Trends in Microbiology

CellPress OPEN ACCESS

Review

Temperature sensing and virulence regulation in pathogenic bacteria

Davide Roncarati [1,*], Andrea Vannini [1], and Vincenzo Scarlato [1]

Pathogenic bacteria can detect a variety of environmental signals, including temperature changes. While sudden and significant temperature variations act as danger signals that trigger a protective heat-shock response, minor temperature fluctuations typically signal to the pathogen that it has moved from one environment to another, such as entering a specific niche within a host during infection. These latter temperature fluctuations are utilized by pathogens to coordinate the expression of crucial virulence factors. Here, we elucidate the critical role of temperature in governing the expression of virulence factors in bacterial pathogens. Moreover, we outline the molecular mechanisms used by pathogens to detect temperature fluctuations, focusing on systems that employ proteins and nucleic acids as sensory devices. We also discuss the potential implications and the extent of the risk that climate change poses to human pathogenic diseases.

Highlights

Temperature is one of many environmental signals bacteria perceive. While a sudden temperature rise signals danger, pathogens very often exploit temperature changes to recognize the surrounding environment and trigger their virulence.

The success of this regulatory strategy is based on the ability of pathogenic bacteria to perceive even small temperature changes when transitioning between environments.

Temperature-regulated virulence genes code for effectors involved in survival and in all the major steps of the pathogenic process, including adhesion to host cells, motility, biofilm formation, immune evasion, and resistance.

Over the course of evolution, a remarkable array of temperature-sensing mechanisms has developed, involving all the major biological macromolecules including protein, DNA, and RNA.

Temperature is not always a danger signal: role of heat-shock proteins beyond protection

All organisms face environmental changes, including temperature variations. The ability to sense and respond to temperature is found in organisms from bacteria to mammals. For many microbes, such as viruses, archaea, bacteria, fungi, and parasites, temperature is a crucial signal affecting growth, development, and disease. These microbes encounter temperature changes due to seasons, global warming, interactions with various hosts, and fevers in infected hosts.

Following a temperature variation, whether an increase or decrease, cells respond by reprogramming gene expression, inducing the production of protective proteins, while slowing down several cellular processes. This response, named the heat-shock response since its discovery in 1962 by Ritossa in *Drosophila melanogaster* [1], is an ancient and highly conserved biological process, and is characterized by the upregulation of a group of proteins, mainly **chaperones** (see Glossary) and proteases – collectively designated heat-shock proteins (HSPs) – which play a protective role and preserve **proteome** homeostasis.

Focusing on bacterial pathogens, it is interesting to note that there are several examples where temperature-regulated HSPs not only assist in protein folding but also play a role in virulence, contributing to the pathogenic process. Indeed, it has been proposed that several pathogens use the major chaperones GroEL and DnaK as surface-exposed **adhesins** during interaction with host cells [2]. This concept is exemplified by *Mycoplasma pneumoniae*, a pathogen causing community-acquired infections in the human respiratory tract. It has been demonstrated that *M. pneumoniae* GroEL and DnaK can bind to human A549 cells, as well as to plasminogen, vitronectin, fibronectin, fibrinogen, lactoferrin, and laminin, suggesting a potential role for both chaperones in adhesion and dissemination during pathogen infections [3]. A similar scenario has been described for distant bacterial spp. like *Salmonella enterica*, *Neisseria meningitidis*, and *Listeria monocytogenes* [4–6]. Furthermore, these chaperones have been shown to trigger

[1]Department of Pharmacy and Biotechnology (FaBiT), Alma Mater Studiorum – University of Bologna, Bologna, Italy

*Correspondence: davide.roncarati@unibo.it (D. Roncarati).

specific signaling in the targeted host cells, such as inducing the synthesis of proinflammatory cytokines and promoting apoptosis [7]. The involvement of temperature-controlled HSPs in virulence and pathogenesis is not limited to the major chaperones GroEL and DnaK. In the highly infectious intracellular pathogen *Francisella tularensis*, the ClpB protein, a member of the Hsp100/Clp family, has two critical roles: it is essential for both heat-shock survival and the proper functioning of the type VI **secretion system**, essential for bacterial virulence [8].

Bacterial pathogens exploit the ambient temperature to recognize their surroundings and adapt their virulence strategy

In the aforementioned examples, where HSPs also play a role in virulence, evoke the concept that the prototypical heat-shock response is not limited to the recovery from sudden temperature stress. Instead, it overlaps and integrates with the capability of bacterial pathogens to perceive temperature fluctuations and adjust their pathogenic behavior appropriately. Several studies employing genome-wide functional genomics approaches based on microarrays and RNA sequencing demonstrated that, following a temperature variation, changes are observed in the abundance of several protein-coding transcripts involved in a variety of cellular processes, in addition to the maintenance of proteome homeostasis, such as metabolism, nutrient acquisition, and virulence. Examples of these include *Salmonella Typhimurium, Vibrio parahaemolyticus, Helicobacter pylori, Staphylococcus aureus, and Yersinia pestis* [9–14]. Furthermore, massive transcriptional reprogramming following a temperature change is observed when the microorganism possesses one or more alternative sigma factors whose regulatory activity depends on the ambient temperature. Several genome-wide studies have highlighted the regulatory cascade driven by these specific sigma factors, impacting the expression of proper **virulence factors** and traits associated with this process (such as nutrient acquisition, enhanced survival, etc.) [15–20]. Such an amount of experimental data allows us to state, as a general principle, that bacterial pathogens exploit host-temperature detection as a signal for triggering the expression of virulence factors, which are molecules that assist the bacterium in colonizing the host at the cellular level. These factors can be either cytosolic, secretory, or membrane-associated and they enable pathogens to attach to host cell surfaces, evade immune defenses, or acquire nutrients that might be otherwise inaccessible. The uncontrolled expression of these virulence factors can prove deleterious for bacteria, leading to the wasteful consumption of metabolic resources and the inappropriate induction of inflammatory and immune responses. Hence, bacteria have developed regulatory mechanisms to limit the expression of virulence factors until they perceive suitable environmental cues within their hosts.

Temperature transition upon host invasion triggers the regulation of crucial virulence traits

Bacterial pathogens experience a temperature shift as they transit from the external environment to the warm-blooded host (36–39°C in mammals), which typically exceeds the former. This variation acts as an invasion signal and prompts the thermally regulated expression of numerous bacterial virulence factors (Figure 1). The first step for bacterial colonization is the adhesion and interaction with host cells, and in many important pathogens this process is thermally controlled. *N. meningitidis* is a commensal of the human nasopharynx and can cause invasive diseases such as sepsis or meningitis [21]. The temperatures that *N. meningitidis* encounters during transmission, colonization, and invasion are significantly diverse, ranging from 25°C in the nasal vestibule to approximately 34°C in the nasopharynx. In addition, during the development of invasive disease, the pathogen meets a core body temperature of 37°C or higher with the febrile response to infection. Considering this complex scenario, it is not surprising that *N. meningitidis* uses temperature sensing to probe its surroundings and control the expression of adhesion factors and other virulence traits accordingly. A comparative proteomic analysis showed differential

Glossary

Adhesins: specialized proteins exposed on the surface of bacterial cells that facilitate adhesion by attaching to the surfaces of other cells or structures.
Capsule: a layer produced by the cell and located on the outside of a bacterium. It may consist of complex carbohydrates, organic acids, and proteins. It performs several functions such as protecting bacteria from toxic compounds and allowing them to escape the host's immune system.
Chaperones: a family of proteins involved in protein quality control by helping misfolded polypeptides refold correctly, preventing protein aggregation, or delivering misfolded or aggregated polypeptides to proteolytic degradation.
Curli: extracellular protein fibers produced by many bacteria, representing a key component of the extracellular matrix. They are produced under biofilm-forming conditions.
Cyclic di-GMP (c-di-GMP): a small signaling molecule formed by two guanosine monophosphate molecules linked by a cyclic phosphate group. It plays a crucial role in the regulation of various bacterial processes, including biofilm formation, motility, virulence, and the transition between sessile and motile lifestyles.
Motility: the ability of bacteria to move independently, typically towards or away from specific stimuli, using mechanisms like flagella-driven swimming or pili-mediated twitching. Cell-invading pathogens manipulate the host cell actin cytoskeleton for actin-based intracellular motility.
Nucleoid-associated proteins: small, basic, and highly abundant DNA-binding proteins that shape bacterial chromatin under changing environmental conditions and regulate gene expression in bacteria.
Persistence: the ability of a subpopulation of bacterial cells to survive exposure to lethal doses of antibiotic concentrations. It is an epigenetic phenomenon where certain bacterial cells become resistant without accumulating genetic mutations.
Proteome: the totality of proteins that is, or can be, expressed by an organism.
Ribosome binding site (RBS): a nucleotide sequence in the mRNA located six to nine nucleotides upstream of the translation start codon. It aids in positioning the first codon at the ribosome's P site to establish the correct reading frame. This occurs through

