the parallel dimeric architecture of the Atg12-Atg5-Atg16 complex does not allow the formation of a concave surface or the symmetry observed in the Sec13-Sec31 heterotetramer of coat protein complex II and in the clathrin heavy chain trimer (triskelion) (30). Thus the Atg12-Atg5-Atg16 complex alone may not function as a structural cage that covers vesicles. Although it would still be possible that it functions together with other coat proteins to construct a coating protein complex, such interaction partners for Atg12-Atg5-Atg16 have not been reported.

**Conserved Surface Residues of Atg16^CCD Are Crucial for Autophagy**—It should be noted that the evolutionary conserved surface residues cluster at the C-terminal half of Atg16^CCD (Fig. 4). Because these residues are not involved in the formation of the dimeric coiled-coil structure of Atg16, they would play important roles in the functions of Atg16 as in vivo mutational analyses have shown that they are required for autophagy (Fig. 5). Since coiled-coil motifs in various proteins have been reported to mediate protein-protein interactions, Atg16^CCD also seems to bind target proteins, where the conserved residues play crucial roles for the binding. Recently, the C-terminal region (residues 141–265) of the coiled-coil domain of human Atg16L, which has sequence similarity to the C-terminal half of Atg16^CCD (Fig. 1A), was reported to directly and specifically interact with the GTP-bound form of Rab33B, a Golgi-resident small GTPase, although its significance in autophagy remains to be elucidated (31). In general, Rab-binding proteins possess two parallel or anti-parallel α-helices, which are directly involved in Rab binding (32). These proteins include GCC185, a trans-Golgi network golgin that binds Rab6 and Sec2, a guanine nucleotide exchange factor for Sec4, a Rab GTPase. It is to be noted that their Rab-binding region has a parallel dimeric coiled-coil structure similar to Atg16 (33, 34) (supplemental Fig. S4). Although Rab33 is not conserved in yeasts, these observations suggest that the conserved surface residues of Atg16 may be responsible for the interaction with Rab family protein(s). It is possible that Rab recruits the Atg12-Atg5-Atg16 complex through the interaction with Atg16 to autophagy-related membranes, whereby the complex exerts its critical functions in autophagosome formation. It would also be possible that unidentified factor(s) other than the Rab family proteins interact with the conserved surface residues of Atg16. The Atg12-Atg5 conjugate might also interact with these conserved residues due to the presence of the flexible linker region of Atg16^47–57^.

Identification and characterization of binding target(s) for Atg16 are required for uncovering the function of Atg16 in autophagy.

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