Journal of Integrative Agriculture 2026, 25(8): 3387–3399

Available online at ScienceDirect

Journal of Integrative Agriculture



RESEARCH ARTICLE

Brucella effector protein BspF manipulates the host cell autophagy by acetylating SNAP29

Ruitong Shen[1, 2*], Yuqi Wang[1, 2*], Qiao Dong[1, 2, 4], Jinying Zhu[1, 2], Yukai Xing[1, 2], Ang Li[1, 2], Gen Lu[1, 2], Sijiao Wu[1, 2], Ze Yu[1, 2], Fangyuan Du[1, 2], Jingbo Gao[1, 2], Qingqing Wei[5], Xiaoyue Chen[1, 2], Jinling Liu[1, 2], Huan Zhang[1, 2#], Zeliang Chen[1, 2, 3#]

[1] *Key Laboratory of Livestock Infectious Disease of Ministry of Education, Shenyang Agricultural University, Shenyang 110866, China*
[2] *Key Laboratory of Ruminant Infectious Disease Prevention and Control (East), Ministry of Agriculture and Rural Affairs, Shenyang Agricultural University, Shenyang 110866, China*
[3] *Department of Epidemiology, School of Public Health, Sun Yat-sen University, Guangzhou 510080, China*
[4] *Liaodong University, Dandong 118001, China*
[5] *First People's Hospital of NingYang, Tai'an 271400, China*

Highlights

- Global host protein acetylation enhances intracellular *Brucella* survival.
- *Brucella* type IV secretion system (T4SS) effector BspF acetylates the host autophagy protein SNAP29 at K103.
- Acetylated SNAP29 disrupts SNARE assembly and blocks host autophagic flux.

Abstract

Brucella spp., an intracellular bacterium, uses its type IV secretion system (T4SS) to regulate host signaling pathways and promote intracellular survival, but the molecular mechanism of this process remains largely unknown. Here we found that increasing the abundance of acetylated protein in host cells promotes the intracellular survival of *Brucella*. Moreover, our results demonstrated that the *Brucella* effector protein BspF can impact protein acetylation modification in host cells by interacting with other intracellular acetyltransferases. We conducted liquid chromatography-tandem mass spectrometry (LC-MS/MS) to characterize the protein acetylation mediated by BspF. We identified that synaptosome associated protein 29 (SNAP29) K103 was acetylated, and that acetylated SNAP29 inhibited its interaction with syntaxin 17 (STX17), thereby regulating the autophagy and providing an environment for the intracellular survival of *Brucella*. Furthermore, our results provide the first report of a bacterial effector using acetylation to affect the SNAP29-STX17-VAMP8 complex, and inhibit the host's defense system. Our results suggest a vital role of SNAP29 acetylation in autophagy of host cells under intracellular infection, by specifically regulating the assembly of soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE).

Keywords: *Brucella*, BspF, acetylation, autophagy, SNAP29

1. Introduction

Post-translational modification (PTM) is a crucial process wherein one or more amino acid residues undergo covalent modifications during or after protein translation. These modifications include phosphorylation, glycosylation, ubiquitination, nitrosylation, methylation, acetylation, lipidation, and proteolysis, affecting almost all aspects of normal cell biology and disease mechanisms. Therefore, identifying and understanding PTMs is crucial in research on disease treatment and prevention. Protein lysine acetylation (denoted by Kac) is a reversible, conserved, and highly regulated type of post-translational modification. It is ubiquitous in both eukaryotes and prokaryotes and can be catalyzed by lysine acetyltransferases and reversibly regulated by lysine deacetylases (Pandey *et al.* 2002; Archambaud *et al.* 2010; Aubry *et al.* 2011; Henriksen *et al.* 2012; Huang and Brumell 2014). Lysine acetylation plays an important role in regulating various cellular processes, such as energy metabolism, mitosis, and autophagy (Pietrocola *et al.* 2015; Menzies *et al.* 2016). Breakthroughs in high-resolution mass spectrometry

Received 4 July 2024; Received in revised form 29 December 2024; Accepted 26 Janurary 2025; Available online 17 February 2025
[#]Correspondence Huan Zhang, E-mail: zhanghuan1988@syau.edu.cn; Zeliang Chen, E-mail: zeliangchen@yahoo.com
[*]These authors contributed equally to this study.

© 2026 CAAS. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). Peer review under responsibility of Editorial Board of *Journal of Integrative Agriculture*.
doi: 10.1016/j.jia.2025.02.011

(MS)-based proteomics have contributed to revealing unknown functions of acetylated proteins mapping in various organisms (Choudhary *et al.* 2014). In addition, protein acetylation has been shown to play a critical role in bacterial chemotaxis, metabolism, and DNA replication (Ren *et al.* 2017).

Brucella spp. are a Gram-negative, intracellular pathogen. It not only replicates and survives in macrophages, but also infects dendritic cells and trophoblast cells (Archambaud *et al.* 2010; Myeni *et al.* 2013; Zhi *et al.* 2019). *Brucella* produces idiosyncratic niches for replication and persistence through a special pathway in host cells (Salcedo *et al.* 2008). During infection, *Brucella*-containing vacuoles (BCV) are formed in host cells to prevent fusion with lysosomes, thereby evading the host immune system (Jiao *et al.* 2021). The main virulence factors of *Brucella* are lipopolysaccharides (LPS), type IV secretion system (T4SS) and BvrR/BvrS system. The *Brucella* T4SS is encoded by the virB operon (virB1-12) and secretes 15 effectors to regulate host inflammatory response and manipulate vesicular transport. The absence of T4SS causes *Brucella* to lose the ability to reach the endoplasmic reticulum of the host cells and subsequent degradation by fusion with lysosomes during endocytosis (Celli *et al.* 2003; von Bargen *et al.* 2012; Jiao *et al.* 2021). *Brucella* secreted protein F (BspF; BAB1_1948), one of the 15 effector proteins required by *Brucella*, consists of 428 amino acids and contains the Gcn5-related N-acetyltransferase (GNAT) family acetyltransferase domain (Myeni *et al.* 2013; Ke *et al.* 2015). BspF can inhibit the protein secretion pathway of host cells (Myeni *et al.* 2013; Ke *et al.* 2015). In addition, BspF promotes *Brucella* replication within replication-permissive vacuoles by interfering with vesicular transport between the trans-Golgi network (TGN) and recycling endocytic compartment (Borghesan *et al.* 2021). Our previous study reported that BspF has decrotonyltransferase activity and regulates the crotonylation level of host cells (Zhu *et al.* 2020).

Autophagy is an important host defense mechanism in the elimination of intracellular microorganisms (Gutierrez *et al.* 2004; Ogawa *et al.* 2005; Guo *et al.* 2012). Moreover, autophagy is a homeostatic and conserved process by which substrates are sequestered in double-membrane bound vesicles (autophagosomes) and transported to the lysosome (Siqueira *et al.* 2018; Keller *et al.* 2020). Conserved autophagy-related proteins regulate autophagy functions (Keller *et al.* 2020). RAB GTPases and soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) mediate the function between the completed autophagosome and the endosomes, multivesicular bodies, or lysosomes (Keller *et al.* 2020). Bacteria have evolved strategies to evade autophagic degradation to undergo their intracellular lifecycles (Miller and Celli 2016). A previous study reported that *Brucella* melitensis 16M can trigger autophagy, thereby promoting its survival in RAW264.7 cells (Guo *et al.* 2012). However, a new study reported that compared with *Brucella* abortus A19, the A19ΔvirB mutant strain showed enhanced autophagy and decreased the survival capability in macrophages and dendritic cells (Zhang *et al.* 2019). These results are consistent with the reports of *Brucella* 2308Δ*vceA*, which demonstrated that compared with *Brucella* 2308, 2308Δ*vceA* promotes autophagy in human trophoblast (HPT-8) cells (Deng *et al.* 2022). These studies suggest that *Brucella* T4SS plays an important role in autophagy, which promotes persistent intracellular infection of *Brucella* (Gutierrez *et al.* 2004; Py *et al.* 2007; Deng *et al.* 2022).

Here, we first demonstrated that the *Brucella* T4SS effector protein BspF can impact protein acetylation modification in host cells and investigate the mechanisms of BspF on host cells autophagy. We performed a proteomic analysis of BspF-catalyzed lysine acetylation using label-free quantitative (LFQ) liquid chromatography-tandem mass spectrometry (LC-MS/MS). Our results showed that BspF causes global acetylation of host cells, and regulates autophagy through its GNAT domain. Further analysis indicated that BspF inhibits the interaction between synaptosome associated protein 29 (SNAP29) and syntaxin 17 (STX17) by acetylating SNAP29, thereby promoting intracellular survival and replication of the bacteria. These findings demonstrate that the BspF is a vital *Brucella* virulence by regulating protein acetylation modification and further manipulating cells autophagy during *Brucella* infection.

2. Methods and materials

2.1. Bacterial strains and cell lines

Brucella abortus 2308 wild-type (WT) and 2308Δ*bspF* mutant strains were used in this study. All *Brucella* strains were grown in tryptic soy agar (TSA) or tryptic soy broth (TSB) (BD, USA) at 37°C in 5% CO_2. *Escherichia coli* Trans1-T1 competent cells were in Luria-Bertani medium (LB) were used for genetic manipulation. HEK-293T and HeLa cells were cultured in DMEM (HyClone; USA) supplemented with 10% (v/v) fetal bovine serum (FBS; Gemini, USA) at 37°C in a humidified atmosphere with 5% CO_2.

2.2. Bacterial infection

Briefly, monolayers of HeLa cells were cultured in 6-well plates for 24 h. Next, the cells were treated with 5 μmol L^{-1} vorinostat (SAHA; CST, USA) for 1.5 h, and then infected with *Brucella* at a multiplicity of infection (MOI) of 200. Culture plates were incubated at 37°C for 2 h, then the infected cells were washed with phosphate buffer saline (PBS). The infected cells were incubated for 1 h in the presence of 50 μg mL^{-1} gentamicin to kill extracellular bacteria. The cultures were then placed in fresh DMEM containing 2% (v/v) FBS and 25 μg mL^{-1} gentamicin, and incubated at 37°C under 5% CO_2. After 24 h, the culture was washed three times with PBS, and the cells were lysed with an appropriate amount of 0.1% (v/v) TritonX-100. The lysates were diluted 10^{-4} and then coated on TSA plates, and the colony-forming units (CFUs) were calculated after incubating at 37°C for 72 h to monitor intracellular replication of *Brucella* strains.

