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Low-temperature sensors in bacteria

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Bacteria are ubiquitous colonizers of various environments and host organisms, and they are therefore often subjected to drastic temperature alterations. Temperature alterations set demands on these colonizers, in that the bacteria need to readjust their biochemical constitution and physiology in order to survive and resume growth at the new temperature. Furthermore, temperature alteration is also a main factor determining the expression or repression of bacterial virulence functions. To cope with temperature variation, bacteria have devices for sensing temperature alterations and a means of translating this sensory event into a pragmatic gene response. While such regulatory cascades may ultimately be complicated, it appears that they contain primary sensor machinery at the top of the cascade. The functional core of such machinery is usually that of a temperature-induced conformational or physico-chemical change in the central constituents of the cell. In a sense, a bacterium can use structural alterations in its biomolecules as the primary thermometers or thermostats.

Keywords: cold; temperature; sensor; bacteria; gene regulation

1. INTRODUCTION

The habitat niches on earth vary considerably in temperature. Accordingly, many biological processes are optimized for different temperatures, the anatomy and physiology of organisms being adapted to their cognate environment. Bacteria are notorious for their ability to colonize different environments. Additionally, the particular niche or lifestyle of many bacteria may be subjected to regular, but sudden, variations in temperature. This reasoning must apply for bacteria adjusting their activities according to seasonal variations, and certainly for pathogens that circulate between the environment, vectors (in some cases) and warm-blooded hosts. Two major problems arise from exposing a cell to a sudden decrease in temperature (Yamanaka *et al.* 1998). First, membrane fluidity decreases, which affects many vital membrane and membrane-associated functions. Second, nucleic acid topology will be stagnated causing halts in processes such as transcription and translation. In parallel, a bacterium departing its host may need to shut off the expression of colonization functions and, in many instances, the accompanying temperature shift acts as the environmental cue that downregulates virulence gene expression. It follows that bacteria need devices for sensing environmental temperature changes in order to adapt their biochemical processes accordingly. We have attempted to summarize the molecular mechanisms that allow temperature sensing in bacteria.

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2. THE STRATEGIES AVAILABLE

A single bacterial strain may have the capacity to grow within a temperature range spanning as much as 50 °C. One such example is the opportunistic pathogen *Listeria monocytogenes*, which has the capacity to grow at temperatures ranging from 1 to 45 °C (Seeliger & Jones 1986). However, the ability to express certain factors by bacteria can be affected by reasonably small temperature changes. Relocation of a culture of *Escherichia coli* adapted to an optimal growth to a sudden temperature increase, or decrease, by some 10–15 °C will result in adaptive shock responses. Such responses involve a remodelling of bacterial gene expression, aimed at adjusting bacterial cell physiology to the new environmental demands (Lemaux *et al.* 1978; Hurme & Rhen 1998; Ramos *et al.* 2001). Less drastic changes in temperature may not induce shock responses, but can be sufficient to modulate the expression of virulence genes, for example in *Shigellae* (Maurelli & Sansonetti 1988) and *Yersiniae* (Straley & Perry 1995). While one might be surprised that organisms built on such minimalist approaches as bacteria respond to temperature changes, the consequence of these observations is that bacteria actually sense temperature shifts in order to control gene expression accordingly.

The very secret of bacterial temperature sensing appears to be located in the ability of the bacterial cell to define and locate those defined changes in its biomolecular constitution that occur as physico-chemical responses to temperature changes. In other words, while the bacterial cell may not be capable of experiencing hot or cold as 'burning' or 'freezing', it is certainly constructed in a way that enables the cell to respond to alterations in temperature (Hurme & Rhen 1998). A temperature-dependent expression and suppression of a given gene can be directed through the altered activity of a single factor or molecule. In many of the examples known, the sensor components

may act as a thermometer or thermostat. Hence, decreased as well as increased temperature can be monitored with the use of the same sensor mechanism.

