CHAPTER 1

THERMOSENSORSY STEMS IN EUBACTERIA

Wolfgang Schumann
Institute of Genetics, University of Bayreuth, Bayreuth, Germany
Email: wschumann@uni-bayreuth.de

Abstract: Four different mechanisms have evolved in eubacteria to comply with changes in the environmental temperature. The underlying genetic mechanisms regulate gene expression at transcriptional, translational and posttranslational level. The high temperature response (HTR) is a reaction on increases in temperature and is mainly used by pathogenic bacteria when they enter their mammalian host. The temperature of 37°C causes induction of the virulent genes the products of which are only needed in this environment. The heat shock response (HSR) is induced by any sudden increase in temperature, allows the bacterial cell to adapt to this environmental stress factor and is shut off after adaptation. In a similar way the low temperature response (LTR) is a reaction to a new environment and leads to the constant expression of appropriate genes. In contrast, the cold shock response (CSR) includes turn off of the cold shock genes after adaptation to the low temperature. Sensors of temperature changes are specific DNA regions, RNA molecules or proteins and conformational changes have been identified as a common motif.

INTRODUCTION

In their natural environment, bacteria are exposed to a variety of environmental insults including sudden changes in osmolarity, in external pH, reactive oxygen species, limitations in nutrient supply and up- and downshifts in temperature.[1] Each stressful situation typically induces a stress response resulting in a characteristic change in the pattern of gene expression. This stress response helps the bacterial cells to restore cellular homeostasis, to protect vital processes and to increase the cellular resistance against subsequent stronger similar stress challenges.

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The habitat niches on earth vary considerably in temperature and therefore, many biological processes are optimized for different temperatures and the physiology of organisms are adapted to their cognate environments. Additionally, the particular niche or lifestyle of many bacteria may be subjected to regular, but sudden, variations in temperature. This reasoning applies for bacteria adjusting their activities according to seasonal variations and certainly for pathogens that circulate between the environment and warm-blooded hosts. Thus, temperature regulation of genes has been the focus of much research and how the temperature signal is sensed and transduced to the biosynthesis machinery has been studied extensively. Here, four different temperature-dependent regulation mechanisms can be distinguished, the heat shock response (HSR), the high temperature response (HTR), the cold-shock response (CSR) and the low-temperature response (LTR). While the first two recognize sudden increases in temperature, the other two respond to a sudden decrease. Furthermore, the heat- and cold-shock responses are transient and include a shut-off after adaptation has occurred even if cells are still exposed to the high or low temperature. The high and low temperature responses are constitutive and persist as long as the bacterial cells are exposed to that temperature. The high temperature response plays an important role for pathogenic bacteria to recognize their mammalian host, where exposure to 37°C induces the virulence genes, which are not needed outside this environmental niche. All four responses are based on genetic programs, which consist of three major steps:

1. Registration of the stress factor by a sensor molecule.
2. The sensor molecule directly or indirectly leads to the induction of a subset of genes called stress genes specific for the inducing stress factor.
3. In the case of a heat- or cold-shock response, expression of the stress genes is reduced after adaptation through a feedback inhibition loop.

How does the sensor register changes in the environmental temperature? Since temperature changes can affect the conformation of virtually any biomolecule, the underlying principle of temperature sensing is based on such conformational changes. Three different thermosensory biomolecules have been described so far: DNA, RNA and proteins. The purpose of this chapter is to describe how these three thermosensors sense temperature changes, thus controlling gene expression at the transcriptional, translational and posttranslational level. Several recent review articles have dealt with one or the other aspect of bacterial thermosensors.[2-6]

DNA ACTING AS THERMOSENSOR

Three different principles have been described involving DNA as thermosensor: DNA supercoiling, promoter-curvature and nucleoid-associated proteins.

DNA Supercoiling

Plasmids from mesophilic and hyper-thermophilic bacteria can undergo a reversible change in their supercoiling level depending on the temperature.[7] A heat shock introduces a transient increase in positive supercoiling leading to plasmid relaxation mediated by DNA gyrase and topoisomerase I.[8] Recovery to the normal supercoiling level is observed within 10 min after the heat shock and is dependent on DNA gyrase, the nucleoid-binding protein HU and the molecular chaperone DnaK.[7] On the contrary, a cold-shock decreases plasmid supercoiling and recovery to the original supercoiling level occurs after about 60 min and may involve DNA gyrase and the HU protein.[9] Since transcription efficiency can be influenced by the DNA topology,[10] the level of DNA supercoiling acts as an important parameter in temperature-dependent gene regulation.

