Microbial Mechanisms of Heat Sensing

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Abstract Temperature is one of the ubiquitous signals that control both the development as well as virulence of various microbial species. Therefore their survival is dependent upon initiating appropriate response upon temperature fluctuations. In particular, pathogenic microbes exploit host-temperature sensing mechanisms for triggering the expression of virulence genes. Many studies have revealed that the biomolecules within a cell such as DNA, RNA, lipids and proteins help in sensing change in temperature, thereby acting as thermosensors. This review shall provide an insight into the different mechanisms of thermosensing and how they aid pathogenic microbes in host invasion.

Keywords Microbes · Pathogen · Temperature · Thermosensors · Virulence

Introduction

Temperature is one of the most important parameters that affects the growth and development of all organisms. Thus, the ability to sense the temperature fluctuations and respond appropriately so as to maintain the internal temperature, has been shared among organisms ranging from bacteria to mammals and has been referred to as thermosensing [1]. It is well known that various microbial species encounter harsh environmental temperatures either due to global warming or when they enter their host species. Therefore, their sustenance is dependent on temperature as a cue which can regulate change in growth, development and pathogenesis [2, 3].

The expression of heat-shock genes or cold shock genes which mostly code for the chaperone proteins is one of the most well known temperature controlled mechanisms [4]. Activation of the chaperone system helps in preventing protein aggregation and in repairing misfolded protein thereby maintaining the protein homeostasis inside the cell [5]. Temperature changes on the other hand can also modulate the expression of virulence genes in pathogenic viruses and bacteria [2]. DNA replication as well as growth properties of viruses have been reported to be influenced by the temperature in diverse host cell types. Similarly, temperature of 37°C serves as a good indicator of host invasion in case of bacteria [3]. Particularly in case of mammalian pathogenic bacteria, elevated temperature indicates successful host invasion leading to activation of virulence genes such as type III secretion system, adhesins and other regulators. Thus, surveying of temperature is critical for not only proper growth of the organism but also for precise coordination of virulence and pathogenesis [3].

At the molecular level, every biomolecule responds to temperature by undergoing conformational change and thus, is employed as a temperature sensor by the cellular machinery. There have been reports of membrane lipids, DNA, RNA and proteins such as chaperone proteins, proteases and kinases being involved in sensing the shift in temperature and regulating the gene expression [6, 7]. Figure 1 depicts the effect of heat stress on different components of the cell which could function as thermosensors. This review provides insight into the different sensing strategies employed by microbes which help in survival under extreme temperatures, particularly heat stress. Moreover, how these strategies help pathogens in host invasion is also discussed and summarized in Table 1.
Membrane Lipids

Acyl-Lipid Desaturase

It has been observed that in various microorganisms, the unsaturated fatty acids get altered in response to temperature fluctuation [8]. This is brought about by various desaturase genes (des), which have been identified to function as potential thermosensors. In cyanobacterium *Synechocystis*, a drop in the temperature (i.e. from 32 to 22 °C) causes an increase in the production of the enzyme acyl-lipid desaturases, encoded by desA, desB and desD genes. The expression of these genes have been found to be enhanced by cold stress and thus, they help in increasing the level of cis-unsaturation of the membrane-bound lipid fatty acids so as to regain the membrane’s fluidity [9, 10]. Similar mechanism has been observed in the case of the bacterium *Bacillus subtilis* when the temperature dropped from 37 to 22 °C. The membrane fluidity was maintained by two proteins i.e. DesK and DesR by regulating the expression of des gene encoding the δ5-acyl-lipid desaturase [11, 12]. DesK is a transmembrane Histidine kinase having cytoplasmic kinase/phosphatase domain which can sense the hardness and thickness of cell membrane [9]. At low temperatures (below 30 °C), the membrane properties promote the Kinase-DesK state which by phosphorylating DesR stimulates the transcription of des gene. On the contrary at high temperatures, the phosphatase-DesK dominates leading to dephosphorylated DesR which results in the termination of des gene transcription [12].

