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CIRCE, a Novel Heat Shock Element Involved in Regulation of Heat Shock Operon *dnaK* of *Bacillus subtilis*

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The *dnaK* and *groESL* operons of *Bacillus subtilis* are preceded by a potential σ^{43} promoter sequence (recognized by the vegetative σ factor) and by an inverted repeat (IR) consisting of 9 bp separated by a 9-bp spacer. Since this IR has been found in many bacterial species, we suspected that it might be involved in heat shock regulation. In order to test this hypothesis, three different mutational alterations of three bases were introduced within the IR preceding the *dnaK* operon. These mutations were crossed into the chromosome of *B. subtilis*, and expression of the *dnaK* and of the unlinked *groESL* operons was studied. The *dnaK* operon exhibited increased expression at low temperature and a reduction in the stimulation after temperature upshift. Furthermore, these mutations reduced expression of the *groESL* operon at low temperature by 50% but did not interfere with stimulation after heat shock. These experiments show that the IR acts as a negative *cis* element of the *dnaK* operon. This conclusion was strengthened by the observation that the IR reduced expression of two different transcriptional fusions significantly after its insertion between the promoter and the reporter gene. Since this IR has been described in many bacterial species as preceding only genes of the *dnaK* and *groESL* operons, both encoding molecular chaperones (39 cases are documented so far), we designated this heat shock element CIRCE (controlling IR of chaperone expression). Furthermore, we suggest that this novel mechanism is more widespread among eubacteria than the regulation mechanism described for *Escherichia coli* and has a more ancient origin.

A temperature upshift from 30°C to 42°C transiently induces the heat shock genes in *Escherichia coli* by activating transcription from promoters specifically recognized by RNA polymerase containing σ^{32} (5, 6) encoded by the *rpoH* gene (14, 25). Enhanced transcription from heat shock promoters is caused by a transient increase in the cellular level of σ^{32} (7, 21) as a result of increased synthesis and stabilization (20). The intracellular concentration of σ^{32} increases 15- to 20-fold within 5 min after temperature upshift and then declines to a new steady-state level. This regulation pathway serves as a paradigm for the eubacteria.

Recently, we started analysis of the regulation of heat shock response in *Bacillus subtilis*. When transcriptional fusions between various *E. coli* heat shock promoters fused to two different reporter genes were analyzed with *B. subtilis*, no temperature-dependent induction could be measured (23). We concluded that, in contrast to vegetative promoters, *E. coli* heat shock promoters are not recognized in *B. subtilis*. Transcriptional analysis of the *dnaK* and *groESL* operons of *B. subtilis* revealed in both cases a DNA sequence resembling the canonical sequence for vegetative promoters preceding the potential transcriptional start sites, which were activated after temperature upshift (18, 24). A close inspection of the DNA sequences around the transcription start sites led to the detection of a perfect inverted repeat (IR) consisting of 9 bp separated by a 9-bp spacer near the 5′ end of the mRNAs within the untranslated regions (18, 24). Comparable results were obtained for the same two operons from *Clostridium acetobutylicum* (12, 13). In addition, a search within the data bases revealed some more examples of the same IR preceding heat shock genes from both gram-positive and gram-negative bacteria, as listed in Table 2 of the article by Wetzstein et al. (24). The occurrence of this IR suggested to us that it might be involved in the regulation of the heat shock response in *B. subtilis*. To prove this hypothesis, different point mutations were introduced within the IR located in front of the *dnaK* operon by oligonucleotide-directed mutagenesis, and these mutations were crossed into the chromosome of *B. subtilis* at the original site. The phenotypes of these mutants were then analyzed. In addition, transcriptional fusions with and without the IR were constructed, and their expression was studied.

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MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. *E. coli* RZ1032 (*ung dut* F′ [9]) and *B. subtilis* 1012 (*leuA8 metB5 hsrM1* [17]) were used throughout most of the experiments. BT01 is a derivative of strain 1012 which carries a chloramphenicol resistance marker inserted into the *Hpa*I site at the beginning of *orf39* (21), and NCC1700 is derived from 1012 by integration of pNEXT33A at the *metD* gene. M13mp19 (11), pMWB5 (24), pDG268 (1), pDG918 (4), and pNEXT33A, which carries a neomycin resistance cassette inserted into the *metD* gene (8), have been described previously. pBT01 is a derivative of pUC18 carrying the 4.0-kb *Hin*dIII-*Eco*RI fragment of the *dnaK* operon (24). Bacteria were routinely grown at 37°C in Luria-Bertani medium. For the induction of the heat shock operons, strain 1012 and its derivatives were shifted from 37°C to 48°C. One hundred micrograms of ampicillin per ml was used for strain RZ1032, and 10 μg of neomycin per ml and 5 μg of chloramphenicol per ml were used for strain 1012 and its derivatives.