Promoter-Curvature

Another important DNA element being able to respond to temperature changes are intrinsic bends. It has been shown that intrinsically curved DNA regions characterized by AT-tracts[11] located upstream of a promoter influence binding of the RNA polymerase.[12] Temperature-induced changes in the topology within these regions directly influence gene expression. One example is the *plc* gene of *Clostridium perfringens* coding for phospholipase C. At low temperature, the altered curvature upstream of its promoter leads to the induction of *plc*. Here, low temperature increases the bending of the AT-tracts thus enhancing the binding affinity for the RNA polymerase.[13,14]

Shigella flexneri is a facultative intracellular pathogen and some genes required for pathogenicity are located within a 31 kb region of the 230 kb plasmid pINV.[15,16] *Shigella* cells are able to penetrate into and replicate within human colonic epithelial cells. Both chromosomal virulence (*vir*) genes and the plasmid pINV are involved in expression of the pathogenicity phenotype in *S. flexneri*.[17] Expression of the invasive phenotype is regulated by the growth temperature.[18] Bacteria growing at 37°C are virulent and able to invade epithelial cells, whereas the same cells are non-invasive when grown at 30°C. Using the method of transposon mutagenesis, a gene has been identified being responsible for the growth-dependent phenotype. When inactivated, cells become virulent even at the low temperature.[19] This gene codes for the H-NS (**h**eat-stable **n**ucleoid-**s**tructuring) protein and silences expression of *virF* coding for a transcriptional activator, which in turn triggers a regulatory cascade involving the activation of other regulatory genes.

At the *virF* promoter, H-NS binds to two sites separated by a region of DNA curvature. Binding to these regions occurs co-operatively at temperatures below 32°C but not at 37°C and bent DNA might act as a sensor of temperature.[20] Experiments have revealed that the intrinsic bent located between the two H-NS binding sites melts abruptly at around 32° allowing the formation of a productive transcription complex[21] (Fig. 1A). Taken together, all experimental data support the hypothesis that the curved DNA tract within the *virF* promoter acts as a thermosensor.

Nucleoid-Associated Proteins

Nucleoid-associated proteins exert genome structuring functions in bacteria. Binding of these proteins to DNA does not only influence its conformation, but also DNA replication, recombination and transcription.[22,23] The best characterized nucleoid-associated protein present in different enteric bacteria is H-NS, which serves as the paradigm of a globular modulator exerting its effect, mostly negative termed silencing, in response to different environmental signals including temperature.[23] H-NS prefers AT-rich sequences and is itself subject to temperature control. While formation of higher-order oligomers and the DNA-binding capacity are reduced at 37°C,[24] the H-NS to DNA ratio increases three- to four-fold during growth at low temperature.[25] Temperature-modulated accessibility of promoter regions occupied by H-NS at low temperature plays a key role of virulence gene