**LpxD1 and LpxD2**

Lipid A is the biologically active component of the Lipopolysaccharide that can trigger an immune response in the host cells. Thus, certain organisms synthesize modified forms of lipid A in response to change in temperature, so as to alter the outer membrane’s integrity and protect itself from host’s immune response [13]. For example, the bacteria *Francisella*, while entering a mammalian host remodels its membrane by altering lipid A using enzyme acyltransferase LpxD1/2 and these have been reported to be differentially upregulated at mammalian temperature (37 °C) and at environmental temperature (21 °C) respectively [14, 15]. Moreover, the modification of lipid A by *Francisella* also helps it to increase the overall charge on its surface and thus repel cationic antimicrobials produced by the host [16]. Similarly studies with *Yersinia pestis*...
| Thermosensor          | Organisms                        | Mode of Action                                                                 | Advantage to Microbe/Pathogenesis                                                                 | References |
|-----------------------|----------------------------------|-------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|------------|
| Membrane lipids       |                                  |                                 |                                                                                                   |            |
| Acyl-lipid desaturase | *Synechocystis*, *Bacillus subtilis* | The Decrease in temperature (below 30°C) leads to activation of Des genes      | Activation of Des genes help in maintaining the membrane fluidity                                 | [10, 12]   |
| Acyltransferase       | *Frisiella*, *Yersinia pestis*    | On encountering mammalian host temperature (37°C), these organisms modify lipid A with the help of acyltransferases | Modified lipid A helps in repelling antimicrobial peptides, reduced activation of macrophages leading to evasion of host defence | [16, 17]   |
| DNA                   |                                  |                                 |                                                                                                   |            |
| Positive supercoiling | *Salmonella*                     | Upon heat exposure, the positive supercoiling of DNA leads to SPI-1 expression | The SPI-1 expression initiates the intestinal invasion                                            | [19]       |
| Promoter curvature    | *Clostridium perfringens*         | The phospholipase C (plc) gene was found to possess three poly (A) tracts forming bent DNA upstream of the promoter. This bent DNA facilitated transcription of the plc gene in a low temperature (25°C) dependent manner | Efficient expression of the plc gene at low temperature facilitates in fulfilling commensal and saprophytic lifestyle | [20]       |
| Nucleoid associated proteins | *Salmonella enterica*, *Shigella flexneri* | At normal temperatures, H-NS represses the expression of the genes required for invasion such as SPI-2 and VirF. Upon host invasion, the rise in temperature (37°C) affects the DNA binding ability of H-NS | The decreased affinity of H-NS proteins for their target sites facilitates the transcription of host invasion genes | [18, 21]   |
| RNA                   |                                  |                                 |                                                                                                   |            |
| ROSE Elements         | *Pseudomonas aeruginosa*          | ROSE element located in the UTR of rhlA and lasl gene regulate their translation. At 30°C, the hairpin loop structure prevent ribosome binding to SD sites. However, heat induced (37°C) structural changes expose the SD sites thereby allowing translation | Higher temperatures help in the production of Quorum-sensing dependent virulence factors          | [29]       |
| fourU Elements        | *Salmonella Typhimurium*, *Yersinia ss* | Hairpin structure control the translation of virulence related genes such as htrA, LcrF | Upon host invasion the increase in temperature leads to disruption of the hairpin structure thereby promoting the translation of these virulent genes | [26, 31]   |
| Cyanobacterial thermometer | *Synechocystis*                  | Single stem loop structure controls the expression of heat shock genes         | Under heat stress conditions, opening of stem loop leads to expression of hspS which aid in protein folding | [32]       |
