The heat shock response of *Escherichia coli*

Florence Arsène, Toshifumi Tomoyasu, Bernd Bukau

Abstract

A large variety of stress conditions including physicochemical factors induce the synthesis of more than 20 heat shock proteins (HSPs). In *E. coli*, the heat shock response to temperature upshift from 30 to 42°C consists of the rapid induction of these HSPs, followed by an adaptation period where the rate of HSP synthesis decreases to reach a new steady-state level. Major HSPs are molecular chaperones, including DnaK, DnaJ and GrpE, and GroEL and GroES, and proteases. They constitute the two major chaperone systems of *E. coli* (15–20% of total protein at 46°C). They are important for cell survival, since they play a role in preventing aggregation and refolding proteins. The *E. coli* heat shock response is positively controlled at the transcriptional level by the product of the *rpoH* gene, the heat shock promoter-specific σ^32^ subunit of RNA polymerase. Because of its rapid turn-over, the cellular concentration of σ^32^ is very low under steady-state conditions (10–30 copies/cell at 30°C) and is limiting for heat shock gene transcription. The heat shock response is induced as a consequence of a rapid increase in σ^32^ levels and stimulation of σ^32^ activity. The shut off of the response occurs as a consequence of declining σ^32^ levels and inhibition of σ^32^ activity. Stress-dependent changes in heat shock gene expression are mediated by the antagonistic action of σ^32^ and negative modulators which act upon σ^32^. These modulators are the DnaK chaperone system which inactivate σ^32^ by direct association and mediate its degradation by proteases. Degradation of σ^32^ is mediated mainly by FtsH (HfB), an ATP-dependent metallo-protease associated with the inner membrane. There is increasing evidence that the sequestration of the DnaK chaperone system through binding to misfolded proteins is a direct determinant of the modulation of the heat shock genes expression. A central open question is the identity of the binding sites within σ^32^ for DnaK, DnaJ, FtsH and the RNA polymerase, and the functional interplay between these sites. We have studied the role of two distinct regions of σ^32^ in its activity and stability control: region C and the C-terminal part. Both regions are involved in RNA polymerase binding.

Keywords: HSPs; Heat shock stress; Sigma-32

1. The heat shock response

The conserved heat shock (stress) response allows cells to adapt to environmental and metabolic changes and to survive stress conditions (Bukau, 1993, 1999; Georgopoulos et al., 1994; Connolly et al., 1999). It is induced by a large variety of stress conditions including physicochemical factors such as heat shock, metabolically harmful substances and complex metabolic processes. In *E. coli*, the heat
shock response to temperature upshift from 30 to 42°C consists in the rapid, up to 15-fold, induction of synthesis of more than 20 heat shock proteins (HSPs), followed by an adaptation period where the rate of HSP synthesis decreases to reach a new steady-state level (Bukau, 1993; Georgopoulos et al., 1994). Major HSPs are molecular chaperones and proteases, including the DnaK and GroE chaperone systems formed by DnaK, DnaJ and GrpE, and GroEL and GroES, respectively. They constitute the two major chaperone systems of *E. coli* as judged by their abundance (15–20% of total protein at 46°C) and importance for cell survival (Georgopoulos et al., 1994; Gross, 1996).

2. Positive regulation by σ^{32}

The *E. coli* heat shock response is positively controlled at the transcriptional level by the product of the rpoH gene, the heat shock promoter-specific σ^{32} subunit of RNA polymerase (Gross, 1996; Connolly et al., 1999) (Fig. 1). σ^{32} is required for induced expression as well as uninduced basal expression of heat shock genes. Some heat shock genes including the groES groEL operon have additional σ^{70}-dependent promoters contributing to the basal levels of their expression. The cellular concentration of σ^{32} is very low under steady-state conditions (10–30 copies/cell at 30°C (Craig and Gross, 1991)) and is limiting for heat shock gene transcription. The heat shock response is induced as a consequence of a rapid increase in σ^{32} levels and, possibly, stimulation of σ^{32} activity. The shut off of the response occurs as a consequence of a decline in σ^{32} levels and inhibition of σ^{32} activity. This mode of regulation of σ^{32} is fast and therefore allows *E. coli* cells to respond rapidly to sudden stress (Connolly et al., 1999).