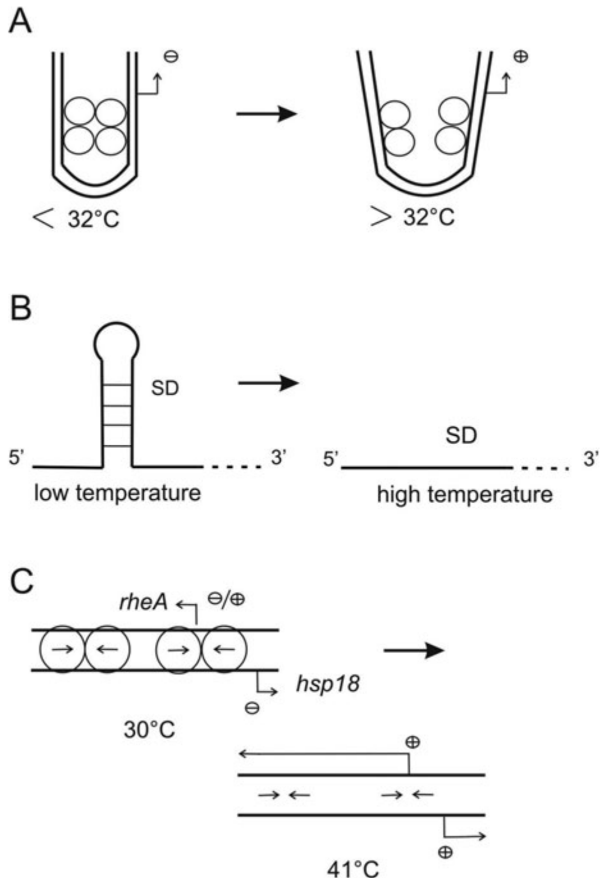

Figure 1. A) Histone-like proteins such as H-NS bind to two different sites on a chromosome or plasmid. At temperatures below 32°C, the DNA is bent in such a way to allow interaction between the two complexes and thus prevent binding of RNA polymerase. At temperatures above 32°C, the bend is reduced in such a way that the two complexes loose interaction and the RNA polymerase can now access the promoter and start transcription. B) At low temperature, the mRNA forms a stem-loop structure sequestering the SD sequence and the ribosomes do not recognize the SD sequence. High temperature will lead to melting of the stem-loop structure allowing access of the ribosomes to the SD sequence. C) At 30°C, the RheA repressor of *Streptomyces albus* binds to two sites as a homodimer thereby preventing expression of the gene *hsp18* coding for a small heat shock protein and regulating its own expression. At 41°C, the repressor undergoes a conformational change causing its dissociation from both sites leading to increased production of the RheA protein itself and of transcription of the *hsp18* gene.

expression in many human pathogens, like *E. coli*, *Salmonella* and *Shigella flexneri*.[26-29] This will be illustrated by two different examples.

Pap pili, encoded by the **p**yelonephritis-**a**ssociated **p**ili (*pap*) operon, are expressed by uropathogenic *E. coli* cells and facilitate the attachment to uroepithelial cells and subsequent colonization of the host upper urinary tract. Pap pili transcription is regulated in response to the growth temperature.[30] Optimal expression occurs at 37°C, with a 52-fold reduction in *papBA* transcription at 23°C[31] and this regulation occurs at the level of transcription.[32] Two proteins have been identified to play an important role in the regulation of transcription of the *papBA* operon, H-NS and RimJ. H-NS prevents transcription at the low temperature[33,34] by binding within the *pap* regulatory region at 23°C but not at 37°C.[31] RimJ is an N-terminal acetyltransferase of the ribosomal protein S5[35] and deletion of the *rimJ* gene leads to a loss of thermoregulation resulting in equivalent *papBA* transcript levels at both 37°C and 23°C.[36] The mechanism by which RimJ represses *papBA* transcription is unknown.

One of the major virulence factors in *Salmonella enterica* is a Type III secretion system (T3SS) encoded in the *Salmonella* pathogenicity island 2 (SPI-2). This horizontally acquired genomic island contains genes whose products activate and assemble the T3SS that is required during intracellular infection and that injects into host cells the effector proteins required for intracellular survival.[37,38] Cells grown at 30°C or lower have been shown to be unable to express the T3SS. Here, virulence gene expression is controlled by Hha and H-NS, two nucleoid proteins silencing the virulence genes at temperatures below 30°C.[39] While H-NS silences expression of the response regulator SsrR, which activates a set of genes responsible for the host infection, Hha silences the SPI-2 gene transcription.

RNA ACTING AS THERMOSENSOR

RNA thermometers have evolved to sense and transduce ambient temperature signals to the translation machinery and most of them are located in the 5′-untranslated region (UTR) of bacterial heat shock and virulence genes (*cis*-acting RNA thermometers), while a few described so far act in *trans* through a small RNA interacting with the appropriate mRNA. At low temperature, the Shine-Dalgarno (SD-) sequence is trapped in a hairpin structure and increasing temperature destabilizes that structure in such a way that the SD-sequence becomes available to the ribosomes allowing translation initiation (Fig. 1B). RNA thermosensors register even subtle changes in temperature and adjust gene expression accordingly. All known RNA thermometers control translation. They control several responses such as the HSR.[40-43]

RNA Thermometer and the HSR

The alternative sigma factor σ32 acts as a key regulator of the HSR in *E. coli*.[44] While at low temperature, cells contain very little sigma-32 (10-30 molecules at 30°C), 5 min after a temperature upshift to 42°C, the amount of σ32 increases about 15-fold. This dramatic increase results from both changes in the stability (will be discussed later) and synthesis of σ32, where synthesis is regulated at the level of mRNA. At lower temperatures, the *rpoH* mRNA is folded into a secondary structure that occludes the SD-sequence and the initiation codon. Here, almost the entire secondary region of the transcript is located in the coding region and not in the 5′-UTR. Two segments called A and B form an extensive RNA secondary structure thus blocking entry of the ribosomes to the SD-sequence. Exposure of cells to the high temperature disrupts the secondary structure and liberates the SD-sequence.[40]

Another RNA thermosensor called ROSE (for **r**epression **o**f heat **s**hock gene **e**xpression) element was discovered in *Bradyrhizobium japonicum*[45] and has been described later in different *Rhizobium* species and in *Agrobacterium tumefaciens*.[41,46] All ROSE elements are located in the 5′-UTR transcripts coding for small heat shock genes, are 70-120 nucleotides long, acquire a complex structure comprising 2-4 stem loops, where the 3′-proximal hairpin contains the SD-sequence and in some cases the AUG start codon as well. Short internal loops and bulges in the computer-predicted final structure are assumed to create a thermolabile structure that melts at increasing temperatures.

