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Current Opinion in Microbiology

The role of acid stress in *Salmonella* pathogenesis

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After uptake by epithelial cells or engulfment by macrophages, *Salmonella* resides in an acidic vacuole. *Salmonella* senses this acidic compartment through the action of the EnvZ/OmpR two-component regulatory system. OmpR, in turn, represses the *cadC/BA* system, preventing neutralization of the bacterial cytoplasm. New, single cell techniques now enable us to observe that in response to acid stress, the pH is low in bacterial cells and acidification is critical for infection. Instead of recovering from acid stress, *Salmonella* uses acid pH as a signal to drive pathogenesis. The relevant molecular mechanisms employed by *Salmonella* to couple acid stress with the expression of virulence genes that promote intracellular survival are explored.

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Current Opinion in Microbiology 2019, **47**:45–51

This review comes from a themed issue on **Host-pathogen interactions: bacteria**

Edited by **Karen M Ottemann** and **Linda J Kenney**

For a complete overview see the Issue and the Editorial

Available online 5th December 2018

https://doi.org/10.1016/j.mib.2018.11.006

Introduction

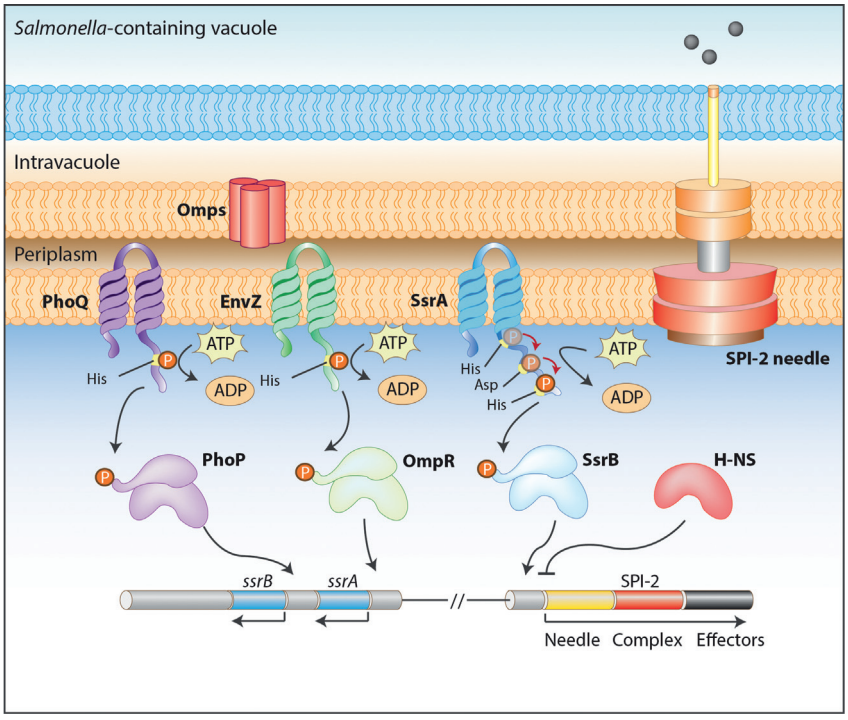
Salmonella infections occur from ingestion of contaminated food or water. The bacteria that survive the extreme acid pH of the stomach travel to the intestine and catalyze their uptake across the intestinal epithelium by activating the expression and assembly of a type three secretion system (T3SS) encoded on *Salmonella* pathogenicity island 1 (SPI-1). Once it is intracellular, *Salmonella* resides in an acidic vacuole that it modifies through the action of effectors. These effectors are secreted by another T3SS located on *Salmonella* pathogenicity island 2 (SPI-2). This article focuses on how *Salmonella* senses the acidic vacuolar environment and the subsequent events that ensue to drive its intravacuolar survival and replication.

The *Salmonella* cytoplasm is acidified in the vacuole

SPI-2 activation requires the expression of the SsrA/B two-component regulatory system (TCRS) that is located on SPI-2 (Figure 1). Expression of *ssrA/B* is activated in the vacuole and requires input from multiple TCRSs, including: the EnvZ/OmpR system [1–3], which activates *ssrA* transcription [4] and produces *ssrA* and *ssrB* [5] and is necessary for full virulence in mice [6,7]; and the PhoQ/P system [8], which activates (boosts) *ssrB* transcription [4]. *ssrB* is in turn auto-regulated [9,10]. The *ssrA/B* gene structure is extremely complex and involves lengthy (>150 nt) untranslated regions in front of both *ssrA* and *ssrB* genes [1]. A separate *ssrB* promoter embedded in the *ssrA* open reading frame is activated by PhoP∼P to enhance SsrB levels [4]. SsrB∼P drives SPI-2 transcription by de-repressing H-NS [11•,12–14] and activating transcription of *ssaB*, *sseA*, *ssaG*, *ssaM* [10] and *ssaR* [15] genes on SPI-2, as well as additional SPI-2 co-regulated genes [3,14].

