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# Thermosensors in eubacteria: role and evolution

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Temperature is an important physical stress factor sensed by bacteria and used to regulate gene expression. Three different macromolecules have been identified being able to sense temperature: DNA, mRNA and proteins. Depending on the induction mechanism, two different pathways have to be distinguished, namely the heat shock response and the high temperature response. While the heat shock response is induced by temperature increments and is transient, the high temperature response needs a specific temperature to become induced and proceeds as long as cells are exposed to that temperature. The heat shock response is induced by denatured proteins and aimed to prevent formation of protein aggregates by refolding or degradation, and the high temperature response is mainly used by pathogenic bacteria to detect entry into a mammalian host followed by induction of their virulence genes. All known high temperature sensors are present in two alternative conformations depending on the temperature. Heat shock sensors are either molecular chaperones or proteases which keep either a positive transcriptional regulator inactive or a negative regulator active or do not attack the regulator, respectively, under physiological conditions. Denatured proteins either titrate the molecular chaperones or activate the protease. The evolution of the different temperature sensors is discussed.

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## 1. Introduction

Temperature is one of the physical parameters under constant vigilance in all living cells. Numerous processes are temperature-controlled in bacteria including the expression of heat and cold shock genes and of virulence genes to mention the most prominent examples (Narberhaus *et al* 2006). How do cells detect changes in temperature? Three different macromolecules have been identified acting as heat sensors: DNA, mRNA and proteins (transcriptional repressors, molecular chaperones and proteases). Depending on the induction mechanism, two different responses are distinguished (table 1): the heat shock response and the high temperature response. The heat shock response is induced by temperature increments. In the classical experiment, *Escherichia coli* cells are transferred from 30°C to 42°C, *Bacillus subtilis* cells from 37°C to 48°C. But the heat shock response is also induced when *E. coli* cells are transferred from 20°C to 35°C, etc. The higher the temperature increment, the more pronounced is the induction level of the

heat shock genes. Another characteristic is the observation that the heat shock response is transient. A feedback mechanism ensures that expression of the heat shock genes returns within about 10 min (in *E. coli* and *B. subtilis*) to a level which is two- to three-fold higher as compared to the basal level present in uninduced cells as long as the cells are kept at the high temperature. In contrast, the high temperature response needs a certain temperature to become effective, e.g., 37°C for pathogenic bacteria to induce expression of their virulent genes. Furthermore, expression of the high temperature genes proceeds as long as the cells are exposed to that temperature; there is no feedback regulation. The purpose of this article is to present an overview on the major mechanisms of sensing increases in temperature and the consequences for the bacterial species. The two classes of thermosensors, direct and indirect, need to be distinguished. While direct thermosensors react to temperature increases by a conformational change, indirect thermosensors detect denatured proteins which are formed after temperature increases.

**Keywords.** Bent DNA; H-NS; HrcA; RNA thermosensor

**Table 1.** Two different genetically regulated responses induced by temperature increases

The heat shock response
response to temperature increments
transient
expression of heat shock genes (proteins)
The high temperature response
response to the absolute temperature
constitutive
expression of high temperature genes (proteins)