A third RNA thermometer is the fourU element. This unusually short thermosensor consists of only 52 nucleotides folding in two hairpins. It was initially described controlling expression of the small heat shock gene *agsA* in *Salmonella*.[47] It consists of two hairpins, where hairpin I might play a structural role during cotranscriptional folding and hairpin II is blocked by a consecutive stretch of four uridine residues used to base-pair with the SD-sequence. Temperature-dependent opening of hairpin II allows binding of the ribosomes to the SD-sequence. A similar structure of four U residues that pair with the SD-sequence has been predicted upstream of the *lcrF* gene in *Y. pestis*.[48] This gene codes for a transcription factor, which is responsible for inducing the expression of plasmid-encoded virulence genes in response to temperature.

RNA Thermometers and the CSR

In *E. coli* and other bacteria, the expression of cold shock genes becomes specifically enhanced or induced de novo during the growth lag following a temperature-downshock from 37°C to 15°C.[49] One of the cold shock genes, *cspA*, codes for the major cold shock protein CspA.[50] CspA and its homologues destabilize secondary structures in both RNA and DNA and are therefore referred to as nucleic acid chaperones.[51] While the *cspA* transcript is unstable at 37°C with a half-life of about 10 sec,[52] it becomes highly stable upon a shift to 15°C. Three-base substitutions around the SD-sequence in the 159-bp 5′-UTR region stabilize the transcript 150-fold, resulting in constitutive expression of *cspA* at 37°C. It has been suggested that at 37°C, the *cspA* transcript adopts a secondary structure which is recognized by RNase E, while it folds into a different secondary structure at 25°C not recognized by this endoribonuclease.[53] Taken together, the *cspA* RNA serves as a cold-shock sensor.

A completely different mechanism has been suggested for cold shock induction of the *pnp* gene of *E. coli* coding for a 3′ to 5′ exonuclease. A more than 10-fold increase in the amount of the *pnp* transcript has been described to occur within the first hour upon a cold shock.[54] While at 37°C only the monocistronic *pnp* transcript is present, a bicistronic mRNA including the coding region of the downstream gene *deaD* encoding a DEAD-box RNA helicase predominates. A Rho-dependent termination site present within the coding region of *pnp* is suppressed upon a cold shock.

In *Borrelia burgdorferi*, the causative agent of Lyme disease, the alternative sigma factor σS plays a central role in the regulation of virulence-associated major outer surface proteins. Translation of the *rpoS* mRNA is stimulated at 37°C by the small DrsA RNA. At 23°C, this noncoding RNA folds into a stable secondary structure, which does not allow base-pairing with the *rpoS* mRNA. It has been suggested that the higher temperature leads to melting of the secondary structure of the DsrA RNA, which is now able to interact specifically with the anti-SD sequence of the *rpoS* transcript. This in turn would stimulate ribosome interaction with the SD-sequence under virulence conditions.[55]

RNA and the LTR

Bacteriophage λ belongs to the group of temperate phages, which have to make a decision whether to enter the lytic or the lysogenic pathway about 10 min after infection. Here, the gene cIII product plays an important role in this decision. It does so by binding to the ATP-dependent FtsH protease, which degrades the cII protein, a transcriptional activator of central importance in the lysogenic pathway.[56,57] High concentrations of cIII promote stabilization of cII thus favouring lysogeny. Two alternative structures of the cIII transcript were first predicted and later verified by structure probing in vitro and in vivo.[43] While one secondary structure sequesters part of the SD-sequence and the start codon, the alternative structures leaves the translation initiation region accessible to the ribosomes to allow translation of cIII. The equilibrium between both structures is temperature-dependent. At high temperature (45°C), the start codon and the SD-sequence are sequestered in a hairpin structure largely preventing synthesis of cIII. This in turn leads to a degradation of cII and the lytic cycle is initiated by these bacterial cells. Under physiological temperature (37°C), the equilibrium is shifted toward the alternative secondary structure in which the ribosome binding site become available leading to the synthesis of cIII followed by initiation of the lysogenic pathway. In the present case, the *cis*-acting RNA thermometer switches on translation with decreasing temperature and does not operate by gradual melting of the secondary structure as in the case of the *rpoH* mRNA. It alternates between two mutually exclusive conformations. What might be the biological reason for temperature control of cIII translation? Phage λ tends to enter the lytic cycle when the host cells are healthy and a sufficient amount of nutrients is available. On the contrary, if the growth conditions are poor, it prefers to integrate its genome into the host chromosome. But under life-threatening conditions such as a severe heat shock (45°C), it might be beneficial for the phage to escape from the host cells.

