CONTROL ELEMENTS IN THE REGULATION OF BACTERIAL HEAT SHOCK RESPONSE

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The heat shock response is a global regulatory network found in all living cells. It involves the induction of many proteins—called heat shock proteins, or Hsps—in response to elevation of temperature (56). Many of the heat shock proteins, such as chaperones and proteases, are important for overcoming changes that involve protein denaturation. The same proteins are also induced by other environmental changes, such as the addition of ethanol, heavy metals, high osmolarity, pollutants, starvation, or interaction with eukaryotic hosts (5, 26, 51, 76, 77). Therefore, the heat shock response can be considered a general stress response. In bacteria, the heat shock response is essential for adaptation to elevated temperatures and to stressful environmental conditions. Induction of this response improves thermotolerance, salt tolerance, and tolerance to heavy metals (29, 37, 38, 58, 78). Moreover, in several bacterial species, heat shock proteins have been shown to play an important role in pathogenesis (31, 36, 39, 44, 66, 67). For example, virulence of *Listeria monocytogenes* involves the heat shock protein lysteriolysin (7, 32, 35, 47, 73), and heat shock proteins are required for binding of *Salmonella typhimurium* to mucosal cells (19) and for survival within macrophages (3). Heat shock response was also implicated in pathogenesis of *Helicobacter pylori* (17), *Mycobacterium leprae*, *Mycobacterium tuberculosis* (48), *Legionella pneumophila* (21, 45), *Chlamydia trachomatis* (9) and *Brucella abortus* (18). Recent results indicated that heat shock proteins are also essential for stationary phase (51) and for differentiation of myxobacteria and *Bacillus subtilis* (16, 80). These findings indicate that the heat shock response is a central control system that is vital for all aspects of bacterial life. Moreover, the heat shock response is critical for bacterial adaptation to changes in the environment, whether as free living organisms or in association with eukaryotic hosts, and is therefore one of the major links between microbial ecology and microbial pathogenesis.

The heat shock response controls the expression of more than 15 genes, possibly as many as 26 genes (12, 56), that code for chaperones, proteases, and regulatory proteins. The induced proteins are similar in all organisms, and several of them are highly conserved in evolution. Two of these proteins, Hsp70 (the product of the bacterial *dnaK* gene) and the

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Hsp10+Hsp60 complex (products of the *groESL* operon), show about 40% homology in amino acids from bacteria to mammals (8, 25). These proteins act as chaperones, maintaining the correct folding of cellular proteins. Because these chaperone proteins are physiologically important, are produced in very high levels under all conditions, and constitute major bacterial antigens, they have been extensively studied in many organisms, including a large number of bacterial species. Therefore, more data are available about the *groE* and *dnaK* genes and on the Hsp60 and Hsp70 proteins than about any other heat shock gene or protein, and the models concerning the heat shock response and its regulation are largely based on these data. The present review is also based mainly on results obtained from studies of the genes and products of the *groE* and *dnaK* operons, the major shock operons. Our understanding of the heat shock response and its control still lacks experimental results from other heat shock genes and operons.

In bacteria, an additional regulatory system exists that is activated by high temperatures. The best-studied gene activated by this system is *htrA*, whose product is essential for bacterial growth only at elevated temperatures (41, 42, 57). This system is activated by σ^E, a second heat shock sigma factor encoded by the *rpoE* gene (20, 28, 59, 62). This gene is also important for pathogenesis and was shown to control mucoidy in cystic fibrosis isolates of *Pseudomonas aeruginosa* (66). This system is much more limited in the number of genes that are involved and the stresses it responds to. The two heat-induced systems are connected in more than one way.

TRANSCRIPTIONAL ACTIVATION OF SPECIFIC HEAT-SHOCK PROMOTERS BY AN ALTERNATIVE SIGMA FACTOR (HEAT-SHOCK SIGMA FACTOR, RpoH, OR σ^{32})

The first and most extensive studies on bacterial heat shock response were performed in *Escherichia coli*. The heat-shock genes in *E. coli* K-12 have specific heat shock promoters, recognized by the heat shock sigma factor (σ^{32}, the product of the *rpoH* gene) that acts as a transcriptional activator (6, 13, 14, 46, 74). Although the *rpoH* gene is transcriptionally activated when the temperature is elevated, the major control of its expression is posttranscriptional, regulated at several levels including proteolysis (34, 46, 54, 83). In *E. coli*, σ^{32} has a short half-life and is degraded by a specific protease, the product of the *hflB* (*ftsH*) gene (23, 27, 34, 75). Damaged proteins produced upon a shift to a higher temperature, or exposure to other conditions that bring about protein denaturation initiate a cascade of events that brings about stabilization of σ^{32} and preferential expression of heat shock genes (10, 11, 22, 24, 33, 34, 37).

