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Archaebacterial heat-shock proteins

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The response to heat shock was examined in seven archaebacterial strains from the genus *Halobacterium*. Upon heat shock each strain preferentially synthesized a limited number of proteins which fell into three narrow mol. wt. ranges. Further examination of the heat-shock response in *H. volcanii* revealed that heat-shock protein (hsp) synthesis was greatest at 60°C. Synthesis of hsps at this induction temperature was both rapid and transient. Cells recovered their normal protein synthesis patterns rapidly upon returning to their normal growth temperature following heat shock. *H. volcanii* cells also responded with a 'heat shock-like' response to salt dilution, a natural environmental stress for these organisms. These results indicate that the heat shock or stress response which is charactertistic of eukaryotic and eubacterial cells is also present among members of the archaebacterial genus *Halobacterium*.

Key words: heat shock/archaebacteria/stress response/evolution

Introduction

The archaebacteria comprise one of the three 'primary kingdoms' of cellular organisms. A variety of evidence suggests that this group is as distinct from the rest of the prokaryotes (the eubacteria) as the latter are from eukaryotes (Woese, 1981), and thus worth intensive investigation by the techniques of comparative molecular biology. We would like to identify a set of genes which are sufficiently conserved as to have identifiable homologues in eubacteria and eukaryotes and whose level of expression can be readily altered by simple environmental manipulation. With such a set, we should be able to extend evolutionary analysis by primary sequence comparison beyond the components of the translation apparatus and begin to identify (by comparative sequencing within the set) likely signals for the initiation and termination of transcription and translation.

We have chosen to look at the response to heat (and salt) shock in the halobacteria, one of the major group of archaebacteria. In eukaryotic cells as diverse as those of humans, plants and yeast, homologous heat-shock proteins (hsps) are made during or after exposure to high temperature, and there is homology between the genes encoding at least one of these conserved eukaryotic hsps and the gene for a thermoinducible eubacterial protein (Craig *et al.*, 1982). In eukaryotes and eubacteria, the expression of heat-shock genes is regulated at the levels of transcription and/or translation in response to temperature and other environmental stresses (for review, see Schlesinger *et al.*, 1982; Tanquay, 1983). Here, we report that halobacteria exhibit a pronounced heat-shock response and suggest that further exploitation of this system is fully justified.

Results

Heat-shock response in halobacteria

We have examined the effect of heat shock on seven halobacterial strains representing a diverse group of species from the genus *Halobacterium*. As in other organisms where a heat-shock response has been described, the response to heat shock in these organisms is characterized by the increased synthesis of a limited number of proteins (hsps). The apparent mol. wts. of the hsps are clustered into three narrow ranges: high (mol. wt. 105 000–75 000), intermediate (mol. wt. 45 000–44 000) and low (mol. wt. 28 000–21 000) (Figure 1). In all of the strains, proteins in the high and low mol. wt. ranges were induced while the intermediate range hsps were found only in the strains *H. trapanicum* and *H. marismortui*. In some strains the similarity between the hsps extends further than these mol. wt. groupings. For example, *H. halobium* and *H. salinarium* have nearly identical patterns of hsps suggesting that these strains and their hsps are closely related. In other strains such close relationships are not observed, but many of these strains do have hsps with similar apparent mol. wts. Further characterization of these hsps will be required to determine whether these proteins are truly related.

H. volcanii hsps

For further analysis of the heat-shock response in halobacteria, we chose *H. volcanii* since its pattern of heat-induced protein synthesis was particularly striking. The temperature response of *H. volcanii* is shown in Figure 2. At temperatures of 42°C or higher there is an increase in the incorporation of label into all cellular proteins, with a pronounced induction of a small subset of these proteins. The induction of all five hsps proceeds in parallel, with the maxima occurring at 60°C. A similar temperature response was observed for the other strains (data not shown).