The small DsrA RNA is an example for a *trans*-acting RNA thermosensor by controlling translation of the *E. coli rpoS* mRNA. In *E. coli* the *rpoS* gene codes for the general stress sigma factor RpoS (σS), the expression of which is controlled at the levels of transcription, translation and protein stability. The amount of active RpoS is adjusted in response to various environmental signals and each step of *rpoS* expression can be affected by one or several environmental stimuli.[58] One of the environmental cues that increase translation of the *rpoS* transcript is low temperature (below 37°C). Here, the small RNA DrsA plays an important role.[59] This *trans*-acting RNA pairs with the leader region of the *rpoS* mRNA to allow a more efficient translation.[60] Temperature affects both the rate of transcription initiation of the *dsrA* gene and the stability of its transcript.[61] The net effect is a 25-fold decrease in full-length *dsrA* transcript at 37°C compared to 25°C. What mechanism is responsible for temperature regulation at the *dsrA* promoter? It could be shown that the sequence of the −10 element and the spacer region are essential elements for the thermal response of the *dsrA* promoter.[62]

PROTEINS ACTING AS THERMOSENSOR

Protein-based thermosensors can either involve temperature-dependent changes in the conformation of the protein itself or in assembly of protein complexes consisting either of identical or different subunits. Protein sensors described so far include transcriptional and translational regulators, molecular chaperones and proteases.

Protein Thermosensors and the HTR

TlpA was the first documented case of a temperature-sensing gene regulator and was presumed to be an ideal sensor of environmental signals. The TlpA protein is encoded by the 96 kb pSLT virulence plasmid of *Salmonella enterica*[63] and characterized by a remarkable long α-helical coiled-coil motif.[64] The N-terminus of TlpA is a sequence specific DNA-binding domain acting as an autoregulatory repressor. TlpA is present in a temperature-dependent two-state equilibrium, between unfolded monomers and highly α-helical coiled-coil oligomers. At physiological temperatures transcription of *tlpA* is low by the repressing activity of TlpA, which in its dimeric and folded coil-coiled conformation is able to bind to the *tlpA* operator. Elevated temperature leads to a shift in the equilibrium that favours the nonfunctional unfolded monomeric form resulting in increased transcription.[64-66] The function of TlpA is unknown, but it does not seem to play a role in the pathogenicity of *Salmonella* per se, but imply an alternative function which is not directly involved in the virulence of *Salmonella*.[67] It might negatively regulate genes to be identified.

The second example of a temperature-sensing autorepressor is the RheA protein identified in *Streptomyces albus*.[68] It negatively regulates expression of *hsp18* coding for a small HSP. While the RheA repressor reduces transcription of its own gene and prevents that of *hsp18* at 30°C, transcription occurs at 41°C (Fig. 1C). Circular dichroism spectroscopy revealed a temperature-dependent transition between an active and an inactive form of RheA.[69]

The *ymoA* gene codes for a small histone-like protein and is involved in thermoregulation of the Type III secretion system (T3SS) of *Yersinia pestis*, which is needed at 37°C, the host temperature, but not at low temperatures. The YmoA protein is highly stable at low temperature and unstable at 37°C. At that temperature, it will be degraded predominantly by the Lon protease and ClpXP acting as a backup system (if Lon is deficient).[70] Since the Lon protease is present and active at all temperatures, degradation might include a conformational change in YmoA at 37°C thus increasing its susceptibility to Lon or ClpXP degradation. Alternatively, an accessory protein might be induced or become activated at 37°C that modifies or targets YmoA for degradation.

Bordetella pertussis, the etiological agent of whooping cough, uses a two-component system comprised of the sensor kinase BvgS and the response regulator BvgA to control expression of virulence genes.[71] Temperature plays an important role in activation of BvgA and may be modulated by sulphate ions and nicotinic acid. Following induction of *bvgAS* at the mammalian body temperature of 37°C, phosphorylation by BvgS allows BvgA binding to promoter regions of virulence genes, such as the adhesin *fimX*.[72] It has been suggested that the transmembrane domain of BvgS senses temperature changes.

