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REVIEW

Microbial thermosensors

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Abstract Temperature is among the most important of the parameters that free-living microbes monitor. Microbial physiology needs to be readjusted in response to sudden temperature changes. When the ambient temperature rises or drops to potentially harmful levels, cells mount protective stress responses—so-called heat or cold shock responses, respectively. Pathogenic microorganisms often respond to a temperature of around 37°C by inducing virulence gene expression. There are two main ways in which temperature can be measured. Often, the consequences of a sudden temperature shift are detected. Such indirect signals are known to be the accumulation of denatured proteins (heat shock) or stalled ribosomes (cold shock). However, this article focuses solely on direct thermosensors. Since the conformation of virtually every biomolecule is susceptible to temperature changes, primary sensors include DNA, RNA, proteins and lipids.

Keywords Heat shock · Cold shock · Temperature · Sensor · Thermometer · Stress response · Virulence · Gene regulation

Temperature as an important environmental signal

All free-living microorganisms are frequently exposed to environmental insults, among them limitations in nutrient supply, sudden changes in osmolarity, and up- or down-shifts in temperature. A rapid protective response to harmful conditions requires the coordinated induction of multiple genes. One of the best-studied examples is the heat shock response in *E. coli* [1]. Global analyses have revealed that more than a hundred genes are under heat shock control [2–4]. Several heat shock proteins (HSPs) are molecular chaperones or proteases. However, there are many other HSPs that cover a wide range of cellular functions in maintaining protein and membrane homeostasis and nucleic acid topology at high temperatures.

An elevated temperature also is a decisive signal for several mammalian pathogens, indicating that they may have successfully invaded a warm-blooded host. This high temperature response should be distinguished from the heat shock response, although it should be noted that many "heat shock" proteins play a role in bacterial virulence and enable, for example, survival in host organisms [5–7]. Not a potentially dangerous temperature, but a moderate temperature of around 37°C (well within the physiological growth range of a mesophilic bacterium) is reached. As a result, virulence genes are induced [8].

However, temperature response is fundamentally different in plant pathogens, such as *Agrobacterium tumefaciens*, *Erwinia* species or *Pseudomonas syringae*. They often prefer low temperatures for successful infection. The transcription of almost all their virulence genes, including determinants that direct bacteria-to-plant gene transfer, plant cell-wall-degrading enzymes, and type III protein secretion machinery, is induced at temperatures well below their respective growth optima [9–14]. Although many of these phytopathogens cause "cold weather" diseases, the ecological rationale for this phenomenon remains to be studied in detail. Since the temperature range for infection differs from that of mammalian pathogens, different thermosensing principles are expected [15].

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Cold is yet another temperature condition that microorganisms frequently encounter in nature. It causes physiological problems that are entirely different from heat stress. The obstacles include reduced enzyme activity, decreased membrane fluidity, and stable RNA structures, which interfere with translation. The induction of cold shock proteins (CSPs) enables efficient translation and maintains membrane integrity after a temperature downshift [16–18].

A prerequisite for mounting an appropriate response to temperature fluctuations is precise thermosensing. How do microbes sense that the temperature has changed? There are multiple ways of achieving this. Often the consequences of a temperature shift rather than the temperature itself provide a signal that the cell recognizes and responds to. Again, the bacterial heat shock response is an informative example. Regardless of whether alternative sigma factors (σ^{32}) or repressor proteins (HrcA, HspR, CtsR) are used to regulate heat shock gene transcription, these factors are tied into complex feedback loops involving molecular chaperones or proteases. The principle of these mechanisms is the focus of several recent review articles [1, 6, 19], and will not be discussed here.

Since virtually every biomolecule responds to temperature shifts by performing conformational changes, this can be exploited for direct temperature sensing mechanisms to control the expression of heat shock, cold shock or virulence genes. The effects of temperature on DNA curvature or supercoiling can influence transcription initiation. The three-dimensional structure of mRNA is sensitive to temperature, providing the means to modulate translation efficiency. Since the tertiary and quaternary structures of proteins are susceptible to temperature changes, and particularly to heat, several protein-dependent thermometers have evolved in nature. The purpose of this review is to present a survey of the direct temperature sensing strategies utilized by bacteria, archaea and unicellular eukaryotes (Table 1). All of the thermosensory elements described in this article control gene expression at the transcriptional, translational or post-translational level.

DNA as a thermosensor

DNA topology

The central genetic dogma states that genetic information is transferred from DNA via RNA to protein [20]. As a consequence, any condition that affects the integrity and topology of DNA will ultimately impact on cellular gene expression. DNA supercoiling is one of the many parameters affected by temperature. This is well documented for plasmids from mesophiles and hyperthermophiles that undergo a reversible change in topology during temperature stress [21]. The extent of supercoiling varies in different organisms. The DNA of all known mesophiles is negatively supercoiled, whereas *Sulfolobus* species growing at temperatures above 80°C contain relaxed or positively supercoiled plasmids. In each case, a heat shock introduces a transient increase in positive supercoiling, which leads to immediate plasmid relaxation in organisms like *E. coli*, *Salmonella* or *Bacillus subtilis* (Fig. 1a). This process is mediated by gyrase and topoisomerase I [22]. Recovery to normal negative supercoiling is observed within 10 min after the heat shock and requires the gyrase in collaboration with the nucleoid-binding protein HU and the chaperone DnaK [21].

Topological dynamics during cold shocks have been analyzed in much less detail. Plasmid supercoiling transiently decreases during cold shock (Fig. 1a). Recovery to the original conformation occurs after 1 h. DNA gyrase and HU may play a role in this process [23].

Similar DNA topology-dependent mechanisms were found in the hyperthermophilic archaea *Sulfolobus* and *Thermococcus* [24]. *Sulfolobus* plasmids reached maximal levels of positive supercoiling after heat shock from 80 to 87°C for 1 h. Negative supercoiling, on the other hand, was maximal after 1 h of cold shock to 65°C. Recovery to normal DNA topology is achieved by enzymes whose activities are regulated by temperature. The reverse gyrase is inhibited at 65°C but greatly stimulated at 85°C. The relaxing activity of topoisomerase VI in *Sulfolobus* is optimal at 75°C but completely abolished at 85°C. The DNA decatenation activity of this enzyme, however, has its optimum temperature at a lower temperature [21]. Various DNA-binding proteins presumably play an important role during recovery. Some of them are very abundant, bind to nonspecific sites, and have bending and unwinding properties.

The available data indicate that the DNA topology in mesophiles and thermophiles acts as a stress sensor under both heat and cold shock conditions [21]. Since transcription efficiency is very sensitive to changes in DNA topology [25], it is easy to see that DNA supercoiling is an important parameter in the thermoregulation of gene expression [26, 27].

