JOURNAL OF BACTERIOLOGY, Nov. 1993, p. 7479–7482
0021-9193/93/227479-04$02.00/0

Vol. 175, No. 22

Heat Shock and Development Induce Synthesis of a Low-Molecular-Weight Stress-Responsive Protein in the Myxobacterium *Stigmatella aurantiaca*

MICHAEL HEIDELBACH,* HEYKO SKLADNY, AND HANS ULRICH SCHAIRER

Zentrum für Molekulare Biologie Heidelberg, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany

Received 7 May 1993/Accepted 14 September 1993

In the fruiting body-forming myxobacterium *Stigmatella aurantiaca* a 21,000-M_r protein, SP21, is synthesized during fruiting, heat shock, and stress induced by oxygen limitation. The corresponding gene was isolated from a gene expression library in λgt11 with an antiserum to the purified protein. The DNA sequence of the gene reveals that SP21 is a member of the α-crystallin family of low-molecular-weight heat shock proteins.

Stigmatella aurantiaca is a gram-negative, rod-shaped myxobacterium that grows on decaying organic matter in soil. The myxobacteria possess a biphasic cell cycle, during which they form a fruiting body. During vegetative growth, the cells glide in swarms upon insoluble organic substrates, part of which they degrade by secreted lytic enzymes. When nutrients are depleted, the cells migrate into aggregation centers from which the fruiting bodies arise. The fruiting body of *S. aurantiaca* consists of a branched stalk supporting the sporangioles, which in turn contain several thousand myxospores. Features observed during eukaryotic multicellular morphogenesis, such as the processing of positional information and cell communication by close contact or diffusible molecules, are predicted to play an important role in fruiting of the myxobacteria (28). Spore formation of *S. aurantiaca* is not strictly coupled to fruiting body formation and can be directly induced in liquid culture by a number of chemicals, the most potent of which are indole and some of its derivatives (7).

During development, a protein with an M_r of 21,000 (SP21) is synthesized; this protein is found in fruiting bodies and chemically induced spores but was not detected in vegetative cells growing under standard conditions. The protein has been purified from chemically induced spores, and the sequence of its N-terminal amino acids has been determined (11). Here we report the isolation of the corresponding gene (*hspA*) from an expression library in λgt11 with an antiserum to purified SP21. The DNA sequence of *hspA* identifies SP21 as a member of the family of low-molecular-weight heat shock proteins (LMW-HSPs). There is striking homology between SP21 and LMW-HSPs of several plants. We demonstrated stress-responsive expression of *hspA* in Western immunoblots upon heat shock and oxygen depletion.

Cloning and sequencing of *hspA*. λgt11 expression libraries were constructed from genomic *S. aurantiaca* DNA partially restricted with *Hpa*II and enriched for the size range of 500 to 2,000 bp by preparative agarose gel electrophoresis. To obtain translational fusions to β-galactosidase in all reading frames, three independent libraries were constructed by using *Hpa*II-*Eco*RI linkers of different lengths (17, 18, or 19 nucleotides) which were ligated to *S. aurantiaca* DNA and subsequently cloned into the *Eco*RI site of the λgt11 vector.

Immunological screening of the recombinant phages was done as described previously (29), using an antiserum to SP21 (11) at a dilution of 1:3,000. Eight independent clones were extracted from the λgt11 expression libraries and purified, and their inserts were subcloned into the *Eco*RI site of pBluescript SKII+ (Stratagene). Southern hybridization of the cloned inserts with one another revealed three distinct classes according to their hybridization patterns. One plasmid (pL4), which contained the 5′ end of *hspA*, was identified by Southern hybridization with a fully degenerated oligonucleotide probe derived from amino acids 19 to 25 of the SP21 polypeptide. It harbored the complete gene for SP21, which in λgt11 is not expressed as a fusion protein but is transcriptionally linked to the β-galactosidase mRNA. The DNA sequences of both strands of the insert were determined by primer walking by the chain termination method (30) with a T7 sequencing kit (Pharmacia). Sequences were composed and analyzed by using the Heidelberg Unix Sequence Analysis Resources.

Properties of *hspA*. The composite sequence of the insert of *hspA* is depicted in Fig. 1. The previously established amino acid sequence of the polypeptide (11) defines the ATG at position 568 to be the start of translation. The expression of *hspA* in λgt11 as an individual cistron implies that the cloned DNA carries the start signals for translation. Therefore, the sequence (GAGGAGGA) 12 bp upstream from the ATG is most probably the ribosome binding site (17). An in-frame opal codon is located at position 1134.