The most evolved temperature-sensing protein is HtrA (for **h**igh **t**emperature **r**equirement) of *E. coli* and also called DegP. This protein was initially identified in *E. coli* as a serine protease belonging to the trypsin clan SA.[73] SA proteases are characterised by a two-domain structure with each domain forming a six-stranded β barrel. The functional unit of HtrA appears to be a trimer forming a funnel-like shape with the proteolytic domain located at its top and the two PDZ domains protruding to the outside. The PDZ domains are highly mobile swinging around to capture substrate proteins and preferentially bind to the C-terminal 3-4 residues of their target proteins. When digestion of β-casein is followed, almost no proteolytic activity is detected below 20°C. At temperatures above 30°C, the proteolytic activity rapidly increases in a nonlinear fashion.[74] As a chaperone, HtrA was shown to refold periplasmic amylase MalS and the artificial substrate citrate synthase. As a protease, HtrA processively degrades misfolded proteins into peptides of defined size by employing a molecular ruler comprised of the PDZ domain 1 and the proteolytic site.[75] In a first step, the C-terminus of an unstructured protein is bound to PDZ domain 1.

In a second step, the first proteolytic cut is introduced by a neighbouring proteolytic site yielding the first product. Next, PDZ domain 1 binds to the new C-terminal end of the remaining substrate and performs a second cut about 12-17 residues into the substrate. This process is repeated until the substrate protein is completely digested.

Protein Thermosensors and the HSR

Two different classes of proteins have been described so far acting as thermosensors upon a sudden heat shock, molecular chaperones and proteases acting at the level of activity and stability, respectively.

One example is the already mentioned σ32 of *E. coli*. Besides being regulated at the level of translation, the sigma factor itself is controlled at the level of activity by DnaK and DnaJ and furthermore at the level of stability by the ATP-dependent metalloprotease FtsH.[76] It has been observed that σ32 is highly unstable at 30°C with a half-life of ~1 min. After a heat shock, σ32 is transiently stabilized with a half-live of ~5 min. Why the sigma factor is unstable at low temperature and by which mechanism it becomes transiently stabilized after a heat shock? Recently, two distinct sites in σ32 have been identified as binding sites for DnaK and DnaJ. DnaJ binding destabilizes a distant region of σ32 in close spatial vicinity of the DnaK-binding site and DnaK destabilizes a region in the N-terminal domain. These conformational changes in the native protein convert it into a substrate for the FtsH protease.[77]

The second example is the HrcA-GroE system of *Bacillus subtilis*. Here, the GroEL chaperone modulates the activity of the HrcA repressor protein. This regulatory protein controls expression of the heptacistronic *dnaK* and the bicistronic *groE* operon[78,79] by binding to an operator called CIRCE (for **c**ontrolling **i**nverted **r**epeat of **c**haperone **e**xpression).[80] It has been suggested that HrcA is present in two conformations, an active and an inactive one and the equilibrium between these two conformers is modulated by GroEL, which shifts this equilibrium toward the active conformation. This model is supported by three sets of experimental data: (1) Whereas an increase in the amount of GroEL reduced the basal level of the proteins encoded by the two operons, a decrease resulted in an increase. (2) In a bandshift assay, purified HrcA retarded more DNA in the presence of GroEL. (3) GroEL specifically binds to immobilized HrcA.[81,82] Based on these observations, the following model has been developed. Both, HrcA synthesized de novo and dissociated from its operator is present in the inactive conformation and interaction with GroEL converts it in its active conformation. After a heat shock, GroEL is titrated by nonnative proteins, leaving HrcA inactive thus leading to the induction of the *dnaK* and *groE* operons. The more nonnative proteins have been removed, the more GroEL will become available to take care of HrcA resulting in a gradual turn-off of the heat shock response.

The third example is the HspR-DnaK system of *Streptomyces coelicolor*. Here, the *dnaK* operon consists of the four genes *dnaK*, *grpE*, *dnaJ* and *hspR*, where *hspR* codes for a repressor protein of its own operon (and some other genes) binding to an operator designated HAIR (for **H**spR-**a**ssociated **i**nverted **r**epeat).[83] Here, the activity of the HspR protein is modulated by the DnaK chaperone.[84] This conclusion is based on four different observations: (1) In a band shift assay, HspR is active only in the presence of DnaK and this activity does not need neither DnaJ nor GrpE. (2) Addition of anti-DnaK monoclonal antibodies to the retarded complex produced a supershift, proving that DnaK is part of the DNA-binding complex. (3) HspR copurified with DnaK in column chromatography.

