Regulation of heat-shock response in bacteria

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ABSTRACT

Bacterial heat-shock response is a global regulatory system required for effective adaptation to changes (stress) in the environment. Several of the important genes involved in this control, such as the genes coding for the chaperones GroE and DnaK (the bacterial homologues of Hsp60 and Hsp70) are localized in operons, with organization typical of the phylogenetic group. In *Escherichia coli,* where it has been studied initially, the expression of the heat-shock operon is transcriptionally controlled by the employment of the heat-shock transcription activator - factor σ32, that recognizes specific heat-shock promoters. Later studies indicated that in most bacteria the control of these heat-shock operons is more complex than in the γ-purple proteobacteria and involves several regulatory elements. One such control element is a repressor that regulates transcription of heat-shock genes by binding to a conserved regulatory inverted repeat (IR=CIRCE) located upstream to heat-shock operons. In addition, this IR determines the stability of the transcript, thus controlling the level of translation. Sequence analyses suggest that the IR-dependent control of heat-shock genes was the first control element and was lost during evolution in several phylogenetic groups, such as the γ-purple proteobacteria.

Introduction

The heat-shock response involves the induction of many proteins - called heat-shock proteins, or Hsp's - in response to elevation of temperature (37). The bacterial heat-shock response is not limited to changes in temperature and is a general stress response, as many of the heat-shock proteins are induced by other environmental changes, such as the addition of ethanol, heavy metals, high osmolarity, pollutants, starvation or interaction with eukaryotic hosts (3, 16, 34, 50, 51). The heat-shock proteins include chaperones and proteases that are presumably essential for overcoming changes that involve protein denaturation. Induction of this response improves thermotolerance, salt tolerance and tolerance to heavy metals (18, 24, 25, 38, 52). Moreover, in several bacterial species heat-shock proteins have been shown to play an important role in pathogenesis (5, 7, 22, 23, 26-29, 31, 41, 47) and survival within macrophages (2). Heat-shock proteins are also essential for stationary phase (34) and for bacterial differentiation in myxobacteria and in *Bacillus subtilis* (12, 53).

The heat-shock response controls the expression of more than 20 genes (9, 37) that code for chaperones, proteases and regulatory proteins. Two of these proteins, Hsp70 (the

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product of the bacterial *dnaK* gene), and the Hsp10+Hsp60 complex (products of the *groESL* operon), act as chaperones, are highly conserved (6, 15), and have been extensively studied in many organisms, including a large variety of bacterial species. The present review deals with the various bacterial strategies for regulating the heat-shock response.

Activation of specific heat-shock promoters by an alternative sigm factor (heat-shock sigma factor, or σ32)

In bacteria, the major control of the expression of heat-shock genes is transcriptional. In *Escherichia coli* the heat-shock response is controlled by a specific sigma factor that activates the transcription of heat-shock genes under the appropriate conditions. This heat-shock sigma factor (σ32) is coded by the *rpoH* gene and binds to specific heat-shock promoters located upstream of heat-shock genes (4, 10, 11, 30, 48). The expression of the *rpoH* gene is under complex regulation (21, 30, 36, 56), and under non heat-shock conditions its product is degraded by a specific protease, the product of the *hflB* (*ftsH*) gene (8, 13, 14, 17, 20, 21, 49). The consensus sequence of the heat-shock promoter has been identified upstream of many heat-shock genes, and no other control elements have been found.

Transcriptional activation of heat-shock genes by release of repression involving an inverted repeat (IR, CIRCE) and a repressor protein (product of the *hrcA* gene)

In low-G+C gram positive bacteria, such as *Bacillus subtilis*, the heat-shock genes are transcribed by the vegetative sigma factor (σ70), and heat-shock induction is mediated by the release of a repressor that under non-heat conditions is bound to an inverted repeat located at the upstream regulatory region of heat-shock operons. This inverted repeat (IR) - also called CIRCE (controlling IR of chaperone expression) - acts as the binding site for the repressor protein Orf39 (or OrfA, in *B. subtilis*), the product of the *hrcA* gene. Deletions of the IR result in constitutive expression of the operon (1, 16, 19, 33, 39, 40, 42, 45, 46, 54, 55, 57). The IR is highly conserved as demonstrated in Fig. 1.and has so far been found only in the upstream region of *groE*, *dnaK* and *dnaJ* operons.

Mycobacterium tuberculosis	cTAGCACTC-N9-GAGTGCTAg
Staphylococcus aureus	TTAGCACTC-N9-aAGTGCTAA
Bacillus subtilis	TTAGCACTC-N9-GAGTGCTAA
Chlamydia pneumoniae	TTAGCACT t-N9-GAGTGCTAA
Brucella abortus	TTAGCACTC-N9-GAGTGCTAA
Bordetella pertusis	TTAGCACTC-N9-GAGTGCTAA

Fig. 1. The conserved inverted repeat in heat-shock operons.

Transcriptional activation of heat-shock genes in α-purple proteobacteria

In bacteria belonging to the α subdivision of proteobacteria - *Agrobacterium tumefaciens*, *Bradyhizobium japonicum* and *Caulobacter crescentus*, the IR element is present in the *groE* operon or in one of the *groE* operons in bacteria that have more than one such operons (32, 46) but not in any of the *dnaK* operons. All the heat-shock operons of α-

purple proteobacteria contain a unique heat-shock promoter, presumbaly activated by a sigma 32-like transcription factor (43). The putative consensus heat-shock promoter is different from both the vegetative and the heat-shock promoter consensus sequences of *E. coli*. The unique heat-shock promoter is transcribed by a heat-shock activator, σ32-like factor that differs from its homologue of the γ-purple proteobacteria in several regulatory aspects, as well as in promoter recognition (35, 36, 43).

Experimental results indicate that the σ32-like transcription factor controls the heat-shock activation of the *dnaK* operons as well as the *groE* operons while the IR functions to repress transcription of the *groE* operon under non heat-shock conditions (45). This situation is different from the low G+C gram positive bacteria where the IR actually controls the heat-shock gene activation.

Post transcriptional control elements

The control mechanisms described above act at the level of transcription. Two additional regulatory elements of the heat-shock response are post-transcriptional. The first mechanism involves regulation of the stability of transcripts containing the IR in their upstream portion. In *B. subtilis* and in *A. tumefaciens* (45, 54), the half life of the *groEL* transcript increased two fold under non heat-shock conditions when deletions were introduced into the IR. The second post-transcriptional control was demonstrated in *A. tumefaciens* and involves specific cleavage of the *groESL* operon transcript (44), leading to differential expression of the two genes of the operon. This mRNA processing is temperature-dependent and is probably the first example of a controlled processing of transcripts in bacteria.

Phylogenetic aspects

The evolution of the various strategies for controlling the heat-shock response is an interesting problem. The phylogenetic analysis based on the non synonymous substitutions of *groE* and *dnaK* indicates that the control system involving the repressor-binding IR (CIRCE) is the ancient control mechanism. It was lost first in the *dnaK* operons, three times in Cyanobacteria, in Streptomyces and in the purple proteobacteria (α, β and γ subdivision). The next event resulted in the loss of the IR from the *groE* operon in one family - the γ2/γ3 subdivision of purple bacteria. The latter family is the only eubacterial family that controls the heat-shock response solely with an alternative sigma factor.

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