2.1. Regulation of synthesis of σ^{32}

The first mechanism of regulation occurs at the level of rpoH transcription. This mechanism has a minor effect on σ^{32} synthesis. However, the regulatory region of rpoH is rather complex and contains at least four promoters, three σ^{70}-dependent promoters and one promoter requiring the σ^{32} factor (Fig. 1). This σ factor is responsible of expression of some genes at high temperature, some of them are periplasmic-folding catalysts (FkpA, OmpK) or proteases (for example degP) (Missiakas and Raina, 1998). The usage of each promoter seems to change according to the temperature (Bukau, 1993; Georgopoulos et al., 1994; Connolly et al., 1999). Moreover, upstream the P5 promoter (σ^{70}-dependent), a functional binding site for CRP and CytR exists, whereas the P3 and P4 promoters are negatively controlled by DnaA. These observations suggest a regulation of rpoH transcription in response to various metabolisms and environmental conditions (Kallipolitis and Valentin-Hansen, 1998).

The second mechanism mediating stress-dependent changes in σ^{32} levels affects the translation of rpoH mRNA (Connolly et al., 1999). rpoH translation is repressed at steady-state conditions and is rapidly derepressed upon shift of the cells from 30 to 42°C. At 2–4 min after temperature upshift, a 12-fold higher translation level compared to the pre-heat
shock condition is reached. Then, translation becomes increasingly repressed during the shut-off phase of the heat shock response to reach a new steady-state level. *rpoH* translation control is mediated through distinct mechanisms involving three *cis*-acting elements of the *rpoH* coding sequence, termed regions A, B and C (Nagai et al., 1991a,b, 1994). Region A is a positive regulatory element comprising the initiation codon and the downstream 20 nucleotides. This region is complementary to the 3’ region of the 16S RNA and probably acts as a translational enhancer (Morita et al., 1999). Region B is a negative regulatory element located within nucleotides 110 and 247. Extensive base pairing between regions A and B has been predicted and was further substantiated by mutational analysis (Morita et al., 1999). The formation of this secondary structure is hypothesized to mediate repression of *rpoH* translation at steady-state conditions, by preventing translation initiation, because of the unaccessibility of the Shine–Dalgarno sequence and initiation codon. The thermal induction of translation results from partial melting of the mRNA secondary structure due to the increased temperature, enhancing the entry of ribosome and translational initiation (Morita et al., 1999). It seems that DnaK, DnaJ, or other cellular components, are not required for this induction. Region C is a negative regulatory element located within nucleotides 364–433 of the *rpoH* coding sequence, involved in repression of *rpoH* translation during the shut-off phase of the heat shock response. It was suggested that region C acts at the protein level to mediate translational repression of *rpoH* (Nagai et al., 1994).

2.2. Regulation of stability and activity of σ^32

A second mechanism mediating stress-dependent changes in σ^32 levels affects stability of σ^32 (Fig. 1) (Gross, 1996; Connolly et al., 1999). During steady-state growth, σ^32 has an extremely short half-life of less than 1 min. Upon temperature upshift from 30 to 42°C, σ^32 becomes transiently stabilized at least 8-fold until the beginning of the shut-off phase of the heat shock response. One protease responsible for σ^32 degradation is the ATP-dependent metalloprotease, FtsH. FtsH is an integral cytoplasmic membrane protein, with the active site located in a cytosolic domain which shares high sequence homology with the conserved family of AAA proteins. It was proposed that σ^32 can be substrate for Lon (La), ClpAP and HslVU (Kanemori et al., 1997). However, since the lack of FtsH stabilizes σ^32 completely in vivo (half-life, 2 h), the degradation of this factor is probably mainly achieved by FtsH (Tatsuta et al., 1998).

The last level of negative regulation of σ^32 involves its activity. Genetic evidence indicates that the DnaK chaperone system mediates the stress-dependent inactivation and, perhaps indirectly, degradation of σ^32 and repression of *rpoH* translation during the shut-off phase of the heat shock response (Gross, 1996; Tatsuta et al., 1998; Connolly et al., 1999).

3. Negative modulation and stress sensing by the DnaK chaperone system

Recently, the role of DnaK and DnaJ in regulation of σ^32 activity and stability was clarified. In contrast to what observed in *ΔftsH* cells, in a ΔdnaK mutant, σ^32 also accumulates but HSP synthesis is also strongly increased. Overproducing of DnaK and DnaJ in the ΔftsH strain, does not lead to detectable changes in the level of σ^32; however, the ability of σ^32 to activate transcription is reduced, suggesting that DnaK inactivates σ^32 in ΔftsH (Tatsuta et al., 1998). Therefore, it was proposed that the activity control of σ^32 required DnaK and DnaJ and is likely to be important for the rapid establishment of appropriate HSP levels after recovery of cells from stress treatment, and the maintenance of homeostasis of heat shock gene expression. DnaK and DnaJ, or its homologue CbpA, could be involved in the FtsH-dependent degradation of σ^32, since in a dnaK mutant, or in the double mutant ΔdnaJ ΔcbpA, the half-life of σ^32 is increased (Tatsuta et al., 1998). However, this role remains unclear since the chaperones have a negative effect in vitro on FtsH degradation (Blaszczak et al., 1999). It is actually supposed that the chaperones affect the stability of σ^32 in vivo, by preventing its binding to the RNA-polymerase, and therefore increasing indirectly σ^32 degradation (Blaszczak et al., 1999).