In *Sulfolobus* it was shown that DNA topology has little effect on transcription levels at the physiological temperature of 75°C. At lower temperatures, however, the transcription of positively supercoiled DNA was blocked at the level of RNA polymerase binding and DNA strand opening [28]. It appears that the polymerase is unable to overcome the thermodynamic barrier imposed by an overwound DNA template.

Local DNA structures also are involved in temperature-mediated gene regulation [29]. A substantial fraction of

Table 1 Overview of molecular thermosensors from prokaryotes and unicellular eukaryotes

Sensor	Mechanism	Signal	Organism	References
DNA topology				
Plasmids	Reduced or increased supercoiling	Low and high temp	Bacteria and archaea	[21]
plc	A tracts in the promoter control RNA polymerase affinity, which is higher at low temperatures because of a higher curvature	Low temp	*Clostridium perfringens*	[29]
desB	Increased supercoiling	Low temp	*Synechocystis*	[33]
SPI-2	Altered DNA binding properties of H-NS	Host temp	*Salmonella enterica*	[37, 47]
virF	Promoter curvature controls access of H-NS and RNA polymerase	Host temp	*Shigella*	[34]
papBA/H-NS	Altered oligomerization of H-NS relieves promoter binding	Host temp	*E. coli*	[45, 46]
RNA				
cis-acting mRNA elements				
rpoH	Secondary structure in the 5′ UTR and coding region controls translation	Heat shock	*E. coli*	[51, 134–136]
ROSE	Secondary structure in the 5′ UTR controls translation	Heat shock	*Bradyrhizobium japonicum*	[52, 55–57]
			Rhizobiaceae	[53]
			Agrobacterium tumefaciens	[137]
			Many α- and γ-proteobacteria	[54]
agsA (fourU)	Secondary structure in the 5′ UTR controls translation	Heat shock	*Salmonella*	[58]
hsp18	Secondary structure in the 5′ UTR controls translation	Heat shock	*Streptomyces albus*	[74]
lcrF (fourU)	Secondary structure in the 5′ UTR controls translation	Host temp	*Yersinia pestis*	[59]
prfA	Secondary structure in the 5′ UTR controls translation	Host temp	*Listeria monocytogenes*	[60]
cIII	Two mutually exclusive mRNA structures in the 5′-UTR control translation	Heat stress	Phage λ	[65]
cspA	Secondary structure in the 5′ UTR controls translation and mRNA stability	Cold shock	*E. coli*	[66, 67]
cspE	Secondary structure in the 5′ UTR mRNA stability	Cold shock	*E. coli*	[68]
trans-acting small non-coding RNAs				
$DsrA_{Bb}$	*rpoS* translation is induced	Host temp	*Borrelia burgdorferi*	[69]
DsrA	*rpoS* translation is induced	Low temp, 23°C, cold shock	*E. coli*	[70, 71, 138, 139]
Proteins				
Transcriptional repressors				
RheA	Reversible transition between active and inactive conformation	Heat shock	*Streptomyces albus*	[73, 74]
TlpA	Reversible dimer to monomer transition	Host temp, heat shock	*Salmonella typhimurium*	[75, 77]
Phr	Dimer binds to heat shock promoters at 95°C and dissociates at 107°C	Heat shock	*Pyrococcus furiosus*	[78]
Sensor kinases				
CorS	Reversible integration of the H-box into the membrane	Low temp (18°C)	*Pseudomonas syringae*	[85, 92]
VirA	Reversible inactivation	Temp above 32°C	*Agrobacterium tumefaciens*	[9]

Table 1 continued

Sensor	Mechanism	Signal	Organism	References
DesK	Membrane-fluidity-dependent shift between kinase and phosphatase activity	Cold shock	*Bacillus subtilis*	[96]
Hik33	Membrane-fluidity-dependent autophosphatase activity	Cold shock	*Synechocystis*	[87, 88]
Other proteins				
Methyl-accepting chemotaxis proteins (MCPs)	Mechanism unknown; response depends on aspartate binding state and protein methylation	Cold, warm	*E. coli*	[107–109, 111–113]
M-like proteins	Dissociation of dimeric coiled-coil molecules into monomers	Host temp	*Streptococci*	[115]
Chaperones and proteases				
Hfq	Binding of the RNA chaperone to *invE* mRNA is higher at low temperatures resulting in transcript degradation	Host temp	*Shigella sonnei*	[48]
GrpE	Reversible melting of α-helices at the dimer interace	Heat shock	*E. coli*	[120]
	Reversible unfolding of globular C-terminal domain		*Thermus thermophilus*	[122, 123]
CGE1	Reversible structural transition in β-domains		*Chlamydomonas reinhardtii*	[124]
Hsp26	Shift from a low-affinity to a high-affinity state	Heat shock	*S. cerevisiae*	[128]
DegP	Reversible switch from chaperone to protease conformation	Heat shock	*E. coli*	[131]
HtrA	A helical lid covers the active site and is lifted up to expose the catalytic and substrate-binding sites		*Thermotoga maritima*	[133]

E. coli promoter was shown to be characterized by intrinsic bends [30]. The presence of intrinsically curved DNA regions (e.g., by AT-rich sequences) influences the promotor's affinity for RNA polymerase [31]. Temperature-induced topological changes in such regions directly affect gene expression. For example, altered DNA curvature upstream of the promoter of the *Clostridium perfringens* phospholipase C (*plc*) gene induces expression at low temperature (Fig. 1b). The RNA polymerase binds to the minor grooves of three A tracts located between −66 and −40 relative to the transcription initiation site of the *plc* gene. At low temperatures, increased bending of these A tracts enhances the binding affinity for the polymerase and facilitates open complex formation [29, 32].

In the cyanobacterium *Synechocystis*, low temperatures increase the supercoiling of the genomic DNA region that contains the regulatory elements of the *desB* gene [33]. The *desB* gene codes for the omega-3 desaturase, a fatty acid desaturase that controls membrane fluidity by introducing double bonds into the acyl chains of lipid-bound fatty acids. Additional mechanisms contribute to maintaining membrane homeostasis at low temperatures (see below).

