

## *Shigella* takes the temperature

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**F**or bacteria, temperature is a fairly reliable cue for entry into a vertebrate host. Many pathogenic bacteria sense elevated temperatures (37°C) and turn on virulence and metabolic genes appropriate for survival and growth within the host. A recent study demonstrated that the expression of a heme uptake protein in *Shigella dysenteriae* and pathogenic *Escherichia coli* was dependent on an RNA thermometer.

When life is short, the ability to conserve resources and quickly adapt to changing conditions is an existential virtue. Pathogenic bacteria have elaborate mechanisms to detect, respond and adapt to life within the distinct milieus of the host and the environment. Sequences in the mRNA of several heat shock proteins and virulence factors, known as RNA thermometers, rapidly regulate translation in response to altered temperatures.<sup>1</sup> These riboswitch sequences in the 5' untranslated regions (UTR) of mRNAs adopt secondary structures that block ribosome access at low temperatures. At higher temperatures, an open conformation allows ribosome access to the Shine Dalgarno sequences and, consequently, translation of the mRNA. Such structures can respond to alterations in temperatures as low as 1°C. RNA thermosensors also play a role in the preferential translation of some transcripts (e.g., cold shock genes) at lower temperatures.

The expression of PrfA, the global regulator of virulence genes in *Listeria monocytogenes*, is regulated via multiple mechanisms, including an RNA thermometer.<sup>1</sup> At low temperatures (30°C), the extended secondary structure of the 127-nucleotide 5' UTR of a subset of

*prfA* transcripts (those expressed from the *prfAP1* promoter) includes a stem-loop configuration that prevents formation of a translation initiation complex. Destabilization of these secondary structures at higher temperatures (37°C, as might occur within a host) allows for translation initiation and increased levels of PrfA.

While strains harboring constitutively active forms of PrfA (PrfA\*, which can function in the absence of a cofactor) are more virulent in mouse models of infection, they exhibit a fitness defect in broth cultures when grown in the presence of wildtype bacteria.<sup>2</sup> PrfA\*-harboring strains have diminished flagella-dependent swimming motility and an impaired ability to utilize carbon sources such as glucose and cellobiose, and cannot survive long periods of starvation. Thus, the tight regulation of PrfA levels and activity is essential for *L. monocytogenes* to transition between pathogenic and saprophytic/environmental lifestyles.

In the pathogenic yersiniae, expression of virulence genes is regulated by the transcriptional regulator LcrF.<sup>3</sup> A hierarchy of protein and RNA thermosensors regulate LcrF expression at body temperature. While *lcrF* transcription is regulated by the thermosensitive modulator YmoA, translation is regulated by an RNA thermometer that includes four consecutive uridine residues (“fourU,” Fig. 1) that pair with the Shine Dalgarno sequence. Opening of this stem-loop structure at 37°C enables ribosome binding and LcrF translation. *Y. pseudotuberculosis* strains with stem-loop stabilizing mutations (closed configuration) did not express detectable LcrF at low or high temperatures, and were attenuated in a mouse model of infection.

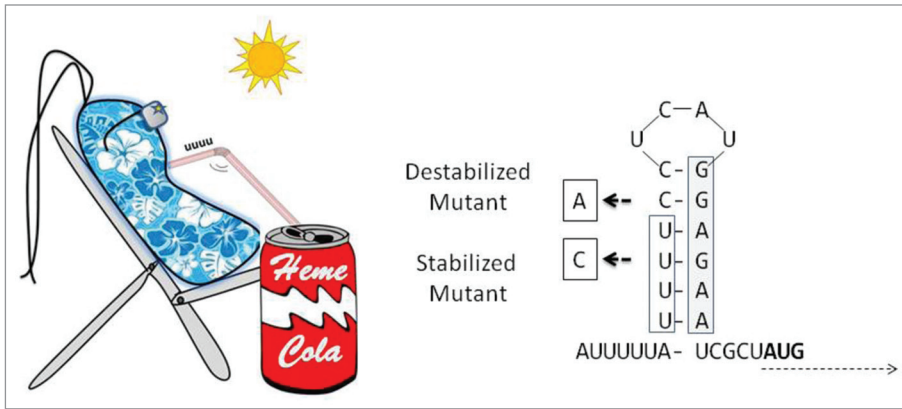
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**Figure 1.** In *S. dysenteriae* and *E. coli*, temperature-dependent opening of a fourU structure in the heme uptake-related *shuA* (or *chuA*) mRNA permits ribosome access to Shine Dalgarno site (boxed) sequences, and consequent translation starting at the downstream AUG site. Mutagenic stabilization of the secondary structure by a U→C mutation prevents ribosome access (and ShuA translation) even at permissive temperatures, while a C→A mutation destabilizes the structure and results in ShuA translation even at low (“Non-permissive”) temperatures.

Interestingly, mutant strains harboring a destabilized secondary structure (open configuration), which expressed LcrF at both low and high temperatures, were also impaired for virulence. This suggests that delicate regulation of LcrF levels, by various mechanisms including RNA thermoregulation, is critical for pathogenesis.

A recent study explores the role of RNA thermoregulation in iron uptake in *Shigella dysenteriae* and uropathogenic *Escherichia coli*.<sup>4</sup> Iron is an essential nutrient for most bacteria and, consistent with this, they elaborate multiple, and apparently redundant, iron uptake systems. Thus, none of the single iron transport system mutants of *Shigella* displayed appreciable defects in intracellular multiplication or cell-to-cell spread, although a mutant deficient in three distinct iron uptake systems failed to replicate within epithelial cells.<sup>5</sup> Given

its toxicity, however, iron uptake systems are also under tight regulatory controls. Iron transport systems in many bacteria, including *Shigella*, are regulated by iron and the Fur repressor. Iron-complexed Fur represses transcription of iron-regulated genes by binding to “Fur boxes” in the corresponding promoter regions. In iron-depleted conditions, Fur is released from the promoter regions, and iron uptake genes are expressed.

Ninety five percent of iron in the human body is sequestered in heme, a source utilized by many pathogenic bacteria. The *S. dysenteriae* outer membrane receptor ShuA (ChuA in *E. coli*) binds heme and facilitates its transport into the periplasm. Other components of the Shu system mediate heme entry into the cytoplasm, as well as its subsequent degradation to yield nutrient iron. Kouse et

al. recently reported that, although iron depletion induced *shuA* transcription, ShuA protein levels were not increased at 25°C.<sup>4</sup> At 37°C, however, ShuA protein levels were also elevated.

A fourU RNA thermometer was shown to regulate the expression of ShuA and ChuA, and transfer of a 32-nucleotide *shuA* 5'UTR region was sufficient to confer temperature-dependent translational regulation to green fluorescent protein expression. A U→C mutation that stabilized the stem-loop structure locked the mRNA in a non-translatable configuration, while a C→A mutation that destabilized the stem-loop structure permitted ShuA translation even at low temperatures. The *shuA* 5'UTR was found to be conserved among pathogenic *E. coli*, and the uropathogenic *E. coli* homolog *chuA* was shown to be similarly induced by temperature elevation.

A cursory analysis of the 8 open reading frames in the heme uptake cluster reveals the presence of a fourU cluster only in the *shuA* 5'UTR. The implication of differential regulation of the cluster, if this is indeed the case, to heme uptake and prevention of heme toxicity is unclear at present. While iron deficiency, a signal for Fur-dependent derepression of uptake systems, could occur both in the host and the environment, the utility of ShuA would only be within a host where heme is available. Using a temperature signal to turn on ShuA expression may not be exactly what Pavlov had in mind when he described “conditional reflexes,” but it surely rings a bell.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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