

# Thermal Control of Microbial Development and Virulence: Molecular Mechanisms of Microbial Temperature Sensing

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**ABSTRACT** Temperature is a critical and ubiquitous environmental signal that governs the development and virulence of diverse microbial species, including viruses, archaea, bacteria, fungi, and parasites. Microbial survival is contingent upon initiating appropriate responses to the cellular stress induced by severe environmental temperature change. In the case of microbial pathogens, development and virulence are often coupled to sensing host physiological temperatures. As such, microbes have developed diverse molecular strategies to sense fluctuations in temperature, and nearly all cellular molecules, including proteins, lipids, RNA, and DNA, can act as thermosensors that detect changes in environmental temperature and initiate relevant cellular responses. The myriad of molecular mechanisms by which microbes sense and respond to temperature reveals an elegant repertoire of strategies to orchestrate cellular signaling, developmental programs, and virulence with spatial and temporal environmental cues.

All organisms must contend with environmental perturbations and fluctuations, including exposure to variations in temperature. The capacity to integrate temperature cues and respond appropriately is shared among organisms ranging from bacteria to mammals. For diverse microbial species, including viruses, archaea, bacteria, fungi, and parasites, temperature represents a critical environmental cue that can mediate changes in growth, development, and pathogenesis. These microbes may experience fluctuations in temperature in the form of seasonal changes in environmental temperature, rising global temperatures, interactions with diverse host species, including endothermic species, and upon febrile episodes encountered in the host in response to infection.

The impact of temperature on viral and bacterial pathogens is well established. It has been known for some time that temperature can profoundly influence the replication of viruses, such as the human influenza virus (1), and that viral growth properties differ significantly at different temperatures in diverse host cell types (2). Further, temperature is a critical environmental trigger for many bacterial species. For bacterial pathogens of mammals, including *Shigella* species, *Yersinia* species, and other pathogens, elevated temperature can signal successful infection of its host and leads to expression of bacterial virulence genes encoding type III secretion systems, adhesins, and other important virulence regulators (3). Conversely, for bacterial pathogens of plants, such as *Agrobacterium tumefaciens*, key virulence factors are often repressed in response to elevated temperature (4, 5), suggesting the importance of temperature in the precise coordination of bacterial virulence and pathogenesis.

Fungal pathogens are also profoundly affected by temperature, which can influence developmental programs as well as virulence. This is illustrated by the dimorphic fungal pathogens for which a temperature-induced morphological transition is required for virulence. These dimorphic fungi, including *Blastomyces dermatitidis* and *Histoplasma capsulatum*, grow as filamentous molds in the soil at ambient temperature and convert to pathogenic yeast after infectious spores are exposed to elevated temperature upon inhalation into the lungs of a mammalian host (6). *In vitro*, transitioning these fungi from ambient temperature to an elevated temperature of 37°C is sufficient to induce this morphogenetic

switch (6). Temperature also influences developmental transitions in the leading human fungal pathogen, *Candida albicans*. Temperature controls the *C. albicans* morphogenetic transition between yeast and filamentous growth (7), phenotypic switching between the white and opaque cellular growth states (8, 9), and resistance to antifungal drugs (10).

Temperature also plays a key role in the virulence and development of diverse species of protozoan parasites. For instance, for the malarial parasite *Plasmodium falciparum*, the developmental transition from sporozoites, the parasite transmission stage, into early exoerythrocytic forms depends on temperature elevation to mammalian physiological temperature (11). Further, there is evidence to suggest that recurrent febrile episodes in malaria patients can accelerate the intraerythrocytic development of the parasite (12). Similarly, for the parasite *Leishmania donovani*, the differentiation from the promastigote to the amastigote life cycle stage correlates with the temperature upshift encountered upon transmission from insect to mammal (13, 14), and temperature also controls the differentiation from tachyzoites to bradyzoites in the parasite *Toxoplasma gondii* (15). Temperature may also influence parasite virulence, as a shift to mammalian physiological temperature contributes to the preservation of sporozoite infectivity of *P. falciparum* (16), highlighting the important role of temperature sensing for both parasite development and virulence.