These modulatory activities result at least in part from reversible association of the DnaK chaperone
system with $\sigma^{32}$ (Gross, 1996; Bukau, 1999; Connolly et al., 1999). DnaK and the co-chaperone DnaJ bind independently to free $\sigma^{32}$. Further analyses led to the proposal of an ATP-controlled $\sigma^{32}$ binding/release cycle in which DnaJ mediates the efficient binding of DnaK to $\sigma^{32}$ in presence of ATP (Laufen et al., 1999). This cycle of $\sigma^{32}$ binding and release is similar to the cycle proposed to operate in other chaperone activities of the DnaK system (Bukau, 1999), implying that $\sigma^{32}$ acts as regular substrate for the DnaK chaperone system. DnaK and DnaJ cooperatively inhibit $\sigma^{32}$ activity in heat shock gene transcription, and GrpE partially reverses this inhibition. This reversible inhibition of $\sigma^{32}$ activity and perhaps stability through transient association of DnaK and DnaJ is proposed to constitute a homeostatic $\sigma^{32}$ activity control system operating in vivo (Fig. 2).

Homeostatic regulation models (Craig and Gross, 1991; Bukau, 1993) propose that induction of a heat shock response after stress treatment relies on the sequestration of the DnaK-system through binding to misfolded proteins accumulating during stress (Fig. 2). HSP-mediated refolding or degradation of misfolded proteins ameliorates the inducing signal and frees the DnaK system to shut off the heat shock response. A common feature of inducers of the *E. coli* heat shock response is their potential to generate misfolded proteins. For instance, the production of heterologous proteins and mutant proteins, and of thermolabile proteins such as firefly luciferase at nonpermissive temperature induces a heat shock response in *E. coli*. The DnaK system is active in refolding or degradation of misfolded proteins, including firefly luciferase inactivated by a heat shock to 42°C (Craig and Gross, 1991; Georgopoulos et al., 1994; Tomoyasu et al., 1998). To further verify the proposed chaperone sequestration models it has been shown, by modulating the expression of the DnaK and DnaJ proteins, that the DnaK chaperone system is limiting for heat shock gene regulation in vivo. Small increases in the chaperone levels resulted in decreased level and activity of $\sigma^{32}$ and a faster shut-off of the heat shock response was observed (Tomoyasu et al., 1998). Finally, the regulation also includes competition with $\sigma^{70}$. The housekeeping $\sigma$ factor is more abundant at 30°C and therefore prevents the binding of $\sigma^{32}$ to the RNA-polymerase. $\sigma^{70}$ tends to aggregate at high temperature leading to an increased ability of $\sigma^{32}$ to bind the RNA-polymerase (Blaszczak et al., 1995). It should be mentioned that sequestration of other regulatory components, such as the FtsH protease, might also contribute to the homeostatic control of heat shock gene expression in *E. coli*. However, the DnaK and DnaJ constitute the primary stress-sensing and transducing system of the *E. coli* heat shock response (Tomoyasu et al., 1998).

4. Functional dissection of $\sigma^{32}$ protein

A molecular understanding of this regulation requires the identification of the chaperone, FtsH and RNA polymerase binding sites within $\sigma^{32}$, and an understanding of the functional interplay between these sites. The structure of $\sigma^{32}$ is shown schematically in Fig. 3. Sigma factors are composed of four regions divided additionally into sub-regions. Characterization of mutants of $\sigma^{70}$ or $\sigma^{32}$ have led to the proposal that the most conserved regions 2.1 and 2.2 are involved in RNAP binding, but it seems that other parts of $\sigma$ factors are probably also involved in this interaction (Lesley and Burgess, 1989; Zhou et al., 1992; Severinova et al., 1996; Joo et al., 1997, 1998). Two regions of $\sigma^{32}$ were recently more studied: region C and the C-terminal part.