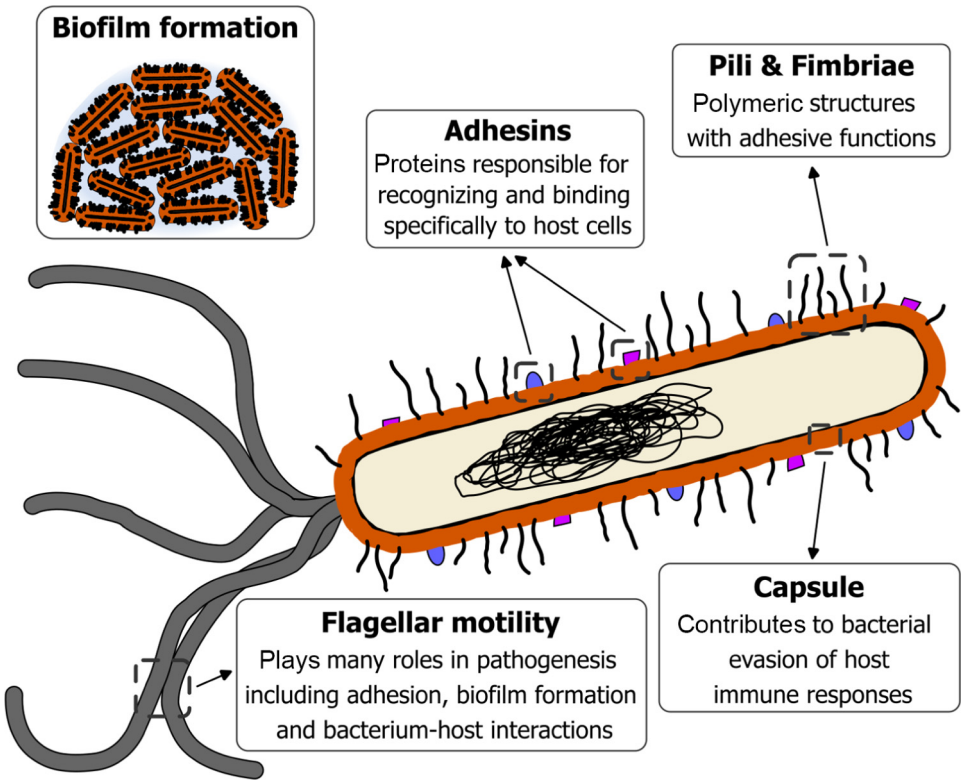

Figure 1. The regulation of key virulence traits is triggered by the temperature change during host invasion. Bacterial pathogens undergo a temperature change as they move from the external environment into their host. This shift serves as an invasion signal, prompting thermal regulation of various bacterial virulence factors, including capsule biosynthesis, flagellar motility, biofilm formation, adhesins and other adhesive structures.

expression of 375 proteins between 32°C and 37°C, predominantly involving those in the periplasm, outer and inner membranes [22]. Among these, the neisserial heparin binding antigen (NHBA), a surface-exposed lipoprotein with multiple functions including bacterial adhesion to epithelial cells [23], was one of the most strongly upregulated proteins at 32°C. Accordingly, the adhesion properties of *N. meningitidis* for mucosal colonization increased at 32°C compared with 37°C [22,24]. Overall, even slight temperature variations of just 5°C, beyond the dramatic shifts experienced in traditional heat shock, result in considerable alterations to the proteome and virulence traits of *N. meningitidis*. In other major pathogens, *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*, the trimeric autotransporter Yersinia adhesin A (YadA) is vital for virulence and its expression is temperature-regulated. When the bacterium enters the host and the temperature shifts to 37°C, YadA expression is triggered [25,26]. Similarly to what has just been described, temperature-dependent regulation of surface-exposed proteins and polymeric structures with adhesive functions (such as pili and fimbriae) has been observed in various pathogenic microorganisms, such as *L. monocytogenes*, pathogenic *Escherichia coli*, *Bordetella pertussis*, *Streptococcus pyogenes*, and others [27,28].

Closely related to adhesion to host cells, the ability to form biofilms is considered an essential factor in the pathogenesis of various bacteria (Figure 1). A biofilm consists of microbial cells growing as a community, adhering to surfaces and surrounded by a matrix of extracellular polymeric substances. The formation of biofilms represents a crucial defense strategy employed by

base-pairing with the 3′-end of the 16S ribosomal RNA in the small ribosomal subunit.
RNA structurome: the complete set of secondary and tertiary structures of all the RNAs expressed by an organism.
Secretion system: a protein complex located to the cell membranes that allows bacteria to translocate specific substrates across the cell envelope. Bacterial pathogens exploit these systems to secrete a broad range of substances (small molecules, proteins, and nucleic acids), which enable the bacterium to colonize and/or manipulate the host.
Transcriptional regulator: a DNA-binding protein that regulates gene transcription in bacteria. Transcriptional regulators bind to specific DNA sequences located in the vicinity of genes' promoters and then either upregulate or downregulate transcript initiation by RNA polymerase.
Virulence factors: molecules, cellular structures, and regulatory systems that assist the bacterium to infect the host, cause disease, and evade the host's immune response. They can be cytosolic, membrane-associated, or secretory.

the majority of microorganisms, offering numerous benefits to cells, such as high infectivity, antibiotic resistance, and **persistence** [29,30]. Biofilm formation is a process heavily influenced by environmental factors, including temperature. It has been reported that temperature affects the formation of biofilm in several pathogens, including *Pseudomonas aeruginosa*, *Burkholderia pseudomallei*, *Vibrio cholerae*, *L. monocytogenes*, *Klebsiella pneumoniae*, and *Campylobacter jejuni* [31–37]. For example, the *rspA* gene of *Salmonella* serovar Typhimurium regulates biofilm formation at different temperatures by modulating the genes involved in the synthesis of cellulose and **curli**, two major biofilm components [38]. However, it is important to consider that bacterial biofilm formation is influenced by numerous factors besides temperature. This could explain the sometimes-conflicting observations regarding the effect of temperature on biofilm formation between assays conducted *in vitro* and *in vivo.*

Another cellular process that impacts bacterial virulence is **motility**, which provides the pathogen with the ability to reach the optimal colonization niche, to maintain and to disperse at the end of the infection cycle [39]. The host's temperature is exploited as a signal to modulate the expression of flagellar genes and, hence, the motility phenotype (Figure 1). For example, the foodborne pathogenic Gram-positive bacterium *L. monocytogenes* employs two different locomotion modes at different environmental temperatures. At 30°C or below (i.e*.,* outside the human host), it uses rotating flagella to move, while at 37°C (i.e*.,* inside the human host), it adopts an actin-based motility. This switch in motility behavior is based on the temperature-coordinated transcriptional regulation of flagellar genes by the repressor MogR and the antirepressor GmaR [40,41]. Specifically, at low temperatures, GmaR antagonizes the repressive function of MogR on the transcription of flagellar genes, allowing the generation of flagella. However, at 37°C GmaR self-aggregates, reducing its ability to neutralize MogR, which then represses the expression of flagellar genes [42]. In *Campylobacter*, the flagellum is made up of two structural proteins, FlaA and FlaB. FlaB gene expression is regulated by a sigma54-dependent promoter and is higher at 42°C compared with 37°C, which enhances flagellar motility at the higher temperature. Additionally, FlaA biosynthesis also peaks at 42°C, further contributing to increased motility, which aligns with the core temperature of the avian host of *Campylobacter* spp. [43,44]. Additional examples of flagella biosynthesis orchestrated by alternative sigma factors in response to changing environmental temperature have been described in several bacterial species, including *E. coli* and *Yersinia* [45–47].

At 37°C, the typical temperature of warm-blooded hosts, several pathogens upregulate crucial virulence factors for defense against the immune system, while also adjusting the expression of molecules that could potentially notify the host of their presence. *S. enterica* serovar Typhi is a human-restricted pathogen that causes typhoid fever and is known for its 'stealthy behavior', meaning that once inside the human body it hides from recognition by the immune system and spreads systemically [48]. The ability to evade immune detection results from the coordinated modulation of virulence factors' expression in response to environmental temperature. At the human core temperature of 37°C, *S.* Typhi produces a greater amount of Vi **capsule** compared with lower temperatures. This molecule envelops the bacterial surface, acting as a virulence factor by inhibiting complement-mediated killing and enhancing resistance against phagocytosis (Figure 1) [49]. Concurrently, at 37°C *S.* Typhi inhibits the expression of flagellin, which otherwise would trigger a potent activation of the immune system. Intriguingly, this opposite temperature-dependent regulation of virulence factors is exerted by the unique *S.* Typhi **transcriptional regulator** TviA, whose mRNA translation efficiency depends on temperature levels [50]. A similar strategy is adopted by *Y. pestis*, the etiologic agent of the plague. As the pathogen transits from ambient temperature to 37°C, it induces the expression of Caf1 capsular antigen, which protects *Y. pestis* from macrophage engulfment [51]. This temperature-dependent regulation is mediated by the dedicated transcriptional regulator Caf1R [52].