Oligonucleotide-directed mutagenesis. Three different point mutations within the IR preceding the *dnaK* operon were isolated by oligonucleotide-directed mutagenesis following the procedure of Kunkel et al. (9). First, the 1.1-kb *Sal*I-*Pvu*II subfragment of pMWB5 was subcloned in M13mp19 cut with *Sal*I-*Sma*I, resulting in M13-IR, which was subsequently propagated in *E. coli* RZ1032. Uracil-containing single-stranded

DNA template was isolated. Synthetic oligonucleotide IR-1 (5′-CTCAATAAGCGAGTTTAAATTCTATAAC-3′) was used to mutagenize M13-IR, resulting in M13-IR-1. Oligonucleotide IR-2 (5′-ATCACCTCTGTTTAAACTCTCAA TA AGC-3′) was used to mutagenize M13-IR and M13-IR-1, resulting in M13-IR-2 and M13-IR-3, respectively. The mutations were confirmed by sequencing of the M13-IR derivatives. Next, the *Sal*I-*Hpa*I fragments were excised from the mutagenized double-stranded forms and exchanged with the same fragment of pBT01, resulting in the plasmids pIR-1, pIR-2, and pIR-3. The correct DNA sequence around the IR was again verified by sequencing. The mutations were then crossed into the chromosome of *B. subtilis* according to the procedure described by Itaya and Tanaka (8). *B. subtilis* BT01 was cotransformed with two plasmids, pNEXT33A and either pIR-1, pIR-2, or pIR-3. Neomycin-resistant transformants were selected and subsequently screened for sensitivity to chloramphenicol, which arises by a double crossover between sequences of the *dnaK* operon inserted into the nonselected plasmid and the homologous chromosomal DNA. The correct arrangement at the IR was confirmed by Southern blotting.

Slot blot analysis. Isolation of total RNA and analyses of mRNA synthesis by slot blotting were performed as described previously (24) by using oligonucleotides complementary to the noncoding strand of *orf39* (5′-ATCCCACCGGCTGT GCCG-3′), *dnaK* (5′-TTATTTTTTGTTTTGGTCGTCGTT-3′), and *groEL* (5′-AGTGCATCGACACCGCG-3′) as probes for hybridization. These oligonucleotides were labeled with digoxigenin-11–ddUTP as described previously (26). Quantification of the amount of mRNA was obtained by scanning the luminographs with an LKB UltroScan-XL laser densitometer (Pharmacia) by using the program GelScan XL (version 2.1).

Measurement of the amount of DnaK protein. The amount of DnaK protein within different *B. subtilis* strains was determined by the technique of rocket immunoelectrophoresis (10). The amount of protein was calculated from the lengths of the rockets by using purified DnaK as a standard.

Construction of transcriptional fusions. Two different plasmids were used for construction of *lacZ* transcriptional fusions. Both plasmids contain a promoterless *E. coli lacZ* gene that uses the translation initiation signals of the *B. subtilis spoVG* gene (16) and a chloramphenicol resistance marker which are bracketed by sequences from the *amyE* locus. A 72-bp oligonucleotide containing the promoter region and the IR preceding the *dnaK* operon (nucleotides 159 to 230 in the numbering system of reference 24) and flanked by *Hin*dIII and *Bam*HI sites was designed. Furthermore, nucleotides 195 to 200 have been replaced by a *Bam*HI site, allowing the recovery of the IR as a *Bam*HI fragment. This 72-bp fragment was ligated into pDG268 (1) cut with *Hin*dIII and *Bam*HI, resulting in pYPS10. To remove the IR from this construction, pYPS10 was digested with *Bam*HI, and the larger fragment was religated (pYPS11). In another construction, the 36-bp *Bam*HI fragment carrying the IR was ligated into the unique *Bam*HI site of pDG918 (4), giving pYPS12. The *Bam*HI site is located 81 bp downstream from the promoter P1 (4). After transformation of *B. subtilis* 1012 with the monomeric forms of the four different plasmids, both the *lacZ* gene and the *cat* marker were integrated by a double-recombinant event into the chromosomal *amyE* locus. β-Galactosidase assays were performed as described previously (15).