| Alternate sigma factor | *E.coli*, *Borrelia burgdorferi* | Secondary structure of mRNA regulate the translation of heat shock genes in *E.coli* and virulence related genes (*OspC* and *OspA*) in *Borrelia burgdorferi* under normal conditions. High temperature destabilizes the structure thereby allowing translation to proceed | Heat induced translation of these genes provides protection against rise in temperature and helps in transcription of virulent genes | [33, 34]   |
| Csp thermometer       | *E.coli*                         | The RNAT in the UTR of CspA genes adopts a stable conformation at 10°C with the SD sequence being exposed. This leads to the translation of CspA protein | CspA protein helps in survival under cold shock conditions                                         | [4]        |
| cIII thermometer      | Bacteriophage *λ*                | The cIII gene product is controlled by RNAT which exists as two mutually exclusive structures. These structures change in a temperature dependent manner | Based on temperature, the phage decides to enter either lytic (virulent) or lysogeny (avirulent) phase | [28]       |
| Thermostensor Organisms | Mode of Action | Advantage to Microbe/Pathogenesis | References |
|-------------------------|---------------|----------------------------------|------------|
| **Other RNAT** | *Yersinia pseudotuberculosis* | The thermolabile stem loop structure regulates the expression of secreted toxin (CNFp) by occluding the RBS at 25 °C and liberating it at 37 °C | The secretion of toxin upon elevated temperature helps in host immune evasion | [37] |
| | *Neisseria meningitidis* | The RNAT present in the UTR of CssA gene allows its translation upon high temperature stress | The expression of this immune evasion factor helps in invading the host | [38] |
| | *Listeria monocytogenes* | Thermolabile RNAT harboring internal loops around AUG and RBS regulates the expression of prfA, which is a master regulator of virulence | Upon encountering 37 °C, prfA protein is translated which is responsible for further expression of nine key virulence genes. This helps in host invasion | [39] |
| | *Salmonella Typhi* | A stem loop structure regulates the expression of tviA gene, which is a transcriptional regulator of virulence factors. Melting of RNAT upon elevated temperature facilitates the translation of tviA | On host invasion, the rise in temperature trigger tviA translation. This leads to Vi capsule formation and suppression of flagellin and type III secretion system which is necessary to evade immune response | [40] |
| | *Streptococcus pneumoniae* | Temperature sensitive RNAT structures regulates the production of polysaccharide capsule | Inflammation triggered rise in temperature in host cells leads to production of capsule and factor H binding protein. These aid in evading the immune response | [41] |
| | *Haemophilus influenza* | Production of factor H binding protein is regulated by temperature sensitive RNAT | Expression of Hsps helps in providing protection against heat stress | [47–49] |
| | *Staphylococcus aureus* | RNAT having two hairpin structures controlled the expression of cidA gene. Low temperatures such as 30 °C facilitates translation of cidA | cidA protein helps in biofilm production and pathogen survival | [42] |
| **Protein thermosensors** | *Salmonella, Yersinia* | TlpA protein in *Salmonella* and RoVA in *Yersinia* binds to DNA at low temperatures (20 °C-25°C) and represses the transcription of virulent genes. However, at 37 °C the conformational change in the protein reduces their affinity for DNA binding, thereby allowing transcription to occur | The expression of virulent genes upon host invasion helps in the invasion of the immune response | [44, 45] |
| | *Bacillus subtilis, Staphylococcus saprophyticus, Chlamydia trachomatis* | CtsR and HrcA proteins bind the promoter of various Hsps and repress the transcription of various Hsps. Heat stress such as 50 °C represses the activity of these proteins thereby allowing transcription of these genes | Expression of Hsps helps in providing protection against heat stress | [47–49] |
| | *E coli* | Tar and Tar receptors sense the change in temperature through the presence of Serine and Aspartate | These receptors provide survival advantage under diverse environmental conditions | [53] |
| | | Chaperones bind unfolded polypeptides and maintain them in a folding-competent state during heat stress | These proteins aid in maintaining homeostasis under heat stress | [54, 60] |
showed that at a temperature range of 21–28 °C, it produced hexacylated lipid A structure whereas at mammalian host temperature (37 °C) tetraacylated lipid A structures were produced. This modification reduced the activation of macrophages thereby enabling successful infection by escaping the host defence [17].