The role of histone-like nucleoid structuring protein (H-NS) and other nucleic acid binding proteins

Apart from directly controlling access to RNA polymerase, bent DNA can also repress transcription in an indirect way by binding specific "silencer" proteins which modulate pre-existing DNA loops [34, 35]. Among these silencer proteins is the nucleoid protein H-NS, a 136-amino-acid protein that plays a fundamental role in bacterial response to temperature. It affects the expression of a large number of unrelated enterobacterial genes coding for housekeeping functions as well as for virulence factors. H-NS prefers AT-rich sequences. The accessibility of its target sequences both in vitro and in vivo varies significantly depending on the temperature, and it has been suggested that this is related to a sharp structural transition in the DNA [36]. H-NS itself is subject to temperature control, since the formation of higher-order oligomers and the DNA binding capacity are reduced at 37°C [37]. The H-NS to DNA ratio is fairly constant except during cold shock, when it increases three- to fourfold [38]. The temperature-modulated accessibility of promoter regions, which are occupied by H-NS at low

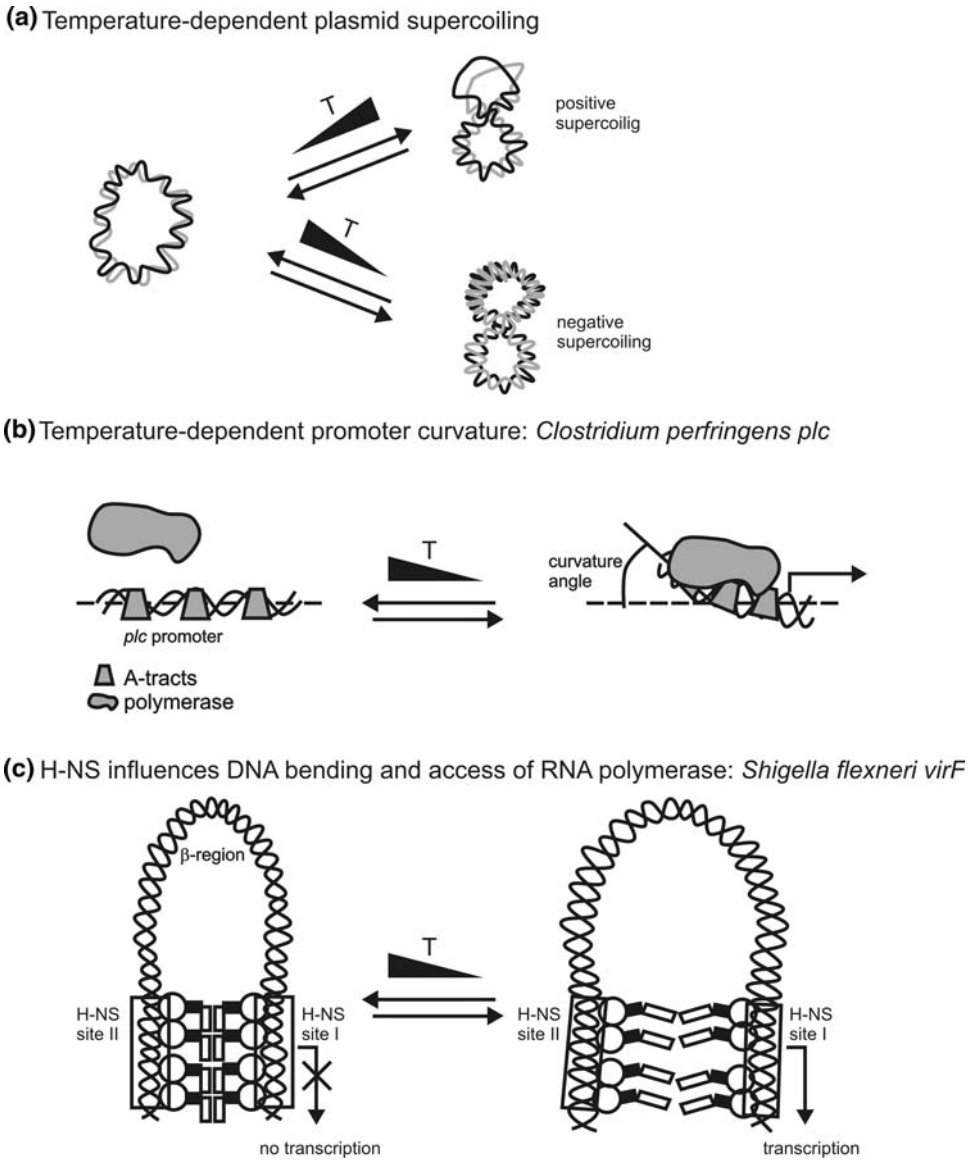

Fig. 1 DNA as thermosensor. **a** Transient relaxation of plasmids occurs upon heat shock. Cold shock causes increased negative supercoiling. **b** Increased DNA bending at phased A tracts at low temperatures improves affinity for the RNA polymerase. **c** DNA topology and DNA binding affinity of H-NS are temperature dependent

temperatures, is a key regulator of virulence gene expression in many human pathogens, like *Yersinia enterolytica*, *Clostridium perfringens* and *E. coli* [32, 39–43]. A recent DNA microarray study demonstrated that H-NS controls 69% of the temperature-regulated genes in *E. coli* K-12. It was shown to be a common regulator of multiple iron and other nutrient acquisition systems preferentially expressed at 37°C and of general stress response, biofilm formation, and cold shock genes highly expressed at 23°C [44]. Another example of H-NS-mediated regulation is the expression of Pap pili, which facilitate the attachment of *E. coli* to uroepithelial cells. Their expression is shut off outside the host at temperatures below 26°C. Conformational changes in H-NS at this temperature are speculated to repress the transcription of *papBA* coding for Pap pilin through the formation of a specific nucleoprotein complex [45, 46].

In *Salmonella*, 77% of the 408 genes that are temperature regulated were found to be either directly or indirectly dependent on H-NS [37]. An example of H-NS-dependent thermoregulation is SPI-2, a horizontally acquired genomic island, which encodes a type III secretion system (T3SS). Bacteria grown at 30°C or lower were unable to express the T3SS, even in the presence of strong inducing signals. Virulence gene expression is controlled by Hha and H-NS, two nucleoid-like proteins which silence the virulence genes at nonpermissive temperatures below 30°C [47]. Thermally induced gene expression is extremely rapid and affects the DNA-binding properties of H-NS and Hha [37]. H-NS silences the expression of the response regulator SsrB, which regulates a set of genes necessary for the host infection. Hha silences the SPI-2 gene expression. Additionally, Hha integrates the host body temperature with

other virulence-inducing signals, such as phosphate and magnesium limitation, to appropriately activate intracellular virulence gene expression [47].