RESULTS

Isolation of point mutations within the IR preceding the *dnaK* operon. To test the hypothesis that the IR is involved in the regulation of the heat shock response, we decided to introduce different point mutations within the IR preceding the *dnaK* operon. Figure 1A displays the genomic organization of this operon, which consists of the four genes *orf39*, *grpE*, *dnaK*, and *dnaJ*. This operon is preceded by a potential σ^{43}-dependent promoter and the IR. Figure 1B presents the DNA sequence around the IR of the *dnaK* operon and below the DNA sequence of three mutated IRs in which nucleotides within the IR have been changed (IR-1 through IR-3 [Fig. 1C]). The rationale behind the isolation of the mutants was as follows. Assuming that this IR is recognized by a protein (see below), we envisage the possibility that this protein will bind separately to these two repeats. Therefore, we decided to isolate three different mutants, thereby altering the left, right, or both IRs. In IR-1 and IR-2, three bases were changed within the left and right arms of the IR, respectively. In IR-3, three nucleotides have been altered in each arm so that the dyad symmetry was restored. In all three cases, nucleotides were changed in such a way that a *Dra*I site was created, facilitating analyses of the mutated DNA.

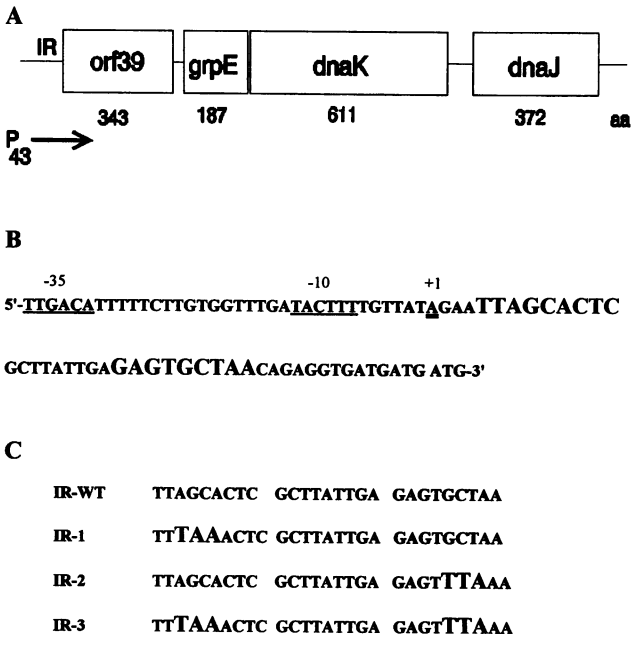

FIG. 1. Point mutations within the IR preceding the *dnaK* operon of *B. subtilis*. (A) Genomic organization of the *dnaK* operon. P_{43}, potential vegetative promoter recognized by σ^{43}. The sizes of the four genes are given in amino acid (aa) residues. (B) DNA sequence around the IR of the *dnaK* operon. The potential vegetative promoter is underlined, the putative transcriptional start site is double underlined and indicated by +1, and the nucleotides of the IR are given in boldface oversized letters. (C) Point mutations within the IR. The altered nucleotides are given in boldface oversized letters.

Transcriptional analysis of the *dnaK* and *groESL* operons. Transcription of the *dnaK* and the unlinked *groESL* loci was analyzed with the three mutants and *B. subtilis* NCC1700, which carries pNEXT33A integrated at the *metD* gene (isogenic wild-type control). mRNA was prepared from the four strains grown at 37°C (0 min) and at 5 and 10 min after temperature upshift to 48°C. Slot blot analysis revealed that, whereas the wild-type strain exhibited a significant increase in the amount of mRNA after temperature upshift, the amount of *dnaK* operon-specific mRNA of the IR mutants was already large under noninducing conditions (Fig. 2). No further signif-

