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MICROREVIEW

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Feeling the heat at the millennium: Thermosensors playing with fire

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Abstract

An outstanding question regards the ability of organisms to sense their environments and respond in a suitable way. Pathogenic bacteria in particular exploit host-temperature sensing as a cue for triggering virulence gene expression. This micro-review does not attempt to fully cover the field of bacterial thermosensors and in detail describe each identified case. Instead, the review focus on the time-period at the end of the 1990's and beginning of the 2000's when several key discoveries were made, identifying protein, DNA and RNA as potential thermosensors controlling gene expression in several different bacterial pathogens in general and on the *prfA* thermosensor of *Listeria monocytogenes* in particular.

KEYWORDS

Listeria monocytogenes, PrfA, RNA-thermosensors, thermosensor, virulence

1 | DISCOVERING HOW BACTERIA FEEL THE HEAT

All living organisms, from humans to bacteria, need to sense and respond to differences in temperature to be able to adjust their behaviour appropriately. Also, because temperatures ranging from 36 to 39°C constitute a hallmark of warm-blooded mammals, temperature sensing can be used to turn on specific regulatory programmes such as bacterial virulence gene expression. During the last two decades, bacterial pathogens were shown to sense differences in temperature by disparate mechanisms involving protein, DNA and RNA (Loh, Righetti, Eichner, Twittenhoff, & Narberhaus, 2018; Shapiro & Cowen, 2012). In all those different cases, temperature is detected through thermally induced structural changes.

In 1997, the laboratory of Mikael Rhen showed that the virulence-plasmid encoded transcriptional repressor TlpA in *Salmonella enterica* serovar Typhimurium harboured an intrinsic α-helical coiled-coil domain showing temperature-dependent structural alterations (Figure 1a) (Hurme, Berndt, Normark, & Rhen, 1997). At lower temperatures (<37°C), TlpA forms a stable coiled-coil structure allowing