The insert of pL4 has a high G+C content (66.5%); there are slight differences in the G+C compositions of *hspA* (63.7%) and the flanking regions (67.6%). The G+C content at the third codon position is above average (78%), while that at the second position is relatively low (45%).

SP21 is composed of 187 amino acids and has a calculated molecular weight of 21,122, which matches the apparent molecular weight in sodium dodecyl sulfate-polyacrylamide gel electrophoresis of 21,000. With the deduced amino acid sequence, the SWISSPROT and PIR protein data bases and the EMBL/GenBank DNA data bases (releases from March 1992) were screened for homologous sequences with the programs Fasta and TFasta (25) and Blastn and TBlastn (1). The search revealed significant homology between SP21 and the family of LMW-HSPs (Table 1). Most striking is the similarity between SP21 and the LMW-HSPs of several plants. SP21 is 38.2% identical to HSP17.4 of *Arabidopsis thaliana*; considering evolutionarily favored amino acid substitutions, the match increases to 55%. The homology of SP21 to some plant and bacterial LMW-HSPs is depicted in Fig. 2 (see Table 1 for identity scores).

* Corresponding author.

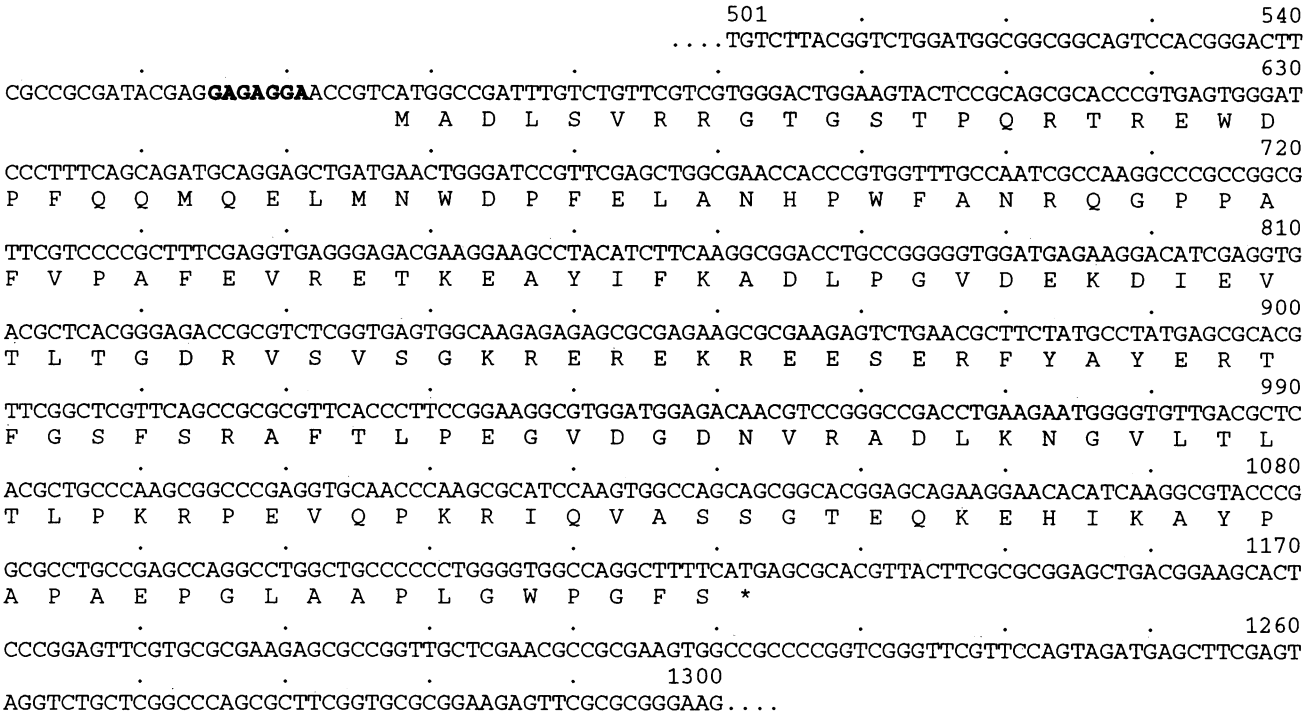

FIG. 1. DNA sequence of *hspA* and deduced amino acid sequence of SP21. Part of the pL4 sequence containing *hspA* is shown. The coding region ranges from nucleotide 568 (ATG) to 1134. Boldface letters indicate the putative ribosome binding site.