## 2. The heat shock response

The heat shock response is a cellular protective and homeostatic response to cope with stress-induced conformational damage of proteins. It occurs when cells growing at a low temperature are shifted to a higher temperature and results in the induction of a set of genes termed heat shock genes coding for heat shock proteins (HSPs). The primary structure of HSPs appears to be highly conserved during evolution, suggesting that they serve similar functions in all organisms (Morimoto *et al* 1990). Many HSPs are molecular chaperones (e.g. DnaK, GroEL, Clp proteins) or ATP-dependent proteases (e.g., Lon, FtsH, ClpAP, ClpXP) and play important roles in protein folding, assembly, transport, repair and turnover under stress and non-stress conditions. The heat shock response has been first described in *Drosophila melanogaster* (Ritossa 1962; Tissières *et al* 1974), then in *E. coli* (Lemaux *et al* 1978; Yamamori and Yura 1982) and has been later shown to be present in all organisms. Indirect sensors of the heat shock response are proteins (molecular chaperones and proteases). They sense denatured polypeptide chains arising upon a sudden increase in temperature and several other stress factor including ethanol. These treatments cause the unfolding of proteins leading to the exposure of hydrophobic regions which are normally buried within the interior of polypeptide chains. These partially or completely unfolded proteins collectively termed as non-native proteins then interact via their exposed hydrophobic regions and form aggregates. Since large aggregates become life-threatening, cells have to prevent their formation by either refolding or degradation. The heat shock response is a four-step reaction: (i) unfolding of proteins where the extent of unfolding depends on the temperature increment; (ii) sensing of the unfolded proteins by molecular chaperones or proteases; (iii) removal of the unfolded proteins by a concerted action of molecular chaperones involved in their refolding and ATP-dependent proteases involved in their degradation; and (iv) shut-off of the heat shock response when all non-native proteins have been removed. In summary, the heat shock response is a response to temperature increments, is transient and results in the expression of heat shock genes which allow cells to remove denatured polypeptide chains (table 1).

## 3. The high temperature response

Pathogenic microorganisms are well adapted to their host ecological niche, but they must also cope with life outside the host. In the case of pathogens of man (and warm-blooded animals) this means that movement occurs between a constant, thermally controlled environment of 37°C in the host and the external biosphere where the temperature fluctuates and is usually less than 37°C. Pathogenic bacteria harbour special genes which promote the initial entry and permit survival of bacteria within the host, as well as genes which result in production of disease and are defined as virulent determinants. Since expression of these virulent genes is needed only after infection of humans, there must be a mechanism triggering their induction. Work carried out over the last two decades has shown that the growth temperature acts as an important regulator (Maurelli 1989). In summary, the high temperature response is a response to the absolute temperature, is constitutive and results in the expression of high temperature genes (table 1). The high temperature proteins are produced as long as the cells are exposed to the enhanced temperature.

## 4. Sensors of high temperature

So far, three sensors of increases in temperature have been described: bent DNA, mRNA and different proteins. The underlying principle for all three sensor types is alterations in their conformation. DNA molecules can contain intrinsic bends which are stabilized by histone-like proteins, and a promoter is located immediately downstream of the bend. These proteins will bind to both sides of the bend and make contact with each other thereby preventing access of the RNA polymerase to a promoter partially overlapping one binding site. This three-dimensional configuration keeps the gene(s) following the promoter in a repressed state. After a shift to 37°C, the intrinsic bends melt and the architecture collapses allowing the RNA polymerase to bind to the promoter resulting in transcription initiation of the downstream gene which in most cases is a transcriptional activator encoded by a high temperature gene. This principle is used by many pathogenic bacteria to induce expression of their virulence genes.

mRNA is another sensor used by many bacterial species for the expression of high temperature genes. These mRNAs are able to form a secondary structure within their 5' untranslated region (UTR) thereby sequestering part or the complete Shine-Dalgarno sequence and often the start codon as well. This in turn, prevents binding of the small ribosomal subunit and thereby translation initiation. At a given temperature the secondary structure reaches its maximum stability and translation of the downstream open reading frame is extremely low. The higher the temperature,

the more unstable the secondary structure and the more protein is produced. At a certain temperature, the secondary structure is completely destroyed resulting in full level of gene expression. In contrast to the above mentioned mechanism where a certain temperature is needed to allow gene expression, the RNA thermometer ensures a graded expression. Each RNA thermometer described so far can control expression of just one single gene.

The third type of temperature sensors are proteins with an intrinsic thermosensor. Here, the temperature within a very small range influences the main characteristic of the protein, DNA-binding in most cases. These proteins are able to recognize their operator sequence at low temperatures while a high temperature will lead to a conformational change preventing binding. As described for the RNA thermosensors, the gradual temperature increase results in a gradual increase in the amount of protein(s) synthesized. Here, one repressor can not only control expression of more than one gene being located on a polycistronic transcript, but can even act as a negative regulator of a regulon with two and more operons.