In our previous studies of EnvZ function, we discovered that EnvZ was responding to cytoplasmic signals, rather than extracellular cues [16,17••] (for a review, see Ref. [18]). As a result of those studies, we suspected that the *Salmonella* cytoplasm might acidify when *Salmonella* was internalized in the acidic vacuole. We transformed *Salmonella* with the I-switch, a FRET, DNA-based biosensor, and used I-switch containing bacteria to infect RAW264.7 macrophages [19•]. As soon as we could measure the pH of *Salmonella* inside cells (approximately 30 min post-infection), the cytoplasm was acidified from an initial pH of 6.8 to pH 5.75, and it further acidified to pH 5.65 over time. Acidification was entirely dependent on OmpR, as the cytoplasm of *ompR* null strains remained at neutral pH. The pH gradient established by the vacuolar H^+-ATPase was essential and inhibition by bafilomycin also blocked acidification [19•]. OmpR bound directly and repressed the *cadC/BA* operon to inhibit neutralization (Figure 2). CadC is a response regulator in the OmpR subfamily and at acid pH, it induces *cadB/A*. CadA is a lysine decarboxylase that consumes a proton during decarboxylation. The antiporter CadB subsequently transports the product, cadaverine, out of the cell, restoring intracellular pH to near neutral. The pH optima of 6.1–6.5 of the CAD system makes it the major amino acid decarboxylation system involved when *Salmonella* is in the vacuole [20]. The model therefore is that

Figure 1



SPI-2 regulation by TCRSs. EnvZ responds to an internal acid stress and interacts with OmpR to promote an activating conformation. OmpR binds and activates *ssrA* transcription, producing both SsrA and SsrB. PhoP boosts SsrB levels during acid stress. SsrB relieves H-NS silencing of SPI-2 genes. SsrB also activates transcription of SPI-2 TT3S structural genes, as well as secreted effectors such as SifA and SseJ.

low external pH normally leads to CadB/A-mediated cytoplasmic neutralization (Figure 2), but this event is repressed by OmpR when *Salmonella* is in the host vacuole. It is now appreciated that EnvZ-directed activation of OmpR plays a major role in enabling *E. coli* and *Salmonella* to survive acid stress and the acidification that also occurs during osmotic stress [19•,21,22••,23].