The transcription of *virF* coding for the primary regulator of invasion functions in the human enteropathogen *Shigella flexneri* is strictly temperature dependent (Fig. 1c). The *virF* promoter is characterized by a major DNA bend halfway between two H-NS binding sites. The temperature-dependent activity of the *virF* promoter is antagonistically mediated by H-NS and FIS, which bind to distinct sites [34]. The promoter curvature serves as a thermosensor. The closed conformation of the *virF* promoter, which keeps the two H-NS binding sites sufficiently close to each other to favor a stable nucleoprotein bridge, opens up with increasing temperature. This allows RNA polymerase to access the *virF* promoter. While the *virF* bending center moves downstream at a rate that is at its maximum around the transition temperature, a binding site for the transcriptional activator FIS is abruptly unmasked. In a fine-tuning manner, FIS induces a twofold increase in *virF* transcription but is not involved in its thermoregulated expression [34].

Additional thermoregulation of *Shigella sonneii vir* genes is mediated by the stability of the *invE* transcript coding for another regulator protein. The mRNA stability is influenced by the temperature-controlled activity of the RNA chaperone Hfq. Binding of purified Hfq protein to *invE* RNA in vitro was shown to be stronger at 30°C than at 37°C [48]. In vivo, Hfq binding is associated with *invE* mRNA degradation.

RNA as thermosensor

Due to its physical properties, RNA is well suited to a role as a post-transcriptional intracellular thermosensor. Temperature-responsive RNAs are often referred to as RNA thermometers [49, 50]. All of the RNA thermometers that have been discovered thus far are translational control elements, and most of them are located in the 5′ UTR of bacterial heat shock and virulence genes (Table 1). The majority function via a conceptually simple mechanism, as follows. At low temperatures, the SD sequence is trapped in a hairpin structure (Fig. 2a). Increasing temperature destabilizes the structure such that the ribosome binding site becomes accessible, allowing translation to be initiated.

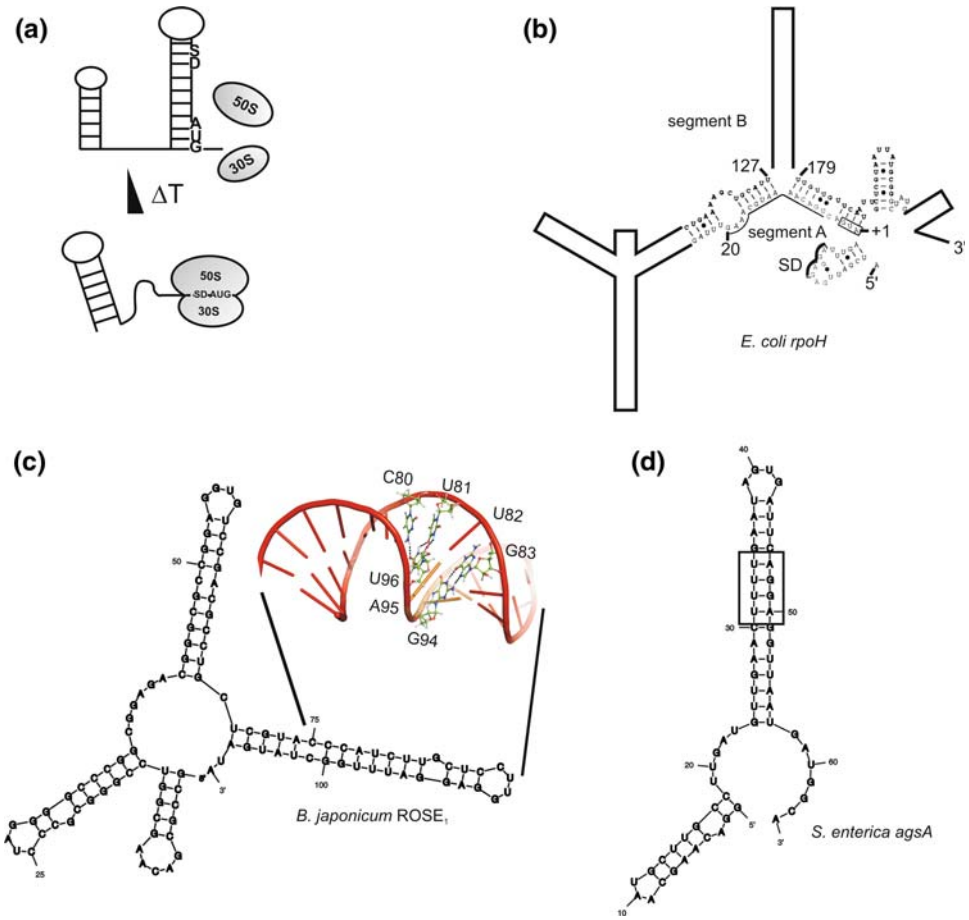

Fig. 2 *cis*-Active RNA thermometers. **a** Principle of RNA thermometers: the Shine–Dalgarno sequence (SD) and AUG start codon in the 5′ UTR of an mRNA are base paired at low temperatures. The melting of the structure at higher temperatures permits the binding of the ribosome (30S and 50S). **b** Thermometer region of the *rpoH* transcript of *E. coli*. **c** ROSE$_1$ element of *Bradyrhizobium japonicum*; *inset* NMR structure of the region indicated. **d** FourU thermometer upstream of the *Samonella enterica agsA* gene

Heat shock

The first RNA thermometer that acts via the melting mechanism was found in the *E. coli rpoH* gene, which codes for the alternative sigma factor σ^{32} or RpoH [51]. In contrast to most other RNA thermometers, almost the entire regulatory region resides in the coding region and not in the 5′ UTR. Two segments (A and B) up to 220 nucleotides into the open reading frame of *rpoH* form an extensive RNA structure that blocks the entry of the ribosome to the SD sequence (Fig. 2b). Disruption of the structure at heat shock temperatures liberates the ribosome binding site and enhances translation of the sigma factor, resulting in the rapid induction of the heat shock response.

Probably the most abundant bacterial RNA thermometer is the repression of heat shock gene expression (ROSE) element. Initially discovered in rhizobia [52, 53], it was later found to be widespread in numerous α- and γ-proteobacteria, including *E. coli* and *Salmonella* [54]. The ROSE element is typically between 60 and 100 nucleotides long and located in the 5′ UTRs of small heat shock genes. It acquires a complex structure comprising 2–4 stem loops (Fig. 2c). The final hairpin contains the SD sequence and in some cases also the AUG start codon. The ROSE element is characterized by only a few conserved nucleotides (UYGCU, in which Y is a pyrimidine) that pair with the SD sequence [50, 54]. Short internal loops and bulges in the computer-predicted final structure are thought to create a thermolabile structure that melts as the temperature increases within the physiological temperature range. The introduction of mismatches into this structure relieves repression at low temperature. Conversely, mutations that stabilize the structure abolish induction at heat stress temperatures [55, 56]. The NMR structure of the temperature-responsive ROSE hairpin revealed that the actual RNA structure differs from the computer prediction [57]. The hairpin containing the SD sequence is made of several noncanonical base pairs, including a G–G pair and a base triple that renders the structure vulnerable to high temperatures (inset in Fig. 2c).