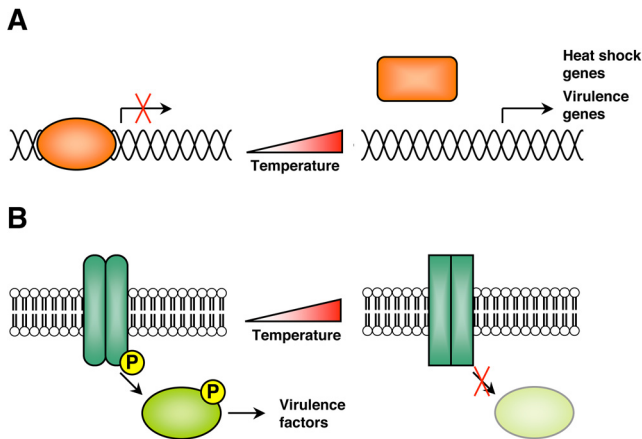
As a consequence of the profound impact of temperature on key developmental, virulence, and survival traits in microbial species, an impressive repertoire of temperature-sensing mechanisms has emerged over evolutionary time. In this review, we discuss the diversity of mechanisms by which microbes sense changes in environmental temperature, highlighting findings from bacterial and fungal species, where these mechanisms have been most ex-

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**FIG 1** Examples of protein temperature sensors. (A) In several species of bacteria, transcriptional repressors are key temperature sensors. These repressors bind DNA associated with heat shock response genes, and upon temperature stress, changes in protein conformation or oligomerization status cause these transcriptional repressors to dissociate from DNA, allowing transcription to occur. Genes that are expressed upon transition to elevated temperature include heat shock genes and virulence genes. (B) In several species of pathogenic bacteria, the histidine kinases of two-component regulatory systems are involved in temperature sensing. Typically, the temperature-sensing kinase activates its downstream effector via autophosphorylation and effector phosphorylation, allowing the effector protein to activate different signaling pathways and virulence factors in response to temperature. Upon elevated temperature, changes in kinase protein conformation will abolish this signaling.

tensively studied. We explore each of the different biomolecules known to be involved in microbial thermosensing, including proteins, lipids, RNA, and DNA. We expand on the role of diverse classes of proteins, including transcription factors, kinases, two-component systems (TCSs), and chaperones in sensing environmental temperature, the function of lipids and membrane fluidity in response to temperature fluctuations, the role of RNA thermometers, and the importance of DNA structure and topology in mediating microbial thermosensing.

### PROTEIN TEMPERATURE SENSORS

Several classes of proteins, including transcriptional regulators, kinases, and chaperones, have been described as temperature sensors (Fig. 1) (17). In numerous Gram-positive bacteria, including *Bacillus subtilis*, *Lactococcus lactis*, and others, the activity of the global transcriptional repressor protein CtsR is regulated by intrinsic heat-sensing capabilities, achieved via a glycine-rich loop, which functions as a protein thermometer (18). When this transcriptional regulator senses temperature stress, it dissociates from DNA associated with heat shock response elements, allowing expression of these transcripts to occur (18). A similar paradigm of temperature-dependent DNA-binding activity has been documented for several other thermosensitive bacterial transcriptional repressors, all of which dissociate from DNA in response to increased temperature (Fig. 1A). Some examples include the TlpA thermosensor in *Salmonella* (19), RovA in *Yersinia* (20), RheA in *Streptomyces albus* (21), and Phr in the archaeal species *Pyrococcus furiosus* (22). GmaR is a transcription factor and thermosensor protein that controls temperature-dependent transcription of motility genes in *Listeria monocytogenes* (23). Currently, GmaR is the only example of a bacterial transcription factor thermosensor

that functions as an antirepressor of temperature-dependent transcription (23).

