Regulation and Function of Heat-Inducible Genes in *Bacillus subtilis*

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In their natural environment, bacteria are frequently exposed to large and rapid temperature changes and, therefore, bacterial cells are equipped with a genetically regulated program to adapt to these temperature changes ensuring growth and survival. The best studied of these programs, the heat shock response, deals with the adaptation to a sudden increase in temperature, which is an important homeostatic mechanism that enables cells from animals, plants, and bacteria to survive a variety of environmental stresses besides heat stress (48, 49). It is characterized by the transiently increased synthesis of a number of proteins collectively designated heat shock proteins (hsps). The strong evolutionary conservation of the heat shock response argues that this response is beneficial for many kinds of cells and has evolved in order to detect and deal with the presence of unfolded, misfolded, damaged, or aggregated polypeptide chains. Furthermore, while some hsps have essential roles in the synthesis, transport, and folding of proteins and are often referred to as molecular chaperones (23), others are ATP-dependent proteases sometimes acting in concert with chaperones (24). In prokaryotes, the major hsps are encoded by single genes expressed at a basal level at all temperatures. Following a temperature upshift, the rates of expression of these genes abruptly accelerate and in general gradually decrease after 5 to 10 min. All bacterial heat shock genes are regulated at the level of transcription either by alternate sigma factors or by repressor proteins (for reviews, see references 27 and 92).

Two major groups of hsps constitute the heat stress stimulon of *Bacillus subtilis:* σ^B-dependent general stress proteins (also known as class II hsps and described in chapter 26), which are induced not only by heat but by a different set of stress and starvation stimuli conferring a nonspecific multiple stress resistance, and heat-specific stress proteins, which may exert a specific protective function against heat stress only. HrcA- and CtsR-dependent proteins (known as class I and class III hsps, respectively) belong to this heat-specific group, but ClpP and ClpC may have an intermediate role between heat-specific and general stress protein because of their double stress control.

CLASS I HEAT SHOCK GENES: THE HrcA REGULON

Discovery

Using gene probes, the *dnaK* and *groEL* genes were first identified and then cloned and sequenced (42, 68, 77, 87). The *groE* operon turned out to be bicistronic, consisting of *groES* and *groEL* (Fig. 1A). This genomic organization is highly conserved among eubacteria and has been described for all species studied so far; some may contain either an additional copy of *groEL*, such as *Streptomyces* spp. (45), and others may inherit up to five copies of the complete *groESL* operon, as described for *Bradyrhizobium japonicum* (18).

Sequencing of the region adjacent to *dnaK* revealed that it is flanked by *grpE* and *dnaJ*, and upstream of *grpE*, a fourth gene named *orf39* was identified coding for a protein of unknown function which turned out to encode the repressor of both the *dnaK* and *groESL* operons (see below). Sequencing of the whole region disclosed three additional *orfs* termed *orf35*, *orf28*, and *orf50* (29). Therefore, the complete *dnaK* operon is heptacistronic (Fig. 1B). While the function of *orf28* and *orf50* remains elusive, the deduced protein sequence of *orf35* exhibits significant homology to the ribosomal protein L11 methyltransferase encoded by *prmA* of *Escherichia coli* (81). The genomic organization of the *dnaK* operons of *Bacillus stearothermophilus*, *Clostridium acetobutylicum*, and *Staphylococcus aureus* is comparable to that of *B. subtilis* (5, 28, 56, 60).

The HrcA-CIRCE Regulation Mechanism

Both the *dnaK* and *groE* operons are preceded by a σ^A-type promoter that is used before and after heat stress (42, 68, 77, 87). Since σ^A is the major sigma factor produced constitutively, some other mechanism(s) must mediate the heat-inducible expression of these two operons. The first hint toward the elucidation of the regulation mechanism was the observation of a perfect inverted repeat of a length of 9 bp separated by a 9-bp spacer, located in both operons between the transcriptional and translational start sites. Since this

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