Another presumably widespread RNA thermometer is the fourU element, which was initially described as being upstream of the small heat shock gene *agsA* in *Salmonella* [58]. The predicted structure contains two hairpins. Four uridine residues in the second hairpin are used to base pair with the SD sequence (Fig. 2d). Structure probing experiments revealed the temperature-controlled opening of the second hairpin, and toeprinting experiments demonstrated that the binding of ribosome to the SD sequence occurs only at heat shock temperatures.

Virulence

Since four uridines can act as a very suitable building block for loosely pairing with AGGA of the SD sequence, fourU thermometers could be frequently used to control bacterial heat shock and virulence genes [58]. The 5′ UTRs of the *Brucella melitensis dnaJ* gene and the *Staphylococcus aureus groES* gene are likely candidates. Back in 1993, an interesting candidate was predicted in the 5′ UTR of the *Yersinia lcrF* (*virF*) gene, which encodes a regulator of the virulence response [59]. Fully consistent with RNA thermometer-mediated control, translation of this gene is inefficient at 26°C and is induced at 37°C.

In *Listeria monocytogenes*, an RNA thermometer controls the translation of the virulence gene activator PrfA [60]. The 5′ UTR of the *prfA* transcript is folded such that the SD sequence is poorly accessible. This structure is stable at 30°C but is destabilized at 37°C, enabling the efficient translation of *prfA* when the pathogen resides in the mammalian host.

Synthetic RNA thermometers

The regulatory principle of RNA thermometers requires only a few nucleotides that loosely pair with the SD sequence itself or the flanking regions in order to interfere with ribosome binding. Thus, there might be many other as-yet undiscovered RNA thermometers in nature. Bioinformatic predictions suggest that this may indeed be the case [61]. Further evidence for this assumption is provided by the fact that efficient artificial RNA thermometers can be readily generated [62–64].

Phage development

Since the melting of the RNA structure proceeds gradually as the temperature increases, all of the natural and synthetic RNA thermometers described above produce a graded response. However, there are also RNA thermometers that exist as two mutually exclusive structures which act in a switch-like fashion. Translation of the cIII mRNA of phage λ is controlled by temperature. The cIII gene product is involved in the lysis–lysogeny decision of the phage. High concentrations of cIII protein at optimal growth temperatures (37°C) favor the lysogenic pathway. During severe heat stress (45°C), phage λ enters the lytic pathway because the amount of cIII is low. Regulation is accomplished by alternative RNA structures upstream of the cIII. High temperatures shift the equilibrium towards the energetically more stable conformation, in which the SD sequence is blocked. At 37°C, the structure with the accessible ribosome binding site is preferred [65].

Cold shock

The expression of some cold shock genes might also be controlled by secondary structures in their 5′ UTRs. A 159-nucleotide-long sequence upstream of the *E. coli cspA* gene has been postulated to act as RNA thermometer that controls the translation efficiency via alternative RNA structures [66]. The RNA structure might also affect the stability of the *cspA* transcript [67]. *E. coli* contains nine paralogs of CspA, among them CspE. The *cspE* gene contains a 5′ UTR of only 43 nucleotides. Alternative RNA structures in this region are thought to control the differential stability of the *cspE* transcript, which is stabilized at 15°C when the gene product is needed [68].

The alternative sigma factor RpoS (σ^S or σ^{38}) plays a central role in the regulation of the virulence-associated major outer surface proteins OspC and OspA in the Lyme disease spirochete *Borrelia burgdorferi*. Temperature is one of the key environmental signals controlling RpoS, and a small noncoding RNA, $DsrA_{Bb}$, regulates the temperature-induced increase in RpoS [69]. Lybecker and Samuels hypothesized that $DsrA_{Bb}$ folds into a stable secondary structure at 23°C, which does not allow for base pairing with the *rpoS* transcript (Fig. 3). After a temperature upshift, the secondary structure of the small RNA is thought to melt, allowing binding to the anti-SD region of the *rpoS* mRNA. This would stimulate translation by releasing the SD sequence and translation start site from a stable secondary structure in the *rpoS* mRNA under virulence conditions (37°C).

In *E. coli*, the small RNA DsrA is essential for the low-temperature expression of *rpoS* during exponential growth [70]. Synthesis of DsrA itself is thermoregulated by an unusual AT-rich promoter, which is transcribed efficiently at 25°C but poorly at 42°C [71]. A processed form of DsrA pairs with the 5′ UTR of the *E. coli rpoS* transcript. This interaction liberates the SD region and induces RpoS synthesis at low temperatures. It remains to be determined if temperature-induced conformational changes in the secondary structure DsrA are involved.

Protein thermosensors

There are a number of structurally and functionally diverse protein-based thermosensors, because the tertiary and quaternary structures of proteins are very susceptible to temperature changes. Temperature shifts can easily shift a protein from an active to inactive conformation, or induce the disassembly of a dimer into a monomer. Protein sensors include transcriptional regulators, chemosensory proteins, chaperones and proteases (Table 1).

Regulators of transcription

The transcription of many heat shock genes in pro- and eukaryotes is tied into complex autoregulatory loops that respond to the accumulation of misfolded proteins [19, 72]. However, the transcription of some bacterial heat shock and virulence genes is under the direct control of temperature-responsive repressor proteins.

RheA, a specific repressor of the small heat shock gene *hsp18*, is one such thermosensor in the filamentous soil bacterium *Streptomyces albus* [73, 74]. Gel retardation experiments have revealed that RheA complexes with the *hsp18-rheA* promoter at low but not at high temperature [73, 74]. In vitro transcription experiments showed that RheA is an autoregulatory protein whose activity is reversibly inhibited by high temperature. Circular dichroism (CD) spectroscopy revealed a temperature-dependent transition between an active and an inactive form of RheA (Fig. 4a). The translation of *hsp18* may be modulated by an RNA thermometer as a second layer of control (Table 1) [74].