After culturing and removal of extracellular bacteria, proteins were extracted from the cells with radio-immunoprecipitation assay (RIPA) buffer for Western blotting. Moreover, Flag-SNAP29 and Myc-STX17 plasmids were co-transfected into HeLa cells for 24 h. Subsequently, the cells were infected with *Brucella* 2308 WT and 2308Δ*bspF* mutant strains for immunoprecipitation. In addition, *Brucella* 2308 WT and 2308Δ*bspF* mutant strains infected HeLa cells

after overexpressing SNAP29 (WT or mutants). After killing extracellular bacteria and culturing cells for 4, 8, 12, or 24 h, cells were lysed with 0.1% (v/v) TritonX-100, then lysates were diluted to 10^{-4} and coated on TSA plates, and the CFUs were calculated after incubation at 37°C for 72 h.

2.3. Immunoprecipitation and co-immunoprecipitation

HEK-293T cells were transfected with the appropriate plasmids for 30 h; HeLa cells were transfected with the appropriate plasmids for 24 h, then cells were infected with *Brucella* 2308 WT or 2308Δ*bspF* mutant strains according to the method described in the Section 2.2. Cells were collected and lysed with 1,000 μL RIPA buffer and protease inhibitor cocktail for 40 min. The lysates were centrifuged at 12,000×g for 15 min at 4°C. After centrifugation, specific primary antibodies were added to the supernatants and incubated for 4 h at 4°C with rotation, and then the 40 μL Protein A+G agarose beads were added and incubated overnight at 4°C with rotation. The beads were then washed three times with ice-cold lysis buffer and boiled at 100°C for 10 min. Finally, the samples were performed in immunoblotting.

The following antibodies were used in immunoprecipitation and co-immunoprecipitation: anti-Flag (4 μL, MBL, Japan, M185) and anti-SNAP29 (8 μL, Proteintech, USA, 12704) antibodies were added to the supernatants and incubated for 4 h at 4°C.

2.4. Immunoblotting assay

Cells were washed with PBS and lysed with RIPA buffer (150 mmol L^{-1} NaCl, 50 mmol L^{-1} Tris-HCl, pH 7.4, 2 mmol L^{-1} Na2EDTA, 10% glycerol, 1% NP-40, and 0.1% SDS) supplemented with a protease inhibitor cocktail. After lysis for 40 min at 4°C with gentle shaking, the supernatants were collected by centrifugation at 12,000×g for 15 min. Samples were subsequently separated by SDS-PAGE and transferred onto polyvinylidene difluoride membranes (PVDF) (Millipore, USA). After blocking with 5% skimmed milk in TBST, the membrane was incubated with primary antibodies overnight at 4°C, followed by incubation with HRP-conjugated secondary antibody for 2 h. The intensity of the western blotting band signal was detected with Clarity ECL reagents (Beyotime, China) according to a gel imager (Aplegen, USA).

The following antibodies were used in this study: anti-LC3B (1:1,000, Sigma, USA, L7543), anti-p62/SQSTM1 (1:1,000, Proteintech, 18420), anti-Beclin 1 (1:1,000, Proteintech, 11306), anti-Kac (1:500, PTM Bio, China, PTM101), anti-H3K14ac (1:1,000, PTM Bio, PTM157), anti-Flag (1:1,000, MBL, M185), anti-HA (1:3,000, MBL, M132-3), anti-VAMP8 (1:1,000, Proteintech, 15546), anti-Syntaxin 17 (1:1,000 Proteintech, 17815), anti-SNAP29 (1:1,000, Proteintech, 12704) and anti-β-actin (1:3,000, Beyotime, China, AF003).

2.5. Acetyltransferase assay

In vitro acetyltransferase assays were subjected to acetyltransferase buffer (50 mmol L^{-1} Tris-Cl, pH 7.5; 100 mmol L^{-1} NaCl, 1 mmol L^{-1} EDTA, and 1 mmol L^{-1} DTT) with 20 μg BspF, along with 0.5 μmol L^{-1} histone octamers, with or without 300 μmol L^{-1} AcCoA (Zhu *et al.* 2020). After 1 h, the reaction was terminated by the addition of 5×SDS loading buffer and subjected to immunoblot analysis using anti-acetyl-lysine antibody (PTM Bio, China).

Alternatively, HA-BspF and empty plasmids were transfected into HEK-293T cells for 30 h. HA-tagged proteins were immunoprecipitated on Protein A/G beads, as described in the Section 2.3. Precipitated immunocomplexes were washed three times with RIPA buffer and then reacted with 0.5 μmol L^{-1} octamers and 300 μmol L^{-1} AcCoA. The reaction mixture was incubated at 37°C for 1 h and was terminated by adding 5×SDS loading buffer. Thereafter, the samples were boiled at 100°C for 5 min, and the eluted proteins were subjected to immunoblot analysis.

2.6. Protein extraction and trypsin digestion

HEK-293T cells were transfected with pCMV-HA-BspF or pCMV-HA empty plasmids for 30 h. The cells were harvested, and acetylation proteomics analysis was conducted with support by Jingjie PTM Biolabs. Samples were sonicated three times on ice using a high intensity ultrasonic processor (Scientz) in lysis buffer (8 mol L^{-1} urea, 1% protease inhibitor cocktail, 3 μmol L^{-1} TSA, 50 mmol L^{-1} NAM and 2 mmol L^{-1} EDTA). The protein was collected from cell debris by centrifugation, and the concentration was determined with a BCA Kit (Beyotime, China), according to manufacturer instructions. For digestion, the protein solution was reduced with 5 mmol L^{-1} DTT for 30 min at 56°C and alkylated with 11 mmol L^{-1} IAA for 15 min at room temperature in the dark. The protein sample was then diluted by adding 100 mmol L^{-1} N H_4HCO_3 to a urea concentration of less than 2 mol L^{-1}. Trypsin was added at a 1:50 trypsin-to-protein mass ratio for the first digestion overnight and at a 1:100 trypsin-to-protein mass ratio for a second 4-h digestion.

2.7. Affinity enrichment of lysine-acetylated peptides

The purification and enrichment of acetylated modified peptides were conducted following published procedures (Zhou *et al.* 2016). Briefly, tryptic peptides were dissolved in NETN buffer (100 mmol L^{-1} NaCl, 1 mmol L^{-1} EDTA, 50 mmol L^{-1} Tris-HCl, 0.5% NP-40, pH 8.0) were incubated with pre-washed anti-acetyl-lysine agarose beads (Cat. no.104, PTM Bio, Hangzhou, China) at 4°C overnight with gentle shaking. The beads were then washed four times with NETN buffer and twice with ddH_2O. The bound peptides were eluted from the beads with 0.1% trifluoroacetic acid (Sigma-Aldrich, USA), following published procedures (Hong *et al.* 2016; Zhou *et al.* 2016). Finally, the eluted fractions were combined and vacuum-dried. For LC-MS/MS analysis, the resulting peptides were cleaned with C18 ZipTips (Millipore, Germany) according to the manufacturer's instructions.

2.8. LC-MS/MS analysis with Q ExactiveTM

The tryptic peptides were dissolved in solvent A (0.1% formic acid and 2% acetonitrile) and directly loaded onto a custom reversed-phase analytical column (15-cm length, 75-μm i.d.). An EASY-nLC 1000 UPLC System was used for the separation of peptides at a constant flow rate of 400 nL min^{-1} in solvent B (0.1% formic acid and 98% acetonitrile solution). The gradient

comprised an increase from 8 to 23% solvent B over 36 min, 23 to 35% over 8 min, and 35 to 80% in 3 min, then a hold at 80% for the last 3 min. The peptides were attached to an NSI source followed by tandem mass spectrometry (MS/MS) in Q ExactiveTM Plus (Thermo, USA) coupled online to the ultra-performance liquid chromatography (UPLC). The electrospray voltage applied was 2.0 kV. Intact peptides and ion fragments were detected and analyzed by Orbitrap at a resolution of 120,000 and 15,000, respectively, with a scan range of 350–1,600 m/z. The data acquisition mode used a data-dependent scanning program that alternated between one MS scan followed by 20 MS/MS scans with a 15.0 s dynamic exclusion. Automatic gain control was set at 5E4.

2.9. Statistical analysis

Immunoblot band density was quantified by ImageJ software. Statistical analysis was performed by IBM SPSS Statistics 22 software. Graph plots were generated by GraphPad Prism 6. Each experiment was performed three times and data was presented as the means±standard errors of the mean (SEMs) of results. The significance of the variability between different groups was determined using one-way analysis of variance (ANOVA) followed by LSD and Duncan's post hoc multiple-comparison test. The ** P-value <0.01 or * P-value <0.05 was considered statistically significant, and P-value >0.05 was considered statically non-significant.

3. Results

3.1. Host protein acetylation promoted intracellular survival of *Brucella*

Lysine acetylation is associated with gene expression, transcription autophagy, apoptosis pathways (Matthias *et al.* 2008; Yang and Seto 2008; Cain *et al.* 2014). Several pathogens can manipulate host cell antibacterial responses and evade the immune system by affecting histone acetylation (Grabiec and Potempa 2018). To characterize the role of acetylation in the *Brucella* spp. life cycle in detail, we first sought to determine whether increasing the abundance of acetylated proteins could promote the survival of *Brucella* in HeLa cells. HeLa cells were treated with SAHA, respectively. Then, the level of acetylation at lysine-14 site on histone 3 (H3K14) in HeLa cells was detected by Western blotting. As shown in Appendix E, the acetylation of H3K14 was markedly increased after treatment with 5, 7, and 9 μmol L^{-1} SAHA compared with the control group. A total of 5 μmol L^{-1} SAHA was finally selected as the optimal concentration for action (Guo *et al.* 2017). HeLa cells were treated with SAHA for 1.5 h and then were infected with *Brucella* 2308 WT strains. The results showed that the intracellular survival of *Brucella* in SAHA-treated cells was significantly higher than that in untreated cells (P<0.01) (Fig. 1-A). These results indicate that acetylation enhances the intracellular survival of *Brucella*.

