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Repurposing of conserved autophagy-related protein ATG8 in a divergent eukaryote

Maude F. Lévêque, Hoa Mai Nguyen, and Sébastien Besteiro

DIMNP- UMR5235, CNRS, Université de Montpellier, Montpellier, France

ABSTRACT

Toxoplasma gondii and other apicomplexan parasites contain a peculiar non-photosynthetic plastid called the apicoplast, which is essential for their survival. The localization of autophagy-related protein ATG8 to the apicoplast in several apicomplexan species and life stages has recently been described, and we have shown this protein is essential for proper inheritance of this complex plastid into daughter cells during cell division. Although the mechanism behind ATG8 association to the apicoplast in *T. gondii* is related to the canonical conjugation system leading to autophagosome formation, its singular role seems independent from the initial catabolic purpose of autophagy. Here we also discuss further the functional evolution and innovative adaptations of the autophagy machinery to maintain this organelle during parasite division.

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The phylum Apicomplexa is an early-branching eukaryotic lineage that contains a number of important human and animal pathogens. Among them are, for instance, *Plasmodium falciparum*, the deadly agent of malaria, and *Toxoplasma gondii*, a rarely life-threatening, but ubiquitous, parasite infecting about one third of the human population. These obligate intracellular parasites possess a number of specific organelles, including a non-photosynthetic plastid called the apicoplast whose origin can be traced to a double endosymbiotic event (Fig. 1A).¹ First, there was a primary endosymbiosis, in which a previously non-photosynthetic eukaryote engulfed and subdued a cyanobacterium. Then the resulting alga has been phagocytosed by another heterotrophic eukaryote, through a secondary endosymbiotic event, to yield a photosynthetic ancestor of Apicomplexa. At some point, members of the apicomplexan lineage became parasitic and lost their ability to photosynthesize. Some, like *Cryptosporidium*, the causative agent of cryptosporidiosis, even subsequently lost the plastid. In spite of having lost a number of biochemical pathways through evolution, the apicoplast remains essential to the survival of many apicomplexan parasites: this organelle is a metabolic hub² that hosts several vital pathways, including type II fatty acid³ or isoprenoid precursors biosynthesis.⁴ Consequently, the

apicoplast is considered as a promising avenue to look for new potential drug targets to combat *Plasmodium*- and *Toxoplasma*-caused diseases.

Recently, the unusual apicoplast localization of an autophagy-related protein ATG8 has been reported in *Plasmodium*,⁵⁻¹¹ as well as in *Toxoplasma*.^{12,13} Macroautophagy (usually simply referred to as autophagy) is a self-degradative process used by eukaryotic cells to get rid of damaged or unwanted components, and recycle cytoplasmic content in response to stress such as nutrient starvation.¹⁴ A characteristic feature of autophagy is the formation of a double-membrane compartment called the autophagosome (Fig. 1B), for sequestering and delivering intracellular components to lysosomes for their degradation and recycling. These structures result from the activation of a highly regulated machinery that promotes initiation and expansion of nascent autophagosomes (also called phagophores, Fig. 1B). Distinct complexes of autophagy-related proteins cooperate in the biogenesis of autophagosomes, but cytosolic protein ATG8¹⁵ and its conjugation system to autophagosomal membranes (Fig. 1B) are particularly important. Once conjugated to autophagosomal membranes, ATG8 is supposedly promoting their elongation and facilitating the completion of the organelle.

CONTACT Sébastien Besteiro  sebastien.besteiro@inserm.fr  DIMNP- UMR5235, CNRS, Université de Montpellier, CC107, 34095 Montpellier Cedex 5, France.