Across species the homology of LMW-HSPs increases towards the C termini of the proteins (22). Remarkably, in SP21 and a subset of plant LMW-HSPs, homologous sequences span the whole protein. The sequence analysis program Tree (6) predicts a closer relationship between SP21 and plant LMW-HSPs than between plant LMW-HSPs and all other eukaryotic LMW-HSPs.

Induction of *hspA* expression. *hspA* expression as a response to stressors (heat shock and anoxia) was investigated. Heat shock was induced by transferring an exponentially growing culture (8×10^7 cells per ml) of *S. aurantiaca* (11) from 32 to 37°C with vigorous shaking. These relatively mild heat shock conditions were sufficient to induce *hspA* expression while the cells remained viable. (At 42°C *hspA* is also induced, but cells die soon after the shift.) About 5 min after the temperature shift, SP21 was detected in a Western blot (Fig. 3) by using the antiserum to SP21 (11). Minor degradation of SP21 resulting in the weak band below M_r 21,000 may be due to proteolysis during sporulation or preparation of the samples. After 40 to 60 min, accumulation ceased and the cellular SP21 concentration reached a plateau.

TABLE 1. Sequence identities of *S. aurantiaca* SP21 to LMW-HSPs belonging to the alpha-crystallin family

Source	Protein	% Identity[a]	Reference
Alfalfa	HSP18.2	37.5	10
A. thaliana	HSP17.4	38.2	34
Soybean	HSP17.5E	37.2	3
Neurospora crassa	HSP30	30.5	27
S. cerevisiae	HSP26	27.3	26
M. leprae	18-kDa antigen	23.3	21
M. tuberculosis[b]	14-kDa antigen	24.5	35
Human	HSP27	16.7	12
Drosophila melanogaster	HSP22	16.5	32

[a] Based on sequences aligned by the program Tree (6).
[b] Amino acid sequence deduced from DNA sequence.

Depletion of oxygen also results in *hspA* expression. Microaerobic or anoxic conditions were achieved by filling an air-tight closed bottle to the top with an exponentially growing culture (5×10^7 cells per ml). Because *S. aurantiaca* is an obligate aerobe, oxygen is consumed rapidly (5). SP21 synthesis was detected in Western blots 4 to 6 h after transfer into the air-tight closed bottle. Whether anoxia itself, a more general down-regulation of metabolism, or a change in the milieu triggers *hspA* expression remains to be determined.

In *S. aurantiaca*, SP21 was recognized as a major constituent of chemically induced spores; it was absent from vegetative cells growing under standard conditions (11). Only a few sequences of bacterial LMW-HSPs, i.e., those from *Clostridium acetobutylicum* (31) and several mycobacterial species (21, 35), are currently known.

Like many eukaryotic LMW-HSPs, SP21 is synthesized in response to a variety of stress conditions and developmental triggers. Developmentally regulated expression of *hspA* during fruiting body formation and indole-induced sporulation has been demonstrated (11). The mechanism by which indole induces sporulation in myxobacteria remains unknown. We speculate that indole either is an analog of a specific signal molecule produced during fruiting body formation or mimicks amino acid starvation by interfering with tryptophan metabolism.