The thermal control of virulence factors by pathogenic bacteria can take place with a coordinated or individual strategy

The examples described earlier illustrate various strategies for achieving thermoregulation of virulence factors. In several cases, pathogens regulate the expression of multiple virulence genes in a coordinated manner by utilizing a single transcriptional regulator. When the expression or activity of this regulator is temperature-dependent, the pathogen can globally adjust the expression of virulence factors in response to the thermal signal, thereby adapting to the novel environment encountered upon entering the host. In *Yersinia* spp., the global virulence regulator RovA, which can act as both a repressor and an activator of transcription, controls numerous virulence genes and is essential for host colonization. In detail, RovA performs a complex regulatory function, directly repressing the transcription of genes involved in biofilm formation such as *hmsT*, a strong biofilm determinant [53–55], and stimulating transcription of the *psaABC* and *psaEF* genes [56] (which respectively code for a cell-surface antigen-adhesin and its transcriptional activators [57]). This protein is sensitive to even narrow temperature fluctuations: at the host body temperature of 37°C, RovA DNA-binding activity – and thus its regulatory function – is impaired. Furthermore, proteolytic degradation of RovA is enhanced at higher temperatures, impacting the stability of the protein [58]. This strategy enables *Yersinia* to coordinate several aspects of virulence according to the perceived temperature [59]. A similar scenario is observed in the pathogen *L. monocytogenes*, where the positive regulator PrfA is crucial for activating the expression of virulence factors, including several protein-encoding genes and at least one regulatory small-RNA, all related to intracellular survival and cell-to-cell spread (Figure 2A) [60].

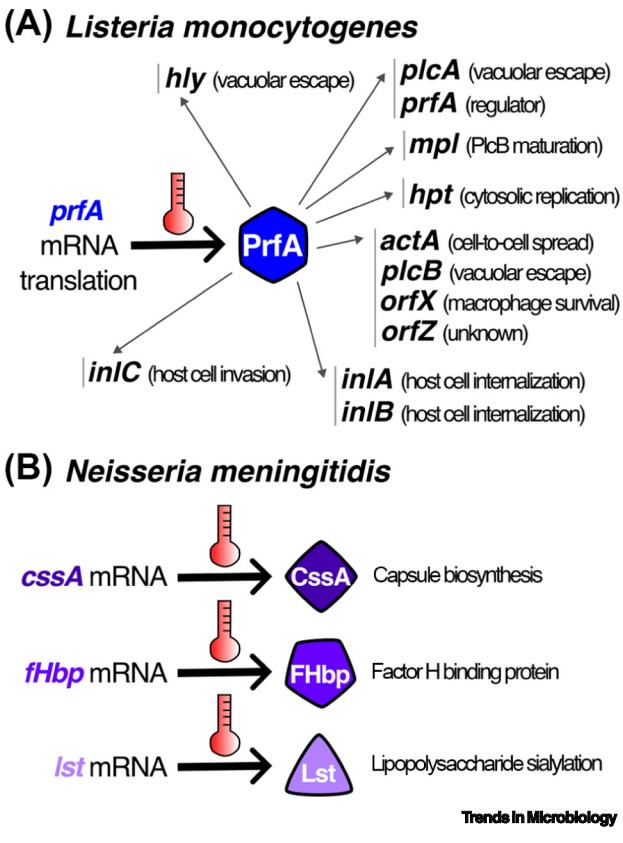

Figure 2. Pathogenic bacteria use various strategies to regulate the expression of virulence factors in response to temperature variations. (A) A single thermosensitive master regulator controls the expression of a cohort of virulence genes. In *Listeria monocytogenes*, temperature variation (symbolized by a red thermometer) affects the translation efficiency of the mRNA encoding PrfA, the master regulator of virulence. The latter controls the transcription of genes coding for virulence factors, including those for cell internalization/invasion, survival inside macrophages, vacuolar escape, and cell-to-cell spread. (B) An alternative thermoregulation strategy: temperature independently affects the regulation of different genes. In *Neisseria meningitidis*, a moderate temperature rise above 37°C impacts independently the mRNA translatability of three genes involved in bacterial resistance against immune killing. In detail, *cssA* codes for the UDP-*N*-acetylglucosamine 2-epimerase enzyme, which catalyzes polysialic acid capsule biosynthesis to prevent detection by the immune system; *fHbp* encodes the Factor H binding protein, providing protection against complement; *lst* codes for a sialyltransferase, an enzyme that transfer sialic acid to surface oligosaccharides, thus preventing killing by complement and neutrophils.

Intriguingly, PrfA expression is temperature-regulated, with minimal levels below 30°C and significantly increased expression above 37°C. Unlike RovA, temperature enhances PrfA mRNA translation, rather than affecting protein activity and stability [61].

However, in some cases, virulence genes are controlled in a temperature-dependent manner without the involvement of temperature-sensitive master regulators, relying instead on direct and individual regulatory mechanisms. For instance, in *N. meningitidis*, temperature independently affects the expression of three genes (*cssA*, *fHbp,* and *lst*) involved in resistance against immune killing (Figure 2A). Specifically, the mRNA of these genes is translated with different efficiency depending on the environmental temperature [62]. Notably, the translatability of these mRNAs changes within a narrow temperature range around and above 37°C, as occurs in the upper airways during infection and upon inflammation. This example highlights that pathogens have evolved regulatory systems that enable them to modulate the expression of virulence factors not only in response to stark temperature increases (as in the transition from an external to an internal host environment), but also following much smaller fluctuations, such as the transition between different hosts, different host tissues, or from physiological temperature to one altered by a febrile-inflammatory state [63].

How pathogenic bacteria are able to perceive temperature changes

The capacity of pathogens to promptly react to temperature fluctuations relies on heat-detecting systems that can perceive environmental signals and trigger suitable response pathways. Given the importance of this signal, a remarkable arsenal of temperature-detecting mechanisms has developed throughout evolution. At the molecular level, each biomolecule reacts to temperature fluctuations through conformational alterations, thus acting as a temperature sensor within the cellular apparatus. To date, many reports have shown the involvement of all major cellular biomolecules in detecting temperature changes and modulating virulence gene expression, including proteins and nucleic acids.

Protein sensors

Proteins are often employed by bacterial pathogens to detect temperature fluctuations and modulate the expression of virulence genes. Generally, an increase in temperature imposes a conformational change in the sensor protein, affecting its activity, stability, or interaction with other functional partners. Such structural transitions are often reversible and typically affect limited regions of the protein.

In cases where the transcriptional regulator of a virulence gene is itself sensitive to temperature changes, there is a direct coupling of temperature sensing with gene regulation (Figure 3A). In the pathogen *S. enterica* serovar Typhi, temperature alters the DNA-binding capability of the coiled-coil domains in their oligomerized form of the virulence regulator TlpA, thereby affecting its regulatory function [64]. Similarly, small fluctuations in temperature influence the DNA-binding activity and stability of the master regulator of virulence genes RovA in *Yersinia* spp. (see previous text). Notably, this strategy of direct gene regulation by temperature-sensitive transcription factors is also used to regulate the classical heat-shock response, as exemplified by the widespread transcription regulators of HSPs, such as HrcA, CtsR, and RheA, whose activity or stability is dependent on environmental temperature. The mechanisms underlying the temperature-dependent regulation carried out by these regulators have been demonstrated for various bacterial pathogens, such as *H. pylori, C. jejuni*, and *Chlamydia trachomatis* for HrcA, *Bacillus subtilis* and *L. monocytogenes* for CtsR, and finally *Streptomyces albus* for RheA [64–71]. It is worth considering that not only the aforementioned heat-shock repressors are used by bacteria as temperature-dependent regulators. Indeed, in some cases, bacteria use alternative sigma