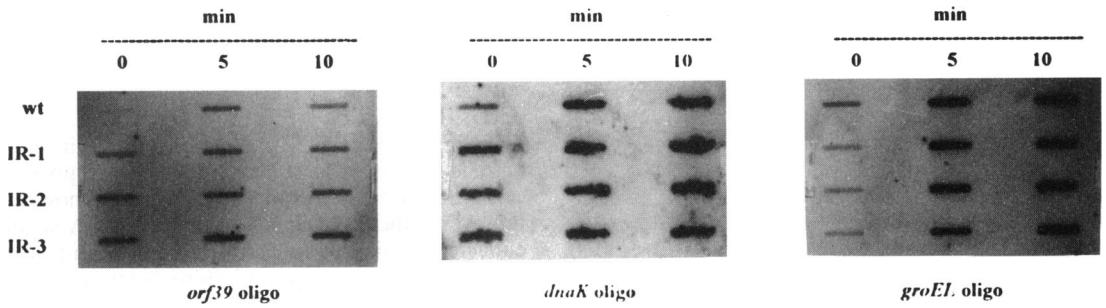

FIG. 2. Concentration of mRNA of the *dnaK* and of the *groEL* operons. Slot blot analysis of RNA isolated before (0 min) and 5 and 10 min after cells have been shifted to 48°C.

icant increase could be observed after a temperature upshift (see below). In contrast, the amounts of *groESL* mRNA under noninducing conditions and after temperature upshift were comparable in the wild-type strain and the three IR mutants (Fig. 2).

To reveal minor differences in the amount of specific mRNA, the bands were quantified by scanning of the luminograph (Table 1). The amount of *orf39*-specific mRNA isolated from the wild-type strain increased 7.4-fold 5 min after temperature upshift, followed by the typical decline. In contrast, the amount of *orf39*-specific mRNA within the three IR mutants reached values between 7.2- and 11.2-fold at 37°C, an amount found with the wild-type strain 5 min after temperature upshift (Table 1). With the mutants, we measured a slight increase in the amount of mRNA after temperature upshift (1.4- to 1.9-fold), followed by a slight decrease, suggesting that the mutated IRs are not completely inactive. Comparable results were obtained for *dnaK*-specific mRNA (Table 1). In contrast, there was a smaller amount of *groEL*-specific mRNA isolated from the three IR mutants than from the wild type under noninducing conditions. In *E. coli*, DnaK and the cochaperones DnaJ and GrpE are involved in the deregulation of the heat shock response (3). The smaller amount of the *groEL* mRNA within the IR mutants present at 37°C could reflect some negative interaction exerted by the *dnaK* operon. Clearly, more experiments are needed to confirm this hypothesis.

Measurement of the amounts of DnaK protein in different *B. subtilis* strains. To verify whether the high constitutive level of mRNA is accompanied by an increased amount of heat shock proteins, the amount of DnaK was measured by the technique of rocket immunoelectrophoresis (10). With different amounts of purified DnaK protein as a standard, the amount of DnaK protein in wild-type cells was calculated to represent 0.6% of the total protein at 37°C, and 15 min after temperature upshift, the amount of DnaK protein had increased to 1.0% of the total protein (Fig. 3). In contrast, in the IR mutants, DnaK protein represented about 1.5% of the total cellular protein, and no significant difference could be measured before and after temperature upshift (Fig. 3). These data confirm our results obtained with the slot blot analysis.

The IR reduces expression of a downstream gene. To further verify that the IR acts as a negative *cis* element, two transcriptional fusions were constructed between the promoter region of the *dnaK* operon and *lacZ* and integrated at the *amyE* locus. Both fusions are distinguished by the presence or absence of the IR. Measurement of the β-galactosidase activity of these operon fusions at 30°C revealed an ~25-fold difference after deletion of the IR (Table 2). We then asked whether the IR can influence the activity of a foreign promoter. To answer this question, the IR was inserted between the promoter region of the *ftsAZ* operon and *lacZ*. As can be seen from Table 2, the IR reduced the expression of the *lacZ* gene about fourfold. These results further demonstrate that the IR acts as a

TABLE 1. Measurements of the amount of mRNA produced at 37°C and after temperature upshift[a]

Element	Probe	RNA produced (arbitrary units) at:		
		0 min	5 min	10 min
Wild type	*orf39*	1	7.4	4.8
IR-1	*orf39*	7.2	10.0	8.4
IR-2	*orf39*	9.4	13.4	12.3
IR-3	*orf39*	11.2	21.0	17.5
Wild type	*dnaK*	1	4.4	4.0
IR-1	*dnaK*	3.0	4.8	5.6
IR-2	*dnaK*	2.4	3.8	5.2
IR-3	*dnaK*	3.3	3.9	4.6
Wild type	*groEL*	1	6.6	7.2
IR-1	*groEL*	0.5	5.6	5.9
IR-2	*groEL*	0.6	5.7	6.9
IR-3	*groEL*	0.5	3.8	6.9

[a] The amount measured with the wild-type strain NCC1700 before induction was set as 1. Luminographs of three different experiments were scanned and yielded comparable results. The data from one of these experiments are given here.

