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Stress Genes and Proteins in the Archaea

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INTRODUCTION

The purpose of this review is to examine the information available on archaeal stress genes and proteins, particularly those of the Hsp70 and Hsp60 families, while critically discussing the data in comparison with what is known for the bacterial and eukaryotic equivalents. The aim was to treat the specific topics of the review embedded in the framework of closely related areas of science. Cross-fertilization between research with archaea and research with bacteria and eukaryotes is highlighted to show how the study of archaea has contributed, and will continue to contribute, to other fields, both basic and applied.

A deliberate effort has been made to simplify the text and make it readable to a general audience. Consequently, terms are explained and the data and theories are presented within a historical perspective. A minimal amount of overlapping between related sections distant from one another in the body of the review is included to enhance the flow, particularly when a later section expands on an earlier one.

A comprehensive search of printed literature and databases was attempted. Colleagues were consulted. The majority of the data are displayed in tables and figures, but only illustrative cases are explained in the text. Reviews rather than original reports are cited for topics related to but not strictly dealing with archaeal genes, proteins, or organisms, to reduce the number of references and save space while providing access to a wealth of published information.

Archaea have been found in a wide variety of ecosystems with very different characteristics, very hot or very cold, temperate, anoxic, oxygenated, etc. (9, 12, 50, 59, 178, 297). Thus, what represents a stressor for a species may be a condition required for the optimal growth of another species. The term "stressor," therefore, must be understood in relation to a particular species or group of organisms that share similar living conditions, for example temperature. In this regard, organisms are classified into psychrophiles (optimal temperature for growth [OTG] 15°C or lower), psychrotolerant organisms (OTG, 20 to 30°C), mesophiles (OTG, 35 to 40°C), thermophiles (OTG, 50 to 70°C), and hyperthermophiles (OTG, 80°C or higher) (186). Temperatures higher or lower than the optimal may cause stress and induce a stress response. A temperature upshift causes a heat shock response (47, 90, 120, 128, 177, 252, 292), whereas a temperature downshift induces a cold stress or cold shock response (255, 275, 304). The latter is not dealt with in this review. Likewise, adaptation to high osmotic and barometric pressures and the response of the cell to their changes (33, 68, 77, 121, 138, 190, 201, 204, 232, 266, 271) are not treated in any detail.

We draw attention, though, to the formation of multicellular structures that result in improved cell resistance to physical, mechanical, and chemical stressors. These structures are of various types and have considerable potential for the biotechnology industry and for the exploration and conquest of inhospitable ecosystems, but their relevance to stress resistance is rarely discussed. We highlight the topics that future studies ought to address in relation to the proteins (and their genes) and other molecules that build the intercellular connective material to keep the cells together in a functional, three-dimensional arrangement.

STRESS, STRESS RESPONSE, STRESS GENES AND PROTEINS, HEAT SHOCK, MOLECULAR CHAPERONES, AND CHAPERONINS

Primer

A cell confronted with an abrupt change in its immediate surroundings suffers stress. The cause, or stressor, may be of various types, for example, physical (temperature elevation) or chemical (increase or decrease in pH, salinity, or oxygen concentration) (47, 113, 252, 292).

A key component of stress is protein denaturation (93, 96, 123, 124, 223, 293). Many proteins lose their native, functional configuration and tend to aggregate. The process may be reversible up to a degree, beyond which it becomes irreversible and generalized within the cell, which ultimately dies.

Another main component of stress is the down-regulation of many housekeeping genes, some of which are actually shut off. Whether this is all due to protein denaturation and represents just the breakdown of the cellular machinery or is an active, induced process by which genes are "told" to slow down or stop has not yet been elucidated. Perhaps both mechanisms, gene failure and regulated shutdown, participate.

Protein damage and gene down-regulation are part of the stress response. There is yet another important component of the stress response, i.e., activation of the stress genes (90, 120, 205, 208, 219, 253, 254, 279). The concentrations of the protein products of these genes increase in response to stressors, protecting the cell from the destructive effects of stress and enhancing post-stress recovery by promoting renaturation (refolding) of partially denatured proteins (93, 101, 223, 293).

Thus, stress inactivates or down-regulates many genes but activates others, whose function is to save the cell. Most stress genes also function in the absence of stress, namely, under normal physiological conditions. The proteins encoded by these genes play critical roles in physiological protein biogenesis. They assist in the folding, translocation, and assembly of other proteins (191, 214, 235, 236, 247). This is the reason why many stress proteins are also called molecular chaperones (72). They help other cellular proteins to (i) fold correctly during and after translation; (ii) migrate to the cell's locale, where they will reside and function; and (iii) assemble into the quaternary structure that will make them useful to the cell when the proteins function as polymers.

Furthermore, some stress proteins participate in the degradation of other polypeptides, for example when these are denatured beyond recovery and could pose a serious threat to the cell if they aggregated (39, 92, 97, 98, 122, 123, 133).

In summary, stress proteins, particularly those that are molecular chaperones, aid and protect other cellular proteins from their birth on, but they also contribute to the elimination of polypeptides that are no longer useful and endanger cell viability.

It is important to bear in mind that not all stress proteins are chaperones and, vice versa, that not every molecular chaperone is a stress protein.

