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MINI REVIEW

Regulation of bacterial heat shock stimulons

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Abstract All organisms developed genetic programs to allow their survival under stressful conditions. In most cases, they increase the amount of a specific class of proteins which deal with the stress factor and allow cells to adapt to life-threatening conditions. One class of stress proteins are the heat shock proteins (HSPs) the amount of which is significantly increased after a sudden temperature rise. How is the heat shock response (HSR) regulated in bacteria? This has been studied in detail in *Escherichia coli*, *Bacillus subtilis* and *Streptomyces* spp. Two major mechanisms have been described so far to regulate expression of the HSGs, namely alternative sigma factors and transcriptional repressors. This review focuses on the regulatory details of the different heat shock regulons in the three well-studied bacterial species.

Keywords Regulon · Repressor · Sigma factor · Chaperones

Abbreviations

CIRCE	Controlling inverted repeat of chaperone expression
ECF	Extracytoplasmic function
HAIR	HspR-associated inverted repeat
HSGs	Heat shock genes
HSPs	Heat shock proteins
HSR	Heat shock response
HTH	Helix-turn-helix
LPS	Lipopolysaccharides
OMPs	Outer membrane proteins
RBS	Ribosome binding site
SD	Shine Dalgarno sequence

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Introduction

When cells of bacteria, archaea or eukaryotes are exposed to a sudden temperature rise, a heat shock, they transiently induce a group of genes called heat shock genes (HSGs) which code for heat shock proteins (HSPs). This heat shock response (HSR) was discovered by F. Ritossa (Ritossa 1996) by chance. He was studying *Drosophila melanogaster* larvae treated with different chemicals and was analyzing the puffing pattern in the giant chromosomes isolated from salivary glands. It was already known at that time that puffs represent transcribed regions of the chromosome. One day, he observed a new puffing pattern he could not explain. It turned out that one of his coworkers had increased the temperature of the incubator hosting the larvae (Ritossa 1962). The HSR had been discovered! The next step was detection of the HSPs by Tissières et al. (1974) followed by the discovery of the alternative sigma factor sigma-32 (Sig32) (also called sigma-H (SigH)) being responsible for transcription of the HSGs in *Escherichia coli*. First, a nonsense mutation has been described affecting the synthesis of a major protein essential for growth at high temperature. The mutation was mapped in a gene called *rpoH* (Cooper and Ruettinger 1975). Next, the product of the *rpoH* gene was identified as the alternative sigma factor Sig32 (Grossman et al. 1984). Meanwhile, the HSR has been studied in many bacteria, and both alternative sigma factors and transcriptional repressor proteins have been identified as major transcriptional regulators (Guisbert et al. 2008; Micevski and Dougan 2013; Schumann 1996, 2003; Yura 1996). In general, these transcriptional regulators normally control expression of several heat shock operons

designated as heat shock regulons. Furthermore, many bacteria code for more than one heat shock regulon regulated by different transcriptional regulators. *E. coli* codes for the SigH and the sigma-E (SigE) heat shock regulons which both together constitute the *E. coli* heat shock stimulon. The present review article will provide an updated version of the transcriptional regulation of heat shock stimulons from three bacterial species serving as paradigms of three important groups, namely *E. coli* as the representative of the Gram-negative, *Bacillus subtilis* as a representative of the low GC and *Streptomyces* of the high GC Gram-positive bacteria.

The heat shock response

After its detection in *Drosophila* and later in *E. coli*, the HSR has been demonstrated to be present in all organisms. What happens after a sudden increase in temperature? Why are HSGs induced? What is the function of the major HSPs? By which mechanism(s) is the HSR induced? A sudden heat shock results in protein unfolding leading to the formation of protein aggregates, and large aggregates can cause cell death. Therefore, the HSR is a ubiquitous strategy to remove denatured proteins and to maintain protein homeostasis in all organisms. It responds to protein unfolding, aggregation and damage by the rapid and transient production of HSPs. Two major classes of HSPs induced in all organisms are chaperones binding to denatured proteins and allowing refolding to their native state and ATP-dependent proteases degrading denatured proteins. In total, both groups of HSPs remove denatured proteins to prevent formation of large protein aggregates and cell death.

I will discuss three different situations. (i) The bacteria grown at physiological temperatures and the HSGs expressed at a basal level. (ii) The bacteria are exposed to a sudden temperature increase, a heat shock, leading to an immediate induction of the HSGs followed by a transient increase in the amount of HSPs. (iii) After adaptation to the increased temperature and removal of the denatured proteins, either by refolding or degradation, the HSR is turned down to a steady-state level somewhat higher than the basal level to allow optimal growth of the bacterial cells under these temperature conditions.