TlpA is an autoregulatory temperature-sensing repressor protein in *Salmonella* [75]. A previously assumed function as a virulence regulator could not be demonstrated in inbred mice [76]. However, a number of studies have clearly established the role of TlpA as a temperature-responsive gene regulator [77]. It is an alpha-helical protein that forms an elongated coiled-coil homodimer. In vitro studies with highly purified TlpA showed that

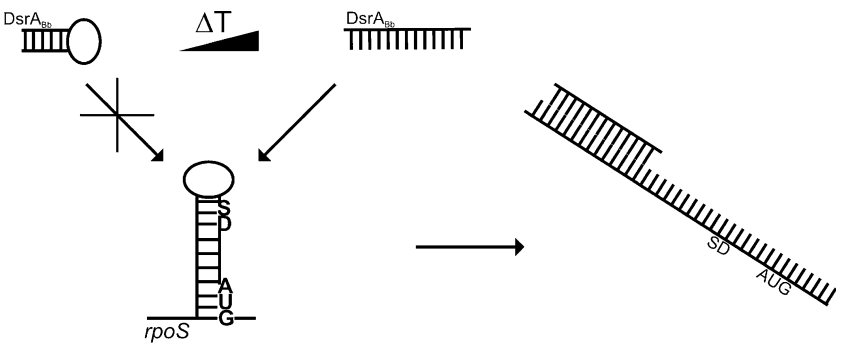

Fig. 3 A *trans*-acting RNA thermometer. The sRNA forms a stable secondary structure, which melts at higher temperatures. The unwound sRNA pairs with the anti-SD sequence in the 5′ UTR of a messenger RNA, making the translation initiation region accessible

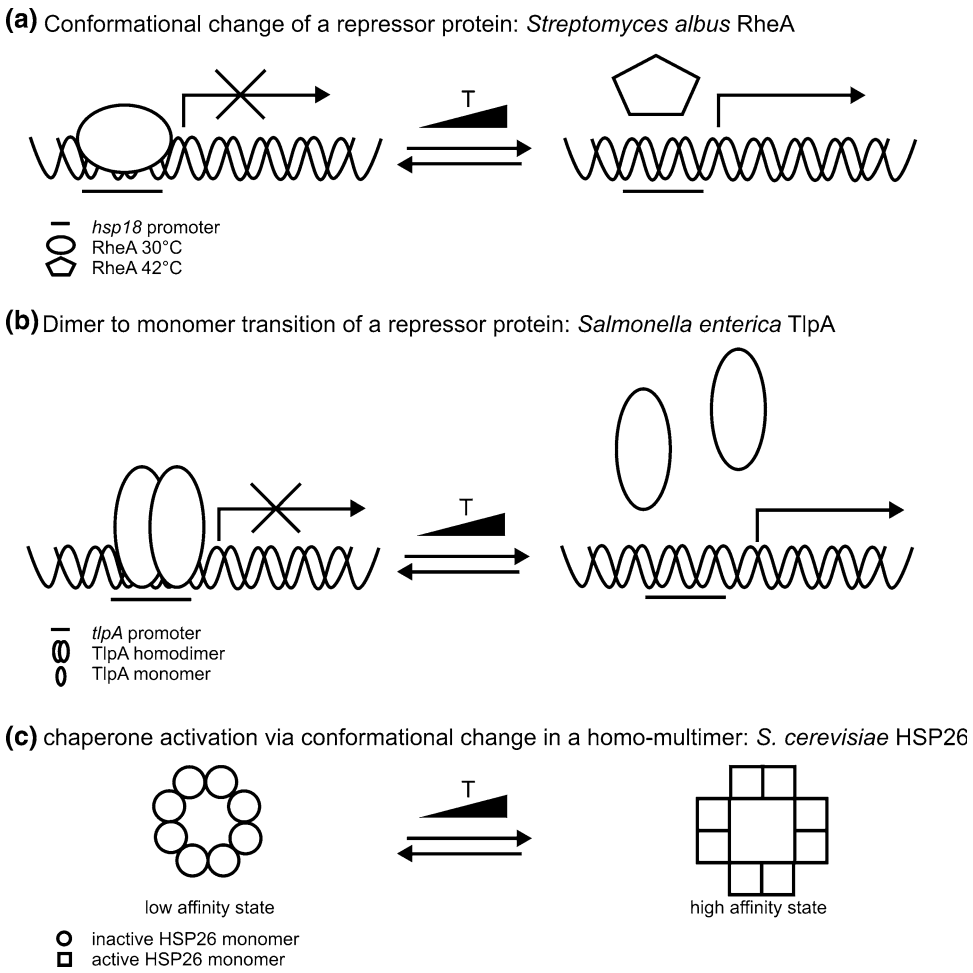

Fig. 4 Protein thermosensors. **a** Heat-induced conformational changes in RheA relieve the repression of the small heat shock gene *hsp18* in *Streptomyces albus*. **b** The *Salmonella enterica* protein TlpA acts as a transcription repressor only in its dimeric state. At higher temperatures, the protein dissociates into monomers that lack DNA binding capacity. **c** The substrate affinity of the yeast chaperone complex HSP26, which consists of 24 subunits, is increased at elevated temperatures

thermosensing is based on a reversible and rapid monomer-to-coiled coil equilibrium [75]. At lower temperatures, the protein forms a dimer that binds the promoter region of its own gene. The subunits dissociate at a host temperature of 37°C, and DNA binding activity is lost (Fig. 4b).

The Phr protein from *Pyrococcus furiosus* is the first heat shock transcription factor in archaea to be documented [78]. It specifically represses the expression of heat shock genes at physiological temperature (95°C) in vitro and in vivo, but is released from the promoters upon heat shock response (107°C). The dimeric protein recognizes a palindromic DNA sequence. Molecular docking and mutational analyses suggested a novel binding mode in which the major specific contacts occur at the minor groove that interacts with the strongly basic wing containing a cluster of three arginine residues. It is presently unclear whether dissociation of Phr is mediated simply by temperature-induced conformation changes or by a more elaborate mechanism [78].

In lower eukaryotes, temperature-dependent gene regulation of morphology and virulence is exhibited by the entire group of systemic dimorphic fungi, such as *Histoplasma capsulatum*, *Blastomyces dermatitidis, Coccidioides* spp., *Paracoccidioides brasiliensis*, *Penicillium marneffei* and *Sporothrix schenkii* [79]. The autoregulatory transcriptional activator Ryp1 in the pathogenic fungus *H. capsulatum* serves as an example. It induces the morphological switch from the filamentous form to the pathogenic yeast form at its host temperature of 37°C [80]. It is not yet clear whether the accumulation or activity is controlled by temperature. In addition, the conserved histidine kinase Drk1 ("dimorphism regulating kinase") was recently identified in *B. dermetitidis* and *H. capsulatum*. Drk1 is required for yeast-like growth at 37°C, suggesting that additional temperature-controlled cell-shape regulating proteins are present in such fungi [81]. Whether one of these proteins is a true thermosensor remains to be shown.