In *E. coli*, transcriptional activation of heat shock genes by σ^{32} is the only known control of the major heat shock operons. In the last few years, it became clear that the regulation of heat shock genes in other bacteria is more complex. For example, in gram-positive bacteria, there are at least three regulons of heat-shock genes, only one of which is activated by a specific heat shock sigma factor, σ^B (26).

TRANSCRIPTIONAL ACTIVATION BY RELEASE OF REPRESSION INVOLVING AN INVERTED REPEAT (IR, CIRCE) AND A REPRESSOR PROTEIN (PRODUCT OF THE *hrcA* GENE)

In most bacterial species there exists another transcriptional control system that regulates expression of one or more of the heat shock genes. This transcriptional control is mediated by 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 a binding site for a protein repressor, Orf39 (or OrfA, in *B. subtilis*), the product of the *hrcA* gene. Deletion of the IR results in constitutive expression of the operon (2, 26, 30, 50, 61, 65, 68, 71, 72, 81, 82, 85).

The IR is highly conserved, as demonstrated in Table 1. So far it was found only in

TABLE 1 The conserved inverted repeat in *groE* 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 pertussis	TTAGCACTC-N9-GAGTGCTAA

the upstream region of *groE*, *dnaK* and *dnaJ* operons or genes, all of them coding for the major chaperones. However, as mentioned above, these major chaperones are highly significant in bacterial physiology and pathogenicity and constitute dominant antigens, and therefore, sequence data are available from bacteria belonging to most phylogenetic groups. Since not much is known about other heat shock genes, it is still impossible to determine if the IR is unique to operons coding for chaperones.

The bacteria that use the IR as a control element can be divided into two groups, with respect to the types of regulatory systems. The first regulatory system has been demonstrated in several gram-positive bacteria of the low-G+C group. In these bacteria all the operons coding for chaperones are transcribed by the vegetative sigma factor—σ^{70} or σ^{A}—and the IR regulates this transcription.

The second regulatory system has been demonstrated in bacteria belonging to the α-purple proteobacteria: *Agrobacterium tumefaciens*, *Bradyrhizobium japonicum*, and *Caulobacter crescentus*. In these bacteria, the heat shock operons contain a specific heat shock promoter that is unique and differs from the vegetative promoter and from the heat shock promoter of *E. coli* (69). This promoter is recognized by a heat shock sigma factor (σ^{32}-like factor) that activates the genes (49, 55, 69). Several, but not all, of the heat shock operons contain the conserved IR in addition and respond to the Orf39 repressor (1, 68). From the available data, it appears that in bacteria that control heat shock transcription by a combination of a heat shock sigma factor and a repressor-binding IR, the latter is not present in *dnaK* operons. In these bacteria, the IR element is present in the *groE* operon or in at least one of the *groE* operons in bacteria that have more than one such operon (49, 72). The significance of this finding is not yet understood.

TRANSCRIPTIONAL ACTIVATION OF HEAT SHOCK GENES IN THE ALPHA SUBDIVISION OF PROTEOBACTERIA (α-PURPLE PROTEOBACTERIA)

The alpha subdivision of proteobacteria, α-purple proteobacteria, contains a large group of well-studied bacteria. These include bacteria of industrial importance, such as *Zymomonas* and *Acetobacter*, and bacteria with unusual cell cycles, such as *Caulobacter*. To this subdivision belong several groups of nitrogen fixers, such as *Rhodospirillum* and *Azospirillum*, as well as the plant symbionts *Rhizobium* and *Bradyrhizobium*. This subdivision also contains important human pathogens, such as *Brucella* and *Rickettsia* and plant pathogens such as *Agrobacterium tumefaciens*.