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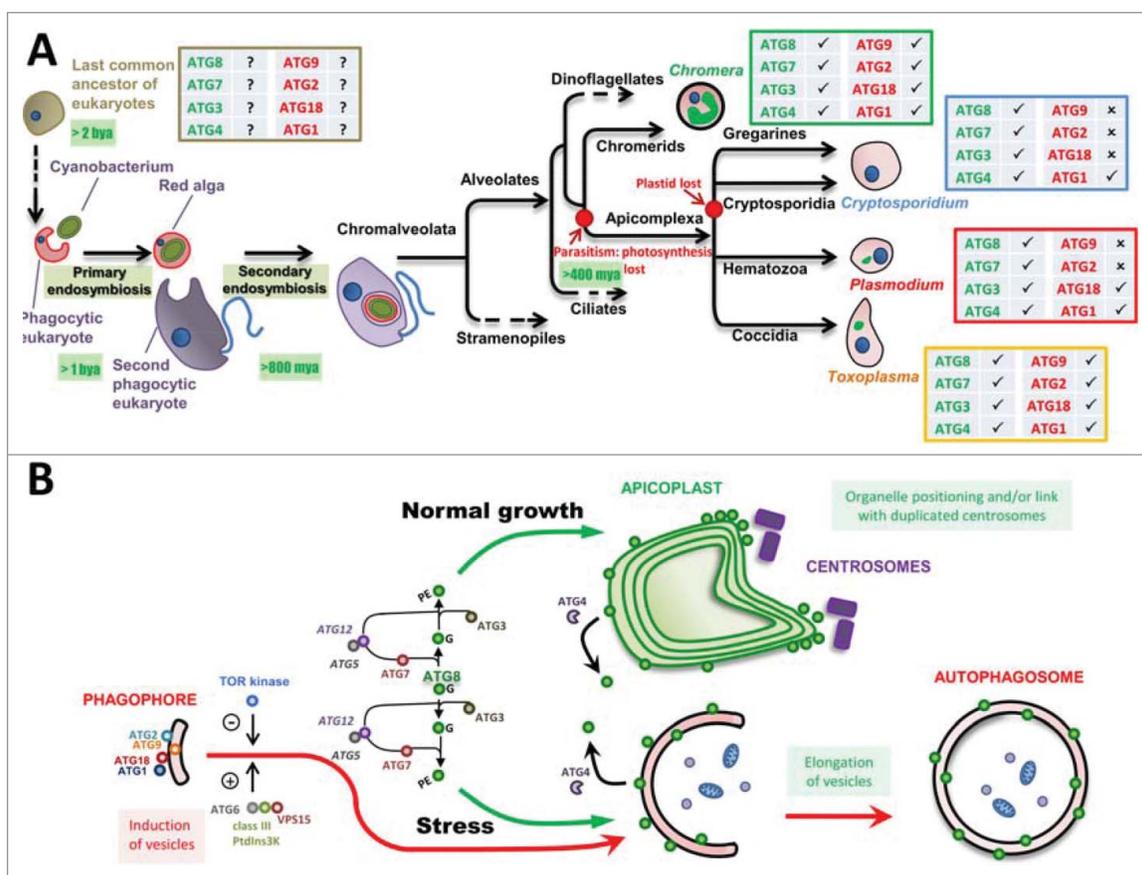


Figure 1. Overview and evolutionary timeline of the autophagy machinery in *T. gondii* and related eukaryotes. (A) Schematic representation of the evolutionary history of apicomplexan parasites. For selected genera, the presence of homologs for autophagy-related genes involved in ATG8 membrane conjugation (green) and in phagophore formation (red) is reported. bya: billion years ago, mya: million years ago. (B) Schematic representation of the partially conserved autophagy machinery and its putative functions in *T. gondii*. Depending on the growth conditions, TgATG8 can either be conjugated to autophagosomal membranes for vesicles elongation, or to the apicoplast outermost membrane for driving organelle inheritance during parasite division. PE: phosphatidylethanolamine (membrane lipid anchor of ATG8), G: C-terminal glycine for lipid conjugation of ATG8.