During indole-induced sporulation, SP21 is detected by Western blot analysis 1 to 2 h after addition of indole (11). *hspA* is expressed much faster after heat shock (ca. 5 min after

```
at17   MSLIPSFFGG....RRSNVFDPF....SLDVWDPFKDFPFNNSALSASFP
gm17   MSLVPSFFGG....RRTNVFDPF....SLDVWDPFEGF..LTPGLTNAPA
sp21   MADLSVRRGTGSTPQRTREWDPFQQMQELMNWDPFE....LANHPWFANR
mt14   MATTLPVQR......HPRSLFPEF.....SELFAAFPSF....AGLRPTF.
ml18                 MLMRT...DPF......RELDRF.....AEQVLGT...

at17   RENSAFVSTRVDWKEPEAHVFKADLPGMK.KEEVKVEIEDDRVLQISGER
gm17   KDVAAFTNAKVDWREPEAHVFKADVPGLK.KEEVKVEVEDGNILQISGER
sp21   QGPPAFVPA.FEVREKEAYIFKADLPGVD.EKDIEVTLTGDRV.SVSGKR
mt14   ..DTRLMRLEDEMKER..YEVRAELPGVDPDKDVDIMVRDGQ.LTIKAER
ml18   .SARPAVMPMDAWREEE.FVVEFDLPGIK.ADSLDIDIERN.VVTVRAER

at17   SVEKEDKNDQWHRLERSSGKFMRRFRLPENKMDQVKAAMENGVLTVTVPK
gm17   SSENEEKSDTWHRVERSSGKFMRRFRLPENKVEEVKASMENGVLSVTVPK
sp21   ERERKREESERFYAYERTFGSFSRAFTLPEGDGDNVRADLKNGVLTLTLPK
mt14   TEQKDFDG....RSEFAYGSFVRTVSLPVGDEDDIKATYDKGILTVSVAV
ml18   PGVDPDR..EMLAAERPRGVFNRQLVLGENDTERILASYQEGVLKLSIPV

at17   EEVKKPEVKTIDISG
gm17   VQESKPEVKSIDISG
sp21   RPEVQPKRIQVASSGTEQKEHIKAYPAPAEPGLAAPLGWPGFS
mt14   SEG.KPTEKHIQIRSTN
ml18   AERAKPRKISVD.RGNNGHQTINKTAHEIIDA
```

FIG. 2. Alignment of SP21 and selected LMW-HSPs. at17, *A. thaliana* HSP17; gm17, *Glycine max* HSP17.5E; sp21, *S. aurantiaca* SP21; mt14, *Mycobacterium tuberculosis* 14-kDa antigen; ml18, *Mycobacterium leprae* 18-kDa antigen. Similar amino acids are boxed; identical amino acids are shaded. The alignment was done by using the program Tree (6).

temperature shift) than after indole induction. This agrees with the findings for other bacteria; e.g., maximum induction of heat shock protein synthesis in *Escherichia coli* occurs within a few minutes of exposure to elevated temperature, with a subsequent decline to a steady-state level which reflects the new growth temperature (9, 19).

The different time courses of SP21 production in response to stress situations such as heat shock, anoxia, or starvation (fruiting body formation) may reflect independent pathways connected by a common crossing point at which the expression of *hspA* is modulated. The existence of a linkage of heat shock and myxospore formation has been demonstrated for *S. aurantiaca* (8) and *Myxococcus xanthus*, a closely related myxobacterium. Heat shock prior to chemical induction of development or starvation leads to accelerated and enhanced spore formation. These effects appear to be dependent on the actual expression of heat shock genes, which might sensitize the cells to other stressors to which they subsequently respond more rapidly (15, 20).

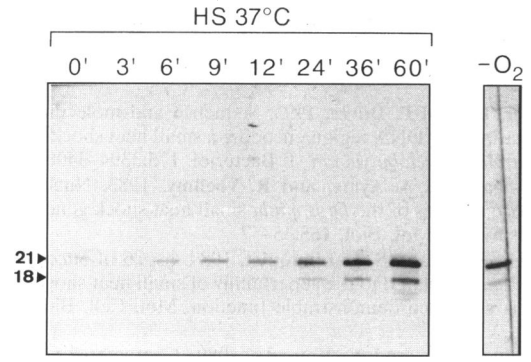

FIG. 3. Western blot analysis of stress-induced *hspA* expression. (Left) The heat shock response of exponentially growing *S. aurantiaca* cells was induced at 37°C, and samples were withdrawn at the time points (minutes) indicated. (Right) An *S. aurantiaca* culture was depleted of oxygen (see text), and a sample was taken after 4 h. The samples were normalized for their protein content. The numbers on the left show molecular weights (in thousands).

The eukaryotic LMW-HSPs are known to form high-molecular-weight complexes, the so-called heat shock granules, during the heat shock response. The purification scheme for SP21 included sedimentation of the protein at 186,000 × *g* within the membrane fraction (11). As the sequence of *hspA* does not indicate a direct interaction of SP21 with the membranes, the ability to sediment SP21 suggests that SP21 also forms aggregates or is organized in larger complexes.

Whereas for mammalian LMW-HSPs a role in the acquisition of thermoresistence has been discussed (2, 16, 24), no biological function could be assigned for the only LMW-HSP of *Saccharomyces cerevisiae* (33). Recent in vitro studies indicate that mammalian LMW-HSP and the alpha-crystallins possess a chaperone-like function in that they prevent thermic aggregation of other proteins (4, 13, 14, 18). Perhaps SP21 has a similar function and stabilizes cellular components during stress and the extensive reconstructing activities accompanying spore formation.