### 5. Sensors of heat shock

Besides these simple thermosensors, more complex ones have evolved to ensure return to the basal level even if the cells are continued to be exposed to the high temperature. Examples are the  $\sigma_{32}$ -DnaK-FtsH and the DegS-RseA systems of *E. coli*, the HrcA-GroE system of *B. subtilis* and the HspR-DnaK system of *Streptomyces*. In all these systems, either a molecular chaperone (DnaK or GroE) or a protease (FtsH) keep a positive transcriptional regulator either inactive ( $\sigma_{32}$ ) or a negative regulator active (HrcA and HspR) or do not attack the regulator (DegS). Upon the sudden appearance of non-native proteins, the molecular chaperones and the protease are titrated by these denatured polypeptide chains leading to the accumulation of active ( $\sigma_{32}$ ) or inactive regulator (HrcA, HspR) or its degradation (RseA).

### 6. Bent DNA molecules as thermosensors

It is now evident that DNA is more than a mere repository of a linear array of coding information, but that the structure of the molecule itself influences information access. DNA bending (curvature), both intrinsic and induced by protein binding, directly affects transcription. DNA with a bent trajectory in its helical axis that is induced solely by innate sequences, such as phased homopolymeric adenine: thymine tracts (AT-tracts), is called intrinsically curved DNA (Ohyama 2001). Under defined environmental conditions, such as a temperature transition from 25 to 37°C, intrinsic DNA curvature in the upstream region of a promoter can

activate virulence-associated genes by increasing the binding affinity of RNA polymerase to the promoter (Nickerson and Achberger 1995) or can repress virulence-associated genes by excluding or trapping RNA polymerase with a bacterial nucleoid-associated silencer protein such as H-NS (Dame *et al* 2002).

The distribution of genomic curvature in mesophilic bacteria exhibits conservative patterns and reveals that a substantial fraction of the promoters is characterized by intrinsic bends located within the promoter or 100-220 bp upstream of the nascent translation start site. Regions of intrinsic bending identified on the chromosome of *E. coli* have been predominantly associated with the 5' regulatory regions of genes (Mizuno 1987; Tanaka *et al* 1991). Bent DNA can be involved in repressing transcription. It acts as a binding site for specific silencer proteins which stabilize or enhance a pre-existing DNA bend, thereby effectively blocking transcription of downstream genes.

The most extensively characterized silencer protein is H-NS which affects a large number of enterobacterial genes coding for housekeeping functions as well as for virulence factors (Atlung and Ingmer 1997; Schröder and Wagner 2002). Two examples will be discussed: expression of the *virF* gene of *Shigella flexneri* and of *Yersinia enterocolitica*.

*Shigella* is a pathogenic microorganism able to invade the intestinal epithelium in humans. The primary event following the shift from the outside environment to the mammalian host is the synthesis of VirF, a transcriptional activator protein which triggers the regulatory cascade resulting in activation of several operons encoding invasion functions (Prosseda *et al* 2002). The activation of the *virF* gene upon entry of *Shigella* into the host cell only occurs at temperatures above 32°C (Colonna *et al* 1995; Prosseda *et al* 1998). At restrictive temperatures H-NS is responsible for repressing *virF* expression by interacting with two sites within the *virF* promoter. H-NS is a 15-kDa chromatin-associated protein found in enterobacteria which bind preferentially to intrinsically curved DNA (Atlung and Ingmer 1997). The major role of H-NS is to modulate the expression of a large number of genes, mostly by negatively affecting transcription. Many of the H-NS-modulated genes are regulated by environmental signals, e.g., increased temperature, and expression of most of these genes is positively regulated by specific transcription factors. The accessibility of the target DNA to H-NS varies as a function of temperature, and it could be shown that a single major bending centre located between the two H-NS binding sites acts as a sensor for temperature (Prosseda *et al* 2004). This intrinsic bent melts abruptly around 32°C allowing RNA polymerase to bind to the *virF* promoter followed by transcription of that gene.