For bacteria, two-component regulatory systems, comprised of a membrane-associated sensor, typically a histidine kinase, and a cytoplasmic response regulator, are key sensors of environmental fluctuations and frequently influence the expression of virulence genes. The sensor kinases of TCSs are frequently found to have temperature-sensing capabilities and thus regulate bacterial virulence in response to temperature fluctuations (Fig. 1B). For instance, the plant pathogen *A. tumefaciens* is virulent only at temperatures below 32°C, and a temperature-sensitive TCS comprised of the sensor kinase VirA and the response regulator VirG modulates its virulence. At temperatures above 32°C, VirA undergoes inactivation, as autophosphorylation of VirA and phosphorylation of VirG are both abolished (5). A similar mechanism is observed with the CorS-CorR TCS in *Pseudomonas syringae*, where temperature-dependent conformational changes in the CorS sensor kinase lead to inactivation at elevated temperatures (24). For the animal pathogen *Edwardsiella tarda*, the PhoP-PhoQ TCS detects changes in temperature to activate the type III and VI secretion systems that are critical for virulence (25). The PhoQ sensor kinase detects temperature via a conformational change in its secondary structure, to activate protein secretion at optimal temperatures between 35°C and 37°C (25). This differential inactivation or activation of TCSs in bacterial species that are pathogens of plants versus pathogens of animals suggests a mechanism by which bacterial pathogens use temperature as a key environmental signal to govern virulence at the optimal temperature of their host. In *Escherichia coli*, additional membrane-associated receptors play a role in temperature sensing. The Tsr and Tar proteins, two transmembrane chemoreceptors, function as thermosensors via differential methylation and changes in protein conformation (26, 27).

Although TCSs are ubiquitous in prokaryotes, they are less frequently found in eukaryotic species. However, histidine kinases, which frequently represent the sensor component of TCSs, have been implicated in environmental sensing as well as developmental transitions in diverse fungi (28). Notably, a histidine kinase sensor regulates temperature sensing and development in two species of dimorphic fungal pathogens. These dimorphic fungi, *B. dermatitidis* and *H. capsulatum*, grow as filamentous molds at ambient temperature and convert to pathogenic yeast after exposure to elevated temperature upon inhalation into the lungs of a mammalian host (6). In *B. dermatitidis* and *H. capsulatum*, the histidine kinase *Drk1* functions as an environmental sensor of temperature and controls morphogenesis, as well as adaptation to environmental stress within the mammalian host (29). *Drk1* is further required for the expression of virulence genes as well as fungal pathogenicity *in vivo*, reinforcing the relationship between fungal thermotolerance and virulence (29).

Chaperone proteins can also act as temperature sensors to control thermal regulation in diverse microbial species. In *Saccharomyces cerevisiae*, the small heat shock protein Hsp26, which functions as part of an oligomer complex, directly senses temperature and shifts from a low- to a high-affinity chaperone state with increased temperature (30). Evidence suggests that the small heat shock protein Hsp21 may play a similar role in temperature sensing in the fungal pathogen *C. albicans*, though the precise mechanism involved remains to be elucidated (31). For the bacteria *E. coli* and *Thermus thermophilus*, the Hsp70 family chaperone

DnaK, its cochaperone DnaJ, and the nucleotide exchange factor GrpE function as a critical thermosensor that detects temperature fluctuations via thermally controlled conformational changes in GrpE (32–35). The chaperone DegP (HtrA) also functions as a unique bacterial thermosensor in both *E. coli* and *Thermotoga maritima*, where the protein transitions between activities as a chaperone and a protease in a temperature-dependent manner (36, 37).

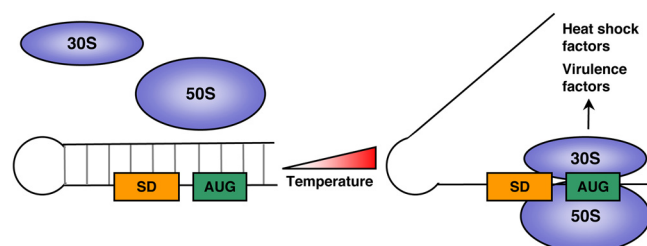
The environmentally responsive chaperone protein HSP90 has long been known to function as a temperature sensor in mammalian cells (38, 39), where global problems in protein folding at elevated temperature titrate HSP90 away from client proteins, such as the heat shock factor HSF1, due to the increase in denatured cellular targets requiring HSP90's chaperone function (40). Although this mechanism of temperature sensing regulated by overwhelming HSP90 function has not been directly validated in microbial species, there is evidence to suggest that Hsp90 may play a similar role as a temperature sensor in the fungal pathogen *C. albicans* (7) and that Hsp90's interaction with Hsf1 may be instrumental to regulating this process (41). Further, in protozoan parasites, including *P. falciparum* and *L. donovani*, evidence suggests that Hsp90 may act as a key thermosensor in mediating temperature-dependent developmental transitions (42).