From one-dimensional gel analyses (Figures 1 and 2) we were unable to determine whether hsps were synthesized at the control temperature. To examine this question, *H. volcanii* cellular proteins were labeled at 37°C and 60°C and analyzed by two-dimensional gel electrophoresis (Figure 3). From these data it was apparent that the heat-shock reponse in these cells was more complex than suggested from the one-dimensional gel analysis. Proteins which corresponded to the major hsps were observed in the cells labeled at 37°C (Figure 3A), indicating that these proteins are synthesized under normal conditions. Moreover, the enhanced resolution of the two-dimensional gel indicated that the 98 000 hsp was composed of two distinct proteins with differing pIs (Figure 3B, proteins 1 and 2). Similarly, the 85 000 hsp appeared to have a second minor component (Figure 3B, protein 3). Three new minor hsps, not resolved by the one-dimensional gels, were also detected (figure 3B, proteins 4, 5 and 6). The effect of

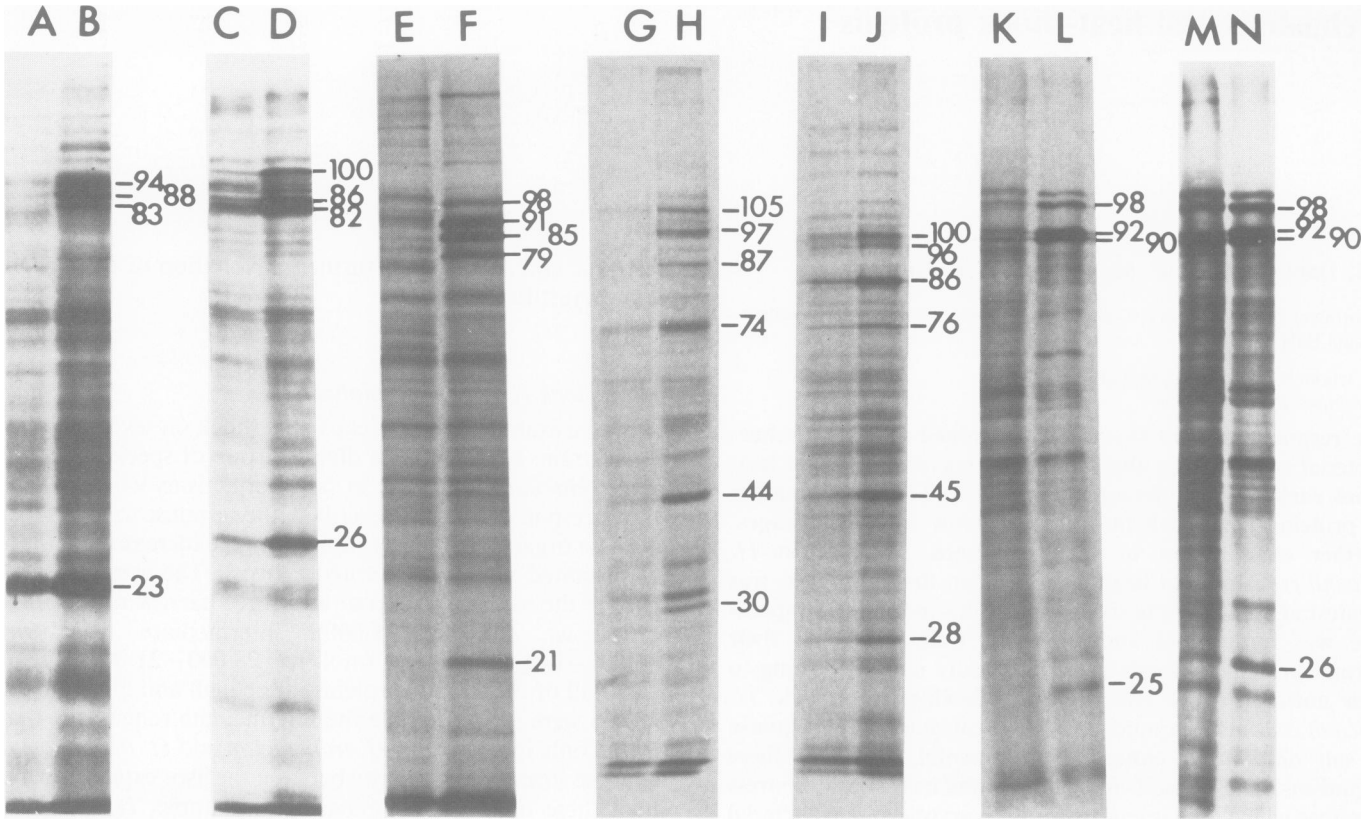

Fig. 1. Heat-shock response of the various halobacterial strains. Cells of each strain were labeled with [^{35}S]methionine for 60 min at 37°C (**lanes A,C,E,G,I,K** and **M**) or 60°C (**lanes B,D,F,H,J,L** and **N**): **lanes A** and **B**, R-4; **lanes C** and **D**, Y-27; **lanes E** and **F**, *H. volcanii*; **lanes G** and **H**, *H. trapanicium*; **lanes I** and **J**, *H. marismortui*; **lanes K** and **L**, *H. halobium*; and **lanes M** and **N**, *H. salinarium*. Labeled proteins were analyzed by SDS-polyacrylamide gel electrophoresis. The apparent mol. wts. (x 10^{-3}) of the hsps are indicated.

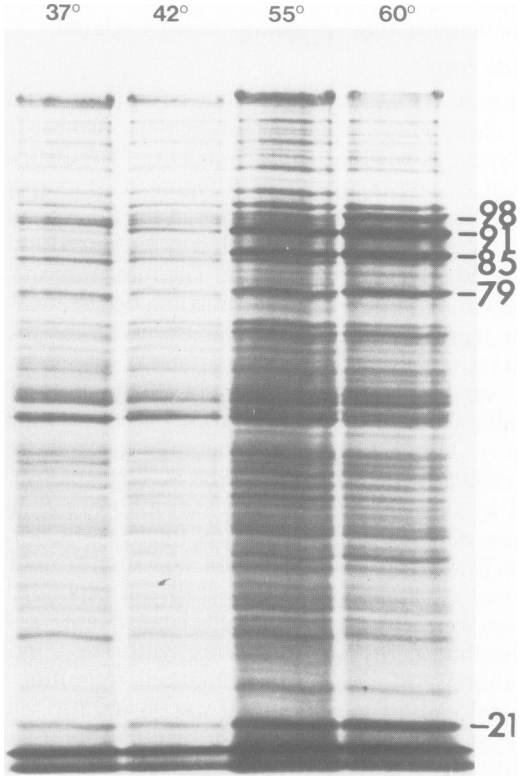