3. THE TOOLS AVAILABLE

If *E. coli* is exposed to an oxidative substance such as hydrogen peroxide, it responds by the activation of a transcriptional regulator protein OxyR (Carmel-Harel & Storz 2000). Activation of OxyR is achieved through the formation of a disulphide bound within the protein (Åslund *et al.* 1999), upon which OxyR induces the expression of a set of genes adapting the bacterial cell to oxidative stress. Thus, the protein itself is capable of sensing a change in bacterial cytoplasmic redox potential and of concomitantly modulating its regulatory capacity because of this change. This illustrates how it is possible both to 'sense' and respond to an abrupt change in a specific environmental factor in a simple, yet elegant mode.

One would expect the bacteria to be similarly elegant when sensing temperature shifts. While the information available is somewhat scant, the picture emerging shows that bacteria use signals generated through changes in nucleic acid or protein conformation, or changes in membrane lipid behaviour, as sensory devices.

(a) *Sensing of temperature through alteration in nucleic acid conformation: DNA*

In the bacterial cell, the genome is not wrapped into a roll of linear DNA. Instead, chromosome and plasmid DNA is contained in a 'twisted' superhelical conformation (Dorman 1996). In bacteria, the degree of superhelicity varies in response to changes in the ambient temperature. In many examples, the expression of many genes is dependent on DNA conformation, and temperature-dependent gene regulation is mastered through changes in DNA supercoiling (Dorman 1991; Grau *et al.* 1994; Hurme & Rhen 1998). This being the case, one may ask how bacteria may control conformational changes in their DNA.

A number of bacterial proteins are known to be involved in creating and maintaining conformational structures in the DNA molecule. At least in *E. coli*, the superhelical tension itself is mainly regulated through the balance of two opposing topoisomerase activities, mainly those of topoisomerases II and I (Drlica 1992; Tse-Dinh *et al.* 1997). In parallel, superhelicity is constrained through the presence of 'nucleoid-associated' proteins, of which H–NS is the best characterized (Williams & Rimsky 1997; Dorman *et al.* 1999).

H–NS is a small protein that binds DNA in a rather non-specific manner, yet H–NS appears to favour binding to curved regions of DNA (Dorman *et al.*, 1999; Bertin *et al.* 2001). Evidently, H–NS can regulate gene expression simply by binding to DNA and also through its ability to affect the level of supercoiling and DNA condensation (Tupper *et al.* 1994). In many instances, H–NS is known to be responsible for the cold repression of bacterial genes (Williams & Rimsky 1997).

In many of the examples explored in more detail, temperature-dependent gene expression is controlled through a concerted action of supercoiling and supercoiling-constraining proteins (Dorman 1996; Hurme & Rhen 1998; Dorman *et al.* 1999). Temperature regulation of virulence gene expression in *Shigella* serves as an illustrative example of the interplay between DNA conformation and a DNA-binding protein. The expression of virulence plasmid functions in *Shigella* is dependent on a transcriptional regulator protein, VirF (Tobe *et al.* 1993). At low temperatures, expression of the *virF* gene is suppressed by H-NS (Tobe *et al.* 1993) and the ability of H–NS to mediate transcriptional repression is dependent on the superhelical state of the promoter region (Falconi *et al.* 1998). Thus, the concerted action of H–NS and the superhelical state contribute to transcriptional repression of *virF*. When increasing the temperature to 37 °C, the ability of H–NS to bind cooperatively to its target sequence at the *virF* promoter sequences decreases, due to a conformational shift in the local DNA topology, allowing transcription of *virF* (Falconi *et al.* 1998). Expression of VirF will lead to expression of the regulator VirB, and subsequently to virulence gene expression. Apparently, a shift to the cold would result in reconstitution of H–NS-mediated repression, downregulation of *virF* and, concomitantly, in repression of virulence gene expression.

(b) *Sensing of temperature through alteration in nucleic acid conformation: RNA*

Theoretically, RNA molecules have a strong potential as temperature sensors, in that they can form pronounced secondary and tertiary structures (Andersen & Delihas 1990), and through their ability to form intermolecular RNA : RNA hybrids (Lease & Belfort 2000). Both of these processes greatly depend on the formation of complementary base pairing, and consequently one would anticipate these to be dependent on environmental temperature.