**DNA**

**Supercoiling**

DNA topology influences transcription and thus, any change in the supercoiling pattern can result in a rapid and efficient alteration of the gene expression during stress conditions [18]. DNA topology has been shown to play an important role in pathogenicity of organisms such as *Salmonella*. Upon encountering exposure to heat, DNA undergoes positive supercoiling thereby triggering SPI-1 gene expression and subsequent initiation of intestinal invasion [19].

**Promoter Curvature**

Temperature sensing can also occur by local DNA structures that affect the transcription of neighbouring genes. Particularly, intrinsically curved DNA regions characterized by AT-rich tracts are often located on the upstream of the promoter region and affect the binding ability of RNA polymerase due to temperature changes [18]. Bacterium *Clostridium perfringens* provides the best example as the promoter of the *plc* gene, which encodes for the enzyme phospholipase C. The promoter was found to have three A tracts which functioned as an RNA polymerase binding site [20, 21]. Low temperature increased the bending of these A tracts and enhanced the binding affinity for the polymerase, thereby facilitating transcription [20].

**Nucleoid-Associated Proteins**

Temperature-dependent local DNA structures can indirectly affect the gene transcription by influencing the interaction of DNA binding proteins that have a regulatory role. These include nucleoid associated proteins such as H-NS, which preferentially binds to AT-rich sequences and functions in a temperature-responsive manner [18]. At temperatures above 37 °C, these proteins are unable to form higher-order oligomers and bind the DNA, whereas at lower temperature (upto 30 °C), the ratio of H-NS proteins to DNA increases three-to-four-fold [22]. This property of H-NS proteins plays a key role in virulence gene expression in many human pathogens.

It has been reported that in *Salmonella enterica*, the genomic island (SPI-2) that codes for Type III secretion system, was thermally regulated by the H-NS proteins. H-NS repressed the expression of SPI-2 at non-permissive temperature (≤ 30 °C) by binding to the DNA. However, at higher temperature, the DNA binding capability of H-NS was affected, leading to the transcription of this gene and promoting intracellular transmission [18, 23] (Fig. 2a). Similarly in the case of pathogenic bacteria *Shigella flexneri*, the virF gene required for invasion was kept repressed at non-permissive temperature (below 32 °C) by H-NS proteins [18, 24]. Moreover, the two H-NS binding sites in the virF promoter were reported to be separated by an
intrinsically bent region and thus higher temperatures by affecting the binding of H-NS to the target sites allowed the transcription of virF genes [21]. Expression of hemolysin gene in *E. coli* was also demonstrated to be regulated by an interplay of DNA bending and H-NS binding proteins [21]. Two H-NS binding sites in hemolysin operon were found to be separated by intrinsic DNA curvature. Therefore the increased flexibility of DNA and higher affinity of H-NS protein at lower temperatures caused the formation of a nucleoprotein complex which repressed the transcription. However, high growth temperature facilitated expression of hemolysin [25]. Thus, H-NS plays an important role in thermoregulation of virulence genes.

**RNA**

RNA thermosensors are basically RNA sequences present in the 5’ UTR of the genes with the ability to form secondary structures that could mask the ribosome binding site (RBS). Heat stress destabilises these structures thereby liberating the RBS and allowing the translation of the gene [26] (Fig. 2b). RNA thermosensors provide faster gene regulation in a cost-efficient manner in comparison to protein based gene regulation. Several temperature sensing RNA sequences known as RNA thermometers (RNAT’s) have been identified in various microorganisms [21].

**ROSE Elements**

ROSE elements (repression of heat shock gene expression), is the most common class of RNAT and were first discovered in *Bradyrhizobium japonicum* [27]. ROSE elements located in the 5’-UTR of the small heat shock genes, form a complex structure comprising 2–4 stem loops consisting of the Shine-Dalgarno sequence (SD sequence) and sometimes even the AUG start codon [21]. Studies have shown that the SD region remained sequestered at 30 °C, but with the gradual rise in temperature such as 37 °C, partial melting of the hairpin occured. Further, temperature of 42 °C caused full liberation of the SD and AUG start codon due to the complete melting of the hairpin structure [26]. Due to its gradual response to temperature alterations in vivo and in vitro, the ROSE element acts like a typical zipper and has been observed in various Rhizobia.
[28]. In opportunistic pathogens such as *Pseudomonas aeruginosa*, studies have identified ROSE regulating the expression of *rhlR* and *lasI* genes, which are required for the virulence [29].