Sensor kinases in plant pathogens

Two component regulatory systems (TCSs) are frequently used by bacteria to adapt cellular functions to changes in

environmental parameters such as osmolarity, pH, light, CO_2, ammonia, oxygen, metal ions, nutrients, host-borne factors and temperature [82–84]. Typical TCSs consist of a membrane-bound sensor histidine kinase that perceives environmental stimuli, and a cytoplasmic response regulator that affects gene expression upon the phosphorylation of an aspartic acid. In some systems, a third component is involved, which can either be an additional histidine kinase, as in the Hik33/Hik19/Rer1 system of *Synechocystis* sp. PCC 6803, or a second response regulator, as in the CorRSP system from the plant pathogen *Pseudomonas syringae*, and the DesKR system of *B. subtilis* [85–88].

Phytopathogenic bacteria use a number of intriguing strategies to colonize their host plants and evade detection by the plant defence system. Such mechanisms include the production of exopolysaccharides, effector proteins, cell-wall-degrading enzymes, and phytotoxins [15]. Several pathovars of *P. syringae* synthesize the non-host-specific phytotoxin coronatine (COR) to enhance virulence [89, 90]. COR is believed to mimic plant signalling, and induces chlorosis, hypertrophy, shrinkage of chloroplasts, and the synthesis of ethylene and proteinase inhibitors [85]. PG4180 synthesizes COR in a temperature-dependent manner with maximum yields at 18°C [91]. At 28°C, the optimal growth temperature of *P. syringae*, COR biosynthesis is negligible [92]. The proteins CorR, S, and P are necessary for this process. The response regulator CorR binds to the promoter region of the *cma* operon responsible for the biosynthesis of coronamic acid, a moiety of COR. The sensor kinase CorS transfers its phosphoryl group to CorR but not to CorP, which correlates with the presence of a receiver aspartate residue in the former but not the latter protein [93]. Despite its high degree of similarity to CorR, CorP lacks a typical DNA-binding motif, suggesting that it just modulates the function of CorR. Topological analyses of the CorS suggest that the sensor kinase is composed of six membrane-spanning domains. The current model holds that autophosphorylation of CorS is controlled by temperature-dependent conformational changes of the H-box containing the conserved histidine. While the H-box is cytoplasmic and functionally active at 18°C, it is proposed that it slips into the membrane and becomes inaccessible to autophosphorylation at 28°C [85].

The plant-pathogenic *Agrobacterium tumefaciens* induces tumors only at temperatures below 32°C. Virulence is controlled by a two-component system consisting of the membrane standing sensor kinase VirA and the response regulator VirG. VirA senses the plant-derived signal molecule acetosyringone and induces *vir* gene expression via VirG. At temperatures of 32°C and higher, VirA undergoes reversible inactivation and thus acts as a thermosensor [9].

Sensor kinases and the protein–lipid connection

The cell envelope is the first cellular compartment to come into contact with the external temperature. Exposure to cold stress or heat shock drastically alters membrane properties, and this must be counteracted quickly in order to maintain membrane integrity and the critical function of membrane proteins [94]. Therefore, membranes may act as thermosensors per se, transducing the signal via membrane-integrated proteins. Sensor kinases are likely candidates for this purpose.

Thermal control of membrane lipid homeostasis in some bacteria does indeed rely on temperature-responsive sensor kinases [95]. As the growth temperature decreases, the fluidity of the membrane is maintained either by de novo synthesis of unsaturated fatty acids or by increasing the proportion of unsaturated fatty acids through the action of membrane lipid desaturases, which introduces double bonds into pre-existing fatty acid site chains. In *B. subtilis,* the DesKR system regulates the expression of the *des* gene coding for a specific desaturase [96]. The sensor kinase DesK consists of four transmembrane domains and a long C-terminal region containing the conserved His residue in the cytoplasmic kinase domain. The activity of the isolated kinase domain is not temperature dependent, suggesting that the membrane portion contains the temperature-sensitive element [86, 97]. It has been proposed that decreased membrane fluidity favors a kinase-dominant state of DesK, which then phosphorylates the response regulator DesR. In turn, DesR forms dimers that activate *des* transcription, resulting in an increase in membrane fluidity. Membranes with higher or normal fluidity then favor a phosphatase-dominant state of DesK, which dephosphorylates DesR. The resulting monomer closes the feedback loop, as is no longer able to activate *des* transcription [98, 99].

The cyanobacterium *Synechocystis* sp. PCC 6803 adapts its membrane fluidity via four acyl-lipid desaturases, designated DesA, DesB, DesC, and DesD [100]. The gene *desC* is expressed constitutively, whereas transcription of *desA*, *desB*, and *desD* is induced after temperature downshift. Two histidine protein kinases, Hik33 and Hik19, control desaturase gene expression [101]. Hik33 (DspA) is integrated into a network of histidine kinases and response regulators that perceive and transduce not only cold shock but also osmotic and oxidative stress conditions [88, 102]. Hik33 was postulated to be a membrane-embedded thermometer. Upon temperature downshift and the subsequent decrease in membrane fluidity, Hik33 autophosphorylates and transfers its phosphate group via Hik19 to the cognate response regulator Rer1. The response regulator, in turn, regulates the transcription of the *desBD* genes. Expression of the *desB* gene is also controlled via supercoiling of its DNA (Table 1) [33].

There may also be a connection between membrane fluidity and heat shock response in *Synechocystis* [103, 104]. The physical order of the membranes (but not modulation of the lipid saturation) had a critical influence on the activation of heat shock genes, in particular the *hsp17* gene. It was suggested that the thylakoid membrane of *Synechocystis* is the cellular thermometer where thermal stress is sensed and transduced into a cellular signal. Hsp17 might be involved in this process, as it shows a preferential interaction with non-bilayer phase-forming lipids. The accumulation of such lipids during heat shock enhances the binding of heat shock proteins to the membrane, which in turn restores membrane function in many organisms [105]. Induction of *Synechocystis* Hsp17 at heat shock temperatures depends on a combination of transcriptional control by the sigma factor SigB [106] and translation control by a novel RNA thermometer (unpublished results).

Methyl-accepting chemotaxis proteins (MCPs)

Escherichia coli responds to small changes in temperature by altering its swimming behavior to migrate in spatial temperature gradients. Four closely related transmembrane proteins have been identified as thermosensors; these function either as warm sensors (Tsr, Tar and Trg) or as cold sensors (Tap and Tar in the presence of attractants) [107–110]. The aspartate chemoreceptor Tar of *E. coli* is best understood mechanistically [111–113]. Tar is a warm sensor in the absence of aspartate, but it is converted to a cold sensor upon adaptation to aspartate or maltose. As a warm sensor, it mediates attractant and repellent responses upon increases and decreases in temperature, respectively. When aspartate is bound, Tar is methylated at one or more of its four methylation sites and becomes a cold sensor, mediating the opposite temperature response to that of the unbound form. Methylation of a single glutamyl residue in a C-terminal α-helix is sufficient to invert the sign of thermosensing [112]. Attractant-independent inversion to a cold sensor was achieved by a single mutation in the second transmembrane region (TM2) of Tar [111]. The molecular mechanism of thermosensing employed by the chemoreceptors remains to be determined.