Fig. 2. Temperature dependence of the heat-shock response in *H. volcanii*. Cells suspended in *H. volcanii* salts + 1% MAM were pre-incubated for 3 min at the indicated temperatures. Cellular proteins were then labeled for 60 min with [^{35}S]methionine at these temperatures. The apparent mol. wts. (x 10^{-3}) of the hsps are indicated.

heat shock on the synthesis of other cellular proteins was more obvious in these gels as well. Many cellular proteins were synthesized at decreased rates or not at all at 60°C, while others remained unaffected.

Induction and reversal of the heat-shock response in H. volcanii

The response of *H. volcanii* cells to heat shock was rapid. When cells were shifted to 60°C and pulse-labeled, hsps were observed as major labeled species after 15 min of exposure to the induction temperature (Figure 4). The synthesis of hsps continued to increase with time of exposure to the heat stress, reaching a maximum at 75 min. Further incubation led to a decreased synthesis of hsps. During the initial phase of the heat-shock response, the labeling of normal cellular proteins was reduced. Recovery from this initial decrease in labeling occurred at later times when the synthesis of hsps had reached its maximum.

Cells also responded quickly when they were returned to 37°C following a short heat pulse. When *H. volcanii* cells were heat pulsed at 60°C for 15 min and returned to 37°C, recovery of the synthesis of normal cellular proteins began immediately (Figure 5). After 135 min of incubation at 37°C, the synthesis of normal cellular proteins returned to control levels. The synthesis of hsps continued during this recovery period and decreased at a slow rate, with detectable levels of the hsps continuing to be synthesized after 135 min at 37°C. The drop in synthesis of the individual hsps appeared to proceed coordinately.