The causative agent of plague, *Yersinia pestis*, contains a homologue of the *Shigella* VirF virulence regulator termed LcrF (Hoe & Gougen 1993; Straley & Perry 1995). Expression of LcrF is thermoregulated. Yet, while the transcription of *lcrF* is the same at 25 °C and 37 °C, the expression of LcrF from the messenger is not. The temperature-dependent expression relies on the *lcrF* mRNA itself being capable of forming an intramolecular stem–loop structure shielding the Shine–Dalgarno sequence, thus preventing translation at 25 °C (Hoe & Gougen 1993). At elevated temperature, the stem–loop structure melts, thus allowing translation. Hence, in the case of LcrF production, the *lcrF* mRNA itself serves as both the messenger and the thermosensor.

Other examples of *cis*-acting mechanisms involved in translational thermoregulation include the expression of the RpoH heat-shock σ-factor in *E. coli* (Kamath-Loeb & Gross 1991; Nagai *et al.* 1991) and the regulation of lysogenic conversion in the *E. coli* phage λ (Altuvia *et al.* 1989). The *rpoH* mRNA includes *cis*-acting sequences involved in translational thermoregulation of RpoH expression. In the case of phage λ, low temperature will favour a conformational state in the *cIII* mRNA that allows translation to proceed. This shows that conformational changes in RNA structures can be used for activating gene expression, both in response to increased and decreased environmental temperature.

In *E. coli* and *Salmonella typhimurium*, at 37 °C, the expression of the alternative σ-factor RpoS increases in the stationary phase of growth. At low growth temperature, however, RpoS can also be expressed in the logarithmic

phase of growth (Sledjeski *et al.* 1996). This cold expression of RpoS is dependent on the expression of the *dsrA* gene encoding a small regulatory RNA (Sledjeski *et al.* 1996; Majdalani *et al.* 1998). The effect of DsrA on RpoS expression seems to be that of stabilization of the *rpoS* mRNA (Majdalani *et al.* 1998). Expression of *drsA* is itself temperature dependent; the *drsA* promoter is more active at low temperature and the *drsA* RNA half-life is drastically prolonged at 25 °C as compared with 37 °C (Repoila & Gottesman 2001). In this case, the temperature-dependent expression of a regulatory RNA may regulate the expression of σ-factor.

According to molecular modelling analyses, DsrA can alter between alternative structures ascribing different regulatory characteristics for the molecule (Majdalani *et al.* 1998; Lease & Belfort 2000). Furthermore, modelling analyses of DsrA suggest that there might be direct interactions with DsrA and the messengers regulated. It remains to be demonstrated if, and how, temperature is capable of directing the structural state of DsrA and whether such structural alterations affect either the half-life or intramolecular base-pairing characteristics of DsrA. Should any such connections be revealed, they would imply that the thermosensory capacity would reside within the *dsrA* sequence itself.

Exposing a culture of *E. coli* to a drastic temperature downshift induces a response termed the cold-shock response (Yamanaka 1999). This response involves the transient expression of a set of proteins aimed at adapting the bacteria to growth at a lower temperature. Such factors include nucleic acid chaperones, a RNA helicase, topoisomerase II gyrase subunit A and H–NS. In contrast to the heat shock, none of these new factors represents a cold-shock σ-factor. In fact, alteration of mRNA stability is considered to represent a major factor for the induction of the cold-shock response. The messenger for the major cold-shock protein, CspA, is transcribed at 37 °C, but hardly detectable due to degradation by RNase E. Upon the cold shock, the *cspA* mRNA becomes stabilized (Brandi *et al.* 1996; Fang *et al.* 1997). Interestingly, the 5′-end of the *cspA* mRNA is involved in its own cold stabilization (Mitta *et al.* 1997), implying that RNA structure can be used for a differential temperature-dependent stabilization of mRNA.

(c) *Sensing of temperature through alteration in protein conformation*

A rapid removal of an *E. coli* culture, propagated at 30 °C, to a new environment at 42 °C will induce the so-called heat-shock response, accompanied by the expression of some 20 proteins (Lemaux *et al.* 1978). Many of the new proteins participate in reconstituting and stabilizing protein structures and in removing misfolded ones. The expression of heat-shock proteins is dependent on the σ-factor RpoH (Yura *et al.* 1993). A special chaperone system, which includes the proteins DnaK, DnaJ and GrpE, is involved in regulating the half-life of RpoH (Gamer *et al.* 1996). It has been suggested that DnaK and DnaJ bind RpoH in a way that inactivates RpoH and predisposes it to proteolytic degradation. Because of heat shock, DnaK and DnaJ bind misfolded proteins, which causes the release and activation of RpoH. Thus, one could implicate the shuffling of DnaK/DnaJ between RpoH and misfolded proteins as the thermosensor regulating expression of heat-shock proteins. This, in turn, demonstrates that the bacteria can utilize changes in protein conformation as a means for temperature sensing.