**fourU Elements**

These are another class of RNAT, based on a stretch of four uridines that pairs with AGGA in the SD sequence [28]. FourU element was first identified to be present upstream of the small heat shock gene aggregation suppressing A (agsA) in *Salmonella enterica* and was found to control its translation initiation [30]. Secondary-structure prediction and experimental RNA probing showed that this element consisted of two hairpins wherein the second hairpin was temperature controlled and it sequestered the SD region through the fourU motif. High temperatures such as 40–45 °C, caused melting of hairpin II thereby allowing the binding of the ribosomes to the SD sequence [18, 30]. Such fourU elements consisting of a single hairpin also controlled the translation of *htrA* gene coding for periplasmic Serine protease in *Salmonella Typhimurium* [26]. Similar control of translation have been reported in the case of virulence factor *LcrF* in *Yersinia ss*, iron-acquisition gene in *Shigella dysenteriae* and transcriptional activator protein *ToxT* in *Vibrio cholerae* [18, 31].

**Cyanobacterial Thermometer**

This is the simplest natural RNAT, being first identified in the upstream region of the heat shock protein 17 gene (*hsp17*) in *Synechocystis* which is only 46 nucleotide long [32]. It was found to form a single stem–loop structure which could open and close in a reversible manner. Thus, with the help of RNAT, cyanobacteria control the expression of small heat shock genes [32].

**Alternate Sigma Factor**

In *E. coli* heat shock response is regulated by alternative sigma factor $\sigma^{32}$, which is encoded by the *rpoH* gene. It was found to function as a thermosensor and was regulated at the translational level by the two distinct regions which resided within the coding region [18]. The open reading frame of *rpoH* formed a highly structured mRNA containing several three way junctions at lower temperature, thereby, preventing the entry of ribosome to the SD sequence [33]. Melting of this RNA structure at higher temperature ($\geq 37$ °C) led to the SD sequence being accessible for translation of the sigma factor which induced the heat shock response [6].

Another alternative sigma factor RpoS ($\sigma^8$ or $\sigma^{38}$) was found to regulate the virulence-associated major outer surface proteins OspC and OspA in the spirochete *Borrelia burgdorferi* [6, 34]. Moreover, a small non-coding RNA, DsrABb, regulated temperature-induced increase in RpoS as with the upshift in temperature, its secondary structure melted, and it interacted with the anti-SD region of the RpoS mRNA. This released the sequestered SD sequence leading to translation under virulence conditions i.e. 37 °C [4, 35].

**Csp Thermometer**

In *E. coli*, cold shock leads to the expression of Cold shock protein A (CspA), an RNA chaperone that binds single-stranded RNAs to prevent the formation of any secondary structures during stress [36]. The expression of CspA was found to be controlled by the help of the RNAT present in the 5' UTR of the gene, thereby attributing thermosensing properties [4]. Lower temperatures (such as 10 °C) favoured mutually exclusive alternative structures causing the rearrangements of the CspA RNAT resulting in the ‘cold shock structure’ being translated efficiently. However, at 37 °C, the translation initiation elements remained buried within a double-stranded structure that hindered the translation efficiency [4].

**cIII Thermosensor**

The fate of Bacteriophage $\lambda$ to enter the lytic phase or lysogenic phase depends on the cIII gene product, which was found to be temperature responsive and controlled by RNAT [28]. The RNAT existed in two mutually exclusive structures i.e. A (translation OFF) and B (translation ON) which functioned in switch-like fashion. The structure B dominated at temperature below 37 °C ensuring the interaction between the anti-SD site and an anti-anti-SD site, thereby making the SD site accessible for translation. The translation of cIII mRNA led to the lysogenic pathway [28]. At 45 °C, the structural rearrangement causes the domination of structure A and thus the SD site gets sequestered due to interaction with the anti-SD site. This prevents the translation of cIII, thereby driving the phage into the lytic pathway [28].