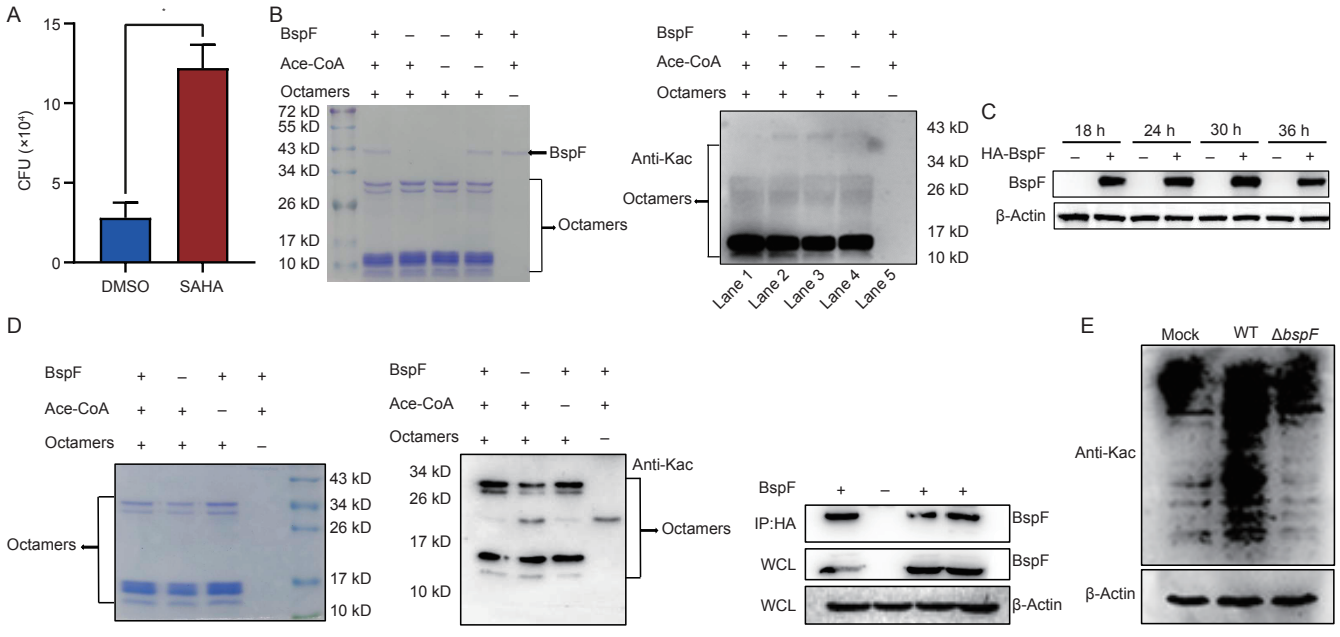


Fig. 1 BspF exhibits acetyltransferase activity. A, acetylation promoted *Brucella* intracellular survival. HeLa cells were infected with *Brucella* 2308 wild type (WT) at a multiplicity of infection (MOI) of 200 for 24 h, with or without SAHA treatment for 1.5 h. Cell lysates were plated on TSA plates to calculate colony-forming units (CFUs) after 72 h at 37°C to assess *Brucella* replication. Data represent mean±SEM. *, P<0.05; **, P<0.01; ***, P<0.001. B, the SDS-PAGE Coomassie staining and Western blot analysis of *in vitro* acetyltransferase activity assay reaction system. The histone octamers were analyzed by anti-acetyl-lysine antibody. C, the abundance of protein expression after transfected BspF. Western blot analysis of BspF protein expression in HEK-293T cells post-transfected by HA-BspF plasmids. The abundance of BspF expression were analyzed by anti-HA antibody. D, the SDS-PAGE Coomassie staining and Western blot analysis of *in vivo* acetyltransferase activity were assayed reaction system. Cells were transfected with HA-BspF plasmids for 30 h. The anti-HA antibody was used for immunoprecipitation. The histone octamers were analyzed by anti-acetyl-lysine antibody. E, BspF can increase the acetylated level of cell proteins. Immunoblotting of acetylation levels are from HeLa cells infected with *Brucella* 2308 WT and 2308Δ*bspF* mutant strains separately. The acetylated level of proteins was analyzed by anti-acetyl-lysine antibody.

3.2. Identification of acetyltransferase activity of BspF *in vitro*

The *Brucella* T4SS effector BspF contains a GNAT family acetyltransferase domain (aa224-350), so we speculated that it may have acetyltransferase activity (Myeni *et al.* 2013; Ke *et al.* 2015; Zhu *et al.* 2020). Based on the regulatory effect of acetylation on the intracellular survival of *Brucella*, we hypothesized that the BspF would catalyze the acetylation of host proteins, thereby playing an important role in the survival of *Brucella*. The SDS-PAGE showed homogeneity of components (octamers or BspF content was consistent in all groups). And Fig. 1-B showed that the acetylation modification level of histone octamer increased in the presence of acetyl-CoA, and the acetylation modification level increased slightly after the addition of BspF.

The results of enzyme activity tests *in vitro* showed that BspF can slightly increase the acetylation modification level of histones. However, the intracellular environment is complex, and it is unclear whether the BspF affects the modification of histones through interaction with other proteins in the cells. In order to understand the effect of BspF on histone modification *in vivo*, we expressed BspF in cells by cell transfection. As shown in Fig. 1-C, the expression of BspF reached its peak at 30 h. Therefore, 30 h after transfection, BspF and its interacting proteins were attached to Beads and incubated with histone octamer to observe the effects of BspF and its immunoprecipitation on histone modification. As shown in Fig. 1-D, the results showed that BspF and its immunoprecipitants could increase the acetylation level of histone octamer. In addition, to detect the effect of the BspF on host cell acetylation modification at the cell level, HeLa cells were infected with *Brucella* 2308 WT strain and 2308Δ*bspF* mutant strain. The overall change of acetylation level of total protein was detected by extracting cell proteins. Results as shown in Fig. 1-E, the protein acetylation modification level of *Brucella* 2308 WT infected cells was significantly higher than that of 2308Δ*bspF* mutant cells infected or uninfected cells. This indicates that BspF significantly elevates the acetylation level of specific host cellular proteins during infection, reinforcing its role as a functional acetyltransferase within the host cell environment.

3.3. BspF acetylated host proteins

To identify the specific substrates of BspF acted on the host cells, this study performed LFQ LC-MS/MS proteomics to compare the differences in acetylation levels between BspF-transfected cells and empty vector-transfected cells (Fig. 2-A). HEK-293T cells were transfected with pCMV-HA-BspF or pCMV-HA empty plasmids for 30 h. Cells were collected and then performed LFQ LC-MS/MS proteomics. A total of 1,075 acetylation sites were mapped to 590 proteins, with 1 to 13 putative acetylation sites in each (Fig. 2-B, Appendix A). Approximately 63.56% of these 590 proteins exhibited only a single acetylated site, and the percentages of proteins with 2, 3, and more acetylated sites were 18.31%, 8.14%, and 9.99%, respectively. A total of 941 of these acetylated sites, pertaining to 540 proteins, were quantified (Appendix E), with 34 sites on 28 proteins showing up-regulation and 23 sites on 23 proteins showing down-regulation (quantification ratio of >2 as the up-regulated and <0.5 as down-regulated threshold) (Fig. 2-C, Appendix A).

To explore the characteristics of the acetylated proteins, we used the wolfpsort software to predict subcellular localization. The results showed that proteins containing differentiated acetylation sites were mainly localized in the cytoplasm, nucleus, and mitochondria (Appendix E). Motif analysis with Motif-x showed that acetylation preferentially occurred on the motifs DKac, KacXXXKac, and GKac, where Kac is lysine acetylation, and X is any amino acid (Appendices E and B). Moreover, we mapped the protein interaction networks of all differentially acetylated proteins (Appendix E).

3.4. BspF inhibited the autophagy in host cells

Non-histone proteins are frequently acetylated and are involved in key cellular processes, including gene transcription, autophagy and metabolism (Narita *et al.* 2019a). Therefore, we hypothesized that BspF could regulate the acetylation of proteins in cells by other means. The acetylated proteomic data showed that three important non-histone proteins, SNAP29, MLLT3, and BAG6, were reliably acetylated, as shown in the proteomics data (Appendix D). BspF catalyzed single acetylation of MLLT3 (K296), SNAP29 (K103), and BAG6 (K1049), implying that it may affect signaling pathways related to these proteins (Appendix G). Notably, SNAP29 is an important regulator of autophagy, which is crucial for the fusion of lysosome and autophagosome (Steegmaier *et al.* 1998; Itakura *et al.* 2012). Thus, we tested whether BspF could regulate autophagy. The autophagy level of cells was detected after infection by *Brucella* 2308 WT strain or 2308Δ*bspF* mutant strain. We evaluated the effect of BspF on autophagy by monitoring changes in LC3-I, LC3-II, Beclin1, and p62. Studies indicate that an increase in LC3-II signifies the formation of autophagosomes, while LC3-I reflects the basal level of autophagic activity; Beclin1, as a key factor in autophagy initiation, is closely associated with autophagic activation; and p62, as an adapter for selective degradation of autophagic substrates, accumulates to indicate impaired autophagic function (Mizushima and Murphy 2020). Thus, variations in these markers can effectively reflect the dynamic state of autophagy. Compared with the *Brucella* 2308 WT strain, LC3II and Beclin1 protein levels were higher in cells infected by the Δ*bspF* mutant ($P>0.05$) (Fig. 3-A). In addition, the level of p62 protein, a characteristic autophagy substrate, was lower in HeLa cells infected by Δ*bspF* mutant than infected by WT strain.

Further study showed that LC3II and Beclin1 protein expression levels were significantly decreased in cells transfected with HA-BspF plasmid, while p62 protein expression level was significantly increased ($P<0.01$) compared with control (Fig. 3-B). These results indicate that *Brucella* can induce autophagy after infection of cells, and the effector protein BspF secreted by *Brucella* has a specific role in blocking autophagy.