Regulation of the heat shock response

The major heat shock regulators are macromolecules acting as molecular thermosensors. The principle of a molecular thermosensor is a switch in the three-dimensional structure of the macromolecule induced at the ambient temperature. While at physiological temperatures, the macromolecule is present in a conformation allowing only a basal level of expression of the HSGs or kept largely inactive by binding to proteins, it adopts a different conformation after a heat shock or is released from its binding partner. Additional mechanisms can fine-tune the HSR as will be described below. Three different thermosensory biomolecules have been described so far: RNA, DNA and proteins (Schumann 2012). RNA and DNA thermosensors can adopt two different secondary structures depending on the temperature, allowing either a basal or a high level of translation or transcription, respectively. RNA thermosensors control the translation of already existing or nascent mRNA molecules by forming a secondary structure that traps the ribosome binding site (RBS), also called Shine-Dalgarno (SD) sequence, at the physiological temperatures. An increase in temperature destabilizes the structure, liberates the RBS and permits formation of the translation initiation complex (Righetti and Narberhaus 2014). One well-studied example of a DNA thermosensor is the transcriptional control exerted by the nucleoid-associated protein regulator H-NS on the *virF* promoter region of the enteropathogen *Shigella flexneri* (Prosseda et al. 2004). At temperatures >32 °C, a temperature-dependent change in the local DNA topology leads to the induction of the *virF* promoter. All known protein thermosensors act as transcriptional repressors. At physiological temperatures, transcriptional repressors bind to their operators and become inactive after a heat shock due to partial unfolding.

A completely different mechanism has been designated as chaperone titration, which involves a direct interaction between chaperones and transcriptional regulators. At physiological temperatures, the chaperones keep most transcriptional repressors in their active conformation or sequester sigma factors. After a heat shock, the chaperones are titrated by denatured proteins which appear immediately within the cells. As a consequence, the transcriptional repressor cannot be folded correctly, will stay in its inactive conformation, and the sigma factors are released and bind to the RNA polymerase core enzyme. The more denatured proteins have been either refolded or degraded by proteases, the more chaperones are able to take care of the transcriptional regulator to allow refolding to its active conformation or to sequester it. This will result in a continuous decrease in the expression of the HSGs to a steady-state level somewhat higher than the basal level as long as the bacterial cells are exposed to increased temperatures.

The *E. coli* heat shock stimulon

All bacteria code for one housekeeping sigma factor (Sig70 in *E. coli*) and several alternative sigma factors. The housekeeping sigma factors are responsible for transcription of the housekeeping genes which are responsible for growth and propagation of the bacterial cell. The alternative sigma factors

are produced or activated when cells undergo particular physiological stresses (Gruber and Gross 2003; Kazmierczak et al. 2005). The *E. coli* K12 strain codes for six, *B. subtilis* for 16 and *Streptomyces coelicolor* for even 62 alternative sigma factors.

So far, two different heat shock regulators, the alternative sigma factors Sig32 and SigE, have been described in *E. coli*. Both sigma factors are cytosolic proteins and present even under physiological conditions, but their interaction with the core RNA polymerase is regulated by different mechanisms. While Sig32 is activated by denatured proteins appearing in the cytoplasm after a heat shock (in the laboratory from 30 to 42 °C), SigE is activated by unfolded proteins in the periplasm (after a heat shock from 30 to 46 °C).

The sigma-32 heat shock regulon

Regulation of Sig32 occurs by three different mechanisms. (i) Translation of its mRNA acting as an RNA thermosensor, (ii) inhibition of its activity by binding to the DnaK/DnaJ/GrpE or GroEL/GroES chaperones and (iii) stability of the Sig32 protein (Fig. 1). The collaboration between the three mechanisms allows the cells to achieve a balanced physiological state, to survive heat stress and to maintain protein homeostasis in living cells.

The steady-state level of Sig32 is low in the absence of heat stress, and low levels of Sig32 (~50 molecules per cell with an half-life of ~1 min) are maintained by the three different mechanisms mentioned above. First, inefficient initiation of translation due to base pairing within the *rpoH* mRNA occluding the Shine-Dalgarno sequence (Fig. 1a). Second, most Sig32 molecules are kept inactive by binding to one of the two major chaperone systems DnaK/DnaJ/GrpE or GroEL/ES (Fig. 1b). In vitro experiments have shown that both chaperone systems inhibit the activity of SigH (Gamer et al. 1992; Guisbert et al. 2004; Gamer et al. 1996; Liberek et al. 1992). Third, the SigH stability is controlled by two different ATP-dependent proteases (Fig. 1c). The first protease identified many years ago is the metalloprotease FtsH anchored in the inner membrane (Herman et al. 1995; Kanemori et al. 1997; Tomoyasu et al. 1995). Quite recently, it has been shown that the signal recognition particle (SRP), which consists of the 4.5S RNA and the Ffh protein forming a complex, recognizes and binds SigH and targets it to the inner membrane, where it is degraded by FtsH (Lim et al. 2013). By using in vivo cross-linking approaches, it could be demonstrated that the homeostatic control region of Sig32 directly interacts with the signal-peptide binding site in the M domain of Ffh, and this interaction is independent of chaperones (Miyazaki et al. 2016). And very recently, a second pathway of degradation has been published. Here, SigH is first covalently bound to the ubiquitin-like protein ThiS followed by degradation by the ClpXP protease (Xu et al. 2015).