It has been suggested that the LMW-HSPs of plants are involved in the regulation of translation during the heat shock response. Nover et al. (23) showed that the heat shock granules of tomato cells contain a particular subset of mRNAs. These authors propose that the heat shock granules in tomato function as a storage compartment for a subset of mRNAs which will be needed after the heat shock.

The marked homology of SP21 to the LMW-HSPs of plants makes it tempting to speculate on the functional relationship of the proteins. Since SP21 is present in the mature fruiting body, it might help pack mRNAs which will be necessary for

the early steps of germination, or SP21 may protect mRNAs of the housekeeping genes during certain periods of development during which other mRNAs are heavily degraded.

S. aurantiaca may be a very helpful model system, because it combines the simplicity of a prokaryote with remarkable cell-cell interactions and multicellular development. These features may help to elucidate the role of LMW-HSPs in development and may allow detection of their function in cell communication. As the fruiting body consists of a stalk, branches, and sporangioles, it might be possible to address questions of "tissue-specific" expression.

To gain more information about the function of the protein and about its role in fruiting body formation, we are attempting to isolate strains carrying mutations in the SP21 gene.

Nucleotide sequence accession number. The *hspA* sequence has been deposited in the EMBL/GenBank data base under accession no. M94510.

We thank R. Rigg for critically reading the manuscript.

This work was supported by the Bundesministerium für Forschung und Technologie, grant BCTO 381/5, and by the Fonds der Chemischen Industrie.