Many isolates of *S. typhimurium* contain a large virulence-associated plasmid that, among other functions, encodes the 371 amino acid protein TlpA (Gulig *et al.* 1993; Hurme *et al.* 1996, 1997). TlpA has the capacity to sense temperature variations and to regulate gene expression accordingly. At low growth temperatures, such as 28 °C, TlpA suppresses its own expression by binding to the promoter region of *tlpA*. A gradual increase in temperature will lead to a concomitant gradual derepression of *tlpA*, whereas at 43 °C the promoter is fully derepressed.

The ability of TlpA both to sense temperature and to regulate gene expression resides in its structural design. Two-thirds of the C-terminal portion of TlpA is contained in an α-helical-coiled-coil structure that constitutes an oligomerization domain. The coiled-coil oligomerization state is dependent on temperature and the TlpA concentration, and only oligomeric TlpA has the capacity to bind DNA. As the temperature increases, the proportion of DNA-binding oligomers decreases, leading to a derepression of the target gene. At moderate temperatures, the concentration of TlpA increases, shifting the balance to the formation of DNA-binding oligomers and, in part, restoring the repression potential of TlpA. Thus, TlpA undergoes a reversible conformational shift in response to temperature alteration, leading to an alteration in the oligomeric structure and subsequently in the regulatory capacity of TlpA (figure 1). The sensory capacity is contained in the coiled-coil structure of TlpA, which illustrates another means of sensing temperature through changes in protein conformation.

While the related virulence-associated plasmids of *Salmonella dublin* and *Salmonella enteritidis* also contain a *tlpA* gene, the large plasmid R27 of *Salmonella typhi* contains a gene apparently coding for a ‘paralogue’ of TlpA. The ORF158 of this plasmid R27 codes for a 405 residue protein showing 22% amino acid identity with TlpA when covering 371 residues (Sheburne *et al.* 2000). Significantly, two-thirds of the C-terminal protein portion is predicted to be in a coiled-coil configuration. Furthermore, the protein includes an N-terminal region showing 45% identity with the predicted TlpA N-terminal DNA-binding motif. While the regulatory capacity of ORF158 has not been defined, it is interesting to note that the conjugative potential of R27 is regulated by temperature, being suppressed at a higher growth temperature.

The coiled-coil structure is a versatile and a rather flexible motif in mediating protein : protein interactions (Lupas 1996). In this context, it is important to note that the H–NS function is also associated with oligomerization and that the H–NS oligomerization domain most evidently relies on the formation of coiled-coil oligomers (Dorman *et al.* 1999; Smyth *et al.* 2000). Furthermore, *E. coli* and many other bacteria in addition to H–NS also express the protein StpA (Dorman *et al.* 1999; Sonnenfield *et al.* 2001). StpA is a prologue of H–NS and thus resembles H–NS in many respects; the proteins are approximately equal in size and show a 58% overall sequence identity. Both proteins include a N-terminal