**Some Examples of Other RNAT Conferring Virulence**

Recent report highlighted that RNAT played a pivotal role in production of secreted toxin cytotox necrotizing factor (CNF$_Y$) in bacterial pathogen *Yersinia pseudotuberculosis* which helps in evading the host immune system [37]. A thermo labile stem loop structure was identified in the 5'UTR of CNF$_Y$ gene in which the RBS was occluded at 25 °C but was liberated at 37 °C. Similarly in case of
Neisseria meningitidis, RNAT controlled the expression of CsaA, which is a bacterial immune evasion factor. The stability of the RNAT present in the 5'UTR region was found to respond to a temperature of 37 °C which caused the opening up of the structure and subsequent access to RBS [38]. In Listeria monocytogenes, RNAT regulated the expression of key transcription factor prfA which functions as a master regulator of virulence [26]. Studies demonstrated that prfA RNAT is highly responsive to a narrow temperature range and thus transition from 30 to 37 °C leads to unfolding of the two internal loops surrounding the RBS and AUG regions. Moreover, distal regions have also been identified to stabilize RNA structure and regulate its translation [39]. Similarly, RNT located in the 5'UTR of transcriptional regulator tvIA of Salmonella Typhi have been shown to regulate virulence and innate immune evasion in response to host temperature. [40], tvIA functions as a transcriptional regulator of virulence factors such as Vi capsule, flagellin and type III secretion system. Thus upon host invasion, the RNAT melts open thereby enabling ribosome access to the SD region and translation of tvIA transcript. tvIA protein in turn promotes more Vi capsule formation and suppression of flagellin and type III secretion system which is necessary to evade recognition by and activation of innate immune response [40]. Moreover, recent studies on meningitis-causing bacteria such as Streptococcus pneumoniae and Haemophilus influenzae have demonstrated that the production of polysaccharide capsule and factor H binding proteins were regulated by temperature responsive RNAT located upstream of the genes. Thus upon sensing the increase in temperature, RNAT provide better protection and immune evasion by expression of these genes [41]. Interestingly, an unconventional RNA thermosensor has also been detected to regulate Staphylococcus aureus cidA gene which is required for biofilm formation and survival of the pathogen. Unlike other bacterial RNAT, this thermosensor was found to facilitate translation at low temperature (i.e. 30 °C) and is speculated to function by a different molecular mechanisms [42]. For a detailed role of RNA thermometers in bacterial pathogenesis readers may refer to [43].

Proteins

Transcription Regulators

Some of the bacterial heat shock and virulence genes have been reported to be controlled by the temperature-responsive repressor proteins. In Salmonella, TlpA was found to be a temperature responsive autoregulatory repressor protein and was observed to be functional at low but not at high temperatures such as those encountered upon host infection [44]. Studies have revealed that at lower temperatures (<37 °C), it dimmerises and binds the DNA thereby blocking transcription whereas at higher temperature the dimmer dissociates leading to gene expression (Fig. 2c). Apart from repressing its own transcription, it also represses the expression of other virulence associated genes [44]. Similarly in pathogens such as Yersinia, transcriptional regulator RovA controls the expression of itself and of the invasin protein required for pathogenesis [45]. At temperature 20–25 °C, RovA was found to be active and bound the target sites (rovA promoter and invA promoter) with high affinity, thereby preventing their transcription. An upshift to 37 °C caused the conformational change in RovA which reduced its DNA binding capacity and thus allowed the expression of target genes.