The heat shock operons of α-purple proteobacteria are activated by a σ^{32}-like transcription factor that recognizes heat shock promoters. This heat shock promoter was identified by comparing nine sequences of known heat shock operons from bacteria belonging to the α-purple proteobacteria (69). These operons include six *groESL* operons from *B. abortus*, *A. tumefaciens*, *C. crescentus*, *B. japonicum*, *Rhizobium meliloti*, and *Zymomonas mobilis* and three *dnaK* operons from *A. tumefaciens*, *C. crescentus*, and *Brucella bovis*. From these data, a consensus promoter sequence could be deduced. This putative consensus

promoter is different from both the vegetative and the heat shock promoter consensus sequences of *E. coli* (Table 2).

The identification of a unique heat shock promoter is compatible with the finding that the heat-shock activator (σ^{32}-like factor) of the α-purple proteobacteria differs from its homolog of the γ-purple proteobacteria in several aspects, including the sites responsible for promoter recognition (Table 2) (53, 54, 69). The promoter-recognition domain of the vegetative sigma factor (σ^{70}) is quite similar between the two groups, as expected from the similarity of the vegetative promoters.

Transcriptional activation of the *dnaK* operons of the α-purple proteobacteria is presumably carried out by the heat shock sigma factor, since no other control element has been identified in any of them. The situation is different in the *groE* operons, specifically in those that contain the CIRCE element. The roles of each of the control elements in the regulation of *groE* transcription was studied in *A. tumefaciens*, a plant pathogen that belongs to the group of α-purple bacteria. Introduction of mutations and deletions that decreased the stability of the putative "stem" formed by the IR resulted in increased transcription of the operon under non-heat-shock conditions but did not decrease the level of heat shock activation (71). This situation differs from that in the gram-positive bacteria, in which the IR actually controls heat shock activation.

We therefore assume that in bacteria that have a complex heat shock control consisting of an alternative sigma factor and a regulatory IR (e.g., α-purple bacteria), the heat shock sigma factor is responsible for heat shock activation of the operon, and the IR control system is involved in maintaining a low level of expression under non-heat-shock conditions.

STABILIZATION OF TRANSCRIPTS OF HEAT SHOCK GENES

The two control mechanisms described above act at the level of transcription. Two additional regulatory elements of the heat shock response are posttranscriptional. The finding that the IR can be transcribed raised the possibility that it is also active at the mRNA level. Evidence for this activity was obtained in *B. subtilis* and *A. tumefaciens* (71, 81). In *A. tumefaciens*, the half-life of the *groEL* transcript increased twofold when deletions were introduced into the IR. The deletions were found to increase the half-life of the transcript under non-heat-shock conditions (71). These results indicate that when it is transcribed, the IR functions at the level of the RNA as well as at the level of the DNA. In both situations, it

TABLE 2 Putative heat-shock promoters and promoter recognition domains of σ^{32} and σ^{70} in α-purple and γ-purple proteobacteria

	Putative promoters		
α-Purple proteobacteria heat shock promoter	CTTG	⟨17/18⟩	CYTAT-T
γ-Purple proteobacteria heat shock promoter	TCTC-CCTTGAA	⟨13/14⟩	CCCAT-AT
γ-Purple proteobacteria vegetative promoter	TTGACA	⟨17⟩	TATAAT

	Promoter recognition domains	
	2.4	4.2
α-Purple proteob. σ^{32}	IKA**S**IQ**E**YILR**SW**S**L**VK**M**G**V**TT	YGVS**R**ERVRQ**I**EK**R**AMKKLR
γ-Purple proteob. σ^{32}	IKA**E**I**H**EYVLR**NW**R**I**VK**VA**TT	YGVS**A**ERVRQ**L**EK**N**AMKKLR
	* * * * **	* * *
α-Purple proteob. σ^{70}	IRQAITRSIADQARTIRIPVHM	F**S**VTRERIRQIEAKALRNV**K**
γ-Purple proteob. σ^{70}	IRQAITRSIADQARTIRIPVHM	F**D**VTRERIRQIEAKALRNV**R**
		* *

decreases the expression of the operon under non-heat-shock conditions.

PROCESSING OF TRANSCRIPTS OF HEAT SHOCK GENES TO FACILITATE DIFFERENTIAL EXPRESSION

An additional posttranscriptional control mechanism was demonstrated in *A. tumefaciens* and involves specific cleavage of the *groESL* operon transcript between the *groES* and the *groEL* genes (70). The resulting *groES* transcript is rapidly degraded, while the *groEL* transcript is stable. The outcome of this cleavage is differential expression of the two genes of the operon. This mRNA processing is temperature dependent and is probably the first example of controlled processing of transcripts in bacteria.