Over the narrow temperature range of 30 to 37°C, pathogenic *Yersinia enterocolitica* undergo a significant shift

in gene expression involving chromosomal and plasmid loci. Within minutes after exposure to 37°C, plasmid-encoded virulence gene transcripts can be detected. Shifting *Y. enterocolitica* from 30 to 37°C has multiple effects including the coordinate repression of flagellum synthesis and induction of a set of plasmid-encoded virulence genes (Cornelis and Wolf-Watz 1997). The plasmid pYV (*Yersinia virulence*) encodes a type III secretion system (*ysc* and *ler* genes) essential for delivery of plasmid-borne anti-host factors collectively referred to as Yops (*Yersinia* outer proteins). Expression of these genes requires the trans-acting DNA binding protein VirF, the synthesis of which is induced by elevated temperature (Michiels *et al* 1991; Rohde *et al* 1999). When *virF* was expressed artificially from the *tac* promoter at 30°C, the *yop* genes failed to become induced suggesting that one or more additional factors are needed (Lambert *et al* 1992). Plasmid pYV undergoes a conformational transition between 30 and 37°C. It contains multiple regions of intrinsic curvature, including *virF*, the transcriptional activator of the virulence genes. It has been shown that bending is pronounced at 4°C and completely lost at 37°C and is maintained in the range of 25 to 30°C (Rohde *et al* 1999).

## 7. mRNA molecules as thermosensors

Besides carrying the coding information for protein synthesis, mRNAs can act as regulators of expression of the encoded message. These sensory RNAs are able to detect changes in the cellular environment through interactions with metabolites (Mandal and Breaker 2004; Winkler and Breaker 2005), proteins (Kaempfer 2003) or small noncoding RNAs (Storz *et al* 2004) or through physical parameters such as temperature acting as RNA thermosensors (Narberhaus *et al* 2006). These RNAs form a secondary structure within their 5'-end that shields the Shine-Dalgarno sequence at physiological temperatures (Yamanaka 1999; Morita *et al* 1999b; Nocker *et al* 2001; Johansson *et al* 2002). Rise in temperature results in the liberation of the Shine-Dalgarno sequence, thereby facilitating ribosome binding and translation initiation. Four different cis-acting RNA thermometers will be discussed: transcripts coding for the bacteriophage  $\lambda$  cIII protein, the heat shock sigma factor  $\sigma 32$  of *E. coli*, small heat shock proteins of *Rhizobiaceae* and activator protein PrfA of *Listeria monocytogenes*.

The cIII protein of phage  $\lambda$  plays a role in the decision of whether this phage enters the lytic or the lysogenic cycle. It serves as an inhibitor of the ATP-dependent metalloprotease FtsH to prevent degradation of the rather unstable transcriptional activator protein cII (Herman *et al* 1997; Shotland *et al* 1997). While high concentrations of cIII result in stabilization of cII thereby favouring lysogenization, low concentrations result in the lytic cycle. Translation

of the cIII transcript is regulated through alternative RNA structures (Altuvia *et al* 1989). One structure sequesters part of the Shine-Dalgarno sequence and the AUG start codon thereby inhibiting translation. The alternative structure leaves the translation initiation region free and accessible to the ribosomes. The equilibrium between both structures is dependent on the growth temperature. High temperatures (45°C) favour an energetically more stable conformation occluding the ribosome-binding site and thereby the lytic pathway. Physiological temperatures (around 37°C) leave the Shine-Dalgarno sequence accessible to the ribosomes resulting in production of cIII supporting the lysogenic cycle. This is the only known example where a high temperature prevents translation. In the other three examples, the high temperature opens the stem of a stem-loop structure allowing the ribosomes to bind to the Shine-Dalgarno sequence.