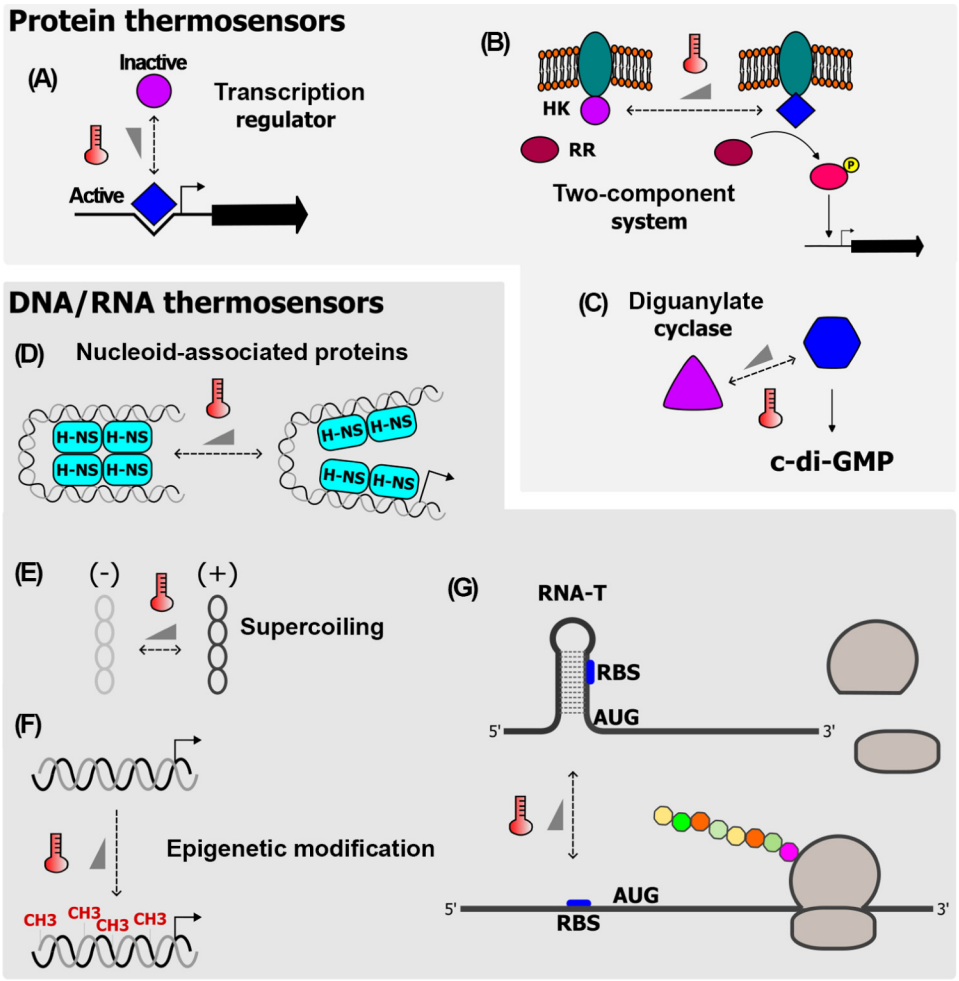

Figure 3. Mechanisms of temperature sensing. Temperature changes can be detected through protein-based (A–C) or nucleic acid-based (D–G) sensors. (A) Transcriptional regulators as temperature sensors. Intrinsic temperature-sensing transcription regulators interconvert between a DNA-binding competent state (blue square) and an inactive isoform (pink circle). The TlpA regulator of *Salmonella enterica* serovar Typhimurium and the RovA transcription factor of *Yersinia* spp. represent primary examples. Throughout the whole figure, the double-headed arrow indicates the reversibility of the structural transition, while the ascending gray triangle indicates an increase in temperature. (B) Two-component systems can combine the detection of temperature changes with the regulation of virulence genes. In *Edwardsiella tarda*, temperature variation triggers a conformational change in the PhoQ membrane-associated histidine kinase (HK, the inactive conformation is represented as a pink circle, with the active isoform shown as a blue square), affecting the phosphorylation and DNA-binding capacity of the PhoP response regulator (RR). (C) In *Pseudomonas aeruginosa*, the enzymatic activity of the c-di-GMP-producing diguanylate cyclase TdcA is affected by temperature fluctuations. (D) Temperature-dependent regulation of *virF* transcription in *Shigella flexneri* involves changes in DNA supercoiling. At low temperatures, the DNA region between the H-NS binding sites bends, enabling interactions between attached H-NS proteins and creating a repressive complex. As temperature rises, this curvature diminishes, disrupting the complex and making the promoter more accessible to RNA polymerase. (E) Temperature impacts DNA supercoiling, especially for plasmids undergoing different topological states. Transcription efficiency is affected by alterations in DNA supercoiling, and temperature changes can impact gene expression, including virulence traits. (F) Temperature can affect the methylation of specific genomic regions. If such methylated sequences (CH3) fall within gene regulatory regions, gene expression would be affected. In *Yersinia enterocolitica* several Dam sites show temperature-dependent methylation patterns within gene regulatory regions. In this case, there are as yet no experimental data on the reversibility of the process. (G) RNA sensing involves temperature-sensitive secondary structures in the mRNA 5'-region. Lower temperatures facilitate structure

(Figure legend continued at the bottom of the next page.)

factors, whose expression is temperature-dependent, to thermoregulate the transcription of entire classes of genes, including virulence-associated genes (reviewed in [72]).

In other situations, the temperature-responsive protein is not the transcriptional regulator directly controlling virulence genes, but rather a component involved in the upstream regulatory cascade. This regulatory strategy is also known as thermotransduction, a process wherein temperature is detected by a sensory receptor, starting a signaling cascade that changes cellular physiology. For example, in the opportunistic human pathogen *Edwardsiella tarda*, the activation of type III and VI secretion systems, essential for virulence, is controlled by the PhoP–PhoQ two-component system and is dependent on environmental temperature. Two-component systems typically consist of a histidine kinase that perceives a specific environmental signal and a corresponding DNA-binding response regulator, responsible for modulating target gene transcription (Figure 3B). In *E. tarda*, the PhoQ sensor kinase is temperature-sensitive and its secondary structure undergoes a conformational rearrangement above a certain temperature. This, in turn, affects PhoP phosphorylation and consequently its regulatory function, resulting in activation of the secretion systems within the optimal temperature range of 35°C to 37°C [73,74]. A second example concerns the recent characterization of the temperature-dependent diguanylate cyclase enzyme TdcA in the pathogen *P. aeruginosa* (Figure 3C). This enzyme produces the second messenger **cyclic di-GMP** (**c-di-GMP**) with catalytic rates increasing more than 100-fold over a temperature change of approximately 10°C [75]. In turn, c-di-GMP levels regulate multiple virulence traits, including biofilm formation, motility, phage resistance, and expression of virulence factors [76–81]. Notably, TdcA orthologs are widespread among bacteria and can be regulated by other environmental signals [75,82]. The thermosensitive catalysis of this pivotal second messenger, highly implicated in virulence, represents a novel mechanism of temperature sensing within bacterial pathogens.

Nucleic acid sensors

Bacterial pathogens can employ nucleic acids to detect temperature variations and other environmental signals that impact the DNA's global structural and topological state, including local curvature. DNA supercoiling and local structure can affect gene transcription by modulating the binding of RNA polymerase and other transcription-related factors (Figure 3D,E). Consequently, DNA can function as a thermometer, detecting temperature stress during heat-shock. Even small temperature changes, such as those occurring during pathogens' invasion of the host, can be detected using DNA as a sensor (reviewed in [83,84]). This mechanism generally relies on the temperature-dependent differential binding of **nucleoid-associated proteins**, such as H-NS, that lose their DNA-binding ability to specific DNA domains as the temperature rises to 37°C, consequently regulating specific sets of genes in response to temperature (Figure 3D) [85,86]. In this respect, the most well-characterized examples concern the H-NS-mediated thermoregulation of a type III secretion system in *S. enterica* and of the invasion regulator VirF in *Shigella flexneri* [87,88]. Interestingly, a recent article describes temperature-dependent differences in DNA methylation affecting gene expression in the pathogen *Y. enterocolitica* (Figure 3F) [89]. Specifically, several Dam sites display temperature-dependent methylation patterns within gene regulatory regions, and some of these genes appear to be temperature-regulated. Although further research is needed to validate these findings, a mechanism based on temperature-dependent epigenetic modification could represent an alternative way of perceiving the environment, involving chemical alteration of DNA sequences rather than structural changes.

formations, masking sequence elements essential for efficient initiation of translation. When the temperature rises, the structure undergoes a rearrangement or resolution, exposing these sequence elements and enhancing translation. This kind of regulatory element is widespread among several pathogens, including Gram-positives (*Listeria monocytogenes*), and Gram-negatives (*Vibrio cholerae, Neisseria meningitidis, Yersinia* spp. and *P. aeruginosa*). Abbreviations: AUG, translation start codon; RBS, ribosome binding site.