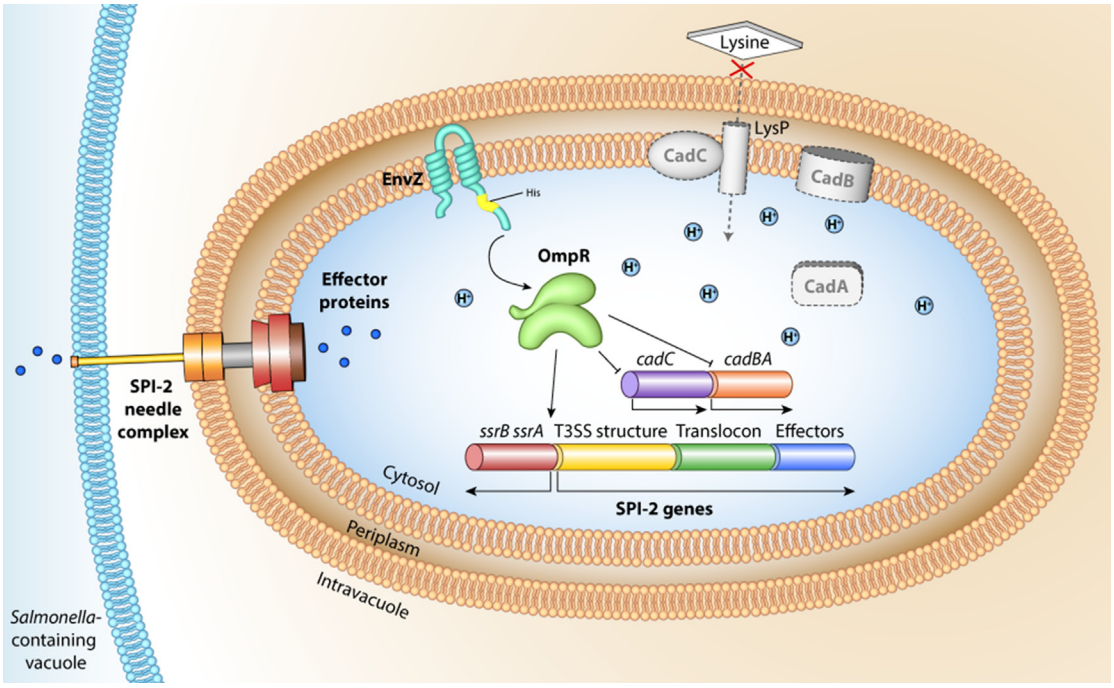
SsrB is a *Salmonella* lifestyle switch

As described above, EnvZ/OmpR plays a major role in allowing the bacterial cell cytoplasm to acidify. This acid stress induces *ssrA* and *ssrB* transcription, driving SsrB∼P activation of SPI-2 genes [1,9,10,14]. In order to count SsrA and SsrB molecules in single cells using superresolution imaging, we constructed chromosomally-encoded, active photoactivatable fusion proteins. For a description of the method and an example using OmpR, see Refs. [24–26]. *In vitro* and *in vivo* measurements of bacteria recovered from HeLa cells demonstrated that the number of SsrA and SsrB molecules increased about three-fold in acid pH compared to neutral pH. Furthermore, the SsrB response regulator was always present at higher concentrations than the SsrA kinase [4]. Increasing the number of SsrA kinase molecules is an important driver of pathogenesis. Recent studies reported that SsrB can act non-canonically at neutral pH (in the absence of phosphorylation) to drive the biofilm pathway by de-repressing H-NS at the promoter of the master regulator *csgD* [11•,27]. Thus, SsrB is an important switch in determining *Salmonella* lifestyles, choosing between an intracellular lifestyle (SPI-2 activation) and an extracellular carrier state forming biofilms on gallstones in the gall bladder [28]. Thus, when the SsrA kinase is present, SsrB ∼P accumulates and it activates the SPI-2 genes. At neutral pH, the SsrA kinase is very low and the unphosphorylated form of SsrB predominates, driving the expression of biofilm genes. Increasing the concentration of SsrA pushes SsrB∼P to activate SPI-2. Validation of recent RNAseq experiments [11•] will provide a deeper understanding of how SsrB controls ancestral genes [29] to promote its various lifestyles. Interestingly, single particle tracking experiments showed that SsrB binding to DNA, as evident by a change in its diffusion coefficient, increased in acid pH by >50% [4]. This was a unique feature of the SsrB response regulator, because DNA binding by OmpR only increased by 5% in acid pH and PhoP binding was insensitive to acid pH.

Cytoplasmic acidification is required for virulence

In the presence of bafilomycin, the H^+-ATPase was inhibited and the vacuole was no longer acidified. Under

Figure 2



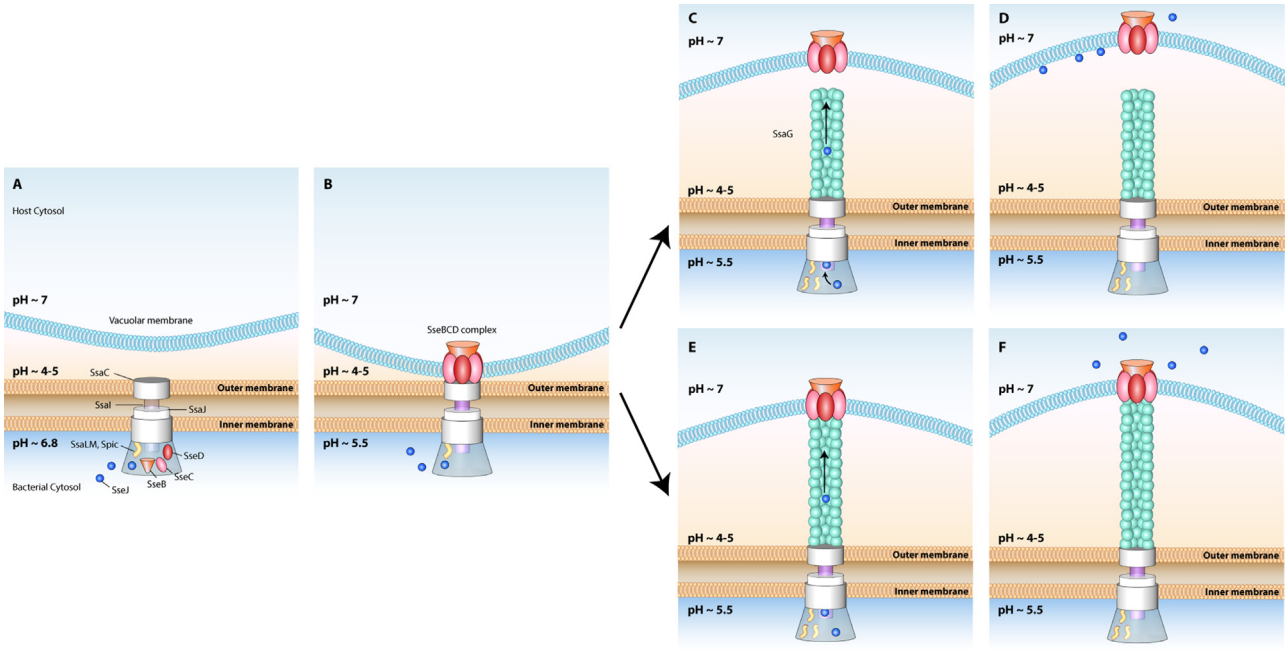
OmpR represses *cadC/BA* to prevent neutralization in the acidic vacuole. Normally, in response to acid stress, CadC activates transcription of *cadBA*. CadA decarboxylates lysine, consuming a proton in the process. The product, cadaverine, is transported out of the cell via the antiporter CadB. In the absence of *cadC/BA* (dashed lines), the *Salmonella* cytoplasm stays acidified. Acidification is required for secretion of SPI-2 effectors. In contrast to *Salmonella*, *Shigella* breaks out of the vacuole and does not reside in an acidic compartment. Hence, it does not require the CAD system. Hence, during its evolution of virulence, *Shigella* has lost the CAD genes [48]. Further, cadaverine was shown to block the activity of *Shigella* enterotoxins [49].