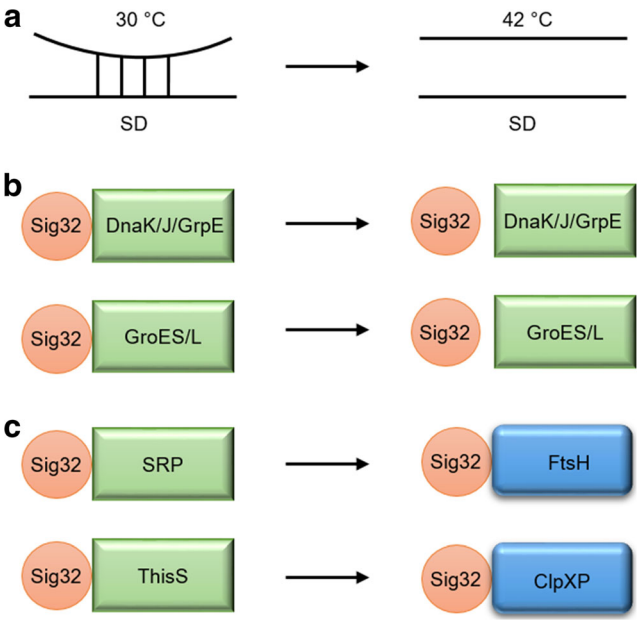

Fig. 1 Regulation of the Sig32 regulon of *E. coli*. **a** The *rpoH* mRNA coding for Sig32 forms a partial secondary structure thereby sequestering the SD sequence at 30 °C allowing only a basal level of translation. After a shift to 42 °C, the RNA strands will be separated allowing a high level of translation. **b** At the low temperature, most Sig32 molecules are sequestered either by the DnaK or the GroEL chaperone system. After a heat shock, the chaperones dissociate from Sig32 bind to the denatured proteins. This mechanism has been called chaperone titration. **c** At 30 °C, most free Sig32 molecules are bound either by the signal recognition particle (SRP) and guided to the FtsH protease or modified by the ubiquitin-like protein ThiS and guided to the ClpXP protease for degradation

Upon a heat shock, the level and activity of Sig32 rapidly increases due to elevated translation of the *rpoH* mRNA. In parallel, the accumulation of unfolded proteins in the cytoplasm sequesters the chaperones transiently (5–10 min) leading to stabilization of Sig32 (Straus et al. 1987). Both mechanisms result in a rapid 12- to 15-fold increase in the levels of Sig32 with an half-life of about 10 min resulting in the induction phase. During the adaptation phase, further synthesis of the heat shock proteins is largely blocked as the activity of Sig32 becomes inhibited by chaperones and its turnover is increased as described above.

The sigma-E heat shock regulon

The alternative sigma factor SigE belongs to the group of extracytoplasmic function (ECF) sigma factors. These sigma factors are capable of responding to a number of signals including cell wall and membrane stress and the oxidation state (Helmann 2002). In *E. coli*, SigE is encoded by the *rpoE* gene being part of the tetracistronic operon *rpoE-rseA-rseB-rseC*.

RseA (regulation of SigE) acts as an antisigma factor of SigE, inserted into the cytoplasmic membrane with one transmembrane domain that tethers SigE to the membrane thereby preventing its interaction with the core RNA polymerase (Missiakas et al. 1997) (Fig. 2). RseB is a periplasmic protein that binds to RseA in a one-to-one binding stoichiometry and acts as a co-antisigma factor (Cezairliyan and Sauer 2007). RseC is an inner-membrane protein able to positively modulate the SigE activity through a so far unknown mechanism (Missiakas et al. 1997).