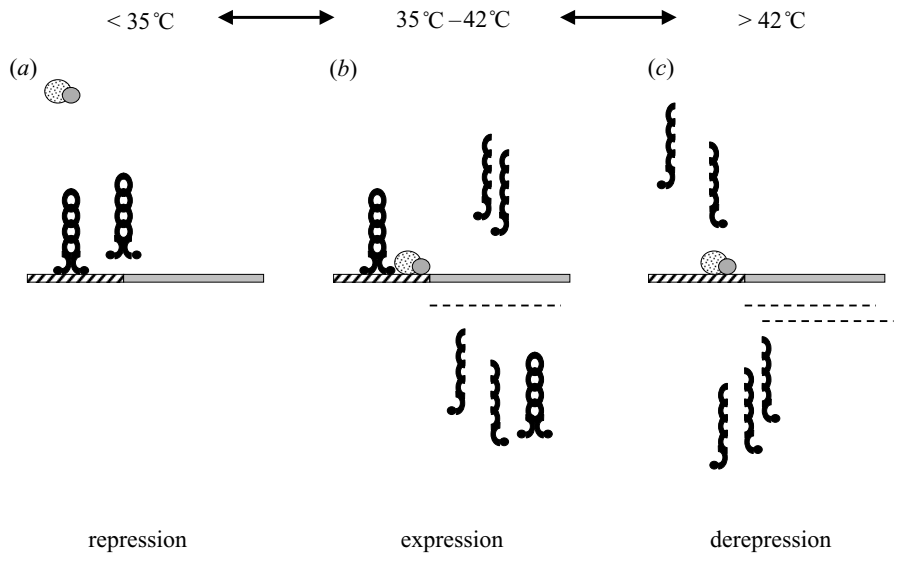

Figure 1. The temperature-directed repression–derepression cycle in TlpA expression. At low temperature (*a*), TlpA is contained in a coiled-coil oligomeric form, capable of binding to the *tlpA* promoter region. This binding prevents transcription and expression of *tlpA*. At intermediate temperature (*b*), part of the oligomeric TlpA is converted into monomers, not capable of binding to DNA. This leads to partial repression of *tlpA* expression, in the formation of more TlpA and TlpA oligomers, which leads to a feedback regulation of the gene. At high temperature (*c*), all of the TlpA is converted into monomers and the *tlpA* gene is fully expressed. A decrease in temperature would complete the cycle by restoring TlpA oligomerization and gene repression. Key: shaded circles, RNA polymerase; chain formation, oligomeric TlpA; bar, *tlpA*; dashed line, mRNA; helix, monomeric TlpA.

coiled-coil oligomerization domain followed by a C-terminal DNA-binding domain, and both proteins bind DNA at curved regions. In fact, in selected cases StpA may compensate for the absence of H–NS in terms of gene regulation (Shi & Bennet 1994). The most interesting aspect resides in the observation that H–NS and StpA may form hetero-oligomers (Dorman *et al.* 1999).

(d) *Alteration in membrane constitution and temperature sensing*

Besides alterations in nucleic acid and protein conformation, the physical state of membranes does change in response to temperature shifts (Vigh *et al.* 1998). Thus, it would not be surprising if bacteria could utilize, for example, changes in membrane fluidity as a thermometer device. *Bacillus subtilis* and the cyanobacterium *Synecocystis* respond to decreased temperature by increasing the *cis*-unsaturation of membrane-lipid fatty acids through expressing acyl-lipid desaturases (Aguilar *et al.* 1998; Suzuki *et al.* 2001). Lipid unsaturation would then restore membrane fluidity at the lower temperature. Hence, appropriate membrane fluidity can be maintained at different environmental temperatures.

In *B. subtilis*, this lipid modification is initiated through the activity of a so-called two-component regulatory system consisting of the DesK and DesR proteins (Aguilar *et al.* 2001). Prokaryotic two-component regulatory systems usually consist of protein pairs, a sensor-kinase and a regulatory protein (Dutta *et al.* 1999). In *B. subtilis*, DesK sits embedded in the cytoplasmic membrane and acts as the sensor-kinase. A temperature decrease-mediated change in the physical state of the membrane is believed to result in functional alteration of DesK activating its potential to phosphorylate the gene regulator DesR (Aguilar *et al.* 2001). Phosphorylated DesR then activates the expression of a Δ5-lipid desaturase. Concomitant desaturation of lipid fatty acids will restore proper membrane fluidity, which in turn will result in a restoration of the activity of DesK (figure 2). At this stage, DesR is moderately dephosphorylated, aborting the transcriptional activation of the genes for the desaturase.

In this example, it appears that the combination of membrane physical state and protein conformation is able to sense temperature and to translate this sensing event into proper gene expression. Interestingly, in *Synecocystis*, a Δ6-desaturase activity is also regulated through a membrane-associated histine kinase Hik33 (Suzuki *et al.* 2001).