The most rapid strategy for altering gene expression in response to temperature fluctuations relies on a *cis*-regulatory element within the mRNA encoding the target protein. Such temperature-responsive RNAs, called RNA-thermometers (RNA-T) or -thermosensors, are RNA control elements located in the 5′-untranslated region (UTR) of heat-shock and virulence genes. The basic mechanism consists in the formation of zipper-like temperature-sensitive secondary structures within the mRNA, particularly in the 5′ region. These structures hinder crucial translation initiation sequence elements, like the **ribosome binding site** (**RBS**), preventing mRNA translation. As temperature increases, the higher thermodynamic energy induces a rearrangement and partial melting of the RNA structure, unmasking the RBS and enhancing mRNA translation (Figure 3G) [90,91]. All the RNA-Ts discovered so far regulate gene expression primarily at the translational level. Nonetheless, regulatory elements were engineered to exploit the temperature-dependent stabilization of secondary RNA structures to control transcription termination in *E. coli* (Box 1) [92].

Several RNA-Ts have been identified and studied in detail, including very complex secondary structures involving >200 nucleotides and much simpler versions, consisting of compact stem-loops. Initially, RNA-Ts were discovered as regulatory elements for temperature-dependent expression of HSP. Subsequently, it became clear that RNA-T extended beyond this role, enabling the thermal-dependent modulation of virulence-associated genes in many pathogenic bacteria. RNA-T provide a rapid response mechanism: the mRNA coding for a virulence factor may be already present within the cell, albeit in an inactive conformation, even before host invasion (i.e., at low temperature). Upon bacterial invasion into the host, the higher temperature triggers conformational changes in the RNA-T, leading rapidly to virulence factor synthesis. Additionally, depending on the extent and base composition of the secondary structure sequestering the RBS, several RNA-Ts have the ability to sense temperature changes of as little as 1°C.

Box 1. Engineered protein- and RNA-based thermosensors as tunable switches for synthetic circuit design

Synthetic biology employs artificial biological tools to manipulate complex systems, essentially through the development of sophisticated gene circuits and biological modules. A key aspect of this approach is designing and developing tools for conditional gene expression, with temperature-responsive biological molecules being particularly useful for this purpose. Using temperature to induce gene expression offers several advantages: it is cost-effective, requires simple instrumentation, avoids the needs for ligands (posing minimal contamination risk), and is reversible.

Many efforts have been devoted to the development of temperature-responsive tunable bioswitches exploiting bacteria- and phage-derived protein- and RNA-based sensors. A prominent example concerns the engineering of the λ-phage cI regulator, which binds as a homodimer to its cognate operator sites. While the wild-type regulator is not affected by temperature, a cI isoform harboring the A66T mutation (called cI857) gains temperature sensitivity due to unstable dimer formation at temperatures above 37°C. The cI857 repression system is valuable for creating genetic circuits with bifunctional dynamic control [113], and additional cI857 variants can also be utilized for temperature-sensitive transcriptional activation [114]. A similar approach has been used to develop an analogous tunable thermal system based on *Salmonella* heat-shock repressor TlpA [115]. RNA thermometers (RNA-Ts) are also being employed for the development of temperature-sensing bioswitches and applied to gene regulation embedded in regulatory circuits. These temperature-responsive riboregulators offer some advantages over protein-based tools: unlike the latter, RNA-Ts typically have a simple structure and mode of action, based on the rapid rearrangement of their secondary and tertiary structures upon temperature change. Furthermore, being a single-stranded nucleic acid, RNA-Ts can be easily engineered to optimize features crucial for regulatory circuits. In particular, genetic engineering to introduce specific base substitutions can be applied to modulate the 'on/off' temperature (i.e., the temperature range, broader or narrower, within which the structure of the RNA-T changes, affecting downstream gene expression) and to modify the way in which the structure is modified by temperature (gradual melting or sharp on/off response). This approach has been followed to implement RNA-only tools to induce or repress gene expression by changing the growth temperature [116–118]. Synthetic RNA-Ts have also been used to build temperature-responsive transcription terminators [70].

Recently, applications of synthetic RNA-Ts in diagnostics and therapeutics have been proposed. For example, two synthetic thermometers, active between 32°C and 46°C, can be used in different in vivo situations to combat infections, such as detecting and responding to host fever and restricting microbial survival to host body temperature, causing self-destruction at room temperature to prevent environmental contamination [115]. Another example concerns a recent study in which an RNA-T-like structure was included in the engineering of bacteria for use in cancer therapy. In brief, the temperature-activated RNA-T-like element delivered a sustained therapeutic output following brief application of focused ultrasound hyperthermia [119].

Nowadays, numerous virulence-associated RNA-Ts have been identified in different bacterial pathogens, mediating the thermoregulation of their mRNAs, such as the aforementioned PrfA in *L. monocytogenes* and CssA, FHbp, Lst in *N. meningitidis* [93]. A major example of an RNA-T coordinating virulence as a consequence of environmental temperature variations can be found in *V. cholerae*. It is a facultative human pathogen residing in aquatic reservoirs. Once inside the host, *V. cholerae* initiates a virulence program that is dependent on the concerted action of different transcriptional regulators, with a major role played by ToxT. This factor possesses an RNA-T in the mRNA 5′-region that folds at temperature below 37°C, impeding efficient *toxT* mRNA translation. At the host temperature of 37°C, the RBS is more exposed, allowing ribosome binding and ToxT expression [94]. Recent studies have shown that in *Yersinia* species, several RNA-Ts combine to thermally control the synthesis, assembly, and functionality of a type III secretion system involved in interfering with the host immune system. Specifically, different RNA-Ts control the translation of virulence master regulator LcrF [95], of the gatekeeper protein YopN [96], and of the structural T3SS components YscJ and YscT [97]. Moreover, while *yopN* RNA-T canonically resides in the 5′-UTR region, RNA-T controlling *yscJ* and *yscT* genes fall within intercistronic regions, extending complexity to this class of regulators. Another very significant example concerns the thermoregulation of the *P. aeruginosa* transcription regulator RlhR, which is involved in the regulation of quorum-sensing. The rhlR gene is part of a tricistronic operon together with two other genes, *rhlAB*, involved in rhamnolipid synthesis. An RNA-T in the 5′ UTR of the first open reading frame, *rhlA*, regulates *rhlR* expression by affecting transcription elongation of the operon. At temperatures below 37°C, the RNA-T suppresses *rhlA* translation, leading to premature transcription termination due to the formation of secondary RNA structures within the *rhlA* coding region. As a result, the downstream genes *rhlB* and *rhlR* are not transcribed. At 37°C, the RNA-T structure denatures, permitting *rhlA* translation and the complete transcription of the tricistronic operon [98].

In recent years, we have witnessed a rapid advancement in genome-wide methodologies for identifying novel RNA-Ts, exploiting their secondary structures. Essentially, transcriptome-wide probing integrates structure probing techniques with next-generation sequencing to uncover RNA structural alterations across various temperatures, defining the temperature-responsive *in vivo* **RNA structurome** of bacterial pathogens [99–101]. Despite the diversity and poor conservation of RNA-T nucleotide sequences and secondary structures, bioinformatic tools have been used to identify novel RNA-Ts across multiple genomes [102–104]. The recently developed Robo-Therm tool combines an adaptive and user-friendly *in silico* motif search with a well-established reporter system [105].

It is important to note that thermosensory RNA elements are not exclusively present in pathogenic bacteria, but can also be found in other organisms, including eukaryotes. However, in the latter, the mechanisms underlying their regulation are only beginning to be understood (for more details, see review [106]).

Concluding remarks and future perspectives

Pathogenic bacteria employ diverse mechanisms to adapt their lifestyle to changing environmental conditions of the specific niches they inhabit during various stages of disease progression. While HSPs shield bacteria from abrupt temperature changes via a homeostatic mechanism, numerous pathogens utilize temperature signals, and other cues, to modulate the production of virulence factors within their host environments.

When considering the intimate link between temperature and virulence, we cannot ignore the implications that climate change and rising environmental temperatures may have on the

Outstanding questions

In some pathogens, HSPs have been shown to play a role in virulence, linking it closely to stress responses. How widespread is this phenomenon in the bacterial world?

What other regulatory systems, triggered by environmental signals other than temperature, can intertwine with thermoregulation to achieve complex control of virulence factors?

How do thermosensing systems operate in other pathogenic microorganisms (like parasites and fungi) in regulating the temperature-dependent expression of virulence traits?

Are RNA-Ts involved only in the regulation of HSP and virulence genes, or are they also involved in other cellular processes/responses?

