論文の内容の要旨

論文題目

A Novel Function of a Transmembrane Protein Yip1A in the Unfolded Protein Response

(小胞体ストレス応答における膜貫通タンパク質 Yip1A の新規機能)

田口 由起

The unfolded protein response (UPR) is an intracellular signaling pathway that is triggered to restore cellular homeostasis when misfolded or unfolded proteins accumulate in the endoplasmic reticulum (ER). The ER is an essential organelle that functions in protein synthesis, folding and secretion, lipid and sterol synthesis, and calcium homeostasis. In mammalian cells, the UPR is composed of three pathways that are initiated by distinct ER sensors: inositol-requiring enzyme 1 (IRE1), protein kinase RNA (PKR)-like ER kinase (PERK), and activating transcription factor-6 (ATF6). Activation of each sensor produces an active transcription factor, which in turn activates downstream target genes. The UPR has been implicated in the pathogenesis of several viral and bacterial infections. These pathogens modulate individual pathways of the UPR in distinct ways to establish more favorable environment for their replication in host cells. Autophagy has also been linked to the survival of several intracellular pathogens. Autophagy is a catabolic process that is induced to maintain homeostasis in response to stresses such as starvation, pathogenic protein aggregation, and invading pathogens. Cytoplasmic contents are sequestered into double-membrane vesicles known

as autophagosomes, which then fuse with endocytic compartments including late endosomes and lysosomes to generate autolysosomes. The sequestered contents are degraded by lysosomal proteases. While autophagy can function as a host defense system against viral and bacterial infections to eliminate these pathogens, certain viruses and bacteria have evolved strategies to subvert the autophagic machinery of host cells for their benefits. However, the precise role of these machineries in the intracellular life of pathogens still needs to be elucidated.

The genus *Brucella* is a serious intracellular pathogen that causes brucellosis in a wide range of animals including humans. Within host cells, *Brucella* spp. reside in a membrane-bound compartment called the *Brucella*-containing vacuole (BCV). The pathogen replicates in the form of ER-derived vacuoles in host cells. The molecular mechanisms by which *Brucella* spp. establish the safe replicative niche remain unknown.

In the present study, I investigated a potential role of the UPR and autophagy in infection of HeLa cells with *Brucella abortus*. Several lines of evidence were provided that demonstrate the mechanism by which *B. abortus* acquires the ER-derived membrane for its replication. First, biochemical analysis of the activation of the UPR sensors revealed that during *Brucella* infection, the IRE1 pathway, but not the PERK and ATF6 pathways, of the UPR was activated in a time-dependent manner, and that the COPII vesicle components Sar1, Sec23, and Sec24D were upregulated. Second, a marked accretion of ER-derived autophagosome-like vacuoles was observed around replicating bacteria using immunofluorescence microscopy and electron microscopy. Third, a novel host factor, Yip1A, was identified for the activation of the IRE1 pathway of the UPR in response to tunicamycin treatment and infection with *B. abortus*. I found that Yip1A is responsible for the phosphorylation of IRE1 through high-order assembly of IRE1 molecules at ER exit sites (ERES) under the UPR conditions. Yip1A is a multi-pass transmembrane protein that belongs to the Yip1 family. Yip1A is localized to ERES, the ERGIC, and the Golgi, and has been implicated in vesicle trafficking between the ER and the Golgi, including COPII vesicle budding at ERES, vesicle tethering to the Golgi membrane, and COPI-independent retrograde vesicle transport. Yip1A has also been implicated in the maintenance of ER morphology. The expression of Yip1A is knocked down by using small interfering RNA (siRNA). In Yip1A-knockdown cells, *B. abortus* failed to generate the ER-derived vacuoles, and

remained in endosomal/lysosomal compartments. These results indicate that the activation of the IRE1 pathway and the subsequent formation of ER-derived autophagosome-like vacuoles are critical for *B. abortus* to establish a safe replication niche, and that Yip1A is indispensable for these processes. Finally, I characterized a possible role of the *Brucella* effector VceC by ectopic expression as well as by introduction of a recombinant VceC protein using a cell-resealing technique. VceC induced both the activation of the IRE1 pathway and the formation of autophagosomes in a Yip1A-dependent manner.

On the basis of these findings, I propose a model for intracellular *Brucella* replication that exploits the host UPR and autophagic machineries to establish ER-derived replicative BCVs (Figure 1A). During infection, *B. abortus* triggers the activation of IRE1, presumably by secreting effector molecules, such as VceC, into the cytoplasm of host cells. IRE1 molecules form high-order complexes with the aid of Yip1A at ERES, and are activated by trans-autophosphorylation. The activated IRE1 in turn triggers the biogenesis of ER-derived autophagosome-like vacuoles. These vacuoles then fuse with endolysosomal vesicles. *B. abortus* might intercept this UPR-induced autophagy process to acquire ER-derived membranes. Given that the bacteria that have reached the ER are located in late endosomal/lysosomal compartments, they would be able to fuse with these vacuoles. Once they have acquired the ER-derived membrane, BCVs retain functional features of the ER, and replication of *B. abortus* in individual vacuoles might be supported through continual accretion of ER membranes derived from the IRE1-specific UPR. In contrast, the knockdown of Yip1A (Figure 1B) or IRE1 (Figure 1C) prevents the activation of IRE1, and therefore ER-derived membranes are not available for *Brucella* replication. Consequently, BCVs remain in endosomal/lysosomal compartments.