these conditions, surprisingly, the SPI-2 T3SS was made and assembled, as visualized by the presence of SseB translocons on the bacterial surface in the macrophage vacuole (see Figure 3) [19•]. In *Salmonella*, the translocon is composed of two hydrophobic proteins, SseC/D and a hydrophilic protein SseB [30]. SseB is produced at neutral pH, but only secreted at acid pH [31]. During a normal infection, when the vacuole and the *Salmonella* cytoplasm were acidified, the translocon moved away from the bacterial surface, but it still remained co-localized with LAMP-1$^+$ endosomes [19•] (Figure 3c). What wasn't clear was whether SseB was still associated with the additional translocon components SseC/D. We presumed that SseB was still associated with SseC and SseD, because it was still co-localized with LAMP-1$^+$ endosomes, a hydrophobic compartment [19•]. However, in other pathogens, translocons can dissociate from one another during infection. For example, in EPEC, the SseB homologue EspA is released by a protease EspC [32]. Another remaining question was whether the translocon was still attached to the needle, SsaG (Figure 3c–f). If SseB were still attached, then the needle would have to elongate, allowing SseB to extend from the bacterial surface (Figure 3e and f). Alternatively, the translocon may function somewhat similarly to *Pseudomonas aueruginosa*. In this example, the translocon PopB/D functioned as a pore-forming toxin after the bacterium had detached from its site of attachment [33••]. This event drove histone H3 modification in the host. Alternatively, if SseB acts like a plug in the translocon pore of SseC/D, then after its removal, effectors may be first secreted into the vacuole and then into the host cytosol (Figure 3d). It still leaves us with the question-what is SseB interacting with? Super-resolution imaging of secreted effectors in the vacuole may help to resolve this issue. Purification of SseC/D complexes and following them during infection will also be informative.

A SPI-2 secretion threshold

Salmonella acidification also occurred in response to osmolytes, but the pH drop was not as significant as it was in response to acid stress [21]. We also compared osmolytes NaCl and sucrose. In the presence of NaCl, the pH_i was 6.7, and this level of acidification did not support SPI-2 secretion, as evident by the lack of accumulation of the effector SseJ in the supernatant [21]. In contrast, in the presence of sucrose, the pH_i was 6.45 and SseJ was secreted, establishing a threshold for SPI-2 secretion between 6.7 and 6.45. At neutral pH, SseJ was produced and it accumulated in the cytoplasm, but it was not

Figure 3



Does SPI-2 secretion involve needle elongation and/or translocon release? **(a)** The T3SS is made and assembled, and SseB appears on the bacterial surface. **(b)** The drop in *Salmonella* cytoplasmic pH triggers unknown events that open the secretion gate. **(c–f)** One of these events may involve needle elongation, because over time, the translocon moves away from the bacterial surface, although it is still in a LAMP-1$^+$ compartment. It is not known whether the translocon detaches during the secretion process (c and d) or whether it is still attached to the SsaG needle (e and f). If SseB is still attached to the needle, the needle must elongate in the vacuole. If SseB detaches from the needle, does the translocon still function as a pore (d)?

secreted. Thus, at neutral pH, the SPI-2 secretion gate was closed. An earlier model proposed that *Salmonella* sensed host **cytosolic** pH, and required a neutralization step to disrupt the gating complex composed of SsaL, SsaM and SpiC [34]. Our results indicated that *Salmonella* sensed **vacuolar** pH and no evidence of a neutralization step was evident [19•]. Understanding the molecular basis of the substrate specificity switch of SPI-2, when secretion switches from apparatus components to effectors, is an active area of investigation, but likely involves an interaction with the needle length control protein SsaP and the effector secretion gate.