To what extent can the climatic variations observed on Earth significantly impact the expression of virulence traits and thus benefit the success of pathogens?

virulence of pathogens. Although there is a general consensus that climate change can impact human pathogenic diseases through several mechanisms, the full extent of this risk remains poorly quantified (see Outstanding questions). Indeed, a recent study based on a systematic literature review of empirical observations showed that climatic hazards can aggravate over 58% of pathogenic human diseases [107]. The results of this study allow us to expand the discussion on pathogenic organisms that may benefit from the climatic changes we are observing. Human pathogenic diseases exhibit extensive taxonomic diversity (including bacteria, viruses, animals, plants, fungi, protozoa, etc.) and various transmission types (such as vector-borne, airborne, direct contact, and more). However, to broaden our perspective further, it is of primary importance to understand the impact of climate change on environmental pathogenic microorganisms that affect plants and animals. A more in-depth comprehension of the thermoregulatory mechanisms in these pathogens will shed light on this issue (see Outstanding questions). Indeed, apart from fostering interactions between individuals and pathogens, climatic hazards can also bolster particular facets of pathogens, like increased reproduction, accelerated life cycles, prolonged exposure periods, and enhanced virulence [107]. For example, it was recently proposed that the fungus *Candida auris*, which normally infects only organisms with a low body temperature, such as reptiles and amphibians, has developed the ability to adapt and survive at higher temperatures (above 37°C). This adaptation has the potential to break the thermal exclusionary zone that otherwise protects humans from infection [108]. Indeed, throughout this review we have often reported that temperature increases enhance the virulence of pathogenic bacteria. Of course, it can be assumed that rising ambient temperatures affect the behavior of the pathogen not once it has already gained access to the host, but in its environmental reservoir. Interestingly, a recent report showed that elevated seawater temperature significantly impacts the expression of genes implicated in adhesion and biofilm formation on plastic surfaces of the marine bacterium *V. parahaemolyticus*, which includes human pathogenic strains [109]. The increased adhesion properties of this pathogen due to elevated water temperature emphasizes the role of climate change in the diffusion of this microorganism. In the context of the aquatic environment, additional factors may contribute to the success of pathogenic microorganisms. For example, *Vibrio vulnificus*, an opportunistic bacterial pathogen that is found in warm and low-salinity waters, can benefit from climate change in multiple ways. In addition to thriving in elevated water temperatures, temperature-induced desalination is enabling *V. vulnificus* to spread in novel marine regions that were previously inhospitable due to high salinity [110]. Meanwhile, increasing temperatures have been shown to significantly impact vector-borne diseases in terrestrial ecosystems. Since vectors are ectothermic (i.e., the regulation of their body temperature depends on external sources), changes in environmental temperature can influence their reproduction, survival, distribution, and ultimately their capacity to transmit pathogens [111]. For instance, rising temperatures have been found to speed up tick development and support the northward migration of tick-carrying mammals, such as the white-footed mouse, which has been linked to the spread of *Borrelia burgdorferi*, the bacterium responsible for Lyme disease, into novel regions [112].

Even though much remains to be understood about the complex interconnections between global warming and pathogenic microorganisms, the observations and available data depict an alarming scenario. They compel us to deepen our studies in this area and to take urgent measures to mitigate climate change.

Declaration of interests

The authors declare no competing interests.

References

1. Ritossa, F. (1962) A new puffing pattern induced by temperature shock and DNP in drosophila. *Experientia* 18, 571–573
2. Henderson, B. and Martin, A. (2011) Bacterial virulence in the moonlight: multitasking bacterial moonlighting proteins are

virulence determinants in infectious disease. *Infect. Immun.* 79, 3476–3491
3. Hagemann, L. *et al.* (2017) The surface-displayed chaperones GroEL and DnaK of *Mycoplasma pneumoniae* interact with human plasminogen and components of the extracellular matrix. *Pathog. Dis.* 75, ftx017
4. Ensgraber, M. and Loos, M. (1992) A 66-kilodalton heat shock protein of *Salmonella typhimurium* is responsible for binding of the bacterium to intestinal mucus. *Infect. Immun.* 60, 3072–3078
5. Knaust, A. *et al.* (2007) Cytosolic proteins contribute to surface plasminogen recruitment of *Neisseria meningitidis*. *J. Bacteriol.* 189, 3246–3255
6. Schaumburg, J. *et al.* (2004) The cell wall subproteome of *Listeria monocytogenes*. *Proteomics* 4, 2991–3006
7. Fourie, K.R. and Wilson, H.L. (2020) Understanding GroEL and DnaK stress response proteins as antigens for bacterial diseases. *Vaccines (Basel)* 8, 773
8. Alam, A. *et al.* (2020) Dissociation between the critical role of ClpB of *Francisella tularensis* for the heat shock response and the DnaK interaction and its important role for efficient type VI secretion and bacterial virulence. *PLoS Pathog.* 16, e1008466
9. Sirsat, S.A. *et al.* (2011) Effect of sublethal heat stress on *Salmonella Typhimurium* virulence. *J. Appl. Microbiol.* 110, 813–822
10. Han, Y. *et al.* (2004) Microarray analysis of temperature-induced transcriptome of *Yersinia pestis*. *Microbiol. Immunol.* 48, 791–805
11. Roncarati, D. *et al.* (2019) *Helicobacter pylori* stress-response: definition of the HrcA regulon. *Microorganisms* 7, 436
12. Li, L. *et al.* (2022) RNA-seq analysis unveils temperature and nutrient adaptation mechanisms relevant for pathogenicity in *Vibrio parahaemolyticus*. *Aquaculture* 558, 738397
13. Bastock, R.A. *et al.* (2021) *Staphylococcus aureus* responds to physiologically relevant temperature changes by altering its global transcript and protein profile. *mSphere* 6, e01303–20
14. Pepe, S. *et al.* (2018) The *Helicobacter pylori* heat-shock repressor hspr: definition of its direct regulon and characterization of the cooperative DNA-binding mechanism on its own promoter. *Front. Microbiol.* 9, 1887
15. Kazmierczak, M.J. *et al.* (2003) *Listeria monocytogenes* sigma B regulates stress response and virulence functions. *J. Bacteriol.* 185, 5722–5734
16. Kim, H. *et al.* (2004) *Listeria monocytogenes* sigmaB contributes to invasion of human intestinal epithelial cells. *Infect. Immun.* 72, 7374–7378
17. Bischoff, M. *et al.* (2004) Microarray-based analysis of the *Staphylococcus aureus* sigmaB regulon. *J. Bacteriol.* 186, 4085–4099
18. Rachid, S. *et al.* (2000) Alternative transcription factor sigma(B) is involved in regulation of biofilm expression in a *Staphylococcus aureus* mucosal isolate. *J. Bacteriol.* 182, 6824–6826
19. Suh, S.J. *et al.* (1999) Effect of rpoS mutation on the stress response and expression of virulence factors in *Pseudomonas aeruginosa*. *J. Bacteriol.* 181, 3890–3897
20. Whiteley, M. *et al.* (2000) Regulation of quorum sensing by RpoS in *Pseudomonas aeruginosa*. *J. Bacteriol.* 182, 4356–4360
21. Rosenstein, Nancy E. *et al.* (2001) Meningococcal disease. *N. Engl. J. Med.* 344, 1378–1388
22. Lappann, M. *et al.* (2016) Impact of moderate temperature changes on *Neisseria meningitidis* adhesion phenotypes and proteome. *Infect. Immun.* 84, 3484–3495
23. Vacca, I. *et al.* (2016) Neisserial heparin binding antigen (NHBA) contributes to the adhesion of *Neisseria meningitidis* to human epithelial cells. *PLoS One* 11, e0162878
24. Borghi, S. *et al.* (2022) Multilayer regulation of *Neisseria meningitidis* NHBA at physiologically relevant temperatures. *Microorganisms* 10, 834
25. Skurnik, M. and Toivanen, P. (1992) LcrF is the temperature-regulated activator of the yadA gene of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. *J. Bacteriol.* 174, 2047–2051
26. Meuskens, I. *et al.* (2022) The trimeric autotransporter adhesin YadA of *Yersinia enterocolitica* serotype O:9 binds glycan moieties. *Front. Microbiol.* 12, 738818
27. Johansson, J. *et al.* (2002) An RNA Thermosensor controls expression of virulence genes in *Listeria monocytogenes*. *Cell* 110, 551–561
28. Pichon, C. *et al.* (2013) The AfaR small RNA controls expression of the AfaD-VIII invasin in pathogenic *Escherichia coli* strains. *Nucleic Acids Res.* 41, 5469–5482
29. Wang, X. *et al.* (2023) Biofilm formation: mechanistic insights and therapeutic targets. *Mol. Biomed.* 4, 49
30. Li, L. *et al.* (2024) Relationship between biofilm formation and antibiotic resistance of *Klebsiella pneumoniae* and updates on antibiofilm therapeutic strategies. *Front. Cell. Infect. Microbiol.* 14, 1324895
31. Kim, S. *et al.* (2020) Thermoregulation of *Pseudomonas aeruginosa* biofilm formation. *Appl. Environ. Microbiol.* 86, e01584–20
32. Plumley, B.A. *et al.* (2017) Thermoregulation of biofilm formation in *Burkholderia pseudomallei* is disrupted by mutation of a putative diguanylate cyclase. *J. Bacteriol.* 199, e00780–16
33. Townsley, L. and Yildiz, F.H. (2015) Temperature affects c-di-GMP signalling and biofilm formation in *Vibrio cholerae*. *Environ. Microbiol.* 17, 4290–4305
34. Stepanović, S. *et al.* (2003) Influence of the incubation temperature, atmosphere and dynamic conditions on biofilm formation by *Salmonella* spp. *Food Microbiol.* 20, 339–343
35. Ochiai, Y. *et al.* (2014) Biofilm formation under different temperature conditions by a single genotype of persistent *Listeria monocytogenes* strains. *J. Food Prot.* 77, 133–140
36. Pokhrel, D. *et al.* (2024) Increase in temperature facilitates *Campylobacter jejuni* biofilm formation under both aerobic and microaerobic incubation. *Poult. Sci.* 103, 103753
37. Müller, J.U. *et al.* (2024) Temperatures above 37°C increase virulence of a convergent *Klebsiella pneumoniae* sequence type 307 strain. *Front. Cell. Infect. Microbiol.* 14, 1411286
38. Pradhan, J. *et al.* (2023) A novel *rspA* gene regulates biofilm formation and virulence of *Salmonella* Typhimurium. *Microb. Pathog.* 185, 106432
39. Chaban, B. *et al.* (2015) The flagellum in bacterial pathogens: for motility and a whole lot more. *Semin. Cell Dev. Biol.* 46, 91–103
40. Gründling, A. *et al.* (2004) *Listeria monocytogenes* regulates flagellar motility gene expression through MogR, a transcriptional repressor required for virulence. *Proc. Natl. Acad. Sci.* 101, 12318–12323
41. Kamp, H.D. and Higgins, D.E. (2011) A protein thermometer controls temperature-dependent transcription of flagellar motility genes in *Listeria monocytogenes*. *PLoS Pathog.* 7, e1002153
42. Cho, S.Y. *et al.* (2022) Structural basis of flagellar motility regulation by the MogR repressor and the GmaR antirepressor in *Listeria monocytogenes*. *Nucleic Acids Res.* 50, 11315–11330
43. Alm, R.A. *et al.* (1993) The *Campylobacter* sigma 54 flaB flagellin promoter is subject to environmental regulation. *J. Bacteriol.* 175, 4448–4455
44. Wösten, M.M.S.M. *et al.* (2010) Temperature-dependent FlgM/FliA complex formation regulates *Campylobacter jejuni* flagella length. *Mol. Microbiol.* 75, 1577–1591
45. Rudenko, I. *et al.* (2019) Inefficient secretion of anti-sigma factor FlgM inhibits bacterial motility at high temperature. *iScience* 16, 145–154
46. Kapatral, V. *et al.* (1996) Temperature-dependent regulation of *Yersinia enterocolitica* Class III flagellar genes. *Mol. Microbiol.* 19, 1061–1071
47. Ding, L. *et al.* (2009) Functional characterization of FlgM in the regulation of flagellar synthesis and motility in *Yersinia pseudotuberculosis*. *Microbiology (Reading)* 155, 1890–1900
48. Dougan, G. and Baker, S. (2014) *Salmonella enterica* serovar typhi and the pathogenesis of typhoid fever. *Ann. Rev. Microbiol.* 68, 317–336
49. Hu, X. *et al.* (2017) Vi capsular polysaccharide: synthesis, virulence, and application. *Crit. Rev. Microbiol.* 43, 440–452
50. Brewer, S.M. *et al.* (2021) A *Salmonella* Typhi RNA thermosensor regulates virulence factors and innate immune evasion in response to host temperature. *PLoS Pathog.* 17, e1009345
51. Peters, D.T. *et al.* (2022) Unraveling the molecular determinants of the anti-phagocytic protein cloak of plague bacteria. *PLoS Pathog.* 18, e1010447
52. Al-Jawdah, A.D. *et al.* (2019) Induction of the immunoprotective coat of *Yersinia pestis* at body temperature is mediated by the Caf1R transcription factor. *BMC Microbiol.* 19, 68