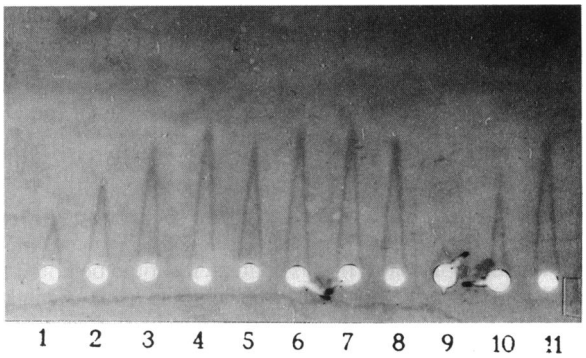

FIG. 3. Concentration of DnaK protein in the wild-type strain and in the three IR mutants measured by the technique of rocket immunoelectrophoresis. Lanes: 1 and 2, wild-type strain at min 0 and 15, respectively; 3 and 4, IR-1 mutant at min 0 and 15, respectively; 5 and 6, IR-2 mutant at min 0 and 15, respectively; 7 and 8, IR-3 mutant at min 0 and 15, respectively. Five micrograms of total cell protein was used in lanes 1 to 8. Ten, 50, and 100 ng of purified DnaK protein were used in lanes 9 to 11, respectively. The amounts of protein were calculated from the lengths of the rockets.

TABLE 2. Analysis of transcriptional fusions[a]

Promoter	CIRCE	β-Galactosidase activity at 30°C (U)
P_{dnaK}	+	2
P_{dnaK}	−	50
P_{ftsAZ}	−	334
P_{ftsAZ}	+	81

[a] Promoter regions of the *dnaK* and *ftsAZ* operons, respectively, have been fused to *lacZ* and integrated at the *amyE* locus. +, CIRCE present; −, CIRCE absent.

negative *cis* element and that the IR can repress the activity of a foreign promoter.

DISCUSSION

Our results show that the IR plays an important role during regulation of the heat shock response of the affected operons. Mutational changes within the left, right, or both parts of the IR preceding the *dnaK* operon abolished its role in acting as a negative *cis* element of expression of this operon at 37°C. These results were further corroborated by analysis of four transcriptional fusions. Two different promoter regions (that of the *dnaK* and P1 of the *ftsAZ* operon) were hooked up to the *lacZ* reporter gene. In both cases, the IR when placed downstream of the promoter reduced expression of the reporter gene, although to different extents. These results confirm that the IR acts as a negative *cis* element, and they further demonstrate that it is active independent of its original promoter region. Unfortunately, heat induction cannot be monitored with these operon fusions, since the amount of β-galactosidase activity drops within 2 min after temperature upshift (data not shown), probably because of protease activity or activities induced under these conditions and recognition of β-galactosidase as a foreign protein in *B. subtilis*. Experiments are in progress to identify a reporter gene with a product which remains stable after heat shock.

The difference in the reduction rates exerted by the IR can be explained by different mechanisms. (i) The distance between the promoter and the IR (10 bp in the case of P_{dnaK} and 81 bp in the case of P_{ftsAZ}) might be a factor. (ii) The difference in reduction rates might be influenced by the promoter itself. (iii) Additional sequences present in the homologous system but absent from the heterologous system could be involved.

These data do not exclude the possibility that additional DNA sequences might be involved in the regulation of heat shock response. We carried out a careful analysis of the DNA sequences around the transcriptional start sites of the *dnaK* and *groESL* operons from positions −100 to +100, and we were unable to detect significant homology beyond that already reported (promoter, IR, Shine-Dalgarno sequence). However, there might be operon-specific sequences involved in the regulation of expression. In this context, it should be noted that the IR of the *dnaK* operon overlaps the Shine-Dalgarno sequence of *orf39* by 2 bp (24), whereas in the case of the *groESL* operon, the IR is separated from the putative Shine-Dalgarno sequence of *groES* by 17 bp (18). A tentative speculation is that the distance between the IR and the Shine-Dalgarno sequence will influence the translation rate of the affected gene. This in turn would imply that the IR is also active at the level of RNA.