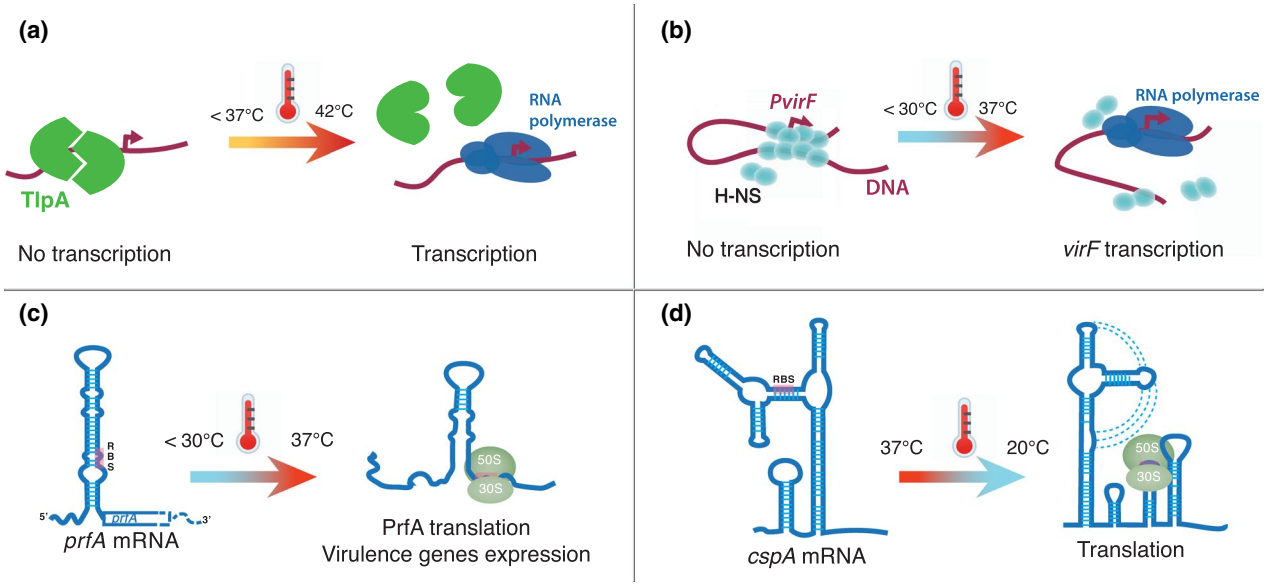

FIGURE 1 Bacterial thermosensors. (a) The TlpA protein thermosensor in *Salmonella enterica* serovar Typhimurium. At lower temperatures (<37°C), the TlpA transcriptional repressor (green) dimerises and binds DNA thereby blocking gene expression. At higher temperatures (>42°C) the coiled-coil domain in TlpA dissociates leading to reduced dimerisation and abrogated DNA binding. (b) Temperature sensing by the *virF* promoter region in *Shigella flexneri*. At low temperatures (<32°C) the *virF* promoter region (red line) adopts a conformation allowing binding of the transcriptional repressor H-NS (light blue) which prevent the *virF* expression. At higher temperatures (>37°C), the DNA restructures, preventing H-NS binding thus allowing *virF* expression. (c) The basic mechanism of *prfA* thermosensing in *Listeria monocytogenes*. The 5′ UTR of the *prfA* mRNA adopts at stem loop structure at low temperatures (<30°C), occluding the ribosome binding site (RBS, pink rectangle). At an increased temperature (~37°C), the hairpin melts, allowing ribosome (green) entry and translation of PrfA. (d) The *Escherichia coli cspA* thermosensor. At high temperatures (~37°C), the *cspA* 5′ UTR forms a structure preventing ribosome binding to bind RBS. At low temperatures (<20°C), the *cspA* 5′ UTR restructures making the RBS accessible for the ribosome

the protein to bind and repress gene expression. By binding to the *tlpA* promoter region, TlpA repress its own expression (Hurme, Berndt, Namork, & Rhen, 1996). TlpA has also been suggested to repress expression of other virulence-associated genes at low temperatures but its absence does not attenuate *S.* Typhimurium virulence (Gal-Mor, Valdez, & Finlay, 2006; Hurme et al., 1997). At an increased temperature (>42°C), the coiled-coil motif unfolds and TlpA is unable to bind DNA and repress gene expression. Additional examples of proteinaceous thermosensors have since then been identified in other pathogens such as the transcriptional regulator RovA in *Yersinia* which positively regulates expression of itself and the gene encoding Invasin, an outer membrane protein binding β1-integrins in the host (Herbst et al., 2009; Nuss et al., 2016; Revell & Miller, 2000). GmaR in *Listeria monocytogenes* acts as an anti-repressor, binding to the transcriptional repressor MogR (Kamp & Higgins, 2011). The GmaR-MogR interaction alleviates expression of motility genes at low temperatures, but due to the temperature-dependent conformational change in GmaR, the GmaR-MogR interaction weakens at high temperatures, leading to decreased motility gene expression.

In 1998, the laboratory of Claudio Gualerzi could show that temperature-induced structural alteration of DNA affects expression of the virulence regulator VirF in *Shigella flexneri* and enteroinvasive *Escherichia coli* (Figure 1b) (Falconi, Colonna, Prosseda, Micheli, & Gualerzi, 1998). At low temperatures (<32°C), the DNA comprising the *virF* promoter region adopts a curved conformation allowing binding of the transcriptional repressor H-NS. At higher temperatures, the DNA structure is altered preventing binding of H-NS. Consequently, the RNA-polymerase gets an increased access to the promoter region and *virF* transcription can commence. A later study has shown that *Clostridium perfringens* also exploits temperature-dependent alterations in the DNA structure to control expression of the gene encoding phospholipase C (Katayama, Matsushita, Jung, Minami, & Okabe, 1999).

Among thermosensors, RNA has been shown to be the most widespread, possibly due to its ability to easily structurally re-arrange. Already in 1989, Altuvia and coworkers showed that phage λ used temperature dependent structural alterations of an RNA-segment to decide whether to undertake a lysogenic or lytic pathway (Altuvia, Kornitzer, Teff, & Oppenheim, 1989). A study published in 1993 presented evidence that expression of the virulence regulators LcrF in *Yersinia pestis* was thermoregulated (Hoe & Goguen, 1993). Hints for RNA-based thermoregulation came from the observation that the amounts of *lcrF* transcript did not correlate with that of the LcrF protein at lower temperatures. In this particularly insightful study, Hoe and Goguen suggested that the Shine-Dalgarno (SD) region of the *lcrF* mRNA in *Yersinia pestis* could be occluded in an RNA hairpin structure at low temperatures. This hairpin would melt with an increased temperature thus allowing binding of the ribosome and translation initiation. Experimental demonstration of this mechanism would have to wait almost two decades when later studies showed that the core part of the hairpin

indeed exists and acts as an RNA thermometer in the closely related species *Yersinia pseudotuberculosis* (Bohme et al., 2012). In 1998, it was shown that translation of the heat shock sigma factor σ^{32} of *E. coli* was controlled by an RNA thermosensor lying partially in the coding region of *rpoH* (Morita et al., 1999). In 2001, the laboratory of Frans Narberhaus showed that expression of heat shock proteins in the plant symbiont *Bradyrhizobium japonicum* was regulated by 5′-UTR located RNA thermosensors, known as ROSE (repression of heat shock gene expression) (Nocker et al., 2001). The results from the latter studies suggested that the SD regions of both *rpoH* and ROSE became more accessible for the ribosome at high temperatures, due to melting of SD:anti-SD interactions. At lower temperatures, these interactions remained stable preventing binding of the ribosome.