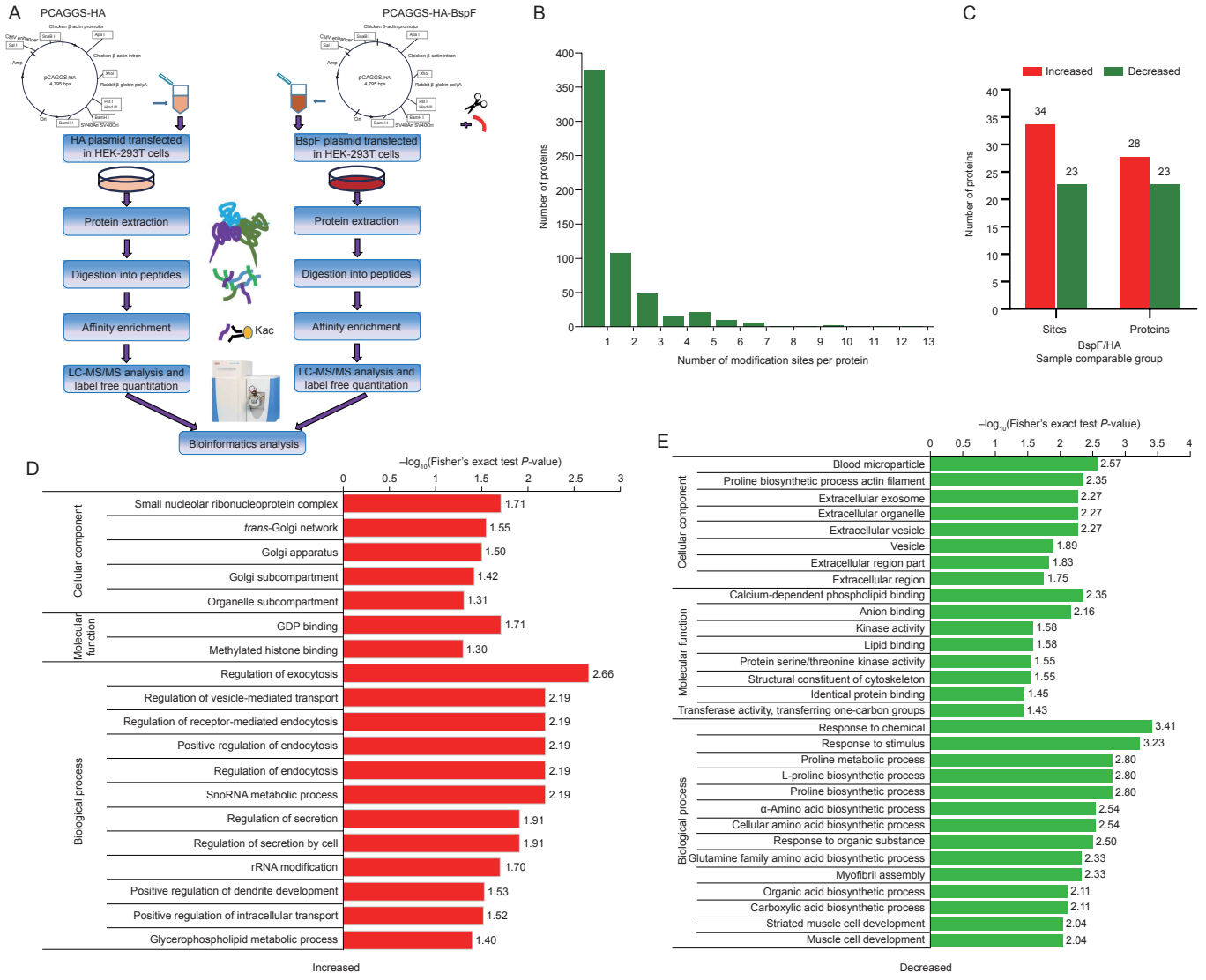


Fig. 2 Overview and characteristics of acetylated proteins in BspF-transfected HEK-293T cells. A, experimental strategy of acetylation modified proteomics in cells. B, distribution of acetylated proteins based on their number of acetylation sites. Column headers show the number of proteins with different acetylation sites. C, differentially acetylated proteins and sites (BspF/HA). D and E, functional characterization of acetylated proteins containing up-regulated (D) and down-regulated (E) Kac sites. The results show biological processes, cellular components, molecular functions.

3.5. BspF regulates autophagy by GNAT domain

To further test whether the function of BspF in regulating cell autophagy is related to its protein acetylation modification, we investigated the role of the GNAT domain of BspF. We transfected BspF, BspFΔGNAT mutant or empty plasmids into HeLa cells for 24 h, and cells were treated with BAF (20 nmol L^{-1}) for 4 h as a control with low basal autophagy. The test results showed that compared with the control group, LC3II and Beclin1 protein expression levels were significantly decreased in cells transfected with HA-BspF plasmid (P<0.05) (Fig. 3-C), while p62 protein expression levels were increased. In cells transfected with HA-BspFΔGNAT mutant, LC3II and Beclin1 protein expression levels were significantly decreased (P<0.05). The expression levels of LC3II, Beclin1 and p62 proteins were basically the same as those in the control group, indicating that BspF regulated autophagy in a manner dependent on GNAT acetyltransferase activity.

3.6. BspF regulates autophagy by acetylating SNAP29

To further explore whether acetylation is involved in the effect of SNAP29 on autophagy, we analyzed the effect of acetylation on SNAP29 function. We mutated SNAP29 lysine (K)-103 to arginine (R)-103 (SNAP29KR) and then transfected mutant plasmids into cells to mimic the deacetylated state of the protein, and mutated SNAP29 Lysine-103 to a glutamine (Q)-103 (SNAP29KQ) for a protein hyperacetylation mimic (Wang Z *et al.* 2019). And only in the presence of BspF, SNAP29WT undergoes acetylation. HeLa cells were co-transfected BspF either SNAP29WT or SNAP29KQ, showing lower LC3 II levels than SNAP29KR transfected cells (P<0.05) (Fig. 3-D). Beclin1 levels showed a similar result. Moreover, we observed a decrease in the cellular p62 levels of exogenously expressed BspF and SNAP29KR cells than other groups (Fig. 3-D). These data support that the GNAT acetyltransferase domain of BspF

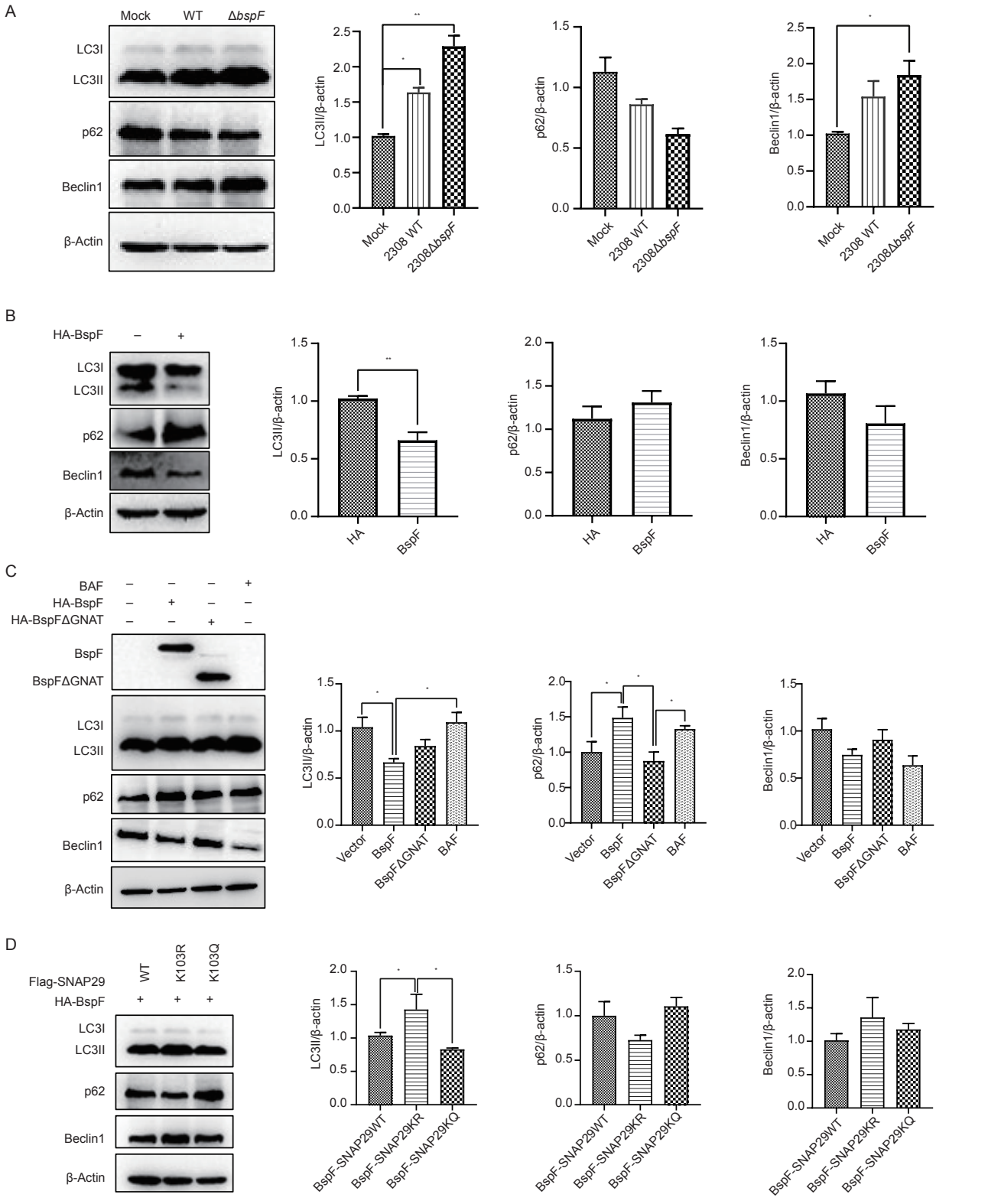


Fig. 3 The GNAT acetyltransferase domain of BspF plays an important role in targeting and acetylating SNAP29 to regulate autophagy. A, immunoblotting of LC3, p62, and Beclin1 proteins in HeLa cells infected with *Brucella* 2308 wild-type (WT) or 2308Δ*bspF* mutant strains for 24 h, and the non-infected cells as control. Data represent mean±SEM; ** and *, significant differences at $P<0.01$ and $P<0.05$, respectively. B, Western blot analysis of LC3, p62, and Beclin1 proteins in HeLa cells were transfected with HA-BspF or empty plasmids for 24 h. Data represent mean±SEM; **, significant difference at $P<0.01$. C, HeLa cells were transfected with BspF (WT or mutant) for 24 h. Empty vector transfection served as a control, and cells were treated with BAF (20 nmol L^{-1}) for 4 h as a control with low basal autophagy. Western blot analysis of LC3, p62, and Beclin1 protein expression. Data represent mean±SEM; *, significant difference at $P<0.05$. D, HA-BspF and Flag-SNAP29 (WT or mutants) plasmids co-transfected in HEK-293T cells for 24 h. The expression level of autophagy proteins was analyzed of lysates by Western blotting. Data represent mean±SEM; *, significant difference at $P<0.05$.

plays an important role in the targeting and acetylating of SNAP29 to regulate autophagy.