What is important to note in this context is that a substance like ethanol or chlorpromazine can also activate components of the heat-shock response (Mizushima *et al.* 1993). While these substances are certainly capable of modulating membrane fluidity, one may speculate whether or not components of the heat-shock response might also utilize the membrane as one additional sensor. In fact, experimental alteration of the photosynthetic thylacoid membranes in *Synecocystis* has been shown to coincide with the expression of *Synecocystis* heat-shock proteins (Vigh *et al.* 1998). This would imply that the thylacoids could also function as temperature sensors and that this sensing could originate from an alteration in the physico-chemical status of these membranes.

4. REGULATORY INTERCONNECTIONS IN THERMOREGULATION

Many of the temperature-induced regulatory circuits described appear either connected to the host cell physiology, and/or interconnected with each other. For example, topoisomerase II (DNA gyrase) activity is depen-

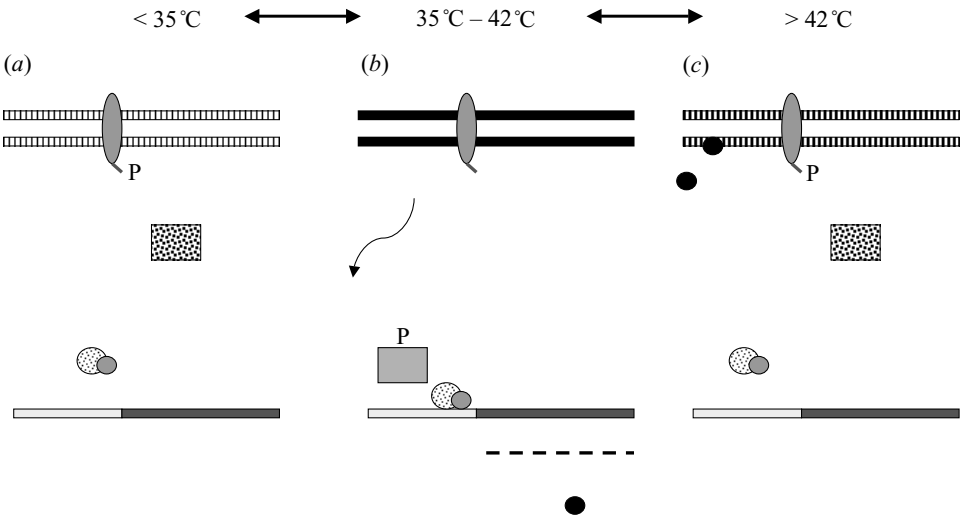

Figure 2. A model for cold-temperature induction of lipid desaturase activity. (*a*) Bacteria are contained at a high growth temperature, with most of their membrane lipids being saturated. (*b*) A sudden decrease in temperature causes a change in membrane fluidity, disturbing membrane functions. The change in membrane fluidity may also cause conformational changes in a sensor kinase (upper part) that enables the protein to phosphorylate a regulator component. The phosphorylated form of the regulator activates genes for lipid desaturases and the lipid desaturases produced reconstitute membrane function by introducing double bonds into membrane lipid fatty acid side chains. The restoration of membrane function (*c*) results in a conversion in the conformational change of the sensor kinase, phosphorylation of the regulator declines and the protein becomes dephosphorylated. Thus, after a pulse of desaturase expression, the activation is terminated and the adaptation cycle is completed. Key: double bars, membrane lipid bilayer; oval, sensor kinase; box, regulator; shaded circle complex, RNA polymerase; bar, desaturase gene; dashed line, mRNA; filled circle, desaturase.

dent on cellular physiology and on a number of factors involved in temperature regulation in general. Gyrase activity is dependent on ATP, hence one could anticipate that the cellular ATP : ADP ratio should affect the state of supercoiling (Drlica 1992). Furthermore, the promoter activity for *gyrAB* (topoisomerase II) is itself reported to be dependent on the DNA superhelical state (Straney *et al.* 1994), whereas the promoter region for the gene encoding topoisomerase I includes the elements of a heat-shock promoter (Qi *et al.* 1997).