SigE is released from its antisigma factor by cell envelope stress involving the stepwise degradation of the antisigma factor by three different proteases acting in a consecutive way (Fig. 2). Two macromolecules have been identified as signals to trigger RseA degradation: outer membrane proteins (OMPs) and lipopolysaccharides (LPS). The DegS protease recognizes the C-terminal YxF motif of unfolded- or unassembled OMPs which will result in cleavage of the RseA antisigma factor in its periplasmic domain (Mecsas et al. 1993; Walsh et al. 2003). In addition to DegS activation, RseA cleavage by DegS needs the dissociation of RseB from RseA (Grigorova et al. 2004; Lima et al. 2013). Next, the RseP protease, anchored in the cytoplasmic membrane with four transmembrane domains, cleaves RseA within or near the transmembrane region resulting in release of the shortened RseA into the cytoplasm (Kanehara et al. 2002, 2003). In the last step, the remaining part of RseA will be completely degraded by one of different cytosolic proteases including ClpXP, ClpAP, Lon or HslUV (Chaba et al. 2007). Finally, SigE will be released into the cytoplasm where it will interact with the RNA polymerase core enzyme guiding it to SigE-dependent promoters leading to the induction of the SigE regulon. When LPS accumulates in the periplasm, it will dissociate RseB from RseA, thereby initiating the RseA proteolysis cascade (Chaba et al. 2011; Lima et al. 2013). SigE activates a total of 89 transcriptional units. When the unfolded OMPs have been removed, the DegS protease will return to its inactive state keeping the antisigma factor RseA intact starting to sequester SigE thus turning off the SigE regulon.

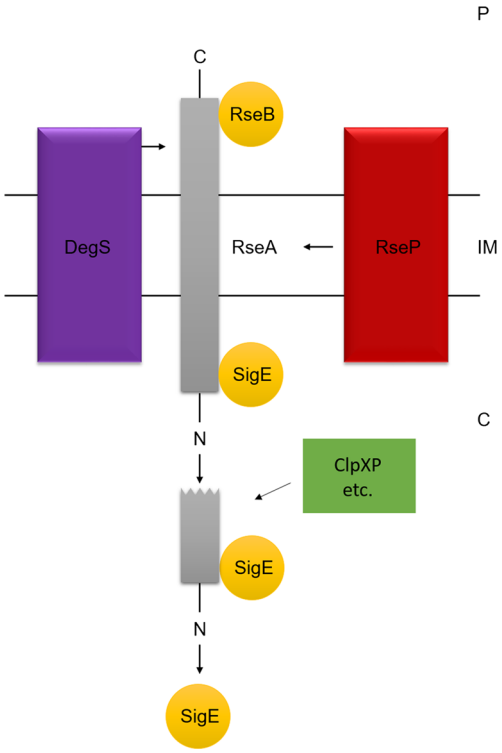

Fig. 2 Regulation of the SigE regulon of *E. coli*. The antisigma factor RseA is inserted into the inner membrane (IM) by one transmembrane domain. The co-antisigma factor RseB binds to its C-terminal part exposed into the periplasm (P), and the N-terminal end extending into the cytoplasm (C) sequesters SigE at low temperatures. After a severe heat shock, the C-terminal ends of partially unfolded outer membrane proteins activate the DegS protease which will cleave within the periplasmic domain of RseA. This leads to a conformational change within its IM domain activating the RseP protease which will cleave within this domain thereby releasing the remaining part of RseA with SigE still bound to it into the cytoplasm, where it will be completely degraded by the ClpXP or some other protease to finally release SigE

The *Bacillus subtilis* heat shock stimulon

B. subtilis is the model organism of the Gram-positive bacteria. Besides its physiology, regulation of sporulation and of competence have been intensively studied (Sonenshein et al. 2002). In 1990, we started to study the HSR in this microorganism. We cloned and sequenced the *dnaK* and the *groESL* operons and detected a repressor gene responsible for negative regulation of both operons (Schmidt et al. 1992; Wetzstein et al. 1992). Later, we could show that the GroESL chaperones are responsible for regulating the activity of the HrcA repressor (Mogk et al. 1997).

Its heat shock response network is composed of the two transcriptional repressors HrcA and CtsR, the alternative sigma factor SigB and the CssRS two-component system (Elsholz et al. 2010a; Hecker et al. 2007; Schumann 2003; Schumann et al. 2002). Some additional genes have been shown to be heat-inducible, but they could not be attributed to one of these four heat shock regulons. Therefore, they must be members of one or more additional heat shock regulons. Two examples are the genes *htpG* coding for a chaperone (Schulz et al. 1997), and *ftsH* encoding a membrane-anchored metalloprotease (Deuerling et al. 1995).