(4) Induction of the DnaK operon is partially decreased in the presence of overproduced DnaK. Based on these results, it has been suggested that DnaK acts as a transcriptional corepressor by directly binding to HspR at its operator site and by activating HspR or keeping it in its active form. As suggested for HrcA and GroEL, the appearance of nonnative proteins after a heat shock will titrate DnaK leading to derepression of the operon.[84]

So far, only one system has been described where a protease acts as a thermosensor. This protease, DegS, is anchored in the inner membrane of *E. coli* cells facing the periplasmic space. It consists of an N-terminal transmembrane domain followed by a central protease domain and a C-terminal PDZ domain.[85] PDZ domains are present in a large number of proteins and are known to recognize specific C-terminal polypeptide sequences.[86] In the case of DegS, the PDZ domain recognizes C-terminal peptides with the Y-X-F motif, common to a number of outer membrane porins (e.g., OmpC). It is assumed that the PDZ domain inhibits the protease domain most probably through direct contact between both domains. Upon appearance of denatured porins exposing their C-terminal tails, the PDZ domain is released from the protease domain and interacts with the Y-X-F motif. Denatured proteins are produced by a severe heat shock or by overproduction of a porin. The free protease domain now attacks the anti-sigma factor RseA. RseA consists of three functional domains, a periplasmic domain, a transmembrane domain and a cytoplasmic domain which sequesters the alternative sigma factor σE.[87,88] The DegS protease efficiently cleaves within in the periplasmic domain of RseA[89] and the remaining part of RseA is subsequently further degraded.[90,91] These proteolytic events destabilize the cytoplasmic domain of RseA, releasing σE to activate transcription of the genes of the σE regulon.[85] Removal of the denatured porins from the periplasm most probably leads to binding of the PDZ domain to the proteolytic domain of DegS resulting in to a shut-off of the heat shock response.

Protein Thermosensors and the LTR

Three different proteins have been reported to be active at low, but not at high temperatures. Example one is the VirA protein encoded by the Ti-plasmid of the soil bacterium *Agrobacterium tumefaciens*. VirA is the sensor kinase of a two-component signal transduction system, which phosphorylates the response regulator VirG which in turn activates a set of *vir* genes. These *vir* genes are involved in the processing and transfer of the T-DNA from the Ti-plasmid into susceptible plant cells.[92] Expression of the virulent genes is specifically inhibited at temperatures above 32°C. At temperature of 32°C and higher, VirA undergoes a reversible inactivation preventing both autophosphorylation and the subsequent transfer of the phosphate to VirG.[93] Why transfer of the T-DNA is inhibited at high temperatures? Since several plant proteins are involved in steps subsequent to T-DNA transfer, one or more of these proteins might be inactive at high temperatures blocking successful integration of the T-DNA into the plant genome.

A second example is the transcriptional activator NifA of *Klebsiella pneumoniae*. In diazotrophic bacteria, the *nif* operons are transcribed by the alternative sigma factor σ54 in conjunction with the transcriptional activator NIFA.[94] NifA binds to upstream activation sequences (UAS) that are located approximately 100 bp upstream of the *nif* promoters and catalyzes isomerization of closed complexes between Eσ54 and the promoters to produce open complexes. Activation occurs only at temperatures below 37°C and it has been suggested that the failure of NifA to bind to its UAS elements at 37°C is due to the fact that the helix-turn-helix motifs in different subunits are not correctly oriented with respect to one another at 37°C.[95] Later, it was shown that the N-terminal domain plays an important role in the temperature sensitivity of the protein.[96]

The third example is the response regulator DegU of *Listeria monocytogenes*. In this bacterial species flagella-based motility is regulated in response to the growth temperature with the permissive temperature being 30°C and below.[97,98] The reason for not becoming flagellated at high temperatures relies on the *flaA* gene coding for the flagellin FlaA that is not expressed under these conditions.[99] Regulation of transcription of flagellar genes relies on three different proteins among them DegU, a response regulator. Since this protein is present at ambient temperatures and phosphorylation is not impaired,[100] its activity has to be modulated in response to the growth temperature. Either DegU is a temperature-sensitive protein being active at low and inactive at high temperatures, or the activity of DegU is regulated by another protein in a temperature-dependent way. Why synthesis of flagella should be prevented at 37°C, the temperature of the mammalian host? Downregulation of *flaA* expression during in vivo infection with *L. monocytogenes* may serve as an adaptive mechanism to avoid host recognition and activation of the host innate immune response.[101,102]