3.7. BspF inhibited interaction between SNAP29 and STX17

In mammalian cells, the SNARE complex is composed of the autophagosome STX17 (Qa SNARE), SNAP29 (Qbc SNARE), and endosome/lysosome R-SNARE VAMP8 mediates the fusion of autophagosomes and endosomes/lysosomes (Itakura *et al.* 2012; Guo *et al.* 2014). A previous study reported that STX17 can be acetylated by host cell acetyltransferase (Shen *et al.* 2021); therefore, we tested whether SNAP29 itself can be acetylated by intracellular acetyltransferase. By transfecting with Flag-SNAP29 plasmids and Myc-STX17 plasmids into Hela cells, our results showed that SNAP29 itself was not acetylated by intracellular acetyltransferase in HeLa cells compared to STX17 (Fig. 4-A and B); it was only specifically acetylated by exogenously expressed BspF. Therefore, we explored the specific mechanism by which *Brucella* secretes BspF in host cells to regulate autophagy. We first examined the formation of the SNAP29-STX17-VAMP8 SNARE complex. The results showed that with an increase in BspF expression, acetylation of SNAP29 increased, but the levels of co-precipitated STX17 markedly decreased (Fig. 4-C). Then, SNAP29 and STX17 were overexpressed, which were then infected with *Brucella* 2308 WT and 2308Δ*bspF* strains. The effect of BspF on the interaction between SNAP29 and STX17 was detected by immunoprecipitation. As shown in Fig. 4-D and E, lower levels of STX17 were co-precipitated with Flag-SNAP29 or endogenous SNAP29 from *Brucella* 2308 WT strain-infected cells than with 2308Δ*bspF* strain-infected or control cells. Similarly, we analyzed the potential effects of BspF on the interaction of SNAP29 with VAMP8. Interestingly, the results showed that BspF did not affect the interaction between VAMP8 and SNAP29 in HEK-293T cells (Fig. 4-F). Therefore, in the presence of BspF, the interaction between SNAP29 and STX17 was reduced, but its interaction with VAMP8 was not affected.

3.8. K103 acetylation by BspF reduced the interaction between SNAP29 and STX17

The above results demonstrated that BspF exerted an acetyltransferase function through the GNAT domain and catalyzed the acetylation of SNAP29 on the K103 site. BspF inhibited the interaction between SNAP29 and STX17 by catalyzing the acetylation of SNAP29, which possibly affected the assembly of the SNARE complex.

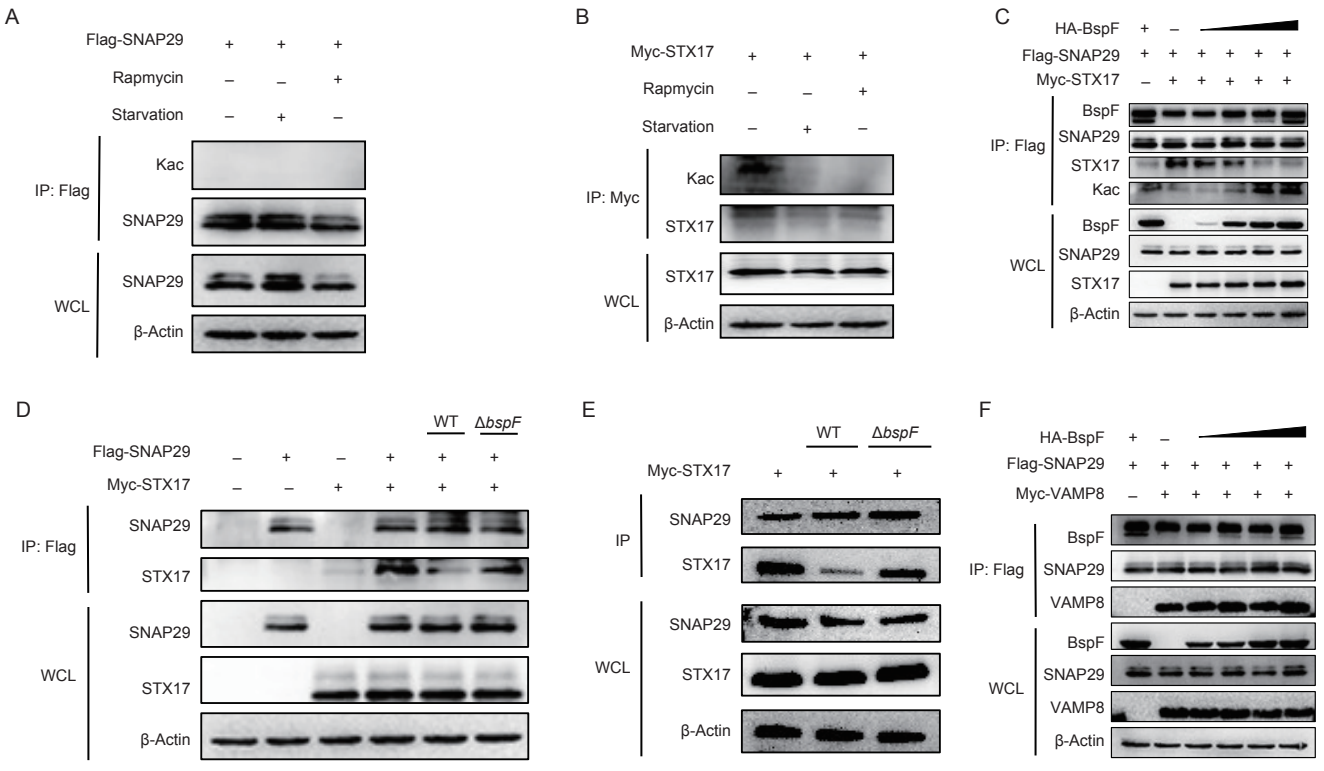


Fig. 4 BspF blocks the interaction of SNAP29 with STX17. A, HeLa cells were transfected with Flag-SNAP29 plasmids and then treated with rapamycin (100 μmol L^{-1}) or serum starvation for 24 h. Flag-SNAP29 was immunoprecipitated with anti-HA antibody, anti-acetyl-lysine and anti-SNAP29 antibodies. B, HeLa cells were transfected with Myc-STX17 plasmids and then processed as in A. C, co-transfection of HA-BspF and Myc-STX17 with Flag-SNAP29 plasmids into HEK-293T cells. Flag-SNAP29 immunoprecipitated with the corresponding antibodies. D, Flag-SNAP29 and Myc-STX17 plasmids were co-transfected into HeLa cells, and then infected with WT or 2308Δ*bspF* mutant strains for 24 h. The cell lysates were immunoprecipitated by the corresponding antibodies. E, Myc-STX17 plasmids were transfected into HeLa cells for 24 h and then infected with WT or Δ*bspF* mutant strains. The cell lysates were analyzed by anti-SNAP29 and anti-STX17 antibodies. F, co-immunoprecipitation of HA-BspF and Myc-VAMP8 with Flag-SNAP29 from cells. The anti-Flag antibody was used for immunoprecipitation, and precipitates were analyzed by anti-HA, anti-VAMP8 and anti-Flag antibodies.

We then tested whether BspF affects the assembly of SNARE by acetylating the K103 site of SNAP29. The effect of SNAP29 acetylation at the K103 site of the interaction between SNAP29 and STX17 was tested. First, we excluded the effect of mutation on the interaction of SNAP29 and BspF or SNAP29 and STX17. The results showed that K103 mutation had no effect on the interaction of SNAP29 with BspF or STX17 (Fig. 5-A and B). BspF, STX17, and wild type plasmids or mutants (KR and KQ) of SNAP29 were co-expressed in HEK-293T cells and used for co-immunoprecipitation assays. SNAP29 specifically precipitated BspF and STX17. In addition, the amount of STX17 precipitated by acetylated SNAP29 (WT and KQ) was significantly lower than that of the acetylation-defective mutant SNAP29 (KR) (Fig. 5-C). Moreover, we co-transfected STX17 plasmids with the expression plasmids of SNAP29 (WT or its mutant) into HeLa cells and then infected with *Brucella* 2308WT or 2308Δ*bspF* mutant strains. In *Brucella* 2308WT infected cells, the interaction of STX17 with acetylated SNAP29 (WT and KQ) was markedly weaker than the acetylation-defective mutant SNAP29KR (Fig. 5-D). Interestingly, the interaction of STX17 with SNAP29WT, SNAP29KR and SNAP29KQ had no difference in *Brucella* 2308Δ*bspF* mutant infected cells (Fig. 5-D). Based on the above results, we demonstrated that the acetylation of SNAP29 by BspF reduced its interaction with STX17.

3.9. BspF promoted *Brucella* survival *in vivo* by acetylating SNAP29

BspF reduced the interaction between SNAP29 and STX17 by catalyzing the acetylation of K103 site of SNAP29. We co-transfected BspF and STX17 with SNAP29 wild-type (WT) plasmid, SNAP29KR or SNAP29KQ mutant in HEK-293T cells, and tested the effect of acetylation at Snap29K103 on the interaction between SNAP29 and STX17. The results showed that the K103 mutation had no effect on the interaction of SNAP29 with BspF or SNAP29 with STX17. In HEK-293T cells co-expressing BspF, STX17 and SNAP29 plasmid, it was found by co-immunoprecipitation experiment that the amount of STX17 deposited by acetylated SNAP29 (WT and KQ) was significantly lower than that of acetylated defective mutant SNAP29 (KR) (Fig. 5-C). In addition, the STX17 plasmid was co-transfected into HeLa cells with an expression plasmid of SNAP29 (WT or its mutant) and then infected with *Brucella* 2308WT or 2308Δ*bspF* mutant strains. In *Brucella* 2308WT infected cells, STX17 bound to acetylated SNAP29 (WT and KQ) was significantly weaker