The HrcA regulon

The HrcA regulon consists of two operons, the heptacistronic *dnaK* (*hrcA-dnaK-dnaJ-grpE-orf35-orf28-orf50*) and the bicistronic *groESL* operon (Homuth et al. 1997; Li and Wong 1992; Schmidt et al. 1992). Both operons are preceded

by a SigA promoter (the *sigA* gene codes for the housekeeping SigA) and followed by a perfect inverted repeat of 9 bp separated by a 9 bp spacer (TTAGCACTC-N9-GAGTGCTAA) designated as CIRCE (controlling inverted repeat of chaperone expression) (Zuber and Schumann 1994). Introduction of several point mutations into the CIRCE element preceding the *dnaK* operon led to high constitutive expression of this operon, but not of the *groESL* operon suggesting that both operons are controlled by a transcriptional repressor (Zuber and Schumann 1994). That the *hrcA* gene codes for this repressor was demonstrated by analysis of an *hrcA* knockout. This knockout mutant caused a high constitutive expression of both operons (Schulz and Schumann 1996).

How is the activity of the HrcA repressor modulated at ambient temperatures (Fig. 3)? We assume that HrcA belongs to the group of protein thermosensors. Immediately after a heat shock, it changes its conformation followed by dissociation from its two CIRCE elements. To become reactivated, it needs to interact with the GroESL chaperone (Mogk et al. 1997). As described above, immediately after a heat shock, the GroESL chaperone is titrated by the denatured proteins. The more denatured proteins are refolded or degraded, the more GroESL chaperones become available to refold HrcA thereby converting it into its active form.

Recently, it has been shown in the case of the HrcA repressor of *Helicobacter pylori* that the chaperone GroESL is able to restore the HrcA binding activity lost upon heat challenge (Roncarati et al. 2014).

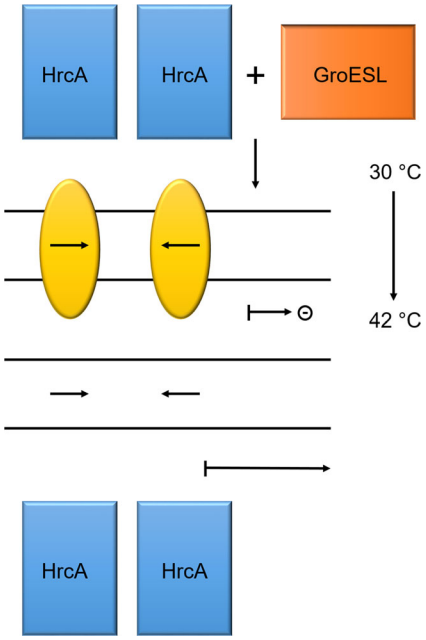

Fig. 3 Regulation of the HrcA regulon of *B. subtilis*. The HrcA repressor needs GroESL for correct folding allowing binding to the perfect inverted repeats, the CIRCE elements at low temperature. After a heat shock, HrcA will undergo a conformational change, dissociate from its inverted repeats and stay in an inactive conformation as long as GroESL is interacting with the denatured proteins

The SigB regulon

Sigma-B (SigB) has been identified as the first alternative sigma factor of bacteria (Haldenwang and Losick 1979). SigB recognizes a particular promoter structure (Haldenwang 1995) and is responsible for the transcription of about 200 protein-coding general stress genes (5 % of the genome) (Hecker et al. 2007; Nannapaneni et al. 2012) and 136 putative regulatory RNAs (Nicolas et al. 2012). Expression of the whole regulon is primarily regulated by controlling the activity of SigB by a complex signal transduction pathway called partner switching mechanism involving positive- and negative-feedback loops placing the involved regulatory genes under autoregulation (Hecker et al. 2007).

The basis of regulation of SigB is a partner-switching cascade utilizing phosphorylation to alter binding partner-specificity of the proteins involved in activation of SigB (Fig. 4). Under physiological conditions, SigB is held in an inactive state by the antisigma factor RsbW (regulation of SigB). Besides binding to SigB, RsbW acts as a kinase and phosphorylates the anti-antisigma factor RsbV thus keeping it in an inactive state. Two different stress regimens can lead to the dephosphorylation of RsbV: environmental or energy stress. Both stresses result in the dephosphorylation of the anti-antisigma factor RsbV by one of two different