The second example is the *rpoH* mRNA of *E. coli* coding for the alternative sigma factor  $\sigma 32$ . At low temperatures, only modest amounts of  $\sigma 32$  are present which, in addition, is rather unstable (Tilly *et al* 1989). At low temperatures (around 30°C and below), the 5'-end forms a rather complicated secondary structure including five stems where two sequester the AUG start codon and part of the Shine-Dalgarno sequence, respectively, largely preventing translation. Disruption of these structures by enhanced temperatures liberates the ribosomal binding site (Morita *et al* 1999a,b).

The third example involves a conserved sequence of about 100 nucleotides present in the 5'UTR of multiple heat shock genes in *Rhizobiaceae* and has been studied in detail in *Bradyrhizobium japonicum* (Narberhaus *et al* 1998; Narberhaus *et al* 1996). This cis-acting regulatory element has been designated ROSE (for repression of heat shock gene expression) and folds into a complex structure occluding both the Shine-Dalgarno sequence and the start codon. Not only the hairpin structure sequestering the ribosome-binding site, but also two additional ones influence the expression level of the heat shock genes suggesting that proper temperature sensing requires the entire and intact structure of the ROSE element. The additional stem-loop structures might serve as zippers ensuring the correct folding of the thermometer as the RNA is being transcribed. The fourth example deals with expression of virulence genes in the food-borne pathogen *L. monocytogenes* which is under the control of the transcriptional activator protein PrfA. This protein is not present at 30°C although its transcript is being made (Renzoni *et al* 1997). A stem-loop structure is present in the leader sequence and responsible for thermoregulation of *prfA* expression (Johansson *et al* 2002). Again, exposure to 37°C is sufficient to open the stem to allow ribosomes to bind, and the PrfA protein turns on transcription of several virulence genes.

## 8. Proteins as thermosensors

The first-documented case of a temperature-sensing protein is TlpA, a 371-residues cytoplasmic protein encoded on the 96-kb pSLT virulence plasmid of *Salmonella enterica* serovar Typhimurium and characterized by a remarkably long  $\alpha$ -helical coiled-coil motif (Hurme *et al* 1996). TlpA can be found in temperature-dependent two-state equilibrium, between unfolded monomers and highly  $\alpha$ -helical coiled-coil oligomers. At physiological temperatures transcription of *tlpA* is autoregulated by the repressing activity of TlpA, which in its dimeric and folded coiled-coil conformation binds to the *tlpA* promoter and represses transcription. Elevated temperatures induce a shift in the equilibrium favouring the non-functional unfolded monomeric form, which leads to increased transcription (Hurme *et al* 1997). TlpA plays a not well-defined role, but it could regulate expression of genes involved in virulence.

The second example for a temperature-sensing protein is the RheA autorepressor. In *Streptomyces albus*, a 18 kDa protein is detected only at high temperatures and plays a key role in thermotolerance (Servant and Mazodier 1995). The *hsp18* gene coding for this small high temperature protein is under negative control by the RheA autorepressor where its gene is located upstream from, and in the opposite orientation to *hsp18*. RheA has been shown to act as a thermosensor by several experimental approaches (Servant *et al* 2000). When DNA mobility-shift assays were carried out, a shift was observed with the DNA/RheA sample at 30°C, but not at higher temperatures. *In vitro* transcription experiments using purified RheA revealed that the protein was able to inhibit its own transcription at 30°C, whereas at 41°C even high concentrations of repressor did not repress transcription. Circular dichroism spectroscopy revealed a reversible temperature-dependent conformational change in RheA that is likely to reflect a transition between its active and inactive form. It follows that elements within the primary amino acid sequence of RheA are sufficient for both maintenance of this repressor in the binding form at low temperature and for its heat-induced conversion to the non-binding form (Servant *et al* 2000).

