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# Ats-1

## A novel bacterial molecule that links autophagy to bacterial nutrition

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**Abbreviations:** Ats-1, *Anaplasma* translocated substrate-1; T4S, type IV secretion; ZFYVE1/DFCP1, Zinc finger FYVE domain-containing protein 1/Double FYVE-containing protein 1; PtdIns3K/PIK3C3, class III phosphatidylinositol 3-kinase; ATG, autophagy-related; ER, endoplasmic reticulum; UVRAG, UV radiation resistance-associated; 3-MA, 3-methyladenine; DFP, diisopropylfluorophosphate

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**Obligatory intracellular life style and a small number of genes for biosynthesis and metabolism necessitate the Gram-negative bacterium, *Anaplasma phagocytophilum*, to depend on the host cell for nutrients. *A. phagocytophilum* resides in a membrane-bound inclusion, and secretes a protein, Ats-1 (*Anaplasma* translocated substrate-1), into the host cell cytoplasm. Ats-1 binds BECN1, a protein critical for autophagy nucleation, and induces autophagosome formation. The autophagosomes traffic to, and fuse with, *A. phagocytophilum* inclusions, delivering autophagic cargo into the inclusions, which can serve as nutrients for bacterial growth. This finding demonstrates that *A. phagocytophilum* subverts host cell autophagic machinery to facilitate infection by secreting a BECN1-binding molecule.**

Macroautophagy (hereafter autophagy), is an essential eukaryotic cellular process to sequester and digest undesirable intracellular objects including intracellular pathogens to protect the whole organism, and thus is considered an important innate immune response mechanism. Autophagosome formation is tightly controlled by sequential assembly of autophagy-related (ATG) proteins. The formation of the PtdIns3K complex, including ATG14-BECN1-PIK3C3/VPS34, is induced at the autophagy-initiation phase. The activation of the enzyme complex leads to formation of a structure called the "omegasome" on a specialized subdomain of the ER, which serves as a cradle for biogenesis of the phagophore. The phagophore elongates and encloses cytoplasmic cargo to form autophagosomes with the help of two ubiquitin-like conjugation systems that produce ATG12–ATG5-ATG16L1 and LC3-II (phosphatidylethanolamine-conjugated LC3), and other ATG proteins. Autophagosomes eventually fuse with lysosomes to become degradative autolysosomes.

*Anaplasma phagocytophilum,* a Gram-negative obligatory intracellular bacterium (rickettsia), primarily infects granulocytes and causes the emerging tick-borne zoonosis called human granulocytic anaplasmosis (HGA). *A. phagocytophilum* has a small genome size of 1.47 Mb, with a limited number of genes for biosynthesis and metabolism, and thus depends on host-synthesized nutrients for growth. *A. phagocytophilum* has the type IV secretion (T4S) system; bacteria use this system to transport macromolecules across the bacterial membrane into eukaryotic cells to dysregulate or modulate target cell functions, resulting in disease development. Ats-1 was discovered as one of the T4S substrates in our previous study. Ats-1 is imported into infected host cell mitochondria and interferes with host cell apoptosis.

Recently we identified an additional function for Ats-1. Ats-1 binds BECN1 and induces autophagosome formation. Yeast two-hybrid screening revealed BECN1, a core component in the autophagy-initiating PtdIns3K complex, is an Ats-1-binding partner, and this interaction was confirmed by co-immunoprecipitation and colocalization of BECN1 and Ats-1 in transfected cells. When ectopically expressed in transfected cells, Ats-1 forms autophagosome-like vesicles, as

they contain ATG14, ZFYVE1/DFCP1 (an ER resident protein and omegasome marker), and LC3 (phagophore/autophagosome marker). The Ats-1 activity to form autophagosomes depends on its interaction with ATG14 via BECN1. Co-immunoprecipitation showed Ats-1 interacts with ATG14, but not with UVRAG (UV radiation resistance-associated) that functions in autophagosome maturation to autolysosomes. The Ats-1 N-terminal deletion mutant, which fails to interact with ATG14, cannot induce autophagosome formation. The induced autophagosomes localize to *A. phagocytophilum* inclusions as demonstrated by the presence of ATG14, ZFYVE1, LC3, and Ats-1. Organelle fractionation studies showed that autophagosomes are increased, and ER fractions are shifted to the *A. phagocytophilum* inclusion fraction in infected cells, compared with those in uninfected cells. The outer membrane of autophagosomes appears to fuse with the *A. phagocytophilum* inclusion membrane, releasing inner membrane-enveloped autophagic bodies inside of the inclusions, because we observed a close contact between the outer membrane of autophagosomes and the *A. phagocytophilum* inclusion membrane, and the presence of LC3-marked vesicles and the single-membrane autophagic body-like structure inside of the inclusion.

We previously showed that autophagy is important for *A. phagocytophilum* growth, as indicated by treatment with pharmacological agents. Stimulation of autophagy by rapamycin enhances *A. phagocytophilum* infection; inhibition of the autophagic pathway by 3-methyladenine (3-MA), a PtdIns3K inhibitor inhibits infection. Ats-1 gave us the key to understand why autophagy is important for *A. phagocytophilum*. Delivery of anti-Ats-1 antibody into infected cells reduces *A. phagocytophilum* infection and autophagosome formation; overexpression of Ats-1 increases infection, indicating that Ats-1-driven autophagosome formation contributes to *A. phagocytophilum* infection. Since Ats-1 binds BECN1 and promotes autophagosome formation, we examined whether BECN1 is required for *A. phagocytophilum* infection in vitro and in vivo. *Becn1* knockdown by siRNA suppresses *A. phagocytophilum* infection; *Becn1*$^{+/-}$ heterozygous-deficient mice are resistant to infection.

Because of the essential role of autophagy in *A. phagocytophilum* infection, the presence of autophagic body-like structures inside of the inclusions, and the dependence of *A. phagocytophilum* growth on host cell metabolites, we tested the hypothesis that autophagosomes provide nutrients for *A. phagocytophilum* growth. Supplementation of excessive amino acids partially overrides the growth inhibition of *A. phagocytophilum* by 3-MA, indicating this hypothesis is true. Degradation of host cell cytoplasmic proteins through proteasomes and lysosomes is not required for *A. phagocytophilum* growth, as it can replicate in the cells treated with the proteasome inhibitor MG132, or the vacuolar-type ATPase inhibitor bafilomycin $A_1$. *A. phagocytophilum* inclusions are devoid of lysosomal markers. Then, how is the autophagic body-like cargo turned into amino acids so that they can be utilized by *A. phagocytophilum*? We speculated that proteases on the surface of *A. phagocytophilum* may digest the host cell cytoplasmic proteins inside of the inclusions. Along these lines, treatment of *A. phagocytophilum*-infected host cells, or pre-treatment of *A. phagocytophilum* with the membrane-permeable serine protease inhibitor DFP inhibits the infection.

Thus, this recent finding provides a new model for how bacteria obtain nutrients from host cells through bacterial molecule-induced autophagy.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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