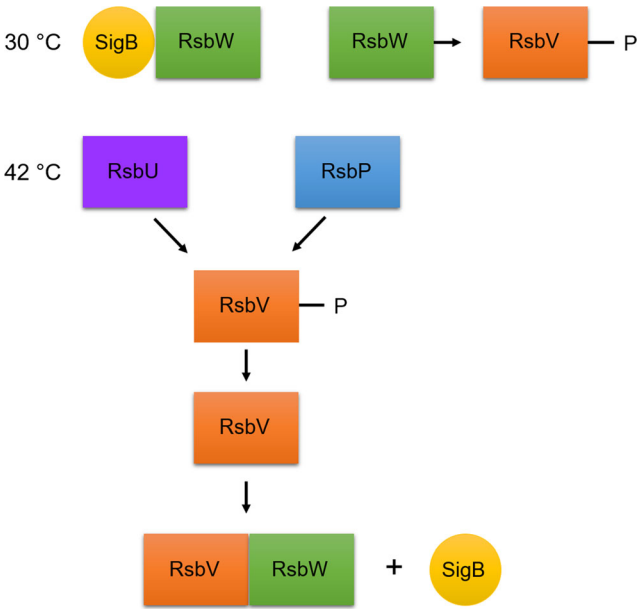

Fig. 4 Regulation of the SigB regulon of *B. subtilis*. At 30 °C, SigB is bound by the RsbW antisigma factor. The kinase activity of RsbW is used to phosphorylate the anti-antisigma factor RsbV to keep it inactive. After environmental stress such as a heat shock, the RsbT phosphatase will become activated which in turn will dephosphorylate RsbV. The anti-antisigma factor attacks the RsbW-SigB complex to release SigB. If cells are exposed to energy stress, the RsbP phosphatase will be activated, etc.

phosphatases depending on the stress regimen. Subsequently, RsbV sequesters RsbW and thus allows SigB to bind to the RNA polymerase core enzyme.

As mentioned above, two different kind of stresses will lead to the activation of SigB. Energy stress affects the RsbQ-RsbP signaling pathway sensing glucose, oxygen, and phosphate starvation and the exposure to agents such as NO, azide, and mycophenolic acid (Hecker et al. 2007). It has been suggested that the RsbQ α/β hydrolase provides a small molecule, which activates the phosphatase RsbP (Nadezhdin et al. 2011).

Environmental stress such as heat shock, salt, acid and ethanol stress transfer their signals via a pathway involving at least ten proteins forming a supramolecular complex known as the stressosome (Marles-Wright and Lewis 2007) to the RsbU phosphatase. This 1.8 MDa stressosome is composed of different types of proteins: one or more members of the partially redundant RsbR co-antagonist family (RsbRA, RB, RC and RD), the RsbS antagonist, and the RsbT serine-threonine kinase (Marles-Wright and Lewis 2007). The four RsbR proteins have different N-terminal non-heme globin domains and conserved C-terminal STAS (sulfate transporter/antisigma factor antagonist) domains, and the smaller RsbS protein is comprised only of one STAS domain (Marles-Wright and Lewis 2007). Twenty molecules of RsbR and ten RsbS dimers multimerize via these STAS domains to form the core of the stressosome, and the RsbT kinase binds to the surface of this structure to the RsbS antagonist. The N-terminal domains of the RsbR dimers are assured to provide sensory input.

If stressosomes sense the appropriate signal, the RsbT kinase is released, which subsequently binds and activates the RsbU environmental phosphatase (Chen et al. 2003; Delumeau et al. 2004). RsbU in turn dephosphorylates the anti-antisigma factor RsbV which attacks the RsbW-SigB complex leading to the release of SigB (Fig. 4).

The CtsR regulon

The CtsR (class three stress repressor) regulon consists of three operons, the tetracistronic *clpC* operon (*ctsR-mcsA-mcsB-clpC*) and the two monocistronic *clpP* and *clpE* operons (Krüger et al. 1996, 1997). CtsR is a classical winged helix-turn-helix (HTH) dimeric DNA-binding protein that recognizes the highly conserved heptanucleotide direct repeat sequence A/GGTCAAANANA/GGTCAA (called CtsR box) overlapping with the transcription initiation site or the −35 and −10 sequences of the promoter of the corresponding operons (Derré et al. 1999b). While the HTH domain binds in the major groove of the DNA, the β-hairpin wing with its tetra-glycine loop contacts the minor groove. CtsR can intrinsically sense the change in temperature via a glycine-rich loop (RGGGGY) present near the DNA-binding winged HTH domain (Derré et al. 2000). Replacement of the glycine residue at position 64 with a more rigid residue greatly impairs the thermosensor ability of CtsR both in vivo and in vitro (Elsholz et al. 2011). Furthermore, the stability of the CtsR repressor is also regulated by ClpE, which functions as a chaperone and is involved in overall protein quality control in the cytoplasm (Fig. 5) (Miethke et al. 2006). The McsA (modulators of CtsR activity) protein contains a zinc-finger motif and stabilizes CtsR under physiological conditions. In its absence, CtsR is no longer active. The McsB protein harbors two activities. It contains an arginine kinase activity (Fuhrmann et al. 2009) and acts as an adaptor protein that delivers phosphorylated CtsR to the ClpCP complex for degradation into small peptides (Kirstein et al. 2007). Under physiological conditions, ClpC inhibits the kinase activity of McsB by sequestering of the protein (Fig. 5) (Elsholz et al. 2011).