Core components of the autophagy pathway are evolutionarily conserved among eukaryotes, including members of the Apicomplexa phylum.¹⁶ However, homology searches revealed they possess an apparently reduced machinery: ATG8 and its membrane-conjugation system are essentially conserved, while several proteins involved in the early steps of autophagosome biogenesis seem to be absent (Fig. 1A). In spite of this, *T. gondii* is apparently able to generate ATG8-decorated autophagosome-like vesicles in response to nutrient stress.^{13,17,18} However, a fully functional catabolic pathway remains to be demonstrated, as no proof of autophagocytosed material recycling has been clearly established yet.

Our recent efforts to investigate TgATG8 function have revealed this protein is crucial for parasite replication inside its host cell:¹⁹ TgATG8 is enriched at the apicoplast during division of the organelle, and plays a role in its proper inheritance into daughter cells. Apicoplast division involves a unique and highly coordinated mechanism to ensure segregation into growing daughter

parasites, through plastid elongation and attachment with duplicated centrosomes.²⁰ In normal growth conditions, TgATG8 is temporally and spatially recruited to the ends of elongating apicoplast prior to cytokinesis, and mediates its centrosome-driven inheritance into the progeny (Fig. 1B). How the protein acts as an intermediate between the dividing apicoplast and the centrosomes remains to be elucidated. TgATG8 might play a role in the correct positioning of the organelle along the cytoskeleton, or to mediate its binding to the centrosomes through yet unknown adaptors.

Our previous work has shown that TgATG8 binding to the outermost membrane of the apicoplast requires the canonical conjugation system normally devoted to autophagosomal membrane-conjugation (Fig. 1B).^{13,18} However, the function of TgATG8 and its associated machinery for this organelle is clearly unrelated to canonical degradative autophagy. This non-canonical function opens the door for a wealth of new and intriguing questions regarding the reassignment of a widely

conserved autophagy pathway and the evolution of plastid-bearing eukaryotic lineages. Because of the endosymbiotic origin of the apicoplast, its outermost membrane is derived from a phagosomal membrane (Fig. 1A). Interestingly, in addition to autophagosomal membranes, members of the ATG8/LC3 family (LC3 is the mammalian ATG8 homolog) are known to be recruited to phagosomal membranes in a non-canonical way.²¹ This ability might thus be an ancient feature, acquired early during evolution.

Organisms in all the sub-domains of the eukaryotic kingdoms contain at least parts of the autophagy machinery, thus it is assumed that the common ancestor of eukaryotes possessed some kind of primitive autophagic capacity (Fig. 1A).²² The formation of autophagosomes is triggered by nutrient starvation in a wide range of phylogenetically-distant eukaryotes, suggesting autophagy has been developed primarily as an adaptive mechanism allowing survival when facing changes in the availability of nutrients in the environment. However, it is possible that part of the machinery was also repurposed for a non-degradative function during evolution, and apicomplexan parasites might represent a striking example of this specialization. Investigating ATG8 recruitment at the plastid membrane of *Chromera*, a recently identified close photosynthetic relative of apicomplexan parasites (Fig. 1A),²³ could be of particular interest to determine if an ancient plastid-related function of ATG8 would be present in these endosymbionts. When looking at components of ATG8 membrane conjugation machinery, it seems very well conserved across the different Apicomplexa subgroups. In contrast, early actors of autophagosome biogenesis (i.e. protein ATG9) are present in *Toxoplasma* and related coccidian, but not found by homology searches in other Apicomplexa (Fig. 1A). Although they might have evolved yet unknown proteins with similar function, this really puts into question the presence of a functional degradative autophagic pathway in these parasites. For instance, it is puzzling to see that gregarines and parasites of the *Cryptosporidium* genus have a poorly conserved machinery for autophagosome formation, arguing against a functional canonical autophagic pathway. On the other hand, they have retained ATG8 and its membrane conjugation machinery, although it would not be needed for an apicoplast-related function as these Apicomplexa have lost the plastid (Fig. 1A). If anything, this highlights the need for further investigations into the canonical and non-canonical functions of the autophagy machinery in these fascinating divergent eukaryotes.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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