The present study revealed a new role for Yip1A in regulating the host UPR and the subsequent ER-derived autophagy during *Brucella* infection. The UPR and autophagy has been implicated in a wide variety of diseases including cancers, diabetes, atherosclerosis, and neurodegenerative diseases. Characterization of the function of Yip1A will provide new insights into the molecular mechanisms of these diseases, and contributes to design therapeutic strategies against these diseases.

テキスト

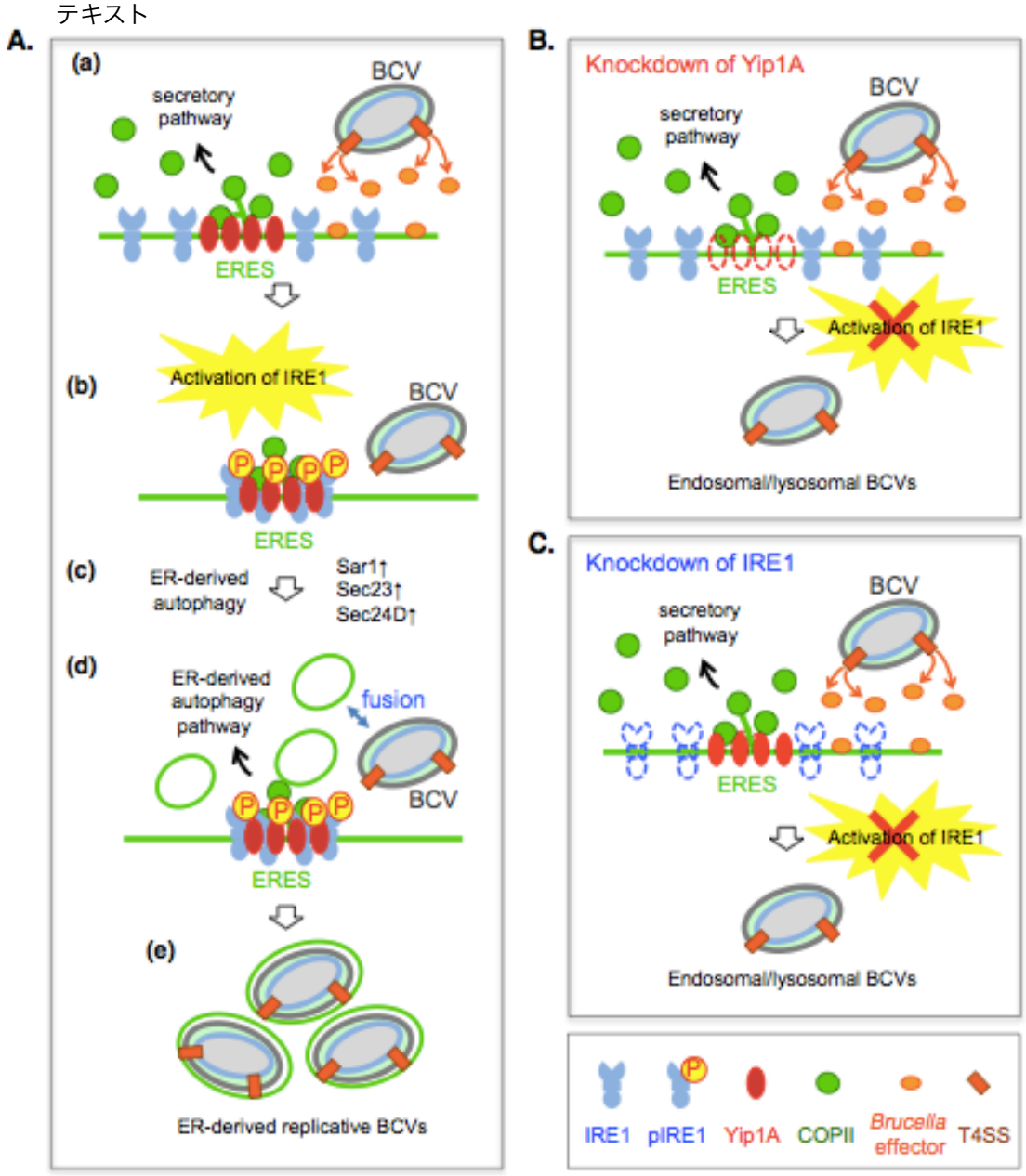

Figure 1