The result of this process is that GroEL is produced at higher levels than GroES. The physiological significance of this is not yet understood. However, many bacteria have molecular mechanisms that result in more GroEL than GroES. This is achieved by having more genes coding for GroEL than for GroES or having several *groE* operons, not all of which contain the *groES* gene (1, 15, 72).

PHYLOGENETIC ASPECTS

An interesting question is how and when the various control systems evolved. For a comprehensive understanding, more data are needed. For example, the genes coding for alternative sigma factors have been cloned and sequenced from only a few bacteria, all belonging to the α-purple bacteria and γ-purple bacteria (4, 52, 53, 60, 63, 79). In addition, it appears that the IR was lost in many *dnaK* operons, but in many cases the upstream sequences available are too short to be certain that this structure is indeed absent. Nevertheless, several interesting conclusions can be drawn from available information, especially from data on the *groE* operons.

Figure 1 shows a phylogenetic tree based on the nonsynonymous substitutions of *groE*. The phylogenetic relationships obtained are the same as these obtained from sequences of the small RNA subunit. The data indicate that the control system involving the repressor-binding IR (CIRCE) is probably the ancient control mechanism and was lost in evolution only twice—once in the $\gamma 2/\gamma 3$-purple bacteria and once in *H. pylori*, of the δ-purple bacteria. It should be noted that in *Campylobacter jejuni*, also of the δ-subdivision of proteobacteria, the IR of the *groE* operon was retained.

UNRESOLVED PROBLEMS AND OPEN QUESTIONS

The most important unresolved problem concerning the heat shock response is what triggers this response at the molecular level. It remains to be determined if—or how—temperature elevation or other environmental stresses affect the regulatory IR, its repressor, or the interaction of the two components. It may be even more difficult to explain the triggering of the σ^{32}-dependent cascade of events. It has been assumed that the transcriptional activator σ^{32} (RpoH) is stabilized at high temperatures, concurrently with the increased level of denatured proteins and decreased availability of the major chaperones. However, studies of mutants with the *rpoH* gene deleted indicate that the heat shock response is already active at temperatures as low as 22°C (84). Clearly, this range of temperatures is too low to support the assumption that only temperature-denatured proteins trigger the heat shock response.

Other questions have to do with the fact that so far most of the information has been obtained on very few heat shock genes and proteins. Additional information is required before comprehensive models can be constructed. For example, one interesting control element is the temperature-dependent cleavage of the *groESL* transcript of *A. tumefaciens* resulting in differential expression of the two genes. Is this mechanism unique to *A. tumefaciens* or to the *groESL* operon? Are there additional controlled transcript-cleavage mechanisms in other bacteria or other operons?