In the beginning of the 2000's, the authors of this review, then in the group of Pascale Cossart, were involved in the identification and characterisation of the RNA thermosensor controlling PrfA expression, the master regulator of virulence gene expression in *L. monocytogenes* (Figure 1c). As a virulence regulator, PrfA activates the expression of virulence genes needed for bacterial attachment and uptake, escape from the phagosome, intracellular survival, cell-to-cell spread and lack of PrfA severely attenuates bacterial infectivity (de las Heras, Cain, Bielecka, & Vázquez-Boland, 2011; Freitag, Port, & Miner, 2009; Radoshevich & Cossart, 2018). We came to study temperature-dependent regulation of *prfA* expression because of peculiar phenotypes that had been observed in the literature and that still remained unexplained at the time. Indeed, it had previously been shown that expression of genes under *prfA* control were not expressed at temperatures below 30°C, whereas the *prfA* mRNA could still be detected (Leimeister-Wachter, Domann, & Chakraborty, 1992). A couple of years later, it was shown in the Cossart's lab that production of the PrfA protein itself could be detected at 37°C but not at low temperatures, reminiscent of LcrF of *Yersinia* (Renzoni, Klarsfeld, Dramsi, & Cossart, 1997). Following up on these observations, reading the latest literature at the library and trying several theories of why the PrfA protein levels were increased at high temperatures (e.g. increased protein stability at 37°C), we came to re-investigate post-transcriptional thermoregulation of *prfA* in the light of what was then the emerging concept of RNA thermosensors (Johansson et al., 2002).

A key observation was made by serendipity, when we could observe that *prfA* expression was still thermoregulated ectopically from a plasmid in *E. coli*, but not when using a plasmid used for PrfA purification where the 5′-UTR was deleted. This suggested that thermoregulation was mediated through the 5′-UTR of the mRNA and that most likely no other factor was needed. Consequently, by structural probing, we could show that the 5′-UTR of the *prfA* mRNA forms a stem loop structure that partially masks the ribosome binding site at 30°C. An increased temperature melts this structure giving access to the ribosome. Destabilising the *prfA* thermosensor structure by introducing point mutations in the stem caused expression of PrfA and of PrfA-regulated genes at lower temperatures, even to the point of rendering *L. monocytogenes* invasive to eukaryotic cells at 30°C, an otherwise non-permissive temperature (Figure 1c).

That a bacterial pathogen could 'know' when to activate its virulence genes simply through direct host-temperature sensing, and that this could be provided by a few nucleotides of RNA was assuredly an appealing idea. Despite being slightly more complicated than first anticipated, through small RNA binding and a possible ribosomal standby site in the coding region, the mechanism of *prfA* thermosensing still holds true as we proposed at the time (Johansson et al., 2002; Loh et al., 2009, 2012). In addition to *Listeria* and *Yersinia*, RNA thermosensors have now been found and characterised in a large number of pathogenic species, such as *Salmonella*, *Neisseria*, *Pseudomonas*, *Vibrio*, *Leptospira* and *Shigella* (Grosso-Becerra et al., 2014; Kouse, Righetti, Kortmann, Narberhaus, & Murphy, 2013; Loh et al., 2013; Matsunaga, Schlax, & Haake, 2013; Rinnenthal, Klinkert, Narberhaus, & Schwalbe, 2010; Weber, Kortmann, Narberhaus, & Klose, 2014).

RNA can be used not only to feel the heat, but also to feel the cold. As a difference to the modest structural differences in 'heat-induced' RNA thermosensors, quite substantial structural alterations are observed in 'cold-induced' RNA thermosensors. In *E. coli*, the 5′ UTR of the *cspA* transcript harbour an RNA thermosensor having a more accessible SD-region at low temperatures (Giuliodori et al., 2010; Zhang et al., 2018). At higher temperatures, the structure is extensively altered, preventing ribosome binding and translation (Figure 1d).

2 | FUTURE VISIONS—THE GLOBAL WARMING OF RNA THERMOMETERS?

How to identify new thermosensors? A fruitful approach might be to use new advantages in RNA sequencing to define the secondary structure of all transcripts in a bacteria (the 'structurome') at one temperature and compare it with another temperature. The Narberhaus laboratory has successfully used such an approach on RNA isolated from *Yersinia pseudotuberculosis* to identify and characterise more than a dozen new thermosensors (Righetti et al., 2016). Using a similar methodology but in vivo, we have recently identified a new 'cold sensing' thermosensor in *Listeria monocytogenes* controlling expression of a cold shock protein (Ignatov and Johansson, unpublished results).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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