Effects of salt dilution on H. volcanii

Both chemical and environmental stresses have been shown to

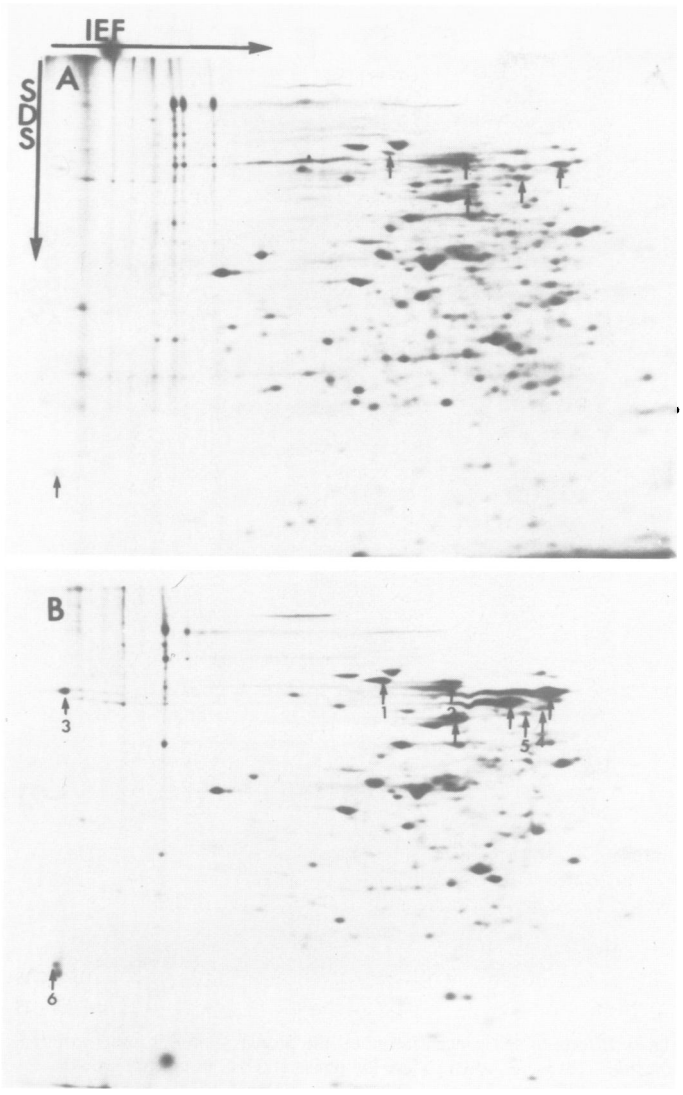

Fig. 3. Two-dimensional gel electrophoresis of *H. volcanii* hsps. *H. volcanii* cellular proteins were labeled for 60 min at 37°C **(panel A)** or 60°C **(panel B)**. The positions of hsps are indicated by arrows. Proteins marked 1–6 are hsps which were not resolved by one-dimensional gel electrophoresis.

elicit a 'heat shock-like' response in a number of organisms (Ashburner and Bonner, 1979; Tanquay, 1983). From these studies it has become apparent that heat shock is representative of a general stress response. Since salt dilution is likely to be a natural stress for the halophilic bacteria, we examined the effects of salt dilution on the protein-labeling patterns of the moderate halophile, *H. volcanii*. This strain is particularly well suited for these experiments since it does not lyse immediately in low salt, as do the extreme halophiles. Figure 6 shows the effect of diluting the salt component of the labeling mixture on the labeling of protein at 37°C. Like that observed during heat shock, many changes in the labeling pattern were observed when the salt concentration was lowered. With decreasing salt concentration, three proteins exhibited increased levels of labeling. Two of these proteins (mol. wt. 91 000 and mol. wt. 79 000) co-migrated with two of the hsps. The induction of the third protein (mol. wt. 17 000) was unique to the salt dilution stress and was not observed during heat shock.