Measuring pH in single bacteria cells

Previous studies that identified acid-sensitive genes in *Salmonella* did not consider the possibility that the source of acid stress might be internal. This was not surprising, because we think of signal transduction as resulting from an extracellular stress driving an intracellular conformational change that activates a response. Furthermore, most of the past measurements of the bacterial response to acid stress reported that *E. coli* responded to acidic pH by a decrease in intracellular pH (pH_i), followed by a rapid recovery (see reviews by [35,36]). However, our discovery that EnvZ was sensing the cytoplasm [16,17••,18] changed our thinking and drove us to measure intracellular responses to acid and osmotic stress [19•,21,23]. This resulted in a paradigm shift, but it required us to look back at the literature in an attempt to understand why our measurements differed from others and led to a different conclusion. In Table 1, the results of this comparison are summarized. In a study of mRNA structure of the acid-induced virulence factor *mgtC* [37], Groisman and colleagues used a plasmid-encoded pH-sensitive GFP to measure the pH of *Salmonella* [38]. The wildtype strain did not acidify, it remained at pH 7.25, even though it was exposed to an external acid pH (pH_e) of 5.1. In a follow-up study, a similar approach reported that an *mgtC* null strain was acidified to $pH_i = 5.9$ [39]. In contrast, in our study, the pH_i of the *mgtC* null strain was identical to the wildtype strain, that is, both strains were acidified to pH 6.1 (see Table 1). How do we reconcile these differing results? We analyzed single cells, and we used either BCECF or the I-switch fluorophores [19•,21]. Our *Salmonella* strain was the same as the one used by Groisman and colleagues, so strain differences do not provide an explanation. The plasmid-inducible pH-sensitive GFP (pHluorin) was driven in some instances by the pBAD promoter [38], which leads to substantial heterogeneity [21,40], which would be masked by population measurements. Finally, the most significant difference was the way that the cells were clamped to generate

Table 1

The strains are listed on the top of the column, along with the clamping method used to measure the pH. The extracellular pH and measured intracellular pH are indicated

Salmonella Typhimurium 14028s	pHluorin [37,39] sodium benzoate clamped	BCECF, I-switch [19,21] nigericin clamped	BCECF [21] sodium benzoate clamped	*E. coli*	MC4100 pHluorin [35,38] sodium benzoate clamped	MG1655 BCECF [21] nigericin clamped
Extracellular pH	7.70	7.20	7.20	Extracellular pH	5.50	5.60
Intracellular pH	8.15	6.80	5.80	Intracellular pH	7.60	6.10
Extracellular pH	5.10	5.60	5.60			
Intracellular pH	7.25	6.10	6.30			

a standard curve. The probe fluorescence is measured after setting the external pH equal to the internal pH over a range of pH values, depending on the sensitivity range of the probe. This generates a standard curve and then in experimental samples, the fluorescence of the probe is measured and a pHi value is deduced from the standard curve. In order to 'clamp' pH_i to pH_e, most previous studies used 20 mM sodium benzoate and claimed that at this concentration, the cytoplasmic pH was equilibrated with the external pH, although no standard curves were shown. In our studies, we clamped cells using the ionophore nigericin [19•,21]. To determine whether the clamping conditions could explain the discrepancy in results, we added sodium benzoate to neutral or acidic bacterial cultures and then measured the pH with the probe BCECF. If the cells were 'clamped' by sodium benzoate, then the pH_i should be equal to the pH_e. The result was truly surprising, because instead of clamping pH_i at external pH, the cells were acidified to pH 5.8. Furthermore, this effect was greatest at neutral pH. This would have led to the erroneous conclusion that bacterial intracellular pH was neutral, when indeed it was very acidic inside. Thus, the discrepancy between our pH measurements and others can be explained. This was further apparent in a recent study that made bulk measurements, clamped with nigericin, and subsequently reported an acidification of the *Salmonella* cytoplasm [41]. Thus, our data indicate that both wildtype and *mgtC* null strains were acidified to the same extent, and MgtC does not play a role in acidification [19•].

Acid pH does not relax the nucleoid

What is the stimulus during acidification that increases SPI-2 gene expression? One model proposed that a decrease in supercoiling occurred, leading to relaxation of the nucleoid [42], and relaxation led to the exposure of OmpR binding sites. This enhanced exposure would promote OmpR regulation of acid-responsive genes. Other data conflicts with this model in two significant ways. Single particle tracking experiments demonstrated that only a very small change in OmpR binding to DNA occurred at acid pH (~5%) [4]. Single particle tracking was certainly capable of detecting enhanced DNA binding, because SsrB binding to DNA increased by more than 50% at pH 5.6 [4]. Perhaps many of the OmpR regulated genes involved in the acid tolerance response are not direct OmpR targets; most of these targets have not been further validated [22••,23,42]. High resolution structured illumination microscopy (SIM) imaging was used to measure the area of the bacterial nucleoid at acid and neutral pH [4,43]. The nucleoid was more compact at acid pH, that is, the nucleoid area was reduced by 20% compared to neutral pH [4]. SIM was able to detect changes in nucleoid area, because in the presence of 100 μg/ml novobiocin, a gyrase inhibitor, the nucleoid was more relaxed [43]. Thus, our results do not support a model that involves nucleoid relaxation at acid pH [4,24,43]. We propose instead that SsrB undergoes a pH-dependent conformational change that increases DNA binding and transcriptional activation of SPI-2 [4], a model that is currently being tested in our laboratory.