## 9. Complex thermosensors

Complex thermosensors sense the appearance of non-native proteins as a consequence of a heat shock or after exposure to other physical and chemical stress factors. These non-native proteins are prone to form aggregates, and large aggregates can cause cell death. Therefore, bacterial cells try to prevent formation of protein aggregates by two complementary strategies: (i) molecular chaperones allowing binding (holder chaperones) and refolding (folder chaperones) are even solubilization of aggregates (disaggregating chaperones),

and (ii) ATP-dependent proteases degrade non-native proteins. What determines the fate of individual non-native proteins is unknown. Selection for refolding or degradation may be either a stochastic process or, alternatively, the degree of unfolding may be important, where slightly damaged proteins can refold and severely damaged proteins will be selected for degradation. Two classes of thermosensors have been identified so far, molecular chaperones and a protease, which modulates the activity of a transcription factor. Four different interactions have been described so far,  $\sigma$ 32-DnaK, HrcA-GroE, HspR-DnaK and DegS-RseA.

$\sigma$ 32 is an alternative sigma factor first described in *E. coli* (Grossman *et al* 1984; Landick *et al* 1984). If *E. coli* cells are growing under physiological conditions, this protein is present only in small amounts due to two different regulation mechanisms. First, the mRNA coding for  $\sigma$ 32 is translated only at a very low rate (*see above*) and second, the half-life of this sigma factor is only about one min due to its rapid degradation preferentially by the ATP-dependent metalloprotease FtsH (Herman *et al* 1995; Tomoyasu *et al* 1995). Since  $\sigma$ 32 is stabilized in the absence of DnaK, this molecular chaperone must influence the sigma factor in such a way to convert it into a substrate for FtsH. After a heat shock, the sudden increase in the amount of non-native proteins will titrate DnaK thereby increasing the stability of  $\sigma$ 32.

HrcA is a transcriptional repressor which controls expression of the *groE* operon in more than 120 bacterial species and the *dnaK* operon and a few additional heat shock genes in some species (Schumann 2003). The DNA-binding activity of HrcA is modulated by the GroE chaperone. If cells are depleted for the GroE proteins, a HrcA-regulated reporter gene is induced suggesting that the continued presence of GroE is necessary to keep HrcA in its active form (Mogk *et al* 1997). As already described for DnaK, non-native proteins will titrate GroE leading to inactive HrcA, and the genes and operons controlled by the repressor are induced. The more non-native proteins are removed from the cytoplasm, the more GroE molecules (and DnaK in the case of  $\sigma$ 32) will be available to return HrcA to its active conformation leading to a gradual turn off of the genes controlled by HrcA.

The HspR repressor encoded by the *hspR* gene is present in *Streptomyces* and controls expression of the *dnaK* operon including its own gene (Bucca *et al* 1997). It has been shown that HspR is unable to bind by itself to its operator but needs DnaK as a corepressor to be kept in its active form (Bucca *et al* 2000). Indeed, the *DnaK* chaperone must be present for HspR to retard a DNA fragment containing the *dnaK* promoter in gel mobility-shift assays. This effect is independent of the co-chaperones DnaJ and GrpE, and does not depend on ATP. Addition of anti-DnaK monoclonal antibodies affected the retardation of the protein-DNA

complex, proving that DnaK is a component of the complex. Again, non-native proteins will titrate DnaK resulting in the induction of the *dnaK* operon.

The last example is the homotrimeric periplasmic protease DegS of *E. coli* which is involved in activation of the alternative heat shock sigma factor  $\sigma E$  (Ades 2004; Alba and Gross 2004). This sigma factor is located in the cytoplasm and since the periplasm is separated from the sigma factor by the cytoplasmic membrane, transmembrane signalling must occur. This is accomplished by the anti-sigma factor RseA, which spans the membrane once and where the C-terminal part is exposed in the periplasm and the N-terminal part in the cytoplasm sequestering  $\sigma E$ . Release of  $\sigma E$  from its anti-sigma factor is triggered by the appearance of unfolded outer membrane proteins, especially porins carrying a certain signature at their immediate C-terminal end (Alba *et al* 2002; Kanehara *et al* 2002). Unfolding occurs either upon exposure to very high temperatures (>45°C) or overproduction of the appropriate outer membrane proteins such as the OmpC porin. The major mechanism used to release  $\sigma E$  from RseA involves degradation of the anti-sigma factor through the activity of two proteases, DegS and RseP, two inner membrane proteases (Alba *et al* 2001; Alba *et al* 2002; Kanehara *et al* 2002). While DegS carries out the first proteolytic step by cleaving RseA within its periplasmic domain, RseP will attack the transmembrane domain immediately afterwards.