53. Bobrov, A.G. *et al.* (2011) Systematic analysis of cyclic di-GMP signalling enzymes and their role in biofilm formation and virulence in *Yersinia pestis*. *Mol. Microbiol.* 79, 533–551
54. Kirillina, O. *et al.* (2004) HmsP, a putative phosphodiesterase, and HmsT, a putative diguanylate cyclase, control Hms-dependent biofilm formation in *Yersinia pestis*. *Mol. Microbiol.* 54, 75–88
55. Liu, L. *et al.* (2015) Reciprocal regulation of *Yersinia pestis* biofilm formation and virulence by RovM and RovA. *Open Biol.* 6, 150198
56. Zhang, Y. *et al.* (2013) Reciprocal regulation of pH 6 Antigen gene loci by PhoP and RovA in *Yersinia pestis* biovar microtus. *Future Microbiol.* 8, 271–280
57. Gao, H. *et al.* (2008) The iron-responsive Fur regulon in *Yersinia pestis*. *J. Bacteriol.* 190, 3063–3075
58. Quade, N. *et al.* (2012) Structural basis for intrinsic thermosensing by the master virulence regulator RovA of *Yersinia*. *J. Biol. Chem.* 287, 35796–35803
59. Nuss, A.M. *et al.* (2016) A precise temperature-responsive bistable switch controlling *Yersinia* virulence. *PLoS Pathog.* 12, e1006091
60. Tiensuu, T. *et al.* (2019) Flick of a switch: regulatory mechanisms allowing *Listeria monocytogenes* to transition from a saprophyte to a killer. *Microbiology (Reading)* 165, 819–833
61. Zhang, H. *et al.* (2020) A tale of two transitions: the unfolding mechanism of the prfA RNA thermosensor. *Biochemistry* 59, 4533–4545
62. Barnwal, R.P. *et al.* (2016) Structure and mechanism of a molecular rheostat, an RNA thermometer that modulates immune evasion by *Neisseria meningitidis*. *Nucleic Acids Res.* 44, 9426–9437
63. Solar Venero, E.C. *et al.* (2024) Fever-like temperature impacts on *Staphylococcus aureus* and *Pseudomonas aeruginosa* interaction, physiology, and virulence both *in vitro* and *in vivo*. *BMC Biol.* 22, 27
64. Hurme, R. *et al.* (1997) A proteinaceous gene regulatory thermometer in *Salmonella*. *Cell* 90, 55–64
65. Roncarati, D. *et al.* (2014) The HrcA repressor is the thermosensor of the heat-shock regulatory circuit in the human pathogen *Helicobacter pylori*. *Mol. Microbiol.* 92, 910–920
66. Versace, G. *et al.* (2021) Feeling the heat: the *Campylobacter jejuni* HrcA transcriptional repressor is an intrinsic protein thermosensor. *Biomolecules* 11, 1413
67. Darsonval, M. *et al.* (2018) CtsR, the master regulator of stress-response in *Oenococcus oeni*, is a heat sensor interacting with ClpL1. *Front. Microbiol.* 9, 3135
68. Servant, P. *et al.* (2000) The RheA repressor is the thermosensor of the HSP18 heat shock response in *Streptomyces albus*. *Proc. Natl. Acad. Sci. USA* 97, 3538–3543
69. Elsholz, A.K.W. *et al.* (2010) CtsR, the Gram-positive master regulator of protein quality control, feels the heat. *EMBO J.* 29, 3621–3629
70. Chastanet, A. *et al.* (2004) clpB, a novel member of the *Listeria monocytogenes* CtsR regulon, is involved in virulence but not in general stress tolerance. *J. Bacteriol.* 186, 1165–1174
71. Hanson, B.R. and Tan, M. (2015) Transcriptional regulation of the *Chlamydia* heat shock stress response in an intracellular infection. *Mol. Microbiol.* 97, 1158–1167
72. Kazmierczak, M.J. *et al.* (2005) Alternative sigma factors and their roles in bacterial virulence. *Microbiol. Mol. Biol. Rev.* 69, 527–543
73. Chakraborty, S. *et al.* (2010) Temperature and Mg^{2+} sensing by a novel PhoP-PhoQ two-component system for regulation of virulence in *Edwardsiella tarda*. *J. Biol. Chem.* 285, 38876–38888
74. Groisman, E.A. *et al.* (2021) How the PhoP/PhoQ system controls virulence and Mg2+ homeostasis: lessons in signal transduction, pathogenesis, physiology, and evolution. *Microbiol. Mol. Biol. Rev.* 85, e0017620
75. Almblad, H. *et al.* (2021) Bacterial cyclic diguanylate signaling networks sense temperature. *Nat. Commun.* 12, 1986
76. Ha, D.-G. and O'Toole, G.A. (2015) c-di-GMP and its effects on biofilm formation and dispersion: a *Pseudomonas aeruginosa* review. *Microbiol. Spectr.* 3, MB-0003-2014
77. Teschler, J.K. *et al.* (2022) Mechanisms underlying *Vibrio cholerae* biofilm formation and dispersion. *Ann. Rev. Microbiol.* 76, 503–532
78. Sun, Y. *et al.* (2020) *Azorhizobium caulinodans* c-di-GMP phosphodiesterase Chp1 involved in motility, EPS production, and nodulation of the host plant. *Appl. Microbiol. Biotechnol.* 104, 2715–2729
79. Sellner, B. *et al.* (2021) A new sugar for an old phage: a c-di-GMP-dependent polysaccharide pathway sensitizes Escherichia coli for bacteriophage infection. *mBio* 12, e0324621
80. Fu, Y. *et al.* (2018) c-di-GMP Regulates various phenotypes and insecticidal activity of Gram-positive *Bacillus thuringiensis*. *Front. Microbiol.* 9, 45
81. Junkermeier, E.H. and Hengge, R. (2021) A novel locally c-di-GMP-controlled exopolysaccharide synthase required for bacteriophage N4 infection of *Escherichia coli*. *mBio* 12, e0324921
82. Yu, Z. *et al.* (2023) Gas and light: triggers of c-di-GMP-mediated regulation. *FEMS Microbiol. Rev.* 47, fuad034
83. Shapiro, R.S. and Cowen, L.E. (2012) Thermal control of microbial development and virulence: molecular mechanisms of microbial temperature sensing. *mBio* 3, e00238-12
84. Roncarati, D. and Scarlato, V. (2017) Regulation of heat-shock genes in bacteria: from signal sensing to gene expression output. *FEMS Microbiol. Rev.* 41, 549–574
85. Ono, S. *et al.* (2005) H-NS is a part of a thermally controlled mechanism for bacterial gene regulation. *Biochem. J.* 391, 203–213
86. Zhao, X. *et al.* (2021) Molecular basis for the adaptive evolution of environment-sensing by H-NS proteins. *Elife* 10, e57467
87. Di Martino, M.L. *et al.* (2016) The multifaceted activity of the VirF regulatory protein in the *Shigella* lifestyle. *Front. Mol. Biosci.* 3, 61
88. Duong, N. *et al.* (2007) Thermosensing coordinates a cis-regulatory module for transcriptional activation of the intracellular virulence system in *Salmonella enterica* serovar Typhimurium. *J. Biol. Chem.* 282, 34077–34084
89. Van Hofwegen, D.J. *et al.* (2023) Comparison of *Yersinia enterocolitica* DNA methylation at ambient and host temperatures. *Epigenomes* 7, 30
90. Sharma, P. *et al.* (1865) RNA thermometers in bacteria: role in thermoregulation. *Biochim. Biophys. Acta Gene Regul. Mech.* 2022, 194871
91. Halder, S. and Bansal, M. (2022) The effect of mutation in the stem of the MicroROSE thermometer on its thermosensing ability: insights from molecular dynamics simulation studies. *RSC Adv.* 12, 11853–11865
92. Roßmanith, J. *et al.* (2018) Design of a temperature-responsive transcription terminator. *ACS Synth. Biol.* 7, 613–621
93. Loh, E. *et al.* (2018) RNA thermometers in bacterial pathogens. *Microbiol. Spectr.* 6. https://doi.org/10.1128/microbiolspec.RWR-0012-2017
94. Weber, G.G. *et al.* (2014) RNA thermometer controls temperature-dependent virulence factor expression in *Vibrio cholerae*. *Proc. Natl. Acad. Sci.* 111, 14241–14246
95. Böhme, K. *et al.* (2012) Concerted actions of a thermo-labile regulator and a unique intergenic RNA thermosensor control *Yersinia* Virulence. *PLoS Pathog.* 8, e1002518
96. Pienkoß, S. *et al.* (2021) The gatekeeper of *Yersinia* type III secretion is under RNA thermometer control. *PLoS Pathog.* 17, e1009650
97. Pienkoß, S. *et al.* (2022) RNA thermometer-coordinated assembly of the *Yersinia* injectisome. *J. Mol. Biol.* 434, 167667
98. Grosso-Becerra, M.V. *et al.* (2014) Regulation of *Pseudomonas aeruginosa* virulence factors by two novel RNA thermometers. *Proc. Natl. Acad. Sci.* 111, 15562–15567
99. Twittenhoff, C. *et al.* (2020) Lead-seq: transcriptome-wide structure probing *in vivo* using lead(II) ions. *Nucleic Acids Res.* 48, e71
100. Jolley, E.A. *et al.* (2023) Transcriptome-wide probing reveals RNA thermometers that regulate translation of glycerol permease genes in *Bacillus subtilis*. *RNA* 29, 1365–1378
101. Kolberg, T. *et al.* (2023) Led-Seq: ligation-enhanced double-end sequence-based structure analysis of RNA. *Nucleic Acids Res.* 51, e63