M-like proteins

Temperature-dependent attachment of streptococci to plasma proteins is mediated by cell surface proteins belonging to the so-called M protein family [114]. They have a coiled-coil conformation in which two α-helical monomers twist around each other, forming a homodimer [115]. Dimer formation of the streptococcal Arp4 protein is a prerequisite for IgA-binding, which is high at 10°C and 20°C but very weak at 37°C. At this temperature, the coiled-coil structure of Arp4 is destabilized.

Chaperones and proteases

Transcription and translation of molecular chaperone and protease genes is induced under heat stress conditions. In addition, the activities of some of the encoded proteins are modulated by the ambient temperature, providing a post-translational mechanism to control the composition of the cellular protein pool [116].

The *E. coli* DnaK chaperone system consists of DnaK, its co-chaperone DnaJ, and the nucleotide exchange factor GrpE. DnaJ (nonnative protein sensor) and GrpE (thermosensor) monitor the cellular situation and thus directly adapt the operational mode of the DnaK system to heat shock conditions [117]. Activity of this system is thermally controlled by conformational changes in GrpE [118]. GrpE is a fascinating molecule with an unusual quaternary structure. The long N-terminal alpha-helix of the protein acts as a thermosensor responsible for the decrease in GrpE-dependent nucleotide exchange that is observed in vitro at temperatures relevant to heat shock [119]. GrpE and DnaK form complexes with a stoichiometry of 2:1. The affinity of GrpE for DnaK is not affected by temperatures above 40°C. However, the paired α-helices of GrpE undergo a helix-to-coil transition, and melting of the helix bundle inactivates nucleotide exchange activity. This retards the release of substrate from the chaperone DnaK at higher temperatures and converts DnaK from an ATP-consuming foldase into a holdase that tightly binds to unfolded polypeptides to maintain them in a folding-competent state under conditions where protein folding is inefficient [120, 121]. Re-association of GrpE at lower temperatures presumably stimulates the release of client proteins from DnaK. A similar thermal transition in GrpE, although occurring in some other domain of the protein, may control DnaK function in *Thermus thermophilus* [122, 123].

The chloroplast homolog of GrpE in the eukaryotic unicellular algae *Chlamydomonas reinhardtii* is termed CGE1 [124]. Bacterial GrpE and chloroplast CGE1 share similar structural and biochemical properties, but some of these—like dimerization—are realized by different domains. Interestingly, CGE1 exists in two isoforms, CGE1a and CGE1b, which are generated by temperature-dependent alternative splicing. CGE1a is predominant at lower temperatures, and CGE1b becomes as abundant as CGE1a at elevated temperatures [125]. CGE1b has ∼25% higher affinity for its chloroplast chaperone partner HSP70B than CGE1a.

The structure and function of small heat shock proteins (sHsps or α-crystalline-type proteins or α-Hsps) are also

temperature dependent. sHsps are ATP-independent chaperones that form large oligomeric structures and bind to non-native proteins in order to prevent their aggregation [126, 127]. Since sHsps are by far the most diverse class of molecular chaperones, the molecular details of their chaperone action differ substantially between individual family members. Typical sHsp oligomers build highly dynamic assemblies, which exchange subunits and alter their oligomeric states in response to temperature. Hsp26 from the yeast *S. cerevisiae* is an excellent example of how temperature can control chaperone function by conformational changes. Hsp26 forms shell-like particles composed of 24 subunits. Local structural rearrangements in the middle domain of Hsp26 occur within a narrow temperature range around 45°C [128]. These changes do not have a major influence on the overall Hsp26 structure, but shift the oligomer from a low- to a high-affinity state (Fig. 4c).

The *E. coli* DegP (HtrA) protein is a remarkable chaperone-protease machine that switches between both activities in a temperature-dependent manner [129]. It is an oligomeric protein that performs important protein quality control functions in the periplasmic space, a compartment devoid of ATP. Initially described as serine protease, DegP was later found to have this activity only at high temperatures, whereas chaperone activity dominates at normal temperatures [130]. Both functions can be separated by a single point mutation that abolishes protease activity without interfering with chaperone function.

The crystal structure of DegP revealed that it forms a hexamer by staggered association of trimeric rings [131]. The proteolytic sites are located in a central cavity that is only accessible laterally. The mobile sidewalls are constructed from 12 PDZ domains, which mediate the opening and closing of the particle and probably the initial binding of substrate. The inner cavity is lined with several hydrophobic patches that may act as docking sites for unfolded polypeptides. In the chaperone conformation, the protease domain of DegP exists in an inactive state in which substrate binding, in addition to catalysis, is abolished. Binding of misfolded proteins was recently shown to transform hexameric DegP into large, catalytically active 12-meric and 24-meric multimers [132]. The inner cavity serves antagonistic functions. Encapsulated folded protomers of outer-membrane proteins are protected from aggregation, whereas misfolded polypeptides are eliminated in that compartment.

The proteolytic activity of *Thermotoga maritima* (Tm HtrA) is also switched on at elevated temperatures, whereas the chaperone function predominates at normal temperatures. Electron paramagnetic resonance and fluorescence spectroscopy experiments revealed that a helical lid (H_L), which covers the active site, is lifted up to expose the catalytic and substrate-binding sites to the solvent at elevated temperatures. The overall structure of the protein was maintained over a wide temperature range [133]. The proteolytic activity of Tm HtrA is switched on by the geometric change that occurs around the H_L, resulting in a substrate-accessible path.

Concluding remarks

Table 1 summarizes the remarkably diverse temperature-sensing mechanisms found in microbes, that have been discussed in this article. The multifaceted response to a simple physical stimulus, temperature, provides a fascinating example of how microorganisms integrate environmental signals and react in a timely and appropriate manner. It is interesting that any potential temperature-responsive checkpoint in the cell—ranging from the membrane to DNA, RNA and protein—is used in nature. In many cases, the molecular details of the temperature sensing are not yet fully understood. Matters are complicated by the fact that primary temperature sensors are difficult to distinguish from sensors that measure indirect effects. Moreover, the thermal response occurs within the physiological growth range and often induces conformational changes that are subtle and reversible. Nevertheless, the known diversity of temperature-sensing mechanisms underscores the critical importance of temperature as an environmental parameter.

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