Since this IR has been described so far as only preceding the *dnaK* and *groESL* operons, which both encode molecular chaperones, we designate this IR as CIRCE (controlling IR of chaperone expression). The IR consists of 9 bp separated by a 9-bp spacer which is highly conserved. It is interesting that the distance between the conserved nucleotides is 9 bp, which corresponds to about one turn of the helix. Up to now, 39 cases of this IR occurring within the different phyla of the eubacteria have been reported (32 cases are reported in the EMBL data base, and an additional 7 cases have been reported by personal communications). To the best of our knowledge, CIRCE represents the most highly conserved DNA sequence among eubacteria. Since it has already been found in the bacterial groups *Chlamydia* spp. and spirochetes, it seems to have a more ancient origin than the regulatory system identified in *E. coli*.

Will CIRCE act by itself, or will it act in concert with a protein? If CIRCE acts without the involvement of a protein, we have to assume a change in the secondary structure dependent on the growth temperature. If it acts at the level of DNA, this secondary structure could be a cruciform structure; if it acts at the level of RNA, it could be a stem-loop structure. We consider it rather unlikely that the activity of this element is governed by temperature. First, we found that CIRCE also precedes the *groESL* operon of *Bacillus stearothermophilus* (19). Here, we could show that a low level of transcription occurs at 55°C and that there is a transient high level of transcription after a temperature upshift to 70°C. Second, induction of the *dnaK* operon of *B. subtilis* can also be accomplished by the addition of 8% ethanol (2). Third, IR-3 would be able to form a secondary structure, but it allows constitutive high-level expression of the downstream operon under noninducing conditions. Fourth, the transient nature of the heat-induced derepression of the *dnaK* and *groESL* operons does not favor this possibility. These four observations argue against temperature-dependent formation of alternate secondary structures without the involvement of a protein. In conclusion, we assume that a protein will bind specifically to the IR sequences, probably by binding separately to the left and right IRs. Since point mutations within these potential binding sites increase expression of the *dnaK* operon, the protein cannot act as an activator but rather acts as a repressor. Will the repressor bind to the IR at the level of DNA, or will it bind at the level of RNA? At the moment, we cannot answer this question. van Asseldonk and coworkers could show that the IR preceding *dnaJ* of *Lactococcus lactis* is located upstream of the potential promoter (22). Deletion of a DNA sequence including the IR located on a multicopy plasmid led to constitutive high-level expression of a reporter gene. At least in this case, the IR must be active at the level of DNA rather than at the level of mRNA. Therefore, we assume that, in *B. subtilis* and in the other bacterial species, CIRCE also acts at the level of DNA. Experiments are in progress to identify the protein binding to CIRCE and to elucidate the mechanism of inactivation and reactivation of this protein upon temperature upshift.

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REFERENCES

1. **Antoniewski, C., B. Savelli, and P. Stragier.** 1990. The *spoIIJ* gene, which regulates early developmental steps in *Bacillus subtilis*, belongs to a class of environmentally responsive genes. J. Bacteriol. **172**:86–93.
2. **Arnosti, D. N., V. L. Singer, and M. J. Chamberlin.** 1986.

Characterization of heat shock in *Bacillus subtilis*. J. Bacteriol. **168:**1243–1249.