How the proteolytic activity of the DegS protease is kept inactive in the absence of unfolded outer membrane proteins? DegS possesses a PDZ interaction domain, and in vitro binding studies revealed that the PDZ domain specifically recognizes a conserved sequence found at the carboxy termini of outer membrane porins (YXF-COOH; Walsh *et al* 2003). It is anticipated that in the absence of unfolded porins, the PDZ domain packs against the protease domain, occluding both the enzyme-active site and the peptide binding site of the PDZ domain. Upon the appearance of unfolded outer membrane porins, the PDZ domain will sense the YXF signature located at their immediate C-terminal end leaving the proteolytic site free. It will attack the largely unstructured C-terminal domain of RseA and cleave at a Val-Ser bond about 30 residues from the C-terminal end of its transmembrane segment. In summary, the DegS protease acts as an indirect thermosensor by sensing unfolded outer membrane porins by virtue of its PDZ domain.

#### 10. The quality control factor HtrA acts as an ATP-independent protease chaperone system

The most evolved temperature-sensing protein is the HtrA (for High Temperature Requirement) protein of *E. coli* also called DegP and has been identified by two phenotypes of corresponding null mutants either unable to grow at elevated temperature (HtrA; Lipinska 2886) or failed to

digest misfolded proteins in the periplasm (DegP; Strauch and Beckwith 1988). This protein was initially identified in *E. coli* as a serine protease belonging to the trypsin clan SA where the order of the catalytic triad is His-Asp-Ser (Clausen *et al* 2004). SA proteases have a two-domain structure with each domain forming a six-stranded  $\beta$  barrel. The N-terminal trypsin domain is followed by two PDZ domains, protein modules that mediate specific protein-protein interactions and bind preferentially to the C-terminal 3–4 residues of the target protein (Saras and Heldin 1996). The proteolytic activity of HtrA is dependent on the temperature of the reaction. When the digestion of  $\beta$ -casein is followed, almost no proteolytic activity is detected below 20°C. At temperatures above 30°C, the proteolytic activity rapidly increases in a non-linear fashion (Spiess *et al* 1999). The functional unit of HtrA appears to be a trimer forming a funnel-like shape with the protease domains located at its top and the PDZ domains protruding to the outside. The PDZ domains are highly mobile, swinging around to capture substrate molecules. Staggered association of two trimeric rings forms the functional HtrA hexamer. Top and bottom of the functional HtrA hexamer are formed by the six protease domains, whereas the twelve PDZ domains generate the mobile sidewalls. The axial pores of the hexamer are completely blocked and the PDZ domains are the only gates allowing lateral access to the central cavity. The PDZ domains mediate the opening and closing of the particle and probably the initial binding of substrate. The inner cavity is lined by several hydrophobic patches that may act as docking sites for unfolded polypeptide chains (Krojer *et al* 2002). The mechanism of the switch from chaperone to protease activity and vice versa remains elusive. The simplest possibility might involve the mobility of the PDZ domains or/and the activity of the proteolytic site which could be temperature-dependent.

In summary, at low temperatures, HtrA has general molecular chaperone activity and is able to stimulate refolding of chemically denatured substrates, whereas its proteolytic activity is almost exclusively present at elevated temperatures. Only in a proteolytically inactive HtrA mutant, HtrAS210A, the chaperone activity is present at low and high temperatures (Spiess *et al* 1999). Therefore, HtrA switches between molecular chaperone and proteolytic activities in a temperature-dependent manner.