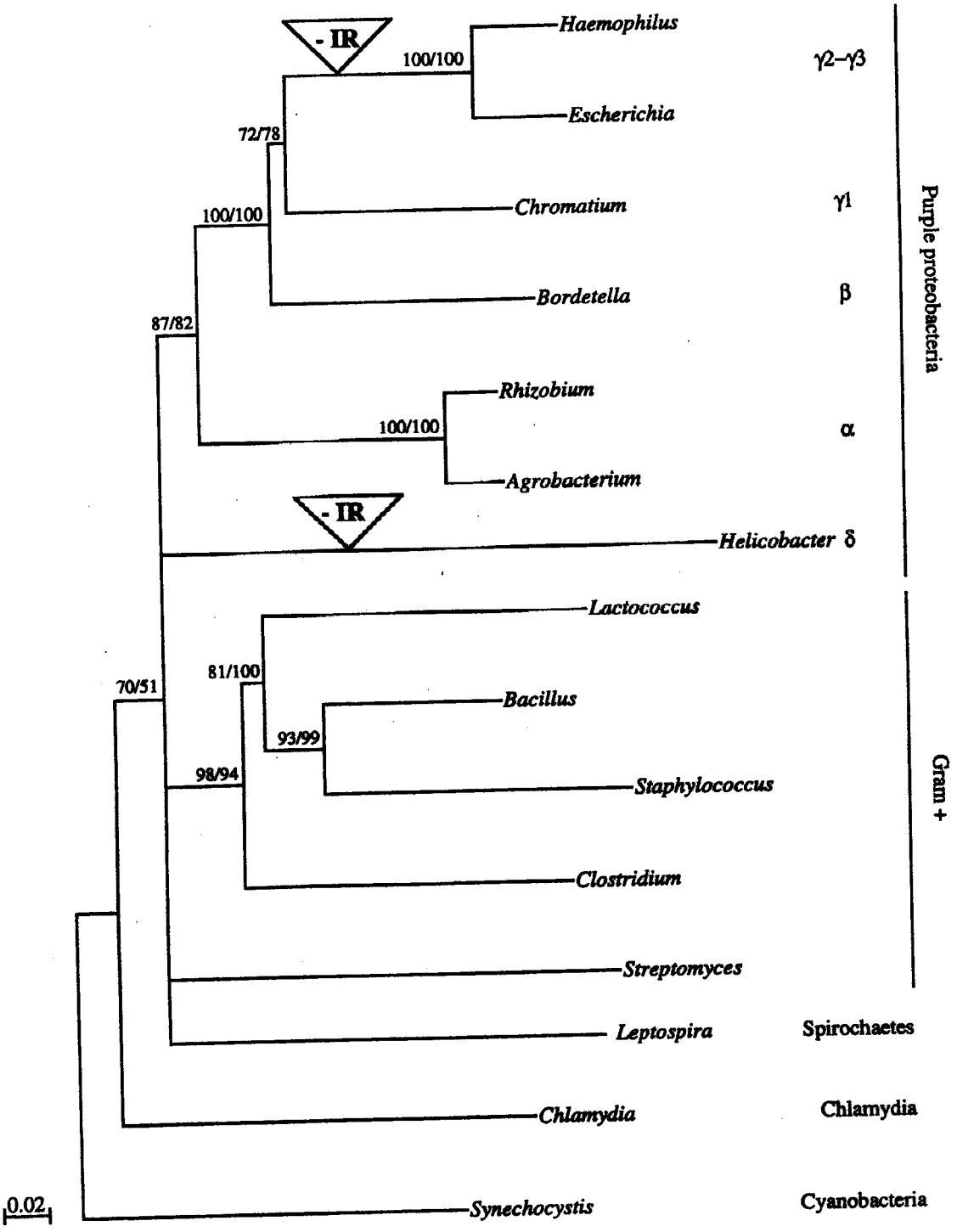

FIGURE 1 *groES/groEL* phylogeny obtained by neighbor-joining (64) with the Ka distance of Li (40). Shown above nodes are the bootstrap values (in %) obtained with 1,000 repetitions for concatenated genes *groES+ groEL* (left of slash) as well as bootstrap values for the same nodes with 16S rRNA sequences (neighbor-joining with the LogDet distance [43]), right of slash. Nodes supported by bootstrap values smaller than 50% with *groE* sequences are resolved differently by *groE* and by 16S rRNA sequences and are indicated as unresolved. Bootstrap values for 16S rRNA sequences are indicated for analysis with complete sequences only (1271 sites), the bootstrap value for the branch leading to *Haemophilus* and *Escherichia* is based on 861 aligned basepairs. Arrows indicate losses of the inverted repeat in *groE*. The scale bar represents 0.02 nonsynonymous substitutions per site.

What is the physiological/molecular reason for the differential expression of these two genes that code for proteins that are presumably working together in complexes? There is no obvious physiological explanation for the excess of GroEL over GroES, yet, as discussed above, such an excess is achieved by several different mechanisms in bacteria.

So far there are extensive experiments on *E. coli*, which uses only σ^{32} activation of heat shock promoters, and on *B. subtilis*, which has at least three regulons for heat shock response genes and in which the operons coding for Hsp60 and Hsp70 are transcribed by the vegetative sigma factor and activated by CIRCE. Many bacterial groups appear to combine the two control elements; the *dnaK* and *groE* operons appear to be activated by an alternative heat shock sigma factor but there is also an IR control element that represses the heat shock genes (or only the *groESL* operon?) in non-heat-shock conditions. Very little information is available about the control of operons other than *groE* and *dnaK* in these organisms.

Several phylogenetic questions are still unresolved, mainly because sequences are available only from a limited number of bacteria. Did the control start with a CIRCE element in all heat shock operons, to be replaced later by σ^{32} activation? When did the alternative heat shock sigma factor emerge? What are the advantages of having each of the control elements involved in the heat shock response?

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