## LIPIDS AND MEMBRANE FLUIDITY AS TEMPERATURE SENSORS

For many microbes, the cellular membrane is among the first to sense fluctuations in environmental temperatures. Exposure to extreme temperatures, hot or cold, can dramatically alter membrane properties, allowing membranes themselves to act as a kind of thermosensor (43). Membrane fluidity, which decreases at lower temperature, is regulated in part by the ratio of unsaturated to saturated fatty acids present in membrane lipids (43). Changes in environmental temperature have been demonstrated to alter both the fatty acid composition and the degree of unsaturation of membrane lipids in diverse bacterial and archaeal species (44, 45, 46). For the bacterium *B. subtilis*, decreased membrane fluidity caused by lower temperature favors a kinase-dominant state of the TCS sensor kinase DesK, which is then able to activate the downstream response regulator DesR. DesR subsequently activates a transcriptional program that is able to restore bacterial membrane fluidity (47, 48). In *Francisella* bacteria, the acyltransferases LpxD1 and LpxD2 control temperature-dependent remodeling of membrane lipid A under different environmental temperature conditions. LpxD2 adds shorter acyl chains to lipid A, allowing growth in colder environments, whereas LpxD1 adds longer acyl chains upon transitioning to higher temperatures experienced in the mammalian host (49). The cyanobacterium *Synechocystis* responds to decreased temperature by upregulating expression of acyl-lipid desaturases, which increase the *cis*-unsaturation of membrane-bound lipid fatty acids, thus restoring membrane fluidity (50, 51).

## RNA THERMOMETERS

Temperature-responsive RNAs, or RNA thermometers, are RNA control elements located in the 5' untranslated region (UTR) of bacterial genes involved in virulence, heat shock, or cold shock (17, 52). The basic principle of RNA thermometers is that the Shine-Dalgarno (SD) sequence (located upstream of the AUG start codon) is base paired to form a hairpin structure with the



**FIG 2** The basic principle of RNA thermometers. For temperature-responsive RNA thermometers, the Shine-Dalgarno (SD) sequence and the AUG start codon are base paired to form a hairpin structure in the 5' UTR of an mRNA transcript at low temperature. Increasing temperature destabilizes the structure, allowing the ribosome-binding site to become accessible, allowing the 30S and 50S ribosomal subunits to bind, and facilitating the initiation of translation. Many transcripts encoding heat shock factors and virulence factors are regulated this way.

AUG start codon in the 5' UTR of an mRNA transcript at low temperature (Fig. 2). Increasing temperature destabilizes the structure, allowing the ribosome-binding site to become accessible and facilitating the initiation of translation (52). By this mechanism, temperature fluctuations can have an instantaneous effect on the expression of RNA thermometer transcripts involved in mediating heat shock response, cold shock response, or virulence, and gene expression correlates with the severity of the temperature change (Fig. 2) (53, 54).

Many RNA thermometers govern the expression of heat shock genes in response to elevated temperature. The most abundant class of bacterial RNA thermometers is the repression of the heat shock gene expression (ROSE) family elements (55, 56), which are found in numerous alpha- and gamma-proteobacteria, including *E. coli* and *Salmonella* species (57). ROSE elements are 60 to 100 nucleotides long, located in the 5' UTR of small heat shock genes, and regulate the translation of these heat shock transcripts in response to temperature change (52). Another class of RNA thermometers that controls the expression of heat shock genes is the fourU class of elements, containing a stretch of four uridines that pair with the SD sequence (58). One of the best examples of a fourU element is upstream of the *agsA* small heat shock gene in *Salmonella enterica*, which forms a translation initiation complex at high but not low temperatures (58). An RNA thermometer regulating expression of heat shock genes has also been identified in the cyanobacterium *Synechocystis*, which controls translation of the *hsp17* heat shock gene in response to elevated temperature stress (59).