Conclusions

When *Salmonella* is in an acidic vacuole, the cytoplasm becomes acidified through the action of EnvZ/OmpR repression of *cadC/BA*. This repression maintains an acidic cytoplasm and then global regulators OmpR and PhoP activate the transcription of *ssrA* and *ssrB*. The number of SsrA and SsrB molecules increases at least three-fold, and DNA binding by SsrB increases by more than 50%. These events relieve H-NS silencing and drive expression of SPI-2 genes, leading to the assembly of the T3SS. A still unknown series of events involving the substrate specificity switch leads to the secretion of virulence factors, including SseJ and SifA into the host cytoplasm [44–46]. The resulting endosomal tubulation (otherwise known as *Salmonella*-induced filaments or Sifs) is a force-driven event requiring the interaction of effectors with the motor protein kinesin [44,47]. This interaction is evident from two-color superresolution images in which kinesin and SseJ are co-localized. In the absence of *sifA*, SseJ does not traffick along tubules, and destabilizes the vacuole [44]. Thus, modification of the vacuole and subsequent Sif formation enables *Salmonella* to replicate within the vacuole and eventually disseminate to systemic sites.

Conflict of interest statement

Nothing declared.

Acknowledgements

I am grateful to my present and past laboratory members for their experimental and intellectual contributions to this work. I also thank Melanie Lee of the MBI SciComm team for illustrations and MP Sheetz for critical comments on the manuscript. Supported by VAIOBX-000372 and NIHAI123640 and a Research Centre of Excellence in Mechanobiology from the Ministry of Education, Singapore.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Feng X, Oropeza R, Kenney LJ: **Dual regulation by phospho-OmpR of *ssrA/B* gene expression in *Salmonella* pathogenicity island 2**. *Mol Microbiol* 2003, **48**:1131-1143.

2. Lee AK, Detweiler CS, Falkow S: **OmpR regulates the two-component system SsrA-SsrB in *Salmonella* pathogenicity island 2**. *J Bacteriol* 2000, **182**:771-781.

3. Garmendia J, Beuzon CR, Ruiz-Albert J, Holden DW: **The roles of SsrA-SsrB and OmpR-EnvZ in the regulation of genes encoding the Salmonella typhimurium SPI-2 type III secretion system**. *Microbiology* 2003, **149**:2385-2396.

4. Liew ATF, Zangoui P, Foo YH, Singh MK, Gao Y *et al.*: *Single Cell, Super-Resolution Imaging Reveals Acid-Dependent SsrB Gene Regulation*. . Submitted for publication 2018.

5. Bustamante VH, Martinez LC, Santana FJ, Knodler LA, Steele-Mortimer O *et al.*: **HilD-mediated transcriptional cross-talk between SPI-1 and SPI-2**. *Proc Natl Acad Sci U S A* 2008, **105**:14591-14596.

6. Chatfield SN, Dorman CJ, Hayward C, Dougan G: **Role of ompR-dependent genes in Salmonella typhimurium virulence: mutants deficient in both ompC and ompF are attenuated in vivo**. *Infect Immun* 1991, **59**:449-452.

7. Dorman CJ, Chatfield S, Higgins CF, Hayward C, Dougan G: **Characterization of porin and ompR mutants of a virulent strain of *Salmonella typhimurium*: *ompR* mutants are attenuated *in vivo***. *Infect Immun* 1989, **57**:2136-2140.

8. Bijlsma JJ, Groisman EA: **The PhoP/PhoQ system controls the intramacrophage type three secretion system of Salmonella enterica**. *Mol Microbiol* 2005, **57**:85-96.

9. Feng X, Walthers D, Oropeza R, Kenney LJ: **The response regulator SsrB activates transcription and binds to a region overlapping OmpR binding sites at Salmonella pathogenicity island 2**. *Mol Microbiol* 2004, **54**:823-835.

10. Walthers D, Carroll RK, Navarre WW, Libby SJ, Fang FC *et al.*: **The response regulator SsrB activates expression of diverse Salmonella pathogenicity island 2 promoters and counters silencing by the nucleoid-associated protein H-NS**. *Mol Microbiol* 2007, **65**:477-493.

11. • Desai SK, Winardhi RS, Periasamy S, Dykas MM, Jie Y *et al.*: **The horizontally-acquired response regulator SsrB drives a Salmonella lifestyle switch by relieving biofilm silencing**. *Elife* 2016, **5**:e10747.

This work demonstrated a dual role for SsrB in*Salmonella* life style by either activating SPI-2 (SsrB∼P) or promoting the carrier state through biofilm formation (SsrB).