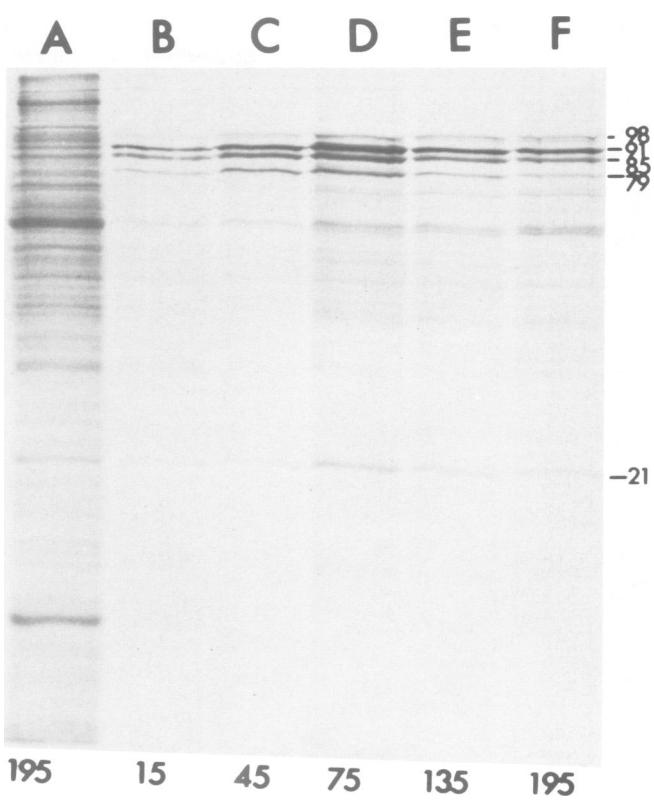

Fig. 4. Time course for the induction of hsp synthesis in *H. volcanii*. Equal portions of cells in salt + 1% MAM were incubated at 37°C **(lane A)** or 60°C **(lanes B–F)**. The cellular proteins were pulse-labeled with [^{35}S]methionine for 15 min at these temperatures. The times indicated are the ends of the various labeling periods. The positions of the major hsps are indicated.

Discussion

The data are now rather compelling for the existence of a common response to stress among all organisms, characterized by a rapid increase in the synthesis of a few proteins. We have demonstrated a similar response in members of the genus *Halobacterium*. When challenged with heat shock, cells from each of the seven strains examined induced the synthesis of a limited number of proteins. The apparent mol. wts. of these proteins fell into three narrow ranges: 105 000–75 000, 45 000–44 000 and 28 000–21 000. The stress response was examined further in *H. volcanii* and found to have the following characteristics: (i) the level of induction of the stress proteins is dependent on the severity of the stress, whether stress is in the form of heat shock or salt dilution, (ii) cells respond quickly to stress, inducing the synthesis of hsps immediately upon exposure to heat, (iii) synthesis of the hsps is transient at high temperatures, reaching a maximum at 75 min, (iv) cells begin to recover their normal cellular protein synthesis pattern immediately when returned to 37°C after a heat shock and (v) stress in the form of salt dilution induces the synthesis of three proteins, two of which have apparent mol. wts. similar to the hsps.

The observations that certain chemicals and environmental stresses other than heat shock induce hsps, and that synthesis and turnover of hsps are influenced by the metabolic state of the cell (Linquist *et al.*, 1982; Lanks, 1983) point towards a more central role for these proteins in cellular homeostasis. The major hsps of *H. volcanii* may also have a function under