Protein Thermosensors and the CSR

Two major problems arise from exposing a cell to a sudden decrease in temperature.[103] First, membrane fluidity decreases, which affects many vital membrane and membrane-associated functions. Second, DNA and RNA topology will be stagnated causing halts in transcription and translation. Furthermore, warm-blooded pathogens leaving its host may need to shut off the expression of virulence gene expression. Therefore, one of the essential processes in the cold-shock response is the adaptation of the membrane to the new temperature. After a temperature-downshift, the physical properties of the cytoplasmic membrane change by undergoing a phase transition from its normal liquid-crystalline phase to a more rigid gel-like phase. In *B. subtilis*, adaptation occurs through two different mechanisms, where one involves desaturation of fatty acid moieties of the membrane. This is accomplished by enzyme fatty acid desaturase, which converts already existing fatty acid moieties into Δ5-unsaturated fatty acids, resulting in higher membrane fluidity.[104] Transcription of the desaturase gene *des* is cold-induced and regulated by the two-component system DesK and DesR.[105] The DesK histidine kinase consists of an N-terminal sensor domain composed of four helical transmembrane domains connected by a C-terminal cytoplasmic domain. Upon sensing the low temperature, the plasticity of the central four-helix bundle domain influences the catalytic activity of the DesK protein, either by modifying the mobility of the ATP-binding domains for autokinase activity or by modulating binding of its response regulator DesR.[106] The phosphorylated DesR binds to a DNA segment upstream of the promoter of the *des* gene and activates its transcription.[107] Upon return of the membrane to the fluid state, DesK becomes a phosphatase, dephosphorylates DesR, which leads to the shut-off of *des* gene activation.

EVOLUTION OF THERMOSENSORS

Based on the suggestion that our DNA world has been preceded by an RNA world, mRNA thermometers can be assumed to have evolved first. In their simplest form, mRNA thermosensors just need a simple hairpin structure, which sequesters the SD-sequence at one temperature and allow access to the ribosomes at another temperature. More sophisticated mRNA thermosensors use more complex secondary structures exemplified by those coding for the heat shock sigma factor σ32 of *E. coli*[40] and those encoding small heat shock proteins in *Rhizobiae*.[45] Here, the additional secondary structures may influence the stability of that sequestering the Shine-Dalgarno sequence. It is important to stress that these mRNA thermosensors allow regulation of just one single gene at the level of translation. Theoretically, mRNA thermosensors could also regulate more than one gene provided translational coupling defined as the interdependence of translation efficiency of neighbouring genes encoded by the same polycistronic mRNA.[108] To regulate more than one gene by a mRNA thermosensor, they code for a transcriptional regulator, either an alternative sigma factor or a transcriptional activator.

DNA thermosensors are based on promoter occlusion. Here, bending of the DNA in the promoter region in conjunction with a silencing protein such as H-NS prevents binding of the RNA polymerase at low temperatures. High temperatures reduce the bending and destroy the whole architecture thus allowing access of the RNA polymerase to the promoter. Protein thermosensors also depend on conformational changes, where the low temperature favours the active, DNA-binding activity and high temperatures the inactive conformation of the protein. So far, only two protein thermosensors have been described the RheA and the TlpA repressor.[69,109] It is astonishing that not more protein thermosensors have evolved since a single point mutation can be sufficient to convert a stable into a temperature-sensitive repressor as exemplified by the cIts857 repressor of phage λ.[110]

The complex thermosensors represent the most sophisticated systems evolved so far. They depend on a molecular chaperone or a protease where both are able to sense denatured proteins. In their absence, they keep a positive regulatory protein inactive (DnaK—σ32) or a negative one active (GroE—HrcA, DnaK—HspR, DegS—RseA) and are titrated by the sudden appearance of nonnative polypeptide chains. The last example is the two-functional HtrA protein, where a switch from a molecular chaperone to a protease is dictated by the temperature.[111]

CONCLUSION

1. Temperature sensing is based on conformational changes of three different biomolecules: DNA, RNA and proteins.
2. Three different principles affect DNA as thermosensor: DNA supercoiling, promoter curvature and nucleoid-associated proteins.
3. RNA termosensors are either based on trapping the Shine-Dalgarno sequence in a secondary structure being destabilized at increasing temperature (*cis*-acting RNA thermometer) or through binding of a small RNA (*trans*-acting RNA thermometer).
4. During the cold shock response, mRNAs acquire secondary structures impairing translation, which is counteracted by specific cold shock proteins.
5. Protein-based thermosensors involve either temperature-dependent change in the conformation of the protein itself or in assembly of protein complexes.
6. Protein thermosensors include transcriptional and translational regulators, molecular chaperones and proteases.

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