12. Lucchini S, Rowley G, Goldberg MD, Hurd D, Harrison M *et al.*: **H-NS mediates the silencing of laterally acquired genes in bacteria**. *PLoS Pathog* 2006, **2**:e81.

13. Navarre WW, Porwollik S, Wang Y, McClelland M, Rosen H *et al.*: **Selective silencing of foreign DNA with low GC content by the H-NS protein in Salmonella**. *Science* 2006, **313**:236-238.

14. Walthers D, Li Y, Liu Y, Anand G, Yan J *et al.*: **Salmonella enterica response regulator SsrB relieves H-NS silencing by displacing H-NS bound in polymerization mode and directly activates transcription**. *J Biol Chem* 2011, **286**:1895-1902.

15. Tomljenovic-Berube AM, Mulder DT, Whiteside MD, Brinkman FS, Coombes BK: **Identification of the regulatory logic controlling Salmonella pathoadaptation by the SsrA-SsrB two-component system**. *PLoS Genet* 2010, **6**:e1000875.

16. Ghosh M, Wang LC, Ramesh R, Morgan LK, Kenney LJ *et al.*: **Lipid-mediated regulation of embedded receptor kinases via parallel allosteric relays**. *Biophys J* 2017, **112**:643-654.

17. •• Wang LC, Morgan LK, Godakumbura P, Kenney LJ, Anand GS: **The inner membrane histidine kinase EnvZ senses osmolality via helix-coil transitions in the cytoplasm**. *EMBO J* 2012, **31**:2648-2659.

This work demonstrated how cytoplasmic signals (osmolytes or acid pH) drive EnvZ activation by influencing a locally disordered region around the phosphorylated histidine to become more helical, activating histidine phosphorylation. It was surprising that histidine kinases could function without being in the membrane.

18. Anand G, Kenney LJ: **The EnvZ/OmpR regulon**. *EcoSal Plus* 2018. Submitted for publication.

19. • Chakraborty S, Mizusaki H, Kenney LJ: **A FRET-based DNA biosensor tracks OmpR-dependent acidification of Salmonella during macrophage infection**. *PLoS Biol* 2015, **13**: e1002116.

In this work, the authors measured the pH of the*Salmonella* cytoplasm in a macrophage vacuole and it was acidified in an OmpR-dependent response by repression of CAD. Acidification was essential for SPI-2 virulence.

20. Cheeseman GC, Fuller R: **Changes in the pH activity profile of the lysine decarboxylase during incubation of Escherichia coli**. *J Appl Bacteriol* 1968, **31**:253-258.

21. Chakraborty S, Winardhi RS, Morgan LK, Yan J, Kenney LJ: **Non-canonical activation of OmpR drives acid and osmotic stress responses in single bacterial cells**. *Nat Commun* 2017, **8**:1587.

22. •• Stincone A, Daudi N, Rahman AS, Antczak P, Henderson I *et al.*: **A systems biology approach sheds new light on Escherichia coli acid resistance**. *Nucleic Acids Res* 2011, **39**:7512-7528.

This work demonstrated a significant role for OmpR in the acid stress response of*E. coli*.

23. Chakraborty S, Kenney LJ: **A new role of OmpR in acid and osmotic stress in E. coli and S. Typhimurium**. *Front Micrbiol* 2018 http://dx.doi.org/10.3389/fmicb.2018.02656.

24. Foo YH, Spahn C, Zhang HF, Heilemann M, Kenney LJ: **Single cell super-resolution imaging of E. coli OmpR during environmental stress**. *Integr Biol* 2015, **7**:1297-1308.

25. Spahn C, Glaesmann M, Gao Y, Foo YH, Lampe M *et al.*: **Erratum to: sequential super-resolution imaging of bacterial regulatory proteins, the nucleoid and the cell membrane in single, fixed E. coli cells**. *Methods Mol Biol* 2017, **1624**:E1.

26. Spahn C, Glaesmann M, Gao Y, Foo YH, Lampe M *et al.*: **Sequential super-resolution imaging of bacterial regulatory proteins: the nucleoid and the cell membrane in single, fixed E. coli cells**. *Methods Mol Biol* 2017, **1624**:269-289.

27. Desai SK, Kenney LJ: **To ∼P or Not to ∼P? Non-canonical activation by two-component response regulators**. *Mol Microbiol* 2017, **103**:203-213.

28. Crawford RW, Rosales-Reyes R, Ramirez-Aguilar Mde L, Chapa-Azuela O, Alpuche-Aranda C *et al.*: **Gallstones play a significant role in Salmonella spp. gallbladder colonization and carriage**. *Proc Natl Acad Sci U S A* 2010, **107**:4353-4358.

29. Brown NF, Rogers LD, Sanderson KL, Gouw JW, Hartland EL *et al.*: **A horizontally acquired transcription factor coordinates Salmonella adaptations to host microenvironments**. *MBio* 2014, **5**:e01727-01714.