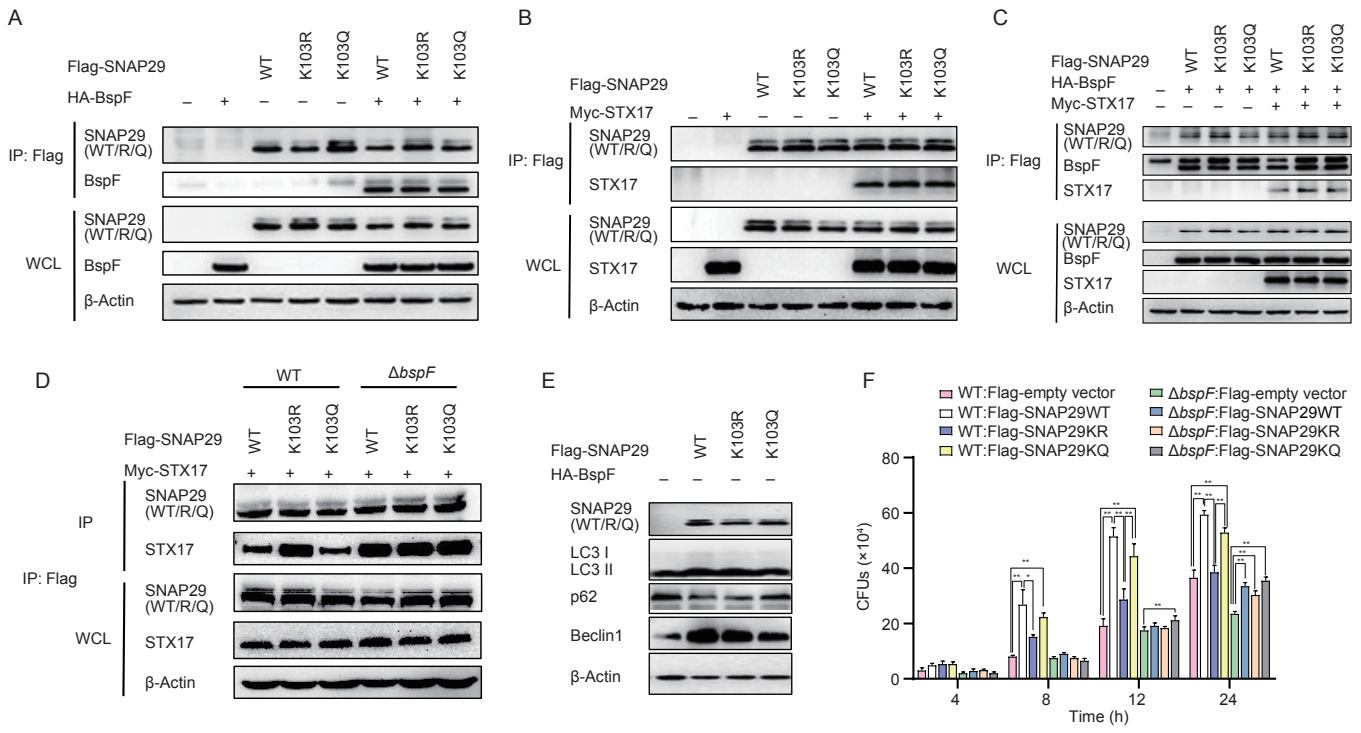


Fig. 5 BspF catalyzes the acetylation of SNAP29 to inhibit its interaction with STX17 and promote intracellular survival of *Brucella*. A, HA-BspF and Flag-SNAP29 (wild-type (WT) or mutants) plasmids co-transfected in HEK-293T cells for 30 h. The immunoprecipitation and the precipitates were analyzed by anti-Flag and anti-HA antibody. B, co-immunoprecipitation of Flag-SNAP29 (WT or mutants) and Myc-STX17 from HEK-293T cells. The anti-Flag antibody was used for immunoprecipitation and precipitates, and precipitates were analyzed by anti-STX17 antibodies. C, HEK-293T cells were transfected with HA-BspF, Flag-SNAP29 (WT or mutants) and Myc-STX17 plasmids for 30 h and then processed as in B. D, Myc-STX17 and Flag-SNAP29 (WT or mutant) plasmids were co-transfected into HeLa cells for 24 h, and then infected with WT or Δ*bspF* mutant strains. The cell lysates were immunoprecipitated by anti-Flag antibody and then analyzed by anti-Flag and anti-STX17 antibodies. E, HeLa cells were transfected with SNAP29 (WT or mutants) for 24 h and then analyzed by anti-LC3, anti-p62, and anti-Beclin1 antibodies. F, HeLa cells were transfected with SNAP29 (WT or mutants) and then infected with and Δ*bspF* mutant strains for 4, 8, 12, and 24 h. Cell lysates were plated on TSA plates to calculate colony-forming units (CFUs) after 72 h at 37°C to assess *Brucella* replication. Data represent mean±SEM; ** and *, significant differences at $P<0.01$ and $P<0.05$, respectively.

than the acetylation-deficient mutant SNAP29KR (Fig. 5-D and E). BspF-catalyzed acetylation of SNAP29 at lysine 103 promotes the intracellular survival of *Brucella* abortus 2308 in HeLa cells (Fig. 5-F). Based on the above results, we demonstrated that BspF promoted *Brucella*'s survival by reducing the interaction between SNAP29 and STX17.

4. Discussion

Acetylation is generally considered as a conservative epigenetic modification that occurs predominantly or completely within the chromatin (Choudhary *et al.* 2014). Non-histone protein acetylation is involved in key cellular processes, such as gene transcription, signal transduction, autophagy, and metabolism (Narita *et al.* 2019b). Recently, a study reported that SAHA significantly impaired macrophage immunity against Mycobacterium tuberculosis infection, therefore increasing susceptibility to tuberculosis (Zhu *et al.* 2019). Moreover, Listeria monocytogenes PGN O-acetyltransferase OatA has been shown to contribute to its ability to escape immunity and promote intracellular survival in macrophages (Aubry *et al.* 2011). Therefore, we explored whether changes in the level of acetylation of host cell proteins affect the survival and replication of *Brucella* spp. Our results showed that SAHA-induced increase in the abundance of acetylated proteins led to an upregulation in the intracellular survival viability of *Brucella* (Fig. 1-A). This result indicated that an increase the abundance of acetylated proteins in host cell is beneficial to the infection and intracellular survival of *Brucella*.

The *Brucella* T4SS is an important virulence factor that plays a key role in evading host innate immunity and achieving intracellular survival and replication (Ke *et al.* 2015). However, the mechanism by which these effectors evade host cells killing remains poorly understood. The *Brucella* T4SS effector BspF has a GNAT acetyltransferase domain and crotonyltransferase activity (Zhu *et al.* 2020). The GNAT superfamily of proteins is widespread in prokaryotes and is involved in regulating stress response, transcription, metabolism processes (Thao *et al.* 2010; Wang *et al.* 2010; Ren *et al.* 2017). ArgA is a member of the GNAT superfamily of acetyltransferases in *Mycobacterium tuberculosis*, and is essential for the growth of this species (Xie *et al.* 2014). Therefore, we speculated that the effector BspF of *Brucella* would exhibit similar acetyltransferase activity. In this study, we confirmed for the first time that BspF can impact protein acetylation modification in host cells by interacting with other intracellular proteases (Fig. 1-B and D). In addition, we found that BspF can cause a significant increase in protein acetylation levels in host cells during *Brucella* infection (Fig. 1-E). Many studies have reported that bacteria affect the acetylation of their own proteins by secreting GNAT acetyltransferase proteins to promote their survival (Barak *et al.* 2004; Yan *et al.* 2008; Ghosh *et al.* 2016; Ren *et al.* 2016). Unlike previous studies, we explored bacteria with proteins containing the GNAT domain to regulate host acetylation to evade host immunity.

To further explore the function of the acetylated proteins in host cells after BspF transfection, we performed GO functional enrichment analysis. The results showed that proteins with up-regulated acetylation were involved in various biological processes, including the regulation of vesicle-mediated transport and intracellular transport, etc. A previous study reported that BspF can inhibit the protein secretion of host cells and promote *Brucella* intracellular growth and persistence (Myeni *et al.* 2013). Recently, Broghesan and colleagues showed that the BspF promotes *Brucella* replication within replication-permissive vacuoles by interfering with vesicular transport between the TGN and recycling endocytic compartment (Borghesan *et al.* 2021). Based on this finding and our results, we speculate that BspF accomplishes this by regulating cell secretion and intracellular transport pathways by modifying the acetylation of host cell proteins. However, the specific mechanism of action requires further study.

Autophagy is an important host defense mechanism in the elimination of intracellular microorganisms (Gutierrez *et al.* 2004; Ogawa *et al.* 2005; Guo *et al.* 2012). Bacterial pathogens have evolved strategies to evade or paralyze the host autophagy system, and studies have reported that *Brucella* can regulate host cell autophagy to promote its intracellular survival (Guo *et al.* 2012; Huang and Brumell 2014; Deng *et al.* 2022). SNAP29 is an important autophagy protein, and is related to phagosomes/autophagosome maturation (Itakura *et al.* 2012; Wesolowski *et al.* 2012), autophagosome membrane docking (Diao *et al.* 2015), exocytosis (Gaudet *et al.* 2011), and vesicle fusion (Steegmaier *et al.* 1998; Gaudet *et al.* 2011). Conserved SNAP29 has two Q-SNARE domains that mediate membrane fusion through domain binding to other Q- and R-SNARE-containing proteins (Morelli *et al.* 2021). In our proteomics study, the SNAP29 protein K103 site underwent acetylation only in the presence of BspF. Therefore, we speculated that BspF participates in regulating the autophagy process by targeting SNAP29. In our results, *Brucella* WT-infected cells resulted in elevated levels of autophagy compared to non-infected cells (Fig. 3-A), which is consistent with other studies (Pizarro-Cerdá *et al.* 1998; Guo *et al.* 2012; Starr *et al.* 2012; Pesce Viglietti *et al.* 2018). Interestingly, compared with *Brucella* WT-infected cells, 2308Δ*bspF* mutant strain-infected cells triggered higher levels of autophagy (Fig. 3-A). Moreover, BspF overexpressed in HeLa cells decreased the levels of autophagy markers such as LC3II and Beclin1 through catalyzing acetylation of SNAP29. These data suggest that BspF targeting SNAP29 and catalyzing its acetylation to inhibit autophagy. Similarly, we found that Deng *et al.* (2022) compared the autophagy of *Brucella* 2308 WT and Δ*vceA* on HPT-8 cells and confirmed that VceA could inhibit autophagy. In addition, Zhang *et al.* (2019) reported that *Brucella* abortus A19Δ*virB* mutant strains showed enhanced autophagy and decreased the survival capability in cells compared to *Brucella* abortus A19 WT strains, suggesting that VirB-encoded T4SS effectors inhibits autophagy and promote intracellular survival. Based on the above results, *Brucella* could activate autophagy after infection to reach autophagosome compartment. *Brucella* translocated BspF into host cells in a T4SS-dependent manner. Subsequently, BspF blocked the autophagy to prevent bacterial clearance by lysosomes. Consistent with

other studies, we found that increasing levels of autophagy could promote *Brucella* intracellular survival. As shown in Fig. 5-E and F, overexpression of SNAP29 or mutants increased the level of autophagy, meanwhile the intracellular survival of *Brucella* markedly increased relative to the empty vector transfected cells. However, after the *Brucella* 2308 WT infection, BspF can catalyze SNAP29WT and SNAP29KQ acetylation, while SNAP29KR does not undergo acetylation, so the level of autophagy in cells transfected with SNAP29KR is higher than SNAP29WT and SNAP29KQ transfected cells. But we found that the intracellular survival of *Brucella* 2308 WT infected cells overexpressing SNAP29KR was lower than SNAP29WT or SNAP29KQ overexpressing cells. Therefore, we speculated that within a certain range, increasing levels of autophagy can promote *Brucella* intracellular survival, and BspF-mediated inhibition of autophagosome-lysosome fusion prevents excessive autophagy and facilitates the intracellular survival of *Brucella*.