Immediately after a heat shock, CtsR is inactivated by a three-step process. Due to its intrinsic heat-sensor ability, it will change its conformation and dissociate from its operators. The β-hairpin loop was identified as the region responsible for thermosensing of CtsR (Elsholz et al. 2010b). Second, McsB is released from ClpC, and phosphorylates CtsR at several conserved arginine residues thereby preventing CtsR from

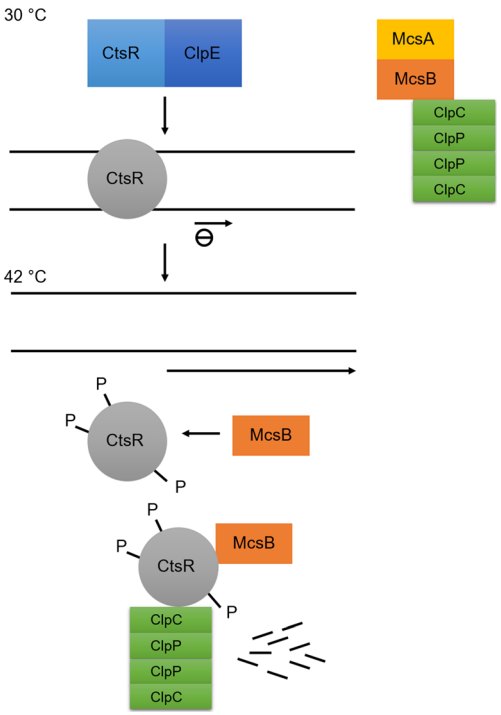

Fig. 5 Regulation of the CtsR regulon. At 30 °C, the CtsR repressor is kept in its active conformation by the ClpE chaperone and binds to its operators. The McsB kinase is kept inactive by forming a complex with McsA, and this complex is anchored on the ClpCP proteasome. After a heat shock, CtsR spontaneously dissociates from its operators, becomes first phosphorylated by McsB to provide return into its active conformation. Next, McsB serves as an adaptor protein and guides CtsR-P to the ClpCP proteasome where it will be degraded to peptides

rebinding to its operators (Elsholz et al. 2011; Fuhrmann et al. 2009) (Kirstein et al. 2005; Krüger et al. 2001). Third, the auto-phosphorylated McsB adaptor protein delivers unbound phosphorylated CtsR-P to the ClpCP protease for degradation (Fig. 5) (Kirstein et al. 2007).

ClpE is a very short-lived protein that is mainly degraded by ClpCP (Gerth et al. 2004), and *clpE* mutants do not exhibit a severe phenotype (Derré et al. 1999a). It is involved in disaggregation of heat-denatured proteins. ClpEP dominates CtsR degradation at the early stage of heat stress ensuring efficient derepression of the genes of the CtsR regulon. Furthermore, ClpE is essential for rerepression during the adaptation period (Miethke et al. 2006).

The CssRS regulon

The CssRS (control of secretion stress regulator and sensor) two-component system is the fourth mechanism by which *B. subtilis* responds to heat and to secretion stress (Westers et al. 2006). CssS is a typical sensor kinase with two transmembrane domains and is induced in response to secretion and heat stress (Darmon et al. 2002; Hyyryläinen et al. 2001; Krobitsch and Lindquist 2000; Noone et al. 2000, 2012). After autophosphorylation, the kinase transfers the phosphoryl group to the response regulator CssR which in turn activates transcription of the two monocistronic genes *htrA* and *htrB*, both coding for chaperone-proteases that either refold or degrade misfolded proteins within the cell envelope. Both proteins have single transmembrane domains and are located at the outer surface of the plasma membrane, although HtrA also accumulates in the culture medium in a truncated from (Antelmann et al. 2003). Details how the CssS sensor kinase becomes activated are unknown so far. This protein contains two transmembrane domains flanking an extracellular loop of 137 amino acids proposed to encode a PAS domain (Chang et al. 2010). It has been shown that this loop is involved in signal perception. As already mentioned the CssRS stimulon consists of three transcriptional units, the bicistronic *cssRS* operon and the two monocistronic *htrA* and *htrB* operons.

The *Streptomyces* heat shock stimulon

Streptomyces spp. are Gram-positive, GC-rich filamentous soil bacteria that belong to the actinomycetales order and possess an about 8 kb linear chromosome (Hopwood 1999). These bacteria constitute an economically important group producing many naturally isolated antibiotics and other therapeutic products. The *Streptomyces* heat shock stimulon consists of three different regulons. All three are under the negative control by three different transcriptional repressors designated as HrcA, RheA and HspR. They all have been studied extensively in *S. albus*.