3. **Georgopoulos, C., D. Ang, K. Liberek, and M. Zylicz.** 1990. Properties of the *Escherichia coli* heat shock proteins and their role in bacteriophage lambda growth, p. 191–221. *In* R. I. Morimoto, A. Tissières, and C. Georgopoulos (ed.), Stress proteins in biology and medicine. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
4. **Gonzy-Tréboul, G., C. Karmazyn-Campelli, and P. Stragier.** 1992. Developmental regulation of transcription of the *Bacillus subtilis ftsAZ* operon. J. Mol. Biol. **224:**967–979.
5. **Gross, C. A., D. B. Straus, J. W. Erickson, and T. Yura.** 1990. The function and regulation of heat shock proteins in *Escherichia coli*, p. 167–189. *In* R. I. Morimoto, A. Tissières, and C. Georgopoulos (ed.), Stress proteins in biology and medicine. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
6. **Grossman, A. D., J. W. Erickson, and C. A. Gross.** 1984. The *htpR* gene product of *E. coli* is a sigma factor for heat-shock promoters. Cell **38:**383–389.
7. **Grossman, A. D., D. B. Strauss, W. A. Walter, and C. A. Gross.** 1987. σ^{32} synthesis can regulate the synthesis of heat shock proteins in *Escherichia coli*. Genes Dev. **1:**179–184.
8. **Itaya, M., and T. Tanaka.** 1990. Gene-directed mutagenesis on the chromosome of *Bacillus subtilis* 168. Mol. Gen. Genet. **223:**268–272.
9. **Kunkel, T. A., J. D. Roberts, and R. A. Zakour.** 1987. Rapid and efficient site-specific mutagenesis without phenotypic selection. Methods Enzymol. **154:**367–383.
10. **Merril, D., T. F. Hartley, and H. N. Claman.** 1967. Electroimmunodiffusion (EID): a simple, rapid method for quantitation of immunoglobulins in dilute biological fluids. J. Lab. Clin. Med. **69:**151–159.
11. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **101:**20–78.
12. **Narberhaus, F., and H. Bahl.** 1992. Cloning, sequencing, and molecular analysis of the *groESL* operon of *Clostridium acetobutylicum*. J. Bacteriol. **174:**3282–3289.
13. **Narberhaus, F., K. Giebeler, and H. Bahl.** 1992. Molecular characterization of the *dnaK* gene region of *Clostridium acetobutylicum*, including *grpE*, *dnaJ*, and a new heat shock gene. J. Bacteriol. **174:**3290–3299.
14. **Neidhardt, F. C., and R. A. VanBogelen.** 1981. Positive regulatory gene for temperature-controlled proteins in *Escherichia coli*. Biochem. Biophys. Res. Commun. **100:**894–900.
15. **Nicholson, W. L., and P. Setlow.** 1990. Sporulation, germination and outgrowth, p. 391–450. *In* C. R. Harwood and S. M. Cutting (ed.), Molecular biological methods for *Bacillus*. John Wiley & Sons, New York.
16. **Perkins, J. B., and P. J. Youngman.** 1986. Construction of Tn*917lacZ*, a transposon derivative that mediates transcriptional gene fusions in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **83:**140–144.
17. **Saito, H., T. Shibata, and T. Ando.** 1979. Mapping of genes determining nonpermissiveness and host-specific restriction to bacteriophages in *Bacillus subtilis* Marburg. Mol. Gen. Genet. **170:**117–122.
18. **Schmidt, A., M. Schiesswohl, U. Völker, M. Hecker, and W. Schumann.** 1992. Cloning, sequencing, mapping, and transcriptional analysis of the *groESL* operon from *Bacillus subtilis*. J. Bacteriol. **174:**3993–3999.
19. **Schön, U., and W. Schumann.** 1993. Molecular cloning, sequencing, and transcriptional analysis of the *groESL* operon from *Bacillus stearothermophilus*. J. Bacteriol. **175:**2465–2469.
20. **Straus, D. B., W. A. Walter, and C. A. Gross.** 1987. The heat-shock response of *E. coli* is regulated by changes in the concentration of σ^{32}. Nature (London) **329:**348–351.
21. **Tzschaschel, B.** Unpublished results.
22. **van Asseldonk, M., A. Simons, H. Visser, W. M. de Vos, and G. Simons.** 1993. Cloning, nucleotide sequence, and regulatory analysis of the *Lactococcus lactis dnaJ* gene. J. Bacteriol. **175:**1637–1644.
23. **Wetzstein, M., and W. Schumann.** 1990. Promoters of major *Escherichia coli* heat shock genes seem non-functional in *Bacillus subtilis*. FEMS Microbiol. Lett. **72:**55–58.
24. **Wetzstein, M., U. Völker, J. Dedio, S. Löbau, U. Zuber, M. Schiesswohl, C. Herget, M. Hecker, and W. Schumann.** 1992. Cloning, sequencing, and molecular analysis of the *dnaK* locus from *Bacillus subtilis*. J. Bacteriol. **174:**3300–3310.
25. **Yamamori, T., and T. Yura.** 1982. Genetic control of heat-shock protein synthesis and its bearing on growth and thermal regulation in *Escherichia coli* K12. Proc. Natl. Acad. Sci. USA **79:**860–864.
26. **Zuber, U., and W. Schumann.** 1991. Tn*5cos*: a useful tool for restriction mapping of large plasmids. Gene **103:**69–72.