Hsps in various gram positive bacteria are jointly regulated by CtsR and HrcA repressor proteins which binds to CtsR box and CIRCE (Controlling inverted repeat of chaperone expression) respectively [46]. In Bacillus subtilis, DNA binding capability of CtsR was found to be functional only at 37 °C and thus, suppressed the transcription under normal conditions. Whereas at heat-shock temperatures (50 °C), CtsR had reduced activity, thereby making the promoter accessible for transcription of target genes [47]. In case of Streptococci, both CtsR and HrcA control the overlapping regulons harboring most of the Hsps [48]. Report by Rossi et al. [46] also indicated that in case of Staphylococcus saprophyticus, both the repressor proteins control the expression of Hsps. Similarly, studies on Chlamydia trachomatis during intracellular infection confirmed the regulation of heat shock genes by HrcA within the infected cell [49]. In Campylobacter jejuni, the DNA binding activity of HrcA is regulated by HspR and GroE proteins under heat shock conditions, which enhances its function as an intrinsic protein thermometer [50].

Methyl-Accepting Chemotaxis Proteins (MCPs)

It has been well known that upon small changes in temperature, Escherichia coli responds by altering its swimming behaviour [51]. TsR and Tar have been documented to be two receptors that sense Serine and Aspartate respectively and are involved in this chemotaxis mechanism [52]. The roles of these receptors have also been observed in sensing temperature, referred to as thermostaxis. Studies have shown that in the absence of both Serine and Aspartate, the receptors lead to the movement towards hotter regions [53]. However, in the presence of Aspartate, Tar promotes the heat seeking response whereas TsR promotes cold-seeking response thereby causing the accumulation of bacteria at intermediate temperatures. Interestingly, in the presence of Serine only, similar
behaviour is observed as both the receptors swap their roles [53]. Thus, it suggests that thermotaxis plays an important role in bacterial survival in diverse environments.

**Chaperones and Proteases**

The chaperone system in *Escherichia coli* consists of DnaK (HSP70) which works along its co-chaperone DnaJ and the nucleotide exchange factor GrpE [54]. Primary function of DnaK involves directing substrates for unfolding, disaggregation, refolding or degradation [54]. The long N-terminal α-helix of GrpE was observed to function as a thermosensor as under heat stress conditions, paired α-helices of GrpE underwent a helix-to-coil transition, which inactivated its nucleotide exchange activity [6]. This in turn inhibited the release of substrate from the chaperone DnaK and resulted in conversion of DnaK from an ATP-consuming foldase into a holdase which tightly binds the unfolded polypeptides [6]. In case of *Bacillus anthracis*, GroEL keeps the protein kinase C (PrkC) in active conformation during the transition stage such as sporulation and infection. Thus, the GroEL helps in regulating signal transduction proteins involved in bacterial pathogenesis [55]. Even ClpC ATPase, which belongs to the heat shock protein family, have been reported to play an important role in *Bacillus anthracis* sporulation pathway. ClpC mediated proteolysis of SpoIAB protein at engulfment stage facilitated spore maturation which is necessary for this pathogen to survive under harsh conditions inside host cells [56]. Apart from Hsp’s, a homolog of cold shock protein, PprM was also found to attribute extreme environment stress tolerance to *Deinococcus radiodurans* [57].

Apart from this, proteases such as DegS also act as thermosensors, embedded in the inner membrane of the cell. With the help of the periplasmic domain, DegS senses the unfolded, misfolded or degraded outer membrane proteins and thus gets activated thereby cleaving the anti-sigma factor RseA [58]. This releases the sigma factor σE leading to the transcription of genes in the σE regulon.

DegP or HtrA, the most evolved temperature sensing protein in *Escherichia coli*, was found to have both the chaperone and the protease activity and remarkably switched between both in a temperature-dependent manner [59, 60]. It has been speculated that at lower temperatures (below 28 °C) the proteolytic Ser remains in an inactive conformation resulting in only chaperone activity. However, the increase in temperature could lead to conformational changes thereby promoting protease activity [60, 61]. Such a mechanism has been also reported in the bacteria *Thermotoga maritima*, which harbours the chaperone-protease Tm HtrA [6]. It was observed to function as a chaperone at normal temperatures but the elevation in temperature caused the conformational changes thereby switching on its protease activity [6].