#### 11. Evolution of the thermosensors

Based on the suggestion that our DNA world has been preceded by an early RNA world, mRNA thermosensors have to be assumed to be the first thermosensors. In their simplest form, mRNA thermosensors just need a single secondary structure which sequesters the ribosome-binding site at one temperature and allow access at another temperature. The

mRNAs coding for the phage  $\lambda$  cIII protein and for the PrfA protein of *L. monocytogenes* are two examples (Altuvia *et al* 1989; Johansson *et al* 2002). More sophisticated mRNA thermosensors use more complex secondary structures such as the mRNAs coding for the heat shock sigma factor  $\sigma_{32}$  and those coding for small heat shock proteins in *Rhizobiae* (Morita *et al* 1999b; Narberhaus *et al* 1998), where the additional secondary structures may influence the stability of that sequestering the Shine-Dalgarno sequence. All these mRNA thermosensors allow regulated expression of just one single gene since they depend on the ribosome-binding site located close to the 5' end. No mRNA thermosensors have been described coding for more than one gene. To increase the number of genes regulated by such an RNA thermosensor, they code for a positive transcriptional regulator, either an alternative sigma factor(s) or a transcriptional activator (PrfA). In principle, it should also be possible to construct a regulatory unit allowing temperature-regulated expression of a polycistronic mRNA. Such a unit has to contain at least two alternative secondary structures, one leading to the formation of a transcriptional terminator at one temperature and the other to a transcriptional antiterminator at the alternative temperature. Such a regulatory unit has never been reported so far.

DNA thermosensors are based on promoter occlusion. Here, bending of the DNA in conjunction with a silencing protein prevents binding of the RNA polymerase at the low temperature. High temperatures reduce the bending thereby destroying the whole architecture at that site allowing access of the RNA polymerase to the promoter. So far, no DNA thermosensor has been described depending solely on the DNA bent. The question has to be asked why such a simple DNA thermosensor does not work. It can be assumed that bending is not strong enough to prevent access of RNA polymerase to the promoter, and that these bends always work together with a silencer protein such as H-NS to occlude the RNA polymerase. But opening of the bent is solely dictated by an increase in temperature which is sensed by the DNA molecule. Protein thermosensors also depend on conformational changes, where the low temperature favours the active and the high temperature the inactive form of the protein. Two such protein thermosensors have been described so far, the RheA and the TlpA repressor (Servant *et al* 2000; Hurme *et al* 1997), which negatively regulate expression of a small heat shock protein (more correct: high temperature protein) and of several virulence genes, respectively. 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 bacteriophage  $\lambda$  *cIts857* repressor (Sussman and Jacob 1962).

The complex thermosensors represent the most sophisticated system having evolved. They depend on either

a molecular chaperone or a protease where both are able to sense denatured polypeptide chains. In the absence of non-native proteins, they either keep a positive regulatory protein inactive (DnaK –  $\sigma_{32}$ ) or a negative one active (GroE – HrcA, DnaK – HspR, DegS – RseA) and are titrated by the sudden appearance of denatured proteins. This heat shock response allows cells to return to the pre-heat shock situation when the non-native proteins have been removed. In contrast, the high temperature response, once induced, will persist as long as the bacteria are exposed to that temperature. This system is used by many pathogenic bacteria infecting warm blooded hosts to sense the new environment. As long as they multiply in the new environment, continued synthesis of their virulence proteins is required. Therefore, the high temperature response is never turned off. The last example is the two-functional HtrA protein, where the switch from a molecular chaperone to a protease is dictated by the temperature. Most interestingly, the protease activity can be eliminated by single point mutation without destroying its chaperone activity (Spiess *et al* 1999). This observation indicates that the high temperature activates the proteolytic activity most probably by a conformational change. It can be assumed that under those conditions the chaperone activity changes from a folding to an unfolding activity to aid degradation of the denatured target proteins.

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