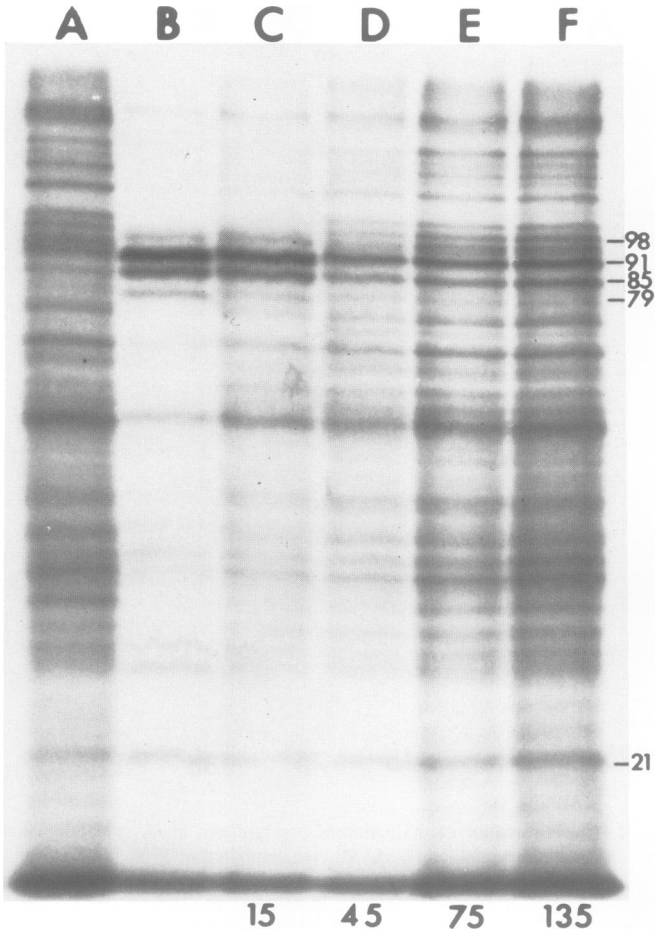

Fig. 5. Recovery from heat shock in *H. volcanii*. Cells in salts + 1% MAM were heat treated at 60°C for 15 min **(lanes B–F)** and returned to 37°C. Cellular proteins were labeled for 15 min with [^{35}S]methionine. The indicated times represent the ends of the labeling periods relative to the time at which cells were shifted to 37°C. **Lanes A** and **B** are control patterns for the pulse labeling of cells at 37°C and 60°C, respectively. Cells in **lane A** were labeled at the same time as the final 37°C recovery time point. Cells in **lane B** were labeled during the initial heat shock period at 60°C. Positions of the major hsps are indicated.

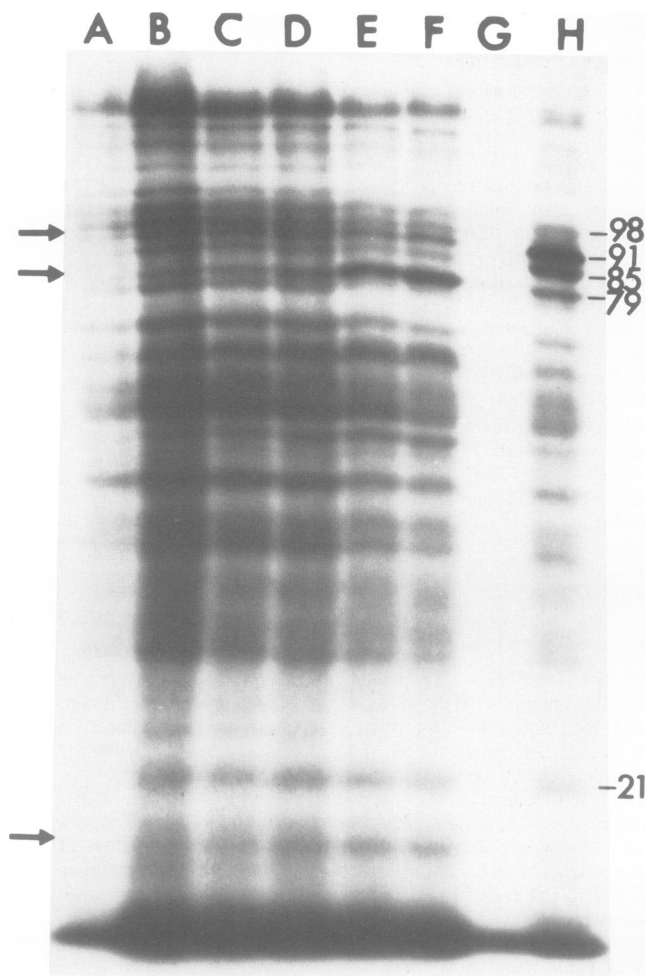

Fig. 6. Effects of salt concentration on the protein synthesis pattern of *H. volcanii*. Cells were grown to late log phase at 37°C in normal growth medium. Cells from 200 μl of culture were isolated, washed with normal *H. volcanii* salts + 1% MAM and resuspended in various dilutions of *H. volcanii* salts containing 1% MAM. Cellular proteins were pulse-labeled at 37°C for 15 min with [^{35}S]methionine and the proteins analyzed by SDS-polyacrylamide gel electrophoresis. **Lane A**, 100% salts; **lane B**, 90% salts; **lane C**, 80% salts; **lane D**, 70% salts; **lane E**, 60% salts; **lane F**, 50% salts; **lane G**, 25% salts; and **lane H**, 60°C heat-shock control. The positions of the major hsps are shown. Arrows indicate cellular proteins which are induced by salt dilution.

normal growth conditions since most are synthesized at detectable levels in the absence of stress. The apparent induction of some hsps by another environmental stress, salt shock, may be an indication that each hsp protein has a unique function and that various stresses preferentially induce the synthesis of only a subset of the 'stress-inducible' proteins.