The HrcA regulon

The HrcA repressor negatively regulates three operons in *S. albus*, the bicistronic *hrcA-dnaJ2* and *groES-groEL1* operons and the monocistronic *groEL2* operon (Grandvalet et al. 1998). All three operons are preceeded by one or two copies of the CIRCE element. Disruption of *hrcA* increased transcription of all three operons. It is assumed that the activity of the HrcA repressor is regulated in a similar way to that proposed for HrcA of *B. subtilis* (Mogk et al. 1997).

The RheA regulon

The RheA regulon of *S. albus* consists so far of two adjacent monocistronic operons. One codes for the small 18 KDa HSP18 protein, and the other for the transcriptional repressor RheA (repressor of hsp 18) which controls transcription of the *hsp18* gene and its nearby own gene negatively (Guglielmi et al. 1991). The *rheA* gene is located 150 bp from, and in opposite orientation of *hsp18*. A *rheA* knockout produces a large amount of the *hsp18* mRNA at physiological temperature (30 °C), but the HSP18 protein is present only after a heat shock. Disruption mutagenesis of *hsp18* exhibited that the HSP18 protein is not essential for growth between 30 and 42 °C but is involved in thermotolerance at extreme temperatures (Servant and Mazodier 1995). These data suggest two independent regulation mechanisms.

The temperature-sensing autorepressor RheA belongs to the group of protein thermosensors. At low temperature, it is present in its active form and regulates its own transcription and that of *hsp18*. After a heat shock, RheA unfolds and adopts a configuration unable to bind its operators. In the absence of the *rheA* gene, the *hsp18* mRNA is present, but is not translated suggesting that the *hsp18* gene is subject to posttranscriptional regulation (Servant and Mazodier 1996). Most probably, the mRNA forms a secondary structure where the 5′ untranslated region folds back and base-pairs with the ribosome-binding site thereby block translation as has been described for the *rpoH* mRNA.

The HspR regulon

The nucleotide sequence of the *dnaK* operon of *S. coelicolor* A3(2) revealed the four genes *dnaK-grpE-dnaJ-hspR* (heat shock protein R), where the HspR protein specifically interacts with three partially related inverted repeat sequences centered at −75, −49, and +4, respectively, relative to the

transcription start site of the operon (Bucca et al. 1995). A similar operon has been identified in *S. albus*, and disruption of the *hspR* gene resulted in a constitutive strong expression of the *dnaK* operon and of the monocistronic *clpB* gene (Grandvalet et al. 1999), indicating that the HspR acts as a transcriptional repressor (Grandvalet et al. 1997). The *clpB* gene is also preceded by an inverted repeat comparable to that found on three copies upstream of the *dnaK* operon, and the consensus sequence of this motif is CTTGAGT-N_7-ACTCAAG. The HspR binding site was designated as HAIR (HspR-associated inverted repeat). In *S. lividans*, the *lon* gene is also negatively regulated by the HspR/HAIR repressor/operator system (Sobczyk et al. 2002).

HspR specifically requires the DnaK protein to interact with the HAIR elements. In gel retardation experiments, the protein-DNA complex can be supershifted by anti-DnaK monoclonal antibodies, demonstrating that DnaK forms an integral component of the complex (Bucca et al. 2000). Since DnaK copurifies with HspR, it was concluded that DnaK functions as a transcriptional co-repressor by binding to HspR at its operator site. Thereby, the DnaK-HspR system represents a novel example of a feedback regulation of gene expression by a molecular chaperone. In the absence of denatured proteins, DnaK forms a complex with HspR able to bind to the HAIR elements. After a heat shock when the amount of denatured proteins increase suddenly, DnaK is titrated by these proteins and is not able to bind to HspR leading to the induction of the HSGs of the HspR regulon.

Concluding remarks

Regulation of the HSR has been studied in *E. coli* first starting in 1975 (Cooper and Ruettinger 1975). Meanwhile, the HSR has been investigated in numerous bacterial species, and two types of regulators have been identified so far: alternative sigma factors and transcriptional repressors. Their activity is regulated either by a conformational change occurring immediately after a heat shock or indirectly by degradation of the protein sequestering the heat shock regulator. In both cases, expression of the HSGs starts immediately after a temperature upshift to prevent formation of protein aggregates which could lead to cell death. It will be interesting to know whether additional types of regulators will be identified during the coming years.

Why do bacteria organize their HSGs into two and more regulons? It has been shown that many HSGs are not only induced by a heat shock, but by additional stressful situations including salt, acid and ethanol stress and starvation for phosphate to mention some prominent stress regimens. Therefore, organization of the HSGs into different regulons allows cells to differentially induce those genes needed during a specific stress regimen.

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