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Structure of autophagy-related protein Atg8 from the silkworm Bombyx mori

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Autophagy-related protein Atg8 is ubiquitous in all eukaryotes. It is involved in the Atg8–PE ubiquitin-like conjugation system, which is essential for autophagosome formation. The structures of Atg8 from different species are very similar and share a ubiquitin-fold domain at the C-terminus. In the 2.40 Å crystal structure of Atg8 from the silkworm Bombyx mori reported here, the ubiquitin fold at the C-terminus is preceded by two additional helices at the N-terminus.

1. Introduction

Autophagy is essential for cell survival in all eukaryotes and is involved in the degradation of cytoplasmic components and intracellular proteins by lysosomes (Reggiori & Klionsky, 2002). Cell signals such as starvation can stimulate autophagy and induce the formation of a double-membrane structure called the autophagosome, which docks and fuses with the lysosome, whereupon the inner contents of the autophagosome are released into the lysosome and are consumed by hydrolases (Shintani & Klionsky, 2004; Reggiori & Klionsky, 2002). Recent studies have revealed a wide variety of physiological roles of autophagy and its relevance to diseases such as liver diseases, muscular disorders, neurodegeneration, pathogen infections and cancer (Cuervo, 2004).

Several autophagy-related genes (ATGs) have been identified in Saccharomyces cerevisiae. Two ubiquitin-like conjugation systems (the Atg8–PE system and the Atg12–Atg5–Atg16 system) are essential for autophagosome formation (Klionsky et al., 2003; Amar et al., 2006). In the Atg8–PE system, the removal of the carboxyl-terminal arginine from newly synthesized Atg8 by a cysteine protease, Atg4, exposes a glycine residue at the carboxyl-terminus. The uncovered glycine is then activated and covalently conjugated to phosphatidylethanolamine (PE) by the E1-like enzyme Atg7 and the E2-like enzyme Atg3 (Ichimura et al., 2000). The Atg8–PE system controls the expansion of the autophagosome precursor, the phagophore (Xie et al., 2008), and directly determines the growth of autophagosomal membranes (Nakatogawa et al., 2007).

Recently, several autophagy-related genes have been identified in Bombyx mori (Zhang et al., 2009). BmATG8 (Genbank accession No. FJ416330), which encodes a homologue of yeast Atg8, was found to be up-regulated in the silk gland in fifth-instar B. mori larvae, suggesting that BmAtg8 may play an important role in maturation of the silk gland (Zhang et al., 2009).

Here, we report the crystal structure of BmAtg8 at 2.40 Å resolution. BmAtg8 consists of the ubiquitin fold at the C-terminus, which is conserved among species from yeast to mammals, with two additional helices at the N-terminus.

2. Materials and methods

2.1. Cloning and protein expression

The gene encoding residues 1–116 of BmAtg8 was amplified by PCR from the total RNA of B. mori. The amplified DNA was cloned...
2.2. Purification

The supernatant was loaded onto 2 ml Ni–NTA affinity resin (GE Healthcare) with lysis buffer at 289 K. After washing with 20 ml 10 mM imidazole in lysis buffer, the target protein of about 14 kDa was eluted with lysis buffer containing 250 mM imidazole and was then further purified on a HiLoad Superdex 75 column (GE Healthcare Bioscience) with lysis buffer at 289 K. The fractions containing the target protein were verified using 15% SDS-PAGE.

2.3. Crystallization and data collection

Purified protein solution (in 20 mM Tris–HCl pH 8.5, 50 mM NaCl) was concentrated to 20 mg ml\(^{-1}\) and was then further purified on a HiLoad Superdex 75 column (GE Healthcare Bioscience) with lysis buffer at 289 K. The fractions containing the target protein were verified using 15% SDS-PAGE.

3.1. Overall structure

The crystal structure of BmAtg8 was refined to a resolution of 2.40 Å. There are two subunits (A and B) in the asymmetric unit, but the symmetry relating them was not imposed during refinement. The majority of residues are fitted well in the electron-density map, except for the N-terminal His tag of both subunits. Statistics of the X-ray data-collection and refinement are summarized in Table 1. The overall structure of BmAtg8 consists of a four-stranded \(p\)-sheet (\(\beta_1\)–\(\beta_4\)) flanked by two pairs of \(\alpha\)-helices (\(\alpha_1\)–\(\alpha_2\) and \(\alpha_3\)–\(\alpha_4\)).
one on each side of the sheet (Fig. 1). The core domain, a ubiquitin fold, consists of four \( \beta \)-strands (\( \beta1-\beta4 \)) with two of the \( \alpha \)-helices (\( \alpha3-\alpha4 \)) on one side of the sheet. In BmAtg8, two additional \( \alpha \)-helices (\( \alpha1 \) and \( \alpha2 \)) cover the other side of the sheet via both polar and hydrophobic interactions.

3.2. Structure comparison

Several crystal structures of Atg8 homologues have been reported. Multiple sequence alignment indicates that Atg8 is highly conserved from yeast to mammals (Fig. 2a). We found that BmAtg8 shows high structural similarity to the search model GABARAP (PDB code 1kjt; Bavro et al., 2001), microtubule-associated protein light chain 3 (LC3; PDB code 1ugm; Sugawara et al., 2004), Trypanosoma brucei Atg8 (TbAtg8; PDB code 3h9d; Koopmann et al., 2009) and S. cerevisiae Atg8 (ScAtg8; PDB code 2zpn; Noda et al., 2008), with root-mean-square deviations (r.m.s.d.s) of 0.5, 0.9, 1.1 and 0.8 Å over 110 \( \alpha \)-helices, respectively (Fig. 2b). The helices \( \alpha1 \) and \( \alpha2 \) sustain their orientation via tight interactions with the ubiquitin core by means of several hydrogen bonds and salt bridges. In detail, residues Phe3, Gln4, Tyr5, Lys6, Ile21, Arg22, Tyr25, Arg28 and Glu34 contribute to the hydrogen bonding. Four salt bridges were found: between Lys6 and Asp100, between Arg14 and Asp102, between Lys15 and Asp100, and between Glu17 and Lys48 (Fig. 2).

Most of these residues are highly conserved from yeast to mammals. This implies that these conserved residues are essential for the protein to maintain its structural integrity and biological function (Sugawara et al., 2004). In BmAtg8, residues His9 and Ser10 on the loop between \( \alpha1 \) and \( \alpha2 \) also contribute to the stabilization of the N-terminal helices. His9 can form hydrogen bonds to Tyr5 and Arg14. Ser10, which is not conserved in the homologues, forms hydrogen bonds to Lys13 and Arg14.

Two hydrophobic pockets are found to exist in all Atg8 homologues. One pocket comprises Glu17, Ile21, Pro30, Ile32, Lys48 and Leu50 and is located between helix \( \alpha2 \) and the core domain and the other comprises Tyr49, Val51, Pro52, Phe60, Leu63 and Ile64 and is located in the ubiquitin fold. Most residues in the hydrophobic treatment.
pockets are also conserved in different species. As reported previously (Noda et al., 2008), these pockets are involved in the interaction between LC3 and p62: the side chains of residues Trp338 and Leu341 in p62 insert deeply into these pockets and an intermolecular β-sheet is formed by p62 and LC3. This suggests that these regions in BmAtg8 are potential binding sites that recognize other protein partners in a similar manner.

In conclusion, BmAtg8 contains a ubiquitin fold which has been found in all Atg8 proteins from yeast to mammals. The high conservation in the structure of Atg8 illustrates that the Atg8 protein plays a central role in the autophagy pathway of eukaryotes.

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