A number of hsps have recently been shown to be conserved over a wide evolutionary span from mammals to eubacteria (Kelly and Schlesinger, 1982; Ingolia *et al.*, 1982; Voellny *et al.*, 1983; Craig *et al.*, 1982). Although similarities in apparent mol. wts. of halobacterial hsps and hsps described for other organisms (Schlesinger *et al.*, 1982) are insufficient to establish evolutionary homology, we have detected DNA sequences related to the *Saccharomyces cerevisiae hsp*70 gene and the related *Escherichia coli dna*K gene in halobacterial genomes by Southern analysis (C.J. Daniels, unpublished observation).

From these initial studies we conclude that a heat-shock or stress response similar to that shown to occur in eukaryotes and eubacteria, is common to members of the archaebacterial genus *Halobacterium*. Further characterization of the genes for the halobacterial hsps is necessary to establish the evolutionary relatedness among hsps from the three kingdoms. It will also provide a useful system for examining gene expression and its regulation in this unique group of organisms.

Materials and methods

Cells and media

The strain *H. salinarium* and two Spanish isolates, Y-27 and R-4, were obtained from M. Kates (University of Ottawa). The strains *H. trapanicium* NCMB 784 and *H. marismortui* were obtained from G.E. Fox (University of Houston). *H. halobium* NRC-1 was obtained from R.D. Simon (University of Rochester) and *H. volcanii* from C.R. Woese (University of Illinois).

H. halobium, H. salinarium, H. trapanicum and *H. marismortui* were grown in a medium containing (per litre): 250 g NaCl, 20 g $MgSO_4.7H_2O$, 3 g Na_3citrate, 2 g KCl, 0.2 g $CaCl_2.2H_2O$, 3 g yeast extract (Difco) and 5 g tryptone (Difco) (Gochnauer and Kushner, 1969). R-4 and Y-27 were grown in medium containing (per litre): 194 g NaCl, 16 g $MgCl_2.6H_2O$, 24 g $MgSO_4.7H_2O$, 1 g $CaCl_2.2H_2O$, 5 g KCl, 0.2 g $NaHCO_3$, 0.5 g NaBr and 10 g yeast extract (Rodriquez-Valera *et al.*, 1980a, 1980b). *H. volcanii* was grown in a medium containing (per litre): 125 g NaCl, 45 g $MgCl_2.6H_2O$, 10 g $MgSO_4.7H_2O$, 10 g KCl, 1.34 g $CaCl_2.2H_2O$, 3 g yeast extract and 5 g tryp-

tone. All strains were grown at 37°C with illumination on a shaking platform (250 r.p.m.).

Labeling of cellular proteins

In all experiments, cells were grown to late log phase (~5 x 10^8 cells/ml). Cells from 200 μl of culture were washed once with 500 μl of a solution containing the respective complete medium in which yeast extract and tryptone were replaced with 1% (w/v) methionine assay mix (Difco) (salts + MAM). Cells were then resuspended in 100 μl of their appropriate salts + MAM mixture. To examine the heat-shock response in the halobacterial strains, portions of cells (100 μl) were pre-incubated at 37°C or 60°C for 3 min. Labeling of cellular proteins was initiated with the addition of 50 μCi of [^{35}S]-methionine (New England Nuclear, 1200 Ci/mmol). Incubations were continued for 60 min. At the end of the incubation period cells were isolated by centrifugation and lysed with the addition of 20 μl of 20 mM Tris-HCl pH 7.5 containing 20 μg/ml RNase and 1.0 μg/ml DNase. Lysates were incubated at 10°C for 10 min. An equal volume of 2 x Laemmli sample buffer (Laemmli, 1970) was added and the solutions were boiled for 5 min. For two-dimensional gel electrophoresis, labeled cells were lysed in 50 μl of lysis solution (O'Farrell, 1975) containing a pH 5–7 mixture of ampholines.