REFERENCES

1. **Altschul, S., W. Gish, W. Miller, E. W. Meyers, and D. J. Lipman.** 1990. Basic logical search tool. J. Mol. Biol. **215:**403–410.
2. **Arrigo, A. P., and M. R. Michel.** 1991. Decreased heat- and tumor necrosis factor-mediated hsp28 phosphorylation in thermotolerant HeLa cells. FEBS Lett. **282:**152–156.
3. **Czarnecka, E., W. B. Gurley, R. T. Nagao, L. A. Mosquera, and J. L. Key.** 1985. DNA sequence and transcript mapping of a soybean gene encoding a small heat shock protein. Proc. Natl. Acad. Sci. USA **82:**3726–3730.
4. **Dejong, W. W., J. A. M. Leunissen, and C. E. M. Voorter.** 1993. Evolution of the alpha-crystallin/small heat-shock protein family. Mol. Biol. Evol. **10:**103–126.
5. **Dworkin, M., and D. J. Niederprüm.** 1964. Electron transport system in vegetative cells and microcysts of *Myxococcus xanthus*. J. Bacteriol. **87:**316–322.
6. **Feng, D. F., and R. F. Doolittle.** 1987. Progressive sequence alignment as a prerequisite to correct phylogenetic trees. J. Mol. Evol. **25:**351–360.
7. **Gerth, K., R. Metzger, and H. Reichenbach.** 1993. Induction of myxospore formation in Stigmatella aurantiaca (myxobacteria). II. Inducers of spore formation and mutants with a changed sporulation behaviour. J. Gen. Microbiol. **139:**865–871.
8. **Gerth, K., and H. Reichenbach.** 1978. Induction of myxospore formation in Stigmatella aurantiaca I. General characterization of the system. Arch. Microbiol. **117:**173–182.
9. **Gross, C. H., D. B. Strans, 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. Tissieres, and C. Georgopoulos (ed.), Stress proteins in biology and medicine. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
10. **Gyorgyey, J., A. Gartner, K. Nemeth, Z. Magyar, H. Hirt, B. E. Heberle, and D. Dudits.** 1991. Alfalfa heat shock genes are differentially expressed during somatic embryogenesis. Plant Mol. Biol. **16:**999–1007.
11. **Heidelbach, M., H. Skladny, and H. U. Schairer.** 1993. Purification and characterization of SP21, a development-specific protein of the myxobacterium *Stigmatella aurantiaca*. J. Bacteriol. **175:**905–908.
12. **Hickey, E., S. E. Brandon, R. Potter, G. Stein, J. Stein, and L. A. Weber.** 1986. Sequence and organization of genes encoding the human 27 kDa heat shock protein. Nucleic Acids Res. **14:**4127–4145.
13. **Horwitz, J.** 1992. Alpha-crystallin can function as a molecular chaperone. Proc. Natl. Acad. Sci. USA **89:**10449–10453.
14. **Jakob, U., M. Gaestel, K. Engel, and J. Buchner.** 1993. Small heat shock proteins are molecular chaperones. J. Biol. Chem. **268:** 1517–1520.
15. **Killeen, K. P., and D. R. Nelson.** 1988. Acceleration of starvation- and glycerol-induced myxospore formation by prior heat shock in *Myxococcus xanthus*. J. Bacteriol. **170:**5200–5207.
16. **Landry, J., P. Chretien, A. Laszlo, and H. Lambert.** 1991. Phosphorylation of HSP27 during development and decay of thermotolerance in Chinese hamster cells. J. Cell Physiol. **147:**93–101.
17. **Lewin, B.** 1987. Genes III. Wiley & Sons Inc., New York.
18. **Merck, K. B., P. J. T. A. Groenen, C. E. M. Voorter, W. A. Dehaardhoekman, J. Horwitz, H. Bloemendal, and W. W. Dejong.** 1993. Structural and functional similarities of bovine alpha-crystallin and mouse small heat-shock protein—a family of chaperones. J. Biol. Chem. **268:**1046–1052.
19. **Neidhardt, F. C., and R. A. vanBogelen.** 1987. Heat shock response, p. 1334–1345. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
20. **Nelson, D. R., and K. P. Killeen.** 1986. Heat shock proteins of vegetative and fruiting *Myxococcus xanthus* cells. J. Bacteriol. **168:**1100–1106.
21. **Nerland, A. H., A. S. Mustafa, D. Sweester, T. Gedol, and R. A. Young.** 1988. A protein antigen of *Mycobacterium leprae* is related to a family of small heat shock proteins. J. Bacteriol. **170:**5919–5921.
22. **Nover, L., and K.-D. Scharf.** 1991. Heat shock proteins, p. 202–211. CRC Press Inc., Boca Raton, Fla.
23. **Nover, L., K.-D. Scharf, and D. Neumann.** 1989. Cytoplasmatic heat shock granules are formed from precursor particles and are associated with a specific set of mRNAs. Mol. Cell. Biol. **9:**1298–1308.
24. **Oesterreich, S., H. Schunck, R. Benndorf, and H. Bielka.** 1991. Cisplatin induces the small heat shock protein hsp25 and thermotolerance in Ehrlich ascites tumor cells. Biochem. Biophys. Res. Commun. **180:**243–248.
25. **Pearson, W. R., and D. J. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85:**2444–2448.
26. **Petko, L., and S. Lindquist.** 1986. Hsp26 is not required for growth at high temperatures, nor for thermotolerance, spore development, or germination. Cell **45:**885–894.
27. **Plesofski-Vig, N., and R. Brambl.** 1990. Gene sequence and analysis of hsp30, a small heat shock protein of *Neurospora crassa* which associates with the mitochondria. J. Biol. Chem. **265:**15432–15440.
28. **Qualls, G. T., K. Stephens, and D. White.** 1978. Light-stimulated morphogenesis in the fruiting myxobacterium, Stigmatella aurantiaca. Science **201:**444–445.
29. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
31. **Sauer, U., and P. Dürre.** 1993. Sequence and molecular characterization of a DNA region encoding a small heat shock protein of *Clostridium acetobutylicum*. J. Bacteriol. **175:**3394–3400.
32. **Southgate, R., A. Ayme, and R. Voellmy.** 1983. Nucleotide sequence analysis of the *Drosophila* small heat shock gene cluster at locus 67B. J. Mol. Biol. **165:**35–57.
33. **Susek, R. E., and S. L. Lindquist.** 1989. hsp26 of *Saccharomyces cerevisiae* is related to the superfamily of small heat shock proteins but is without a demonstrable function. Mol. Cell. Biol. **9:**5265–5271.
34. **Takahashi, T., and Y. Komeda.** 1989. Characterization of two genes encoding small heat-shock proteins in Arabidopsis thaliana. Mol. Gen. Genet. **219:**365–372.
35. **Verbon, A., R. A. Hartskeerl, A. Schuitema, A. H. J. Kolk, D. B. Young, and R. Lathigra.** 1992. The 14,000-molecular-weight antigen of *Mycobacterium tuberculosis* is related to the alpha-crystallin family of low-molecular-weight heat shock proteins. J. Bacteriol. **174:**1352–1359.