102. Waldminghaus, T. *et al.* (2007) Genome-wide bioinformatic prediction and experimental evaluation of potential RNA thermometers. *Mol. Gen. Genomics.* 278, 555–564
103. Chursov, A. *et al.* (2013) RNAtips: analysis of temperature-induced changes of RNA secondary structure. *Nucleic Acids Res.* 41, W486–W491
104. Churkin, A. *et al.* (2014) RNAthermsw: direct temperature simulations for predicting the location of RNA thermometers. *PLoS One* 9, e94340
105. Sharts, D.M. *et al.* (2024) Robo-Therm, a pipeline to RNA thermometer discovery and validation. *RNA* 30, 760–769
106. Thomas, S.E. *et al.* (2022) RNA structure mediated thermoregulation: what can we learn from plants? *Front. Plant Sci.* 13, 938570
107. Mora, C. *et al.* (2022) Over half of known human pathogenic diseases can be aggravated by climate change. *Nat. Clim. Chang.* 12, 869–875
108. Casadevall, A. *et al.* (2019) On the emergence of *Candida auris*: climate change, azoles, swamps, and birds. *mBio* 10, e01397–19
109. Billaud, M. *et al.* (2022) An increase of seawater temperature upregulates the expression of *Vibrio parahaemolyticus* virulence factors implicated in adhesion and biofilm formation. *Front. Microbiol.* 13, 840628
110. Archer, E.J. *et al.* (2023) Climate warming and increasing *Vibrio vulnificus* infections in North America. *Sci. Rep.* 13, 3893
111. de Souza, W.M. and Weaver, S.C. (2024) Effects of climate change and human activities on vector-borne diseases. *Nat. Rev. Microbiol.* 22, 476–491
112. Nuttall, P.A. (2022) Climate change impacts on ticks and tick-borne infections. *Biologia* 77, 1503–1512
113. Wang, X. *et al.* (2021) Reversible thermal regulation for bifunctional dynamic control of gene expression in *Escherichia coli*. *Nat. Commun.* 12, 1411
114. Xiong, L.L. *et al.* (2022) Tunable temperature-sensitive transcriptional activation based on lambda repressor. *ACS Synth. Biol.* 11, 2518–2522
115. Piraner, D.I. *et al.* (2017) Tunable thermal bioswitches for *in vivo* control of microbial therapeutics. *Nat. Chem. Biol.* 13, 75–80
116. Neupert, J. *et al.* (2008) Design of simple synthetic RNA thermometers for temperature-controlled gene expression in *Escherichia coli*. *Nucleic Acids Res.* 36, e124
117. Hoynes-O'Connor, A. *et al.* (2015) *De novo* design of heat-repressible RNA thermosensors in *E. coli*. *Nucleic Acids Res.* 43, 6166–6179
118. Sen, S. *et al.* (2017) Design of a toolbox of RNA thermometers. *ACS Synth. Biol.* 6, 1461–1470
119. Abedi, M.H. *et al.* (2022) Ultrasound-controllable engineered bacteria for cancer immunotherapy. *Nat. Commun.* 13, 1585