In the *H. volcanii* time course experiments, equal portions (100 μl) of washed cells were incubated at 37°C or 60°C for 3 min. Cells were then pulse-labeled at these temperatures with [^{35}S]methionine for 15 min at the times indicated. Cells were processed for electrophoresis as described above. In the *H. volcanii* heat-shock recovery experiment, equal portions (100 μl) of washed cells were pre-incubated at 60°C for 15 min and then returned to 37°C. Cells were then pulse-labeled with [^{35}S]methionine for 15 min at the indicated times after their return to 37°C, and processed for electrophoresis as above.

Gel electrophoresis

One-dimensional gel electrophoresis was carried out on 8% SDS-polyacrylamide gels (Laemmli, 1970) containing 0.5% (w/v) linear polyacrylamide. In all experiments, equal volumes of labeled extracts were loaded onto the gels. Two-dimensional gel electrophoresis was performed using a pH 5–7 isoelectric focusing gel as described by O'Farrell (1975). Gels were fixed and stained in a solution containing 50% (v/v) methanol, 10% (v/v) acetic acid and 0.1% (w/v) Coomassie Blue. De-stained gels were treated with Enhance (New England Nuclear), dried and exposed to Kodak XAR-5 film at −70°C using DuPont Lightning Plus intensifying screens. Purified *E. coli* RNA polymerase (beta, beta'-subunits 160 000 and 150 000), phosphorylase *b* (93 000), bovine serum albumin (68 000), ovalbumin (43 000), trypsinogen (24 000) and lysozyme (14 300) were used as mol. wt. markers.

Acknowledgements

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References

Ashburner,M. and Bonner,J.J. (1979) *Cell,* **17**, 241-254.

Craig,E.A., Ingolia,T.D., Slater,M.R., Manseau,L. and Bardwell,J. (1982) in Schlesinger,M.J., Ashburner,M. and Tissieres,A. (eds.), *Heat Shock from Bacteria to Man,* Cold Spring Harbor Laboratory Press, NY, pp.11-18.

Gochnauer,M.B. and Kushner,D.J. (1969) *Can. J. Microbiol.,* **15**, 1157-1163.

Ingolia,T.D., Slater,M.R. and Craig,E.A. (1982) *Mol. Cell Biol.,* **2**, 1388-1398.

Kelly,P.M. and Schlesinger,M.J. (1982) *Mol. Cell Biol.,* **2**, 267-274.

Laemmli,U.K. (1970) *Nature,* **227**, 680-685.

Lanks,K.W. (1983) *Proc. Natl. Acad. Sci. USA,* **80**, 5325-5329.

Linquist,S., DiDomenico,B., Bugaisky,G., Kurtz,S., Petko,L. and Sonoda, S. (1982) in Schlesinger,M.J., Ashburner,M. and Tissieres,A. (eds.), *Heat Shock from Bacteria to Man,* Cold Spring Harbor Laboratory Press, NY, pp. 167-175.

O'Farrell,P.H. (1975) *J. Biol. Chem.,* **250**, 4007-4021.

Rodriquez-Valera,F., Ruiz-Berraquero,F. and Ramos-Cormenzana,A. (1980a) *J. Gen. Microbiol.,* **119**, 535-538.

Rodriquez-Valera,F., Ruiz-Berraquero,F. and Ramos-Cormenzana,A. (1980b) *Can. J. Microbiol.,* **26**, 1259-1263.

Schlesinger,M.J., Ashburner,M. and Tissieres,A., eds. (1982) *Heat Shock from Bacteria to Man,* published by Cold Spring Harbor Laboratory Press, NY.

Tanquay,R.M. (1983) *Can. J. Biochem. Cell Biol.,* **61**, 387-394.

Voellny,R., Bromley,P. and Kochner,H.P. (1983) *J. Biol. Chem.,* **258**, 3516-3522.

Woese,C.R. (1981) *Sci. Am.,* **244(6)**, 94-106.

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