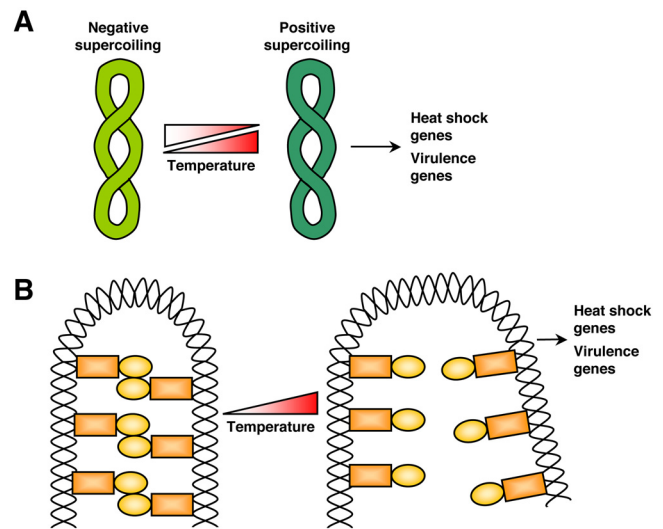
In response to low temperature, the expression of cold shock genes can also be controlled via RNA thermometers, although this phenomenon has not been explored as extensively as RNA thermometers controlling heat shock (60, 61). In *E. coli*, a sequence upstream of the cold shock protein CspA has been postulated to function as an RNA thermometer, governing translation efficiency of this transcript (62). The mRNA of *cspA* forms different structures at 37°C compared to cold shock temperatures, and the structure produced under cold shock conditions is translated more efficiently, suggesting a mechanism by which this bacterium responds to cold shock conditions (63). Other factors involved in bacterial response to cold shock are postulated to be regulated by RNA thermometers, including the cold shock factor CspE and the small RNA involved in low-temperature growth, DsrA (64–66), although the precise mechanisms remain to be elucidated.

RNA thermometers can also control the expression of virulence genes, when an increase in temperature to 37°C signals that the bacterium has successfully invaded its host. For the plague bacterium *Yersinia pestis*, LcrF is a crucial virulence factor that regulates the expression of adhesin proteins at 37°C but not at ambient temperature (67). The 5' UTR of LcrF contains a fourU RNA thermometer, which enables translation of this virulence factor specifically at host temperature (68, 58). A similar fourU RNA thermometer controls key virulence factors in *Yersinia pseudotuberculosis* in response to host temperature (69), as *Y. pseudotuberculosis* expressing a stabilized RNA thermometer variant is avirulent in a murine infection model (69). In *L. monocytogenes*, an RNA thermometer controls translation of the virulence factor PrfA, which activates several virulence genes, including adhesins, phagosome escape factors, and immune-modulating factors (70). The 5' UTR of the *prfA* transcript is folded such that the SD sequence is poorly accessible at 30°C but is destabilized at 37°C, enabling translation of this virulence factor in response to host temperature (70). An RNA thermometer-like mechanism also controls the life cycle of phage lambda, as temperature controls translation of the cIII mRNA, involved in the lysis-lysogeny decision (71). High concentrations of cIII protein at optimal growth temperatures (37°C) favor lysogeny, while under heat stress conditions (45°C), cIII is low and the lytic life cycle is favored (71). Regulation of this temperature-dependent transition is accomplished by alternative RNA structures in the 5' UTR of cIII, which change in response to temperature fluctuations (71). Thus, temperature-sensitive RNA thermometers can have a profound impact on virulence and development in response to temperature fluctuations.

### DNA STRUCTURE AND TOPOLOGY AS A THERMOSENSOR

Conditions that influence the integrity and topology of DNA, including changes in temperature, ultimately impact on gene expression (Fig. 3). In this way, DNA can act as a thermosensor of environmental temperature change. DNA supercoiling of bacterial and archaeal plasmids is one of the primary parameters of DNA topology that is affected by temperature, as has been documented for numerous species that grow at moderate temperatures (mesophiles) or at high temperatures (hyperthermophiles) (Fig. 3A) (72). In these species, changes in DNA supercoiling can function as a sensor of temperature stress under both heat shock and cold shock conditions, and DNA transcription efficiency is highly sensitive to changes in DNA supercoiling (72). For mesophiles, such as bacterial species *E. coli* and *Salmonella*, DNA is negatively supercoiled, and heat stress induces a transient increase in positive supercoiling, leading to plasmid relaxation, while cold shock leads to a transient decrease in supercoiling (Fig. 3A) (73, 74). For hyperthermophiles, such as the archaeal species of *Sulfolobus* and *Thermococcus*, DNA is positively supercoiled, and heat stress induces increased positive supercoiling, while colder temperatures induce negative supercoiling (72, 75). Transcription efficiency is extremely sensitive to changes in DNA supercoiling, and temperature changes can profoundly influence gene expression, including the expression of key virulence determinants in these bacterial and archaeal species (Fig. 3A) (76, 77).