**Miscellaneous**

**Kinases**

Two-component system (TCS) in bacteria comprises a pair of sensor and response regulator proteins which help in responding to a variety of environmental stimuli [62]. The sensor protein is usually membrane-bound Histidine kinase, which upon environmental stimuli auto phosphorylates and transfers the phosphate group to its response regulator protein. The response regulator then activates the transcription of target genes. [51, 62].

In *Bacillus subtilis*, DesK, which is a Histidine kinase, was found to play an important role in maintaining membrane fluidity at low temperatures [63]. Interestingly, although it lacked the extracytoplasmic signal-sensing domain, it sensed the signal via its 10 transmembrane helices. Studies have further proposed that DesK sensed the membrane status such as thickness, fluidity and water permeability and transduced the signal [63]. Apart from this, Dimorphism regulating kinase (Drk1) represents a variant of TCS as it consists of both the sensor and the receiver domain. It is one of the conserved Histidine kinases present in the dimorphic fungi such as *Histoplasma capsulatum* and *Blastomyces dermatitidis*. Drk1 has been shown to act as a temperature sensor as it controls morphogenesis as well as adaptation of these fungi to various stresses present inside the mammalian host [2].

There have also been reports depicting the presence of two-component system in plant pathogens like *Agrobacterium tumefaciens* which control virulence. It consists of the sensor kinase VirA which phosphorylates the response regulator VirG, which in turn activates other sets of vir genes [51]. The vir genes aid in the excision and transfer of T-DNA from the *Agrobacterium* to the plant host [6]. Various experiments revealed that the expression of virulence occurred only at temperatures below 32 °C and temperatures above this led to its reversible inactivation of VirA and thus prevented VirG from being phosphorylated [6].

In *Synechocystis sp.*, the membrane fluidity changes under cold temperatures were observed to be controlled by desaturases such as DesB which has been shown to be regulated by Histidine kinases i.e. Hik33 and Hik19 [64, 65]. Hik33 possibly perceived the cold-induced membrane rigidification because of the change in its thickness through the help of its transmembrane domains. The signal is then relayed via Ree26 response regulator to activate the cold-inducible genes [65].
Type VI Secretion System (T6SS)

The type VI secretion system (T6SS) is responsible for mediating interspecies competition, virulence, and natural transformation in *Vibrio cholerae* [66]. It has been reported to consist of a base which spans the cell envelope, an inner tube that is composed of hemolysin-coregulated protein (Hcp) polymers, and an outer contractile sheath that is formed by VipA and VipB [66]. Further, it was speculated that temperature affected T6SS to a great extent as the message abundance, protein production and secretion, and activity of Hcp were highest at 25 °C when compared to 37 °C [66].

Conclusion and Future Prospects

Heat stress resulting from elevated ambient temperature is one of the most environmental stresses faced by the microbes, particularly when they enter mammalian hosts [67]. Thus, the ability to sense and adapt to environmental temperature perturbations represent a life-or-death challenge for several species [68]. Moreover, thermosensors play a vital role in survival and pathogenesis of various microbes as upon host infection the rise in temperature leads to the activation of various virulence factors that help them in immune invasion. Hence, understanding their molecular strategies becomes important as they can be used as potential targets for drug discovery and vaccine development. Thermosensors such as RNA and DNA provide a rapid and specific control upon temperature change whereas protein thermosensors help in bringing about a global change. However, not all thermosensors discovered so far have been elucidated completely. The recent advancements in the field of RNA sequencing can be used to define secondary structures of all transcripts in bacteria and can be compared at different temperatures [44]. Narberhaus and co-workers have successfully used a similar approach on RNA isolated from *Yersinia pseudotuberculosis* to identify and characterise more than a dozen new thermosensors [69]. Thus, these thermosensing mechanisms will not only aid in elucidating similar strategies in eukaryotes but also help in understanding how temperature orchestrates cellular signaling and development.

Declarations

Conflict of interest The authors declare that the research was conducted in absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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