A plausible explanation for regulatory interconnections could be that some components involved in temperature regulation are also used for responses to signals other than temperature. In *E. coli*, topoisomerase II activity is enhanced both after exposure to heat and cold shock, and DnaK has been shown to interact with topoisomerase II, thereby stimulating its enzymatic activity (Ogata *et al.* 1996; Tanji *et al.* 1992). Additionally, environmental osmomolarity also affects DNA supercoiling and gene regulation.

Another possible purpose for the apparently complicated regulatory networks involved in thermoregulation could reside in the need to fine-tune the responses. In this respect, a complicated, but illustrative, example originates from the regulation of *hns* and *stpA*. While H–NS can repress *stpA*, both DrsA and StpA have been reported to repress production of H–NS (Dorman *et al.* 1999). In addition, as hetero-oligomers formed between H–NS and StpA evidently have different regulatory characteristics, the formation of H–NS : StpA hybrids may provide bacteria with a variable set of regulatory proteins with the ability to respond differently to environmental changes, such as temperature.

5. REVERSIBILITY AND TEMPORALITY IN THERMOREGULATION

The pragmatic thermoresponse should be one that is reversible and controlled. This statement certainly must apply to the regulation of virulence genes among pathogens cycling between host and environmental niches. Once such a pathogen enters a warm-blooded host, thermal cues could function for the induction of host colonization and virulence factors. Upon leaving the host, the expression of such factors is useless and the response is turned off because of a reversal in the ambient temperature.

Even when contained in a given environment, there might be a need for transient gene expression. An excellent example of this is the modulation of membrane-lipid fatty acid saturation in *B. subtilis*. According to the model adapted here (Aguilar *et al.* 2001; figure 2), a shift to low growth temperature will activate the expression of lipid desaturase activity. However, the mechanism used for sensing the decrease in temperature is autoregulated and shuts off the induction of desaturase activity as soon as the desired membrane physico-chemical state is restored.

The complexity of thermosensing and thermoregulation may reflect the demands to handle and fine-tune responses to an important environmental factor in a dynamic fashion. However, ultimately, it seems that basic and uncomplicated biochemical processes are used as primary sensors and, for that purpose, changes in the nucleic acid, protein or membrane physico-chemical state appear highly suitable.

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Discussion

D. J. Bowles (*Centre for Novel Agricultural Products, Department of Biology, University of York, York, UK*). At what temperature ranges do you think that these changes that you are talking about come into play?

M. Rhen. Most of the examples were picked up from virulent bacteria, so basically it is a transition from the environment to 37 °C, but what we know from the lab experiments is that the temperature range does not need to be very large. As little as a 4 to 5 °C change can be enough.

P. Quick (*Department of Animal and Plant Sciences, University of Sheffield, Sheffield, UK*). Given these thermal changes in protein structure that you are talking about, do we need to invoke a membrane-based response at all?

M. Rhen. Not really, but life is not built up in a way like that. We do know, for a fact, that these transitions in lipid fluidity are being used as a sensory device. Of course, one can easily image how that will affect the conformation of membrane proteins if lipids are changed. You can always argue that there may be different temperature ranges where it is optimized. For coiled-coil structures, there might have been different evolutionary events that have led to structural changes. By varying the structure at the core, you could either up- or downregulate the melting point, thereby setting different temperature windows for sensing. Yes, I am surprised that it is not being used more often.

Anon. Introduction of negative supercool in the DNA induces maybe some cold-shock proteins. If you raised the temperature, and reduce the negative supercooling of the DNA, are any genes known to be induced under other conditions?

M. Rhen. The enzymes that participate and generate a negative supercoiling and relax the supercoiling are highly regulated in response to temperature. So the topoisomerase II promoter itself is sensitive to supercoiling, whereas the topoisomerase I promoter contains elements of a heat-shock promoter, so that heat will induce topoisomerase I. If we take the example of the reporter plasmid, and the supercoiled state of that plasmid, we noticed that soon after either a cold or heat shock there is a relaxation in the supercoiled state that subsequently is rapidly restored. This restoration is, in part, managed through the cold-shock and heat-shock expressers themselves. The heat-shock proteins actually activate the topoisomerase II by binding to it. So, again, it is very dynamic and operates through a feedback regulatory process.

GLOSSARY

ADP: adenosine diphosphate
ATP: adenosine triphosphate
RNase: ribonuclease
ORF: open reading frame