4.1. Role of region C

Region C corresponds to a segment of nine amino acids between residues 133 and 140 of $\sigma^{32}$ (RKLFFNLR), located between the conserved regions 2 and 3 of $\sigma^{32}$ (Fig. 3), and is almost entirely conserved within $\sigma^{32}$ homologous but not within
other σ factors and therefore termed the RpoH box (Nakahigashi et al., 1995). This specific conservation strongly suggests a regulatory function of the RpoH box. This region C was characterized by analyzing the in vivo half-life of protein fusions between N-terminal fragments of σ^32 and β-galactosidase. The stability of such fusions strongly increased when a stretch of 12 residues (R122–Q144), is deleted or replaced by another reading frame (Nagai et al., 1994).

Using a peptide library approach, a consensus for a DnaK binding site was proposed by Rudiger et al. It is composed of four to five hydrophobic residues flanked by positively charged amino acids (Rüdiger et al., 1997). By screening of a σ^32-derived peptide library for chaperone binding sites, and using peptides corresponding to region C, two high-affinity binding sites were shown to be present in the σ^32 region C, indicating a regulatory importance of the RpoH box (McCarty et al., 1996). Based on these results, it was proposed that binding of DnaK to region C is central to a conserved chaperone-dependent regulatory mechanism, allowing to transduce information about the stress status of the cell to the heat shock gene transcription machinery. Peptides corresponding to region C are able to bind DnaK and are substrate for FtsH in vitro. We first define the mutation to affect DnaK binding using these peptides (Arsène et al., 1999). Replacement of a single hydrophobic residue in each DnaK binding site by negatively charged residues (I123D; F137E) strongly decreased the binding of these peptides to DnaK and degradation by FtsH. However, introduction of these and additional region C alterations into the σ^32 protein did not affect σ^32 degradation in vivo and in vitro and DnaK binding in vitro. Thus, in contrast to the expectations, no evidence for a role of region C in chaperone binding and degradation by FtsH was found (Arsène et al., 1999). Instead, region C was shown to be involved in high affinity binding of σ^32 to RNA polymerase, thereby providing to σ^32 a competitive advantage over other σ factors (especially σ^70) in association to RNA polymerase, and contributing indirectly to the rapidity of the heat shock response (Joo et al., 1998; Arsène et al., 1999).

4.2. Role of the C-terminal part

The second region of interest was the C-terminal part of σ^32. Herman et al. (1998) have suggested that FtsH protease can recognize C-terminal protease recognition signal (SsrA) for degradation of protein as it was proposed for other protease such as Clp and Tsp proteases (Maurizi et al., 1999). Blaszcza et al. (1999) analyzed the effect of C-terminal deletions of

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**Fig. 3.** Schematized representation of σ^32. σ^32 is shown schematically with indicated locations of the conserved regions 1–4 and details of region C including the RpoH box. Sequences of two potential DnaK binding sites identified by peptide scanning are indicated.
15 or 20 amino acids and investigated the stability of these truncated proteins. They proposed that the C-terminal region of σ32 is important for FtsH recognition and degradation. We also constructed five-, 11-, 15- and 21-amino acid C-terminally truncated versions of σ32. Unfortunately, some of our data did not support the results of Blaszczak et al. (1999), since our constructs are degraded by FtsH and Lon proteases in vitro with the same kinetics as the wild type σ32 (unpublished result). The C-terminal deletions are still able to bind DnaK (Bblaszczak et al., 1999; our unpublished data) and all truncated proteins shared DnaK-dependent degradation in vivo (our unpublished result). The reasons for the contradiction in these results are still unclear. However, all C-terminal truncated proteins are affected in RNA-polymerase binding, suggesting that this part is important for σ32 activity (Blaszczak et al., 1999; our results).

In conclusion, neither region C nor the C-terminal part are directly required for DnaK binding. However, both regions are required to increase RNA-polymerase binding efficiency and, therefore, are indirectly important for the regulation of σ32 activity. The ability of region C to increase the affinity of σ32 for RNAP has physiological consequences. In fact, only 10–30 molecules of σ32 that exist in a cell growing at 30°C are sufficient to produce HSPs that account for at least 5% of total cytosolic protein (Gross, 1996; Connolly et al., 1999). This should be due to a higher affinity of σ32 to bind RNAP as compared to the other σ factors. Region C may provide competitive advantages of both, σ32 over other σ factors for RNAP binding, and RNAP over DnaK, DnaJ and FtsH for σ32 binding. Region C thereby increases the efficiency and speed by which the heat shock response is induced upon temperature upshift, and the efficiency of heat shock gene transcription at steady-state conditions. It is now important to characterize regions involved directly in the DnaK-mediated control, since it would complete the regulation model of the heat shock response in E. coli.

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