EDITORIAL

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Small RNA in the acid tolerance response of *Salmonella* and their role in virulence

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Salmonella are enterobacterial pathogens that are a major cause of food-borne illness and have been found at different sites in the body during infection and at different stages of food processing, reflecting the adaptability of these organisms. They are major food contaminants and thus are exposed to a broad spectrum of nutrient availability that they must negotiate in order to survive.[1] On ingestion, *S.* Typhimurium can penetrate and bypass the intestinal epithelial cell lining of the intestine through a mechanism that requires the expression of a type 3 secretion system (T3SS) that is encoded by a set of horizontally acquired genes in the *Salmonella* Pathogenicity Island 1 (SPI1). In patients that are immunocompromised, *S.* Typhimurium causes a systemic infection producing typhoid like fever symptoms due to its ability to survive and replicate within macrophages. This survival is in turn facilitated by a second T3SS encoded within an island called the *Salmonella* Pathogenicity Island 2 (SPI2).[2,3]

Salmonella have been extensively studied as model organisms for virulence, pathogenesis, genomic evolution and gene regulation. However, most of these studies have focused on proteins and their involvement. It is only recently that *Salmonella* has become a model for RNA-mediated regulation. The non coding RNA spectrum of *Salmonella* includes small RNAs (sRNA) that serve as post transcriptional regulators, *cis*-regulatory elements that serve as sensors and protein binding RNAs. *S.* Typhimurium was found to express about 140 sRNAs at early stationary phase including 60 newly identified sRNAs. There is no doubt that many more sRNAs are yet to be identified under various conditions be it stress or environmental.[4]

Salmonella Typhimurium is exposed to a wide range of acidic environments inside their host as well as in their natural habitat.[5] Being an intracellular parasite it infects the intestinal epithelium and is capable of multiplying in non-phagocytic cells and also survives in macrophages. Reports indicate the presence of an acid tolerance response in *S.* Typhimurium which protects the organism at lower pH.[5] *Salmonella* prefer neutral pH conditions but can adapt to much lower pH levels around 3. The ATR mechanism includes 2 stages: (a) Pre-acid shock and (b)Post-acid shock. The former is induced at pH 5.8 hence triggering production of an inducible ATR specific pH homeostasis system functional when external pH falls below pH 4.0; latter occurs following acid shift to 4.5 or below hence accounting for the name. Several acid shock proteins (ASPs) are synthesized during the post-acid shock for a transient time period which disappear after 30–40 minutes leading toward the inability of cells to survive at subsequent lower pH levels (3.3).[6] It has also been shown that the log-phase ATR could be induced by allowing the cells to grow for about 60–90mins at a pH of 4.4. This also induces protective tolerance to cells when they are exposed to a pH of 3.1.[7]

The four levels of acid tolerance presented in increasing order of tolerance they provide are: log phase cells, acid-adapted stationary phase cells, stationary phase cells, and acid-adapted cells. Two-dimensional analysis has shown that 60 ASPs are induced during the log phase while 45 ASPs are induced following adaptation of stationary phase cells.[8]

It has long been known that an enhanced tolerance to pH stress would play a role in virulence particularly during uptake and survival of *Salmonella* in macrophages. An enhanced survival ability of these microbes within macrophages would result in a greater propensity for systemic infection and it is this relation between acid tolerance and virulence that is of interest to us.

Small non-coding RNAs have emerged in the last 2 decades as major regulators of expression at the global level. Small non-coding RNAs (sRNAs) are recognized as important regulators in all kingdoms of life. They do not commonly contain expressed open reading frames and usually are conserved in closely related species. In bacteria, sRNAs usually 50–500 nucleotides long and regulate gene expression either by pairing to mRNAs and affecting their stability and/or translation or by binding to proteins and modifying their activity.[9] The major classes, mediate their effects through cis or trans binding to their target mRNA, sometimes involving Hfq (RNA Chaperone) and bringing about either activation or repression of their targets. Binding is hypothesized to block entry of the ribosome (translational repression) or to unravel secondary structures which sequester or block the ribosome binding site of the mRNA (translational activation). Regulation is often coupled to nuclease mediated cleavage of the mRNA.[10,11]

Various studies on the transcriptome of *Salmonella* have implicated a number of known and novel sRNAs in acid tolerance. To name a few, DsrA, RprA, ArcZ and GcvB have been known to play roles in acid stress.[12] Others, such as IsrM, IsrC, IsrE etc. have been

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shown to play vital roles in virulence. Mutants of the above sRNAs have been shown to lack certain virulence properties. However, it is only recently that focused large scale studies are being conceived to throw light on the diverse roles played by these regulators. This link between acid tolerance and virulence is of particular interest when considering Enterobacteria as these 2 properties are vital for their survival and propagation in a host.

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