Next, we want to explore the specific mechanism by which BspF regulates autophagy. Without the T4SS, *Brucella* cannot reach the endoplasmic reticulum of the host cell and is subsequently degraded by fusion with lysosomes during endocytosis (von Bargen *et al.* 2012). The fusions of endosomes/lysosomes with autophagosomes are mediated by the SNARE complex, which consists of STX17 (Qa SNARE), SNAP29 (Qbc SNARE), and endosomal/lysosomal R-SNARE VAMP8 (Itakura *et al.* 2012; Ding *et al.* 2014). Some viruses and bacteria have been reported to promote their survival by interacting with SNAP29 to inhibit autophagosome-lysosomal fusion (Ding *et al.* 2014; Lai *et al.* 2017; Corona *et al.* 2018; Mohamud *et al.* 2018; Valleau *et al.* 2018; Wang Z *et al.* 2019). Our research demonstrated that in the presence of BspF the binding of SNAP29 to STX17 was significantly reduced. In terms of bacterial infection, *Brucella* 2308 WT-infected cells significantly reduced the binding of SNAP29 to STX17. In contrast, *Brucella* 2308Δ*bspF* mutant strain infection seemed to promote the interaction between SNAP29 and STX17. Based on the domain analysis of SNAP29 protein sequences, the lysine-103 site is located on the SNARE domain of SNAP29. Our results also demonstrate that SNAP29 acetylation affects the interaction between SNAP29 and STX17, thereby affecting SNARE complex formation (Fig. 5-C and D). In addition, the competitive binding of BspF to SNAP29 may also resulted in decreased SNAP29 binding to STX17. Our intracellular survival assay results further showed that SNAP29WT or SNAP29KQ promoted *Brucella* intracellular survival in HeLa cells, mainly depending on BspF. Based on our research, we speculated that acetylation at the K103 may create steric hindrance and competitive binding of BspF to SNAP29, resulting in a reduced amount of STX17 interacting with SNAP29. These two approaches work together to affect SNARE assembly and function, thus inhibiting phagosome-lysosome fusion to promote intracellular survival, however, further experimental studies are needed to verify this conjecture.

5. Conclusion

In conclusion, our study is the first to show that the *Brucella* effector BspF has an effect on protein acetylation modification that can target SNAP29 and catalyze its acetylation to inhibit binding with STX17, thereby promoting *Brucella* intracellular survival and replication. The GNATs superfamily of proteins is widespread in prokaryotes, such as *Mycobacterium tuberculosis* and *Salmonella enterica* species. Therefore, other pathogens may use a similar mechanism to evade host immunity and promote intracellular survival. In the future, we will further study whether this acetylation mechanism is similarly employed by other pathogens. In addition, Borghesan *et al.* (2021) showed that the BspF promotes *Brucella* replication within replication-permissive vacuoles by interfering with vesicular transport between the TGN and recycling endocytic compartment. Due to SNAP29 playing an important role in Golgi trafficking and secretion (Itakura *et al.* 2012), we will continue to explore whether BspF regulates Golgi and vesicular trafficking by acetylating SNAP29. Taken together, our findings reveal that effector BspF plays an important role in regulating host cell processes and promoting intracellular survival of *Brucella*. Our study may be used to inform the development of a treatment strategy for *Brucella* infection.

Acknowledgements

This work was supported by the Educational Department of Liaoning Province, China (JYTQN2024017).

Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

Ethical statements

All experiments described in this article were conducted in compliance with relevant safety regulations and did not involve the use of animals.

Appendices associated with this paper are available at https://doi.org/10.1016/j.jia.2025.02.011

References

Archambaud C, Salcedo S P, Lelouard H, Devilard E, de Bovis B, Van Rooijen N, Gorvel J P, Malissen B. 2010. Contrasting roles of macrophages and dendritic cells in controlling initial pulmonary *Brucella* infection. *European Journal of Immunology*, **40**, 3458–3471.

Aubry C, Goulard C, Nahori M A, Cayet N, Decalf J, Sachse M, Boneca I G, Cossart P, Dussurget O. 2011. OatA, a peptidoglycan O-acetyltransferase involved in *Listeria monocytogenes* immune escape, is critical for virulence. *The Journal of Infectious Diseases*, **204**, 731–740.

Barak R, Prasad K, Shainskaya A, Wolfe A J, Eisenbach M. 2004. Acetylation of the chemotaxis response regulator CheY by acetyl-CoA synthetase purified from *Escherichia coli*. *Journal of Molecular Biology*, **342**, 383–401.

von Bargen K, Gorvel J P, Salcedo S P. 2012. Internal affairs: Investigating the *Brucella* intracellular lifestyle. *FEMS Microbiology Reviews*, **36**, 533–562.

Borghesan E, Smith E P, Myeni S, Binder K, Knodler L A, Celli J. 2021. A *Brucella* effector modulates the Arf6-Rab8a GTPase cascade to promote intravacuolar replication. *The EMBO Journal*, **40**, e107664.

Cain J A, Solis N, Cordwell S J. 2014. Beyond gene expression: The impact of protein post-translational modifications in bacteria. *Journal of Proteomics*, **97**, 265–286.

Celli J, de Chastellier C, Franchini D M, Pizarro-Cerda J, Moreno E, Gorvel J P. 2003. *Brucella* evades macrophage killing *via* VirB-dependent sustained interactions with the endoplasmic reticulum. *The Journal of Experimental Medicine*, **198**, 545–556.

Choudhary C, Weinert B T, Nishida Y, Verdin E, Mann M. 2014. The growing landscape of lysine acetylation links metabolism and cell signalling. *Nature Reviews Molecular Cell Biology*, **15**, 536–550.

Corona A K, Saulsbery H M, Corona Velazquez A F, Jackson W T. 2018. Enteroviruses remodel autophagic trafficking through regulation of host SNARE proteins to promote virus replication and cell exit. *Cell Reports*, **22**, 3304–3314.

Deng X, He J, Wang Y, Yang Q, Yi J, Zhang H, Wang Y, Miao Y, Wang Z, Chen C. 2022. Deletion of the type IV secretion system promoter VirB in *Brucella* abortus A19 strain attenuated the virulence of the bacteria and promotes autophagy. *Canadian Journal of Microbiology*, **68**, 165–176.

Diao J, Liu R, Rong Y, Zhao M, Zhang J, Lai Y, Zhou Q, Wilz L M, Li J, Vivona S, Pfuetzner R A, Brunger A T, Zhong Q. 2015. ATG14 promotes membrane tethering and fusion of autophagosomes to endolysosomes. *Nature*, **520**, 563–566.

Ding B, Zhang G, Yang X, Zhang S, Chen L, Yan Q, Xu M, Banerjee A K, Chen M. 2014. Phosphoprotein of human parainfluenza virus type 3 blocks autophagosome-lysosome fusion to increase virus production. *Cell Host & Microbe*, **15**, 564–577.

Gaudet P, Livstone M S, Lewis S E, Thomas P D. 2011. Phylogenetic-based propagation of functional annotations within the Gene Ontology consortium. *Briefings in Bioinformatics*, **12**, 449–462.

Ghosh S, Padmanabhan B, Anand C, Nagaraja V. 2016. Lysine acetylation of the *Mycobacterium tuberculosis* HU protein modulates its DNA binding and genome organization. *Molecular Microbiology*, **100**, 577–588.

Grabiec A M, Potempa J. 2018. Epigenetic regulation in bacterial infections: Targeting histone deacetylases. *Critical Reviews in Microbiology*, **44**, 336–350.

Guo B, Liang Q, Li L, Hu Z, Wu F, Zhang P, Ma Y, Zhao B, Kovács A L, Zhang Z, Feng D, Chen S, Zhang H. 2014. O-GlcNAc-modification of SNAP-29 regulates autophagosome maturation. *Nature Cell Biology*, **16**, 1215–1226.

Guo F, Zhang H, Chen C, Hu S, Wang Y, Qiao J, Ren Y, Zhang K, Wang Y, Du G. 2012. Autophagy favors *Brucella* melitensis survival in infected macrophages. *Cellular & Molecular Biology Letters*, **17**, 249–257.

Guo H, Zhang J, Wang Y, Bu C, Zhou Y, Fang Q. 2017. Comparative proteomic analysis of lysine acetylation in fish CIK cells infected with aquareovirus. *International Journal of Molecular Sciences*, **18**, 2419.

Gutierrez M G, Master S S, Singh S B, Taylor G A, Colombo M I, Deretic V. 2004. Autophagy is a defense mechanism inhibiting BCG and *Mycobacterium tuberculosis* survival in infected macrophages. *Cell*, **119**, 753–766.

Henriksen P, Wagner S A, Weinert B T, Sharma S, Bacinskaja G, Rehman M, Juffer A H, Walther T C, Lisby M, Choudhary C. 2012. Proteome-wide analysis of lysine acetylation suggests its broad regulatory scope in *Saccharomyces cerevisiae*. *Molecular & Cellular Proteomics*, **11**, 1510–1522.

Hong Y, Cao X, Han Q, Yuan C, Zhang M, Han Y, Zhu C, Lin T, Lu K, Li H, Fu Z, Lin J. 2016. Proteome-wide analysis of lysine acetylation in adult *Schistosoma japonicum* worm. *Journal of Proteomics*, **148**, 202–212.

Huang J, Brumell J H. 2014. Bacteria-autophagy interplay: A battle for survival. *Nature Reviews Microbiology*, **12**, 101–114.

Itakura E, Kishi-Itakura C, Mizushima N. 2012. The hairpin-type tail-anchored SNARE syntaxin 17 targets to autophagosomes for fusion with endosomes/lysosomes. *Cell*, **151**, 1256–1269.