Another mechanism by which DNA topology is influenced by temperature is by local DNA structures. The promoters of bacterial species such as *E. coli* contain intrinsically curved DNA regions due to AT-rich sequences (78, 79). Temperature fluctuations in-



**FIG 3** DNA structure and topology as a thermosensor. (A) For mesophilic bacterial species, plasmid DNA is negatively supercoiled and heat stress induces a transient increase in positive supercoiling, leading to plasmid relaxation, while cold shock leads to a transient decrease in supercoiling. Transcription efficiency is sensitive to changes in DNA supercoiling, and temperature changes can influence gene expression in these species, including the expression of virulence determinants and heat shock factors. (B) The histone-like nucleoid structuring protein (H-NS) binds DNA and represses transcription in numerous bacterial species. H-NS regulation of DNA is sensitive to fluctuations in temperature, and the formation of higher-order oligomers and overall DNA binding capacity are reduced in response to elevated host temperature. Expression of many virulence genes and heat shock genes are regulated in response to host temperature in this manner.

duce topological changes in these regions, affecting RNA polymerase binding and gene expression. In the cyanobacterium *Synechocystis*, DNA curvature found in certain promoter regions is influenced by temperature changes and regulates the expression of genes important for membrane fluidity (80). DNA curvature also influences the expression of phospholipase genes in response to temperature fluctuations in the bacterium *Clostridium perfringens* (81), suggesting a mechanism by which these species rapidly adapt to environmental temperature perturbations.

Finally, DNA-mediated temperature sensing can be achieved through the binding of silencer proteins, such as the histone-like nucleoid structuring protein (H-NS), which binds DNA and represses transcription in numerous bacterial species. H-NS regulation of DNA is exquisitely sensitive to fluctuations in temperature, as the formation of higher-order oligomers and overall DNA binding capacity are reduced in response to sensing host temperature (37°C) (Fig. 3B) (82). This temperature-dependent accessibility of promoter regions occupied by H-NS is a highly conserved mechanism of ensuring that virulence genes are expressed in response to host temperature. For example, in *S. enterica*, expression of a type III secretion system that is critical for virulence is repressed by H-NS at temperatures below 30°C (83). Similarly, in *Shigella flexneri*, the *virF* gene, encoding an invasion regulator, is expressed in a host temperature-dependent manner, controlled by the binding of H-NS (84). Overall, approximately 69% and 77% of temperature-regulated genes are controlled by H-NS in *E. coli* and *Salmonella*, respectively (82, 85), demonstrating the fundamental importance of H-NS in coordinating gene expression with environmental temperature.

## CONCLUSION

Temperature is a universal environmental stimulus that influences all microbes, and fluctuations in temperature may be experienced by environmental change or upon infection of a host. Accordingly, these changes in temperature may activate heat shock or cold shock stress response pathways to cope with severe changes in environmental temperature or may inform microbial pathogens of successful host infection and initiate virulence programs. As demonstrated here, diverse and disparate microbes, including bacteria, archaea, and fungi, have all evolved molecular strategies to sense temperature and activate appropriate response pathways. Many of the canonical mechanisms of temperature sensing have been elucidated in bacterial species, and future work may inform how these mechanisms, or perhaps novel mechanisms of thermosensing, operate in microbes for which temperature is an important environmental cue, including fungi and parasites. Temperature-dependent control of microbial development, virulence, and survival may have even broader implications for the origin of mammalian endothermy, which may have evolved to optimally restrict pathogens such as fungi, many of which lose growth capacity above ambient temperatures (86, 87). The stunning complexity of molecular mechanisms used to sense and control temperature fluctuations reflects on the pervasive impact of temperature as one of the most powerful selective forces in nature.

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