30. Chakravortty D, Rohde M, Jager L, Deiwick J, Hensel M: **Formation of a novel surface structure encoded by Salmonella pathogenicity island 2**. *EMBO J* 2005, **24**:2043-2052.

31. Beuzon CR, Banks G, Deiwick J, Hensel M, Holden DW: **pH-dependent secretion of SseB, a product of the SPI-2 type III secretion system of Salmonella typhimurium**. *Mol Microbiol* 1999, **33**:806-816.

32. Guignot J, Segura A, Tran Van Nhieu G: **The serine protease EspC from enteropathogenic Escherichia coli regulates pore formation and cytotoxicity mediated by the type III secretion system**. *PLoS Pathog* 2015, **11**:e1005013.

33. •• Dortet L, Lombardi C, Cretin F, Dessen A, Filloux A: **Pore-forming activity of the Pseudomonas aeruginosa type III secretion system translocon alters the host epigenome**. *Nat Microbiol* 2018, **3**:378-386.
In this work, the authors demonstrated that the translocon was detached from the type III secretion system and functioned as a pore forming toxin that drove host responses.

34. Yu XJ, McGourty K, Liu M, Unsworth KE, Holden DW: **pH sensing by intracellular Salmonella induces effector translocation**. *Science* 2010, **328**:1040-1043.

35. Slonczewski JL, Fujisawa M, Dopson M, Krulwich TA: **Cytoplasmic pH measurement and homeostasis in bacteria and archaea**. *Adv Microb Physiol* 2009, **55**:1-79 317.

36. Krulwich TA, Sachs G, Padan E: **Molecular aspects of bacterial pH sensing and homeostasis**. *Nat Rev Microbiol* 2011, **9**:330-343.

37. Lee EJ, Groisman EA: **Control of a Salmonella virulence locus by an ATP-sensing leader messenger RNA**. *Nature* 2012, **486**:271-275.

38. Wilks JC, Slonczewski JL: **pH of the cytoplasm and periplasm of Escherichia coli: rapid measurement by green fluorescent protein fluorimetry**. *J Bacteriol* 2007, **189**:5601-5607.

39. Lee EJ, Pontes MH, Groisman EA: **A bacterial virulence protein promotes pathogenicity by inhibiting the bacterium's own F1Fo ATP synthase**. *Cell* 2013, **154**:146-156.

40. Siegele DA, Hu JC: **Gene expression from plasmids containing the araBAD promoter at subsaturating inducer concentrations represents mixed populations**. *Proc Natl Acad Sci U S A* 1997, **94**:8168-8172.

41. Choi J, Groisman EA: **Acidic pH sensing in the bacterial cytoplasm is required for Salmonella virulence**. *Mol Microbiol* 2016, **101**:1024-1038.

42. Quinn HJ, Cameron AD, Dorman CJ: **Bacterial regulon evolution: distinct responses and roles for the identical OmpR proteins of Salmonella Typhimurium and Escherichia coli in the acid stress response**. *PLoS Genet* 2014, **10**:e1004215.

43. Gao Y, Foo YH, Winardhi RS, Tang Q, Yan J *et al.*: **Charged residues in the H-NS linker drive DNA binding and gene silencing in single cells**. *Proc Natl Acad Sci U S A* 2017, **114**:12560-12565.

44. Gao Y, Spahn C, Heilemann M, Kenney LJ: **The pearling transition provides evidence of force-driven endosomal tubulation during Salmonella infection**. *mBio* 2018, **9(3)**: e01083-18 http://dx.doi.org/10.1128/mBio.01083-18.

45. Ohlson MB, Fluhr K, Birmingham CL, Brumell JH, Miller SI: **SseJ deacylase activity by Salmonella enterica serovar Typhimurium promotes virulence in mice**. *Infect Immun* 2005, **73**:6249-6259.

46. Stein MA, Leung KY, Zwick M, Garcia-del Portillo F, Finlay BB: **Identification of a Salmonella virulence gene required for formation of filamentous structures containing lysosomal membrane glycoproteins within epithelial cells**. *Mol Microbiol* 1996, **20**:151-164.

47. Boucrot E, Henry T, Borg JP, Gorvel JP, Meresse S: **The intracellular fate of Salmonella depends on the recruitment of kinesin**. *Science* 2005, **308**:1174-1178.

48. Day WA Jr, Fernandez RE, Maurelli AT: **Pathoadaptive mutations that enhance virulence: genetic organization of the cadA regions of Shigella spp**. *Infect Immun* 2001, **69**:7471-7480.

49. Maurelli AT, Fernandez RE, Bloch CA, Rode CK, Fasano A: **"Black holes" and bacterial pathogenicity: a large genomic deletion that enhances the virulence of Shigella spp. and enteroinvasive Escherichia coli**. *Proc Natl Acad Sci U S A* 1998, **95**:3943-3948.