Jiao H, Zhou Z, Li B, Xiao Y, Li M, Zeng H, Guo X, Gu G. 2021. The mechanism of facultative intracellular parasitism of *Brucella*. *International Journal of Molecular Sciences*, **22**, 3673.

Ke Y, Wang Y, Li W, Chen Z. 2015. Type IV secretion system of *Brucella* spp. and its effectors. *Frontiers in Cellular and Infection Microbiology*, **5**, 72.

Keller M D, Torres V J, Cadwell K. 2020. Autophagy and microbial pathogenesis. *Cell Death & Differentiation*, **27**, 872–886.

Lai J K F, Sam I C, Verlhac P, Baguet J, Eskelinen E L, Faure M, Chan Y F. 2017. 2BC non-structural protein of enterovirus A71 interacts with SNARE proteins to trigger autolysosome formation. *Viruses*, **9**, 169.

Matthias P, Yoshida M, Khochbin S. 2008. HDAC6 a new cellular stress surveillance factor. *Cell Cycle*, **7**, 7–10.

Menzies K J, Zhang H, Katsyuba E, Auwerx J. 2016. Protein acetylation in metabolism-metabolites and cofactors. *Nature Reviews Endocrinology*, **12**, 43–60.

Miller C, Celli J. 2016. Avoidance and subversion of eukaryotic homeostatic autophagy mechanisms by bacterial pathogens. *Journal of Molecular Biology*, **428**, 3387–3398.

Mizushima N, Murphy L O. 2020. Autophagy assays for biological discovery and therapeutic development. *Trends in Biochemical Sciences*, **45**, 1080–1093.

Mohamud Y, Shi J, Qu J, Poon T, Xue Y C, Deng H, Zhang J, Luo H. 2018. Enteroviral infection inhibits autophagic flux *via* disruption of the SNARE complex to enhance viral replication. *Cell Reports*, **22**, 3292–3303.

Morelli E, Speranza E A, Pellegrino E, Beznoussenko G V, Carminati F, Garré M, Mironov A A, Onorati M, Vaccari T. 2021. Activity of the SNARE protein SNAP29 at the endoplasmic reticulum and golgi apparatus. *Frontiers in Cell and Developmental Biology*, **9**, 637565.

Myeni S, Child R, Ng T W, Kupko J J, Wehrly T D, Porcella S F, Knodler L A, Celli J. 2013. *Brucella* modulates secretory trafficking *via* multiple type IV secretion effector proteins. *PLoS Pathogens*, **9**, e1003556.

Narita T, Weinert B T, Choudhary C. 2019a. Author Correction: Functions and mechanisms of non-histone protein acetylation. *Nature Reviews Molecular Cell Biology*, **20**, 508.

Narita T, Weinert B T, Choudhary C. 2019b. Functions and mechanisms of non-histone protein acetylation. *Nature Reviews Molecular Cell Biology*, **20**, 156–174.

Ogawa M, Yoshimori T, Suzuki T, Sagara H, Mizushima N, Sasakawa C. 2005. Escape of intracellular *Shigella* from autophagy. *Science*, **307**, 727–731.

Pandey R, Müller A, Napoli C A, Selinger D A, Pikaard C S, Richards E J, Bender J, Mount D W, Jorgensen R A. 2002. Analysis of histone acetyltransferase and histone deacetylase families of *Arabidopsis thaliana* suggests functional diversification of chromatin modification among multicellular eukaryotes. *Nucleic Acids Research*, **30**, 5036–5055.

Pesce Viglietti A I, Gentilini M V, Arriola Benitez P C, Giambartolomei G H, Delpino M V. 2018. Abortus modulates osteoblast function through the induction of autophagy. *Frontiers in Cellular and Infection Microbiology*, **8**, 425.

Pietrocola F, Galluzzi L, Bravo-San Pedro J M, Madeo F, Kroemer G. 2015. Acetyl coenzyme A: A central metabolite and second messenger. *Cell Metabolism*, **21**, 805–821.

Pizarro-Cerdá J, Méresse S, Parton R G, van der Goot G, Sola-Landa A, Lopez-Goñi I, Moreno E, Gorvel J P. 1998. *Brucella* abortus transits through the autophagic pathway and replicates in the endoplasmic reticulum of nonprofessional phagocytes. *Infection and Immunity*, **66**, 5711–5724.

Py B F, Lipinski M M, Yuan J. 2007. Autophagy limits *Listeria monocytogenes* intracellular growth in the early phase of primary infection. *Autophagy*, **3**, 117–125.

Ren J, Sang Y, Lu J, Yao Y F. 2017. Protein acetylation and its role in bacterial virulence. *Trends in Microbiology*, **25**, 768–779.

Ren J, Sang Y, Tan Y, Tao J, Ni J, Liu S, Fan X, Zhao W, Lu J, Wu W, Yao Y F. 2016. Acetylation of lysine 201 inhibits the DNA-binding ability of PhoP to regulate salmonella virulence. *PLoS Pathogens*, **12**, e1005458.

Salcedo S P, Marchesini M I, Lelouard H, Fugier E, Jolly G, Balor S, Muller A, Lapaque N, Demaria O, Alexopoulou L, Comerci D J, Ugalde R A, Pierre P, Gorvel J P. 2008. *Brucella* control of dendritic cell maturation is dependent on the TIR-containing protein Btp1. *PLoS Pathogens*, **4**, e21.

Shen Q, Shi Y, Liu J, Su H, Huang J, Zhang Y, Peng C, Zhou T, Sun Q, Wan W, Liu W. 2021. Acetylation of STX17 (syntaxin 17) controls autophagosome maturation. *Autophagy*, **17**, 1157–1169.

Siqueira M D S, Ribeiro R M, Travassos L H. 2018. Autophagy and its interaction with intracellular bacterial pathogens. *Frontiers in*

Immunology, **9**, 935.
Starr T, Child R, Wehrly T D, Hansen B, Hwang S, López-Otin C, Virgin H W, Celli J. 2012. Selective subversion of autophagy complexes facilitates completion of the *Brucella* intracellular cycle. *Cell Host & Microbe*, **11**, 33–45.
Steegmaier M, Yang B, Yoo J S, Huang B, Shen M, Yu S, Luo Y, Scheller R H. 1998. Three novel proteins of the syntaxin/SNAP-25 family. *Journal of Biological Chemistry*, **273**, 34171–34179.
Thao S, Chen C S, Zhu H, Escalante-Semerena J C. 2010. Nε-lysine acetylation of a bacterial transcription factor inhibits Its DNA-binding activity. *PLoS ONE*, **5**, e15123.
Valleau D, Little D J, Borek D, Skarina T, Quaile A T, Di Leo R, Houliston S, Lemak A, Arrowsmith C H, Coombes B K, Savchenko A. 2018. Functional diversification of the NleG effector family in enterohemorrhagic *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America*, **115**, 10004–10009.
Wang K, Ma H, Liu H, Ye W, Li Z, Cheng L, Zhang L, Lei Y, Shen L, Zhang F. 2019. The glycoprotein and nucleocapsid protein of hantaviruses manipulate autophagy flux to restrain host innate immune responses. *Cell Reports*, **27**, 2075-2091.e2075.
Wang Q, Zhang Y, Yang C, Xiong H, Lin Y, Yao J, Li H, Xie L, Zhao W, Yao Y, Ning Z B, Zeng R, Xiong Y, Guan K L, Zhao S, Zhao G P. 2010. Acetylation of metabolic enzymes coordinates carbon source utilization and metabolic flux. *Science*, **327**, 1004–1007.
Wang Z, Yang X, Liu C, Li X, Zhang B, Wang B, Zhang Y, Song C, Zhang T, Liu M, Liu B, Ren M, Jiang H, Zou J, Liu X, Zhang H, Zhu W G, Yin Y, Zhang Z, Gu W, Luo J. 2019. Acetylation of PHF5A modulates stress responses and colorectal carcinogenesis through alternative splicing-mediated upregulation of KDM3A. *Molecular Cell*, **74**, 1250-1263.e1256.
Wesolowski J, Caldwell V, Paumet F. 2012. A novel function for SNAP29 (synaptosomal-associated protein of 29 kDa) in mast cell phagocytosis. *PLoS ONE*, **7**, e49886.
Xie L, Zeng J, Luo H, Pan W, Xie J. 2014. The roles of bacterial GCN5-related N-acetyltransferases. *Critical Reviews in Eukaryotic Gene Expression*, **24**, 77–87.
Yan J, Barak R, Liarzi O, Shainskaya A, Eisenbach M. 2008. *In vivo* acetylation of CheY, a response regulator in chemotaxis of Escherichia coli. *Journal of Molecular Biology*, **376**, 1260–1271.
Yang X J, Seto E. 2008. The Rpd3/Hda1 family of lysine deacetylases: From bacteria and yeast to mice and men. *Nature Reviews Molecular Cell Biology*, **9**, 206–218.
Zhang J, Li M, Li Z, Shi J, Zhang Y, Deng X, Liu L, Wang Z, Qi Y, Zhang H. 2019. Deletion of the type IV secretion system effector vcea promotes autophagy and inhibits apoptosis in *Brucella*-infected human trophoblast cells. *Current Microbiology*, **76**, 510–519.
Zhi F, Zhou D, Bai F, Li J, Xiang C, Zhang G, Jin Y, Wang A. 2019. VceC mediated IRE1 pathway and inhibited CHOP-induced apoptosis to support *Brucella* replication in goat trophoblast cells. *International Journal of Molecular Sciences*, **20**, 4104.
Zhou S, Yang Q, Yin C, Liu L, Liang W. 2016. Systematic analysis of the lysine acetylome in *Fusarium graminearum*. *BMC Genomics*, **17**, 1019.
Zhu C, Cai Y, Zhu J, Zhang L, Xing A, Pan L, Jia H, Mo S, Feng C G, Shen H, Chen X, Zhang Z. 2019. Histone deacetylase inhibitors impair the host immune response against *Mycobacterium tuberculosis* infection. *Tuberculosis* (Edinb), **118**, 101861.
Zhu J, Dong Q, Dong C, Zhang X, Zhang H, Chen Z. 2020. Global lysine crotonylation alterations of host cell proteins caused by *Brucella* effector BspF. *Frontiers in Cellular and Infection Microbiology*, **10**, 603457.

Executive Editor-in-Chief Dongming Zhao
Managing Editor Yanyan Sun