The wide spectrum of activities of stress proteins is not limited to the chaperoning of other proteins as described above. These activities also include other functions, for example, modulation of their own synthesis (6, 20, 90), regulation of the stress kinase JNK (85), association with enzymes (for purposes yet to be determined) (43), and participation in signal

TABLE 1. Classification of Hsp into families according to their molecular mass

Name[a]	Mass (kDa)	Found in archaea
Heavy (high mol wt, Hsp100)	100 or higher	No[b]
Hsp90	81–99	No
Hsp70 (chaperones)	65–80	Yes
Hsp60 (chaperonins)	55–64	Yes
Hsp40(DnaJ)	35–54	Yes
Small Hsp (sHsp)	<35	Yes
Other (proteases, etc.)	Various	Yes

[a] Synonyms are given in parentheses. For additional information and a brief history, see references 103, 177, and 235 and references therein.

[b] Not yet investigated, or investigated but not yet found, or found but incompletely characterized (see also references 47 and 152).

transduction pathways (175) and in rRNA processing (249). It is therefore clear that stress proteins are multifunctional and ubiquitous. They play their roles in all cells, cell compartments, and organelles and are said to be promiscuous because they interact with a great variety of other molecules.

This diversity of functions is reflected in the structural features of the stress proteins, which are composed of domains and motifs with specific roles. As we discuss below, characterization of these domains and motifs has helped in the classification of newly found genes and proteins, identification of stress proteins in their various anatomical locations, and determination of their evolutionary origins.

Stress versus Heat Shock

Stress genes and proteins are often named heat shock genes and proteins in today's literature, for historical reasons. Because of this, they are represented by the acronyms *hsp* and Hsp, respectively.

hsp and Hsp were first observed in *Drosophila* exposed to a temperature higher than the optimum for growth (27°C) (reference 177 and references therein). The genes activated by the temperature upshift were called heat shock genes, and their products were called heat shock proteins. In this review, we use the terms "stress" and "heat shock" interchangeably to qualify the words response, gene, and protein, although we favor the use of "stress" rather than "heat shock" and reserve the latter for the specific instances in which the stressor is a temperature elevation.

Hsp (and their genes) are classified into groups or families according to their molecular mass in kilodaltons (Table 1). The proteins of the 55- to 64-kDa group, or Hsp60 family, are also called chaperonins and are included within the molecular chaperones, generally speaking. More specifically, the latter term is applied to the Hsp70 family. The genes and proteins belonging to the Hsp60 and Hsp70 families have been extensively studied in many bacterial and eukaryotic species.

Phylogenetic Domains

The classification of all living cells into three main evolutionary lines, or phylogenetic domains, *Bacteria* (eubacteria), *Archaea* (formerly archaebacteria), and *Eucarya* (eukaryotes) (11, 297, 298, 300), is still useful despite its limitations and the challenges generated by new findings and contrasting theories (66, 67, 80, 103, 105, 106, 109–111, 188, 189, 192, 198, 234, 286, 298). It helps us to visualize how evolution produced what we see today and to track down genes from the past to the present.

The overwhelming majority of information available on stress genes and proteins comes from studies of bacteria (e.g., *Escherichia coli* and *Bacillus subtilis*) and eukaryotes (e.g., *Drosophila melanogaster*, *Saccharomyces cerevisiae*, plants, and mammals including humans). The study of stress genes and proteins in organisms of the phylogenetic domain Archaea began only a decade ago and is much less advanced than in the other two domains.

hsp70(*dnaK*) LOCUS

Structure and Organization

The terms *hsp70* for the gene and Hsp70 for the protein are used for eukaryotes, while the same gene and protein are called *dnaK* and DnaK, respectively, in bacteria. We use *hsp70* and Hsp70 in most cases, regardless of the origin, for simplicity and because these terms are more widely known than *dnaK* and DnaK, and also because archaea are not bacteria.

The first *hsp70* gene identified by cloning and sequencing within the domain Archaea was reported in 1991 (182). The gene was found in the mesophilic methanogen *Methanosarcina mazei* S-6 (OTG, 37°C). Shortly thereafter, in 1992, a homolog was cloned and sequenced from another archaeon, *Halobacterium* (*Haloarcula*) *marismortui* (110). This organism is also mesophilic but belongs to a group, the extreme halophiles, different from that of *M. mazei* S-6.

For a while, the above genes were the only two archaeal *hsp70* genes known. In 1994, two additional homologs were reported: one in another mesophilic extreme halophile, *Halobacterium cutirubrum* (OTG, 45°C), and the other in a thermophile, *Thermoplasma acidophilum* (OTG, 55°C) (111).

These findings seemed to indicate that the *hsp70* gene was present in archaea, confirming the widely held notion that this gene is one of the most highly conserved, occurring in all organisms. This idea was challenged in 1996, when the sequencing of the whole genome of *Methanococcus jannaschii* (OTG, 85°C) revealed the absence of the *hsp70* gene in this archaeon (31), a result that confirmed observations in other laboratories (47, 164).

More recently, in 1997, the sequencing of the thermophilic methanogen *Methanobacterium thermoautotrophicum* ΔH (OTG, 55°C) was published (263). The *hsp70* gene is present in this methanogen, which in this regard is therefore similar to the mesophilic *M. mazei* S-6 but different from the hyperthermophilic methanogen *M. jannaschii*.

While the sequencing of the *M. thermoautotrophicum* ΔH was under way, another *hsp70* gene was cloned and sequenced from a second *Methanosarcina* species, the thermophilic species *Methanosarcina thermophila* TM-1 (OTG, 50°C) (126). So by this time, it was clear that at least some mesophilic and thermophilic methanogens do have the gene but that some (perhaps all) hyperthermophiles do not.

After the discovery of an archaeal *hsp70* gene in *M. mazei* S-6 in 1991, more sequencing up- and downstream of this gene revealed the other genes that accompany *hsp70*(*dnaK*) in bacteria: *hsp40*(*dnaJ*) and *hsp23*(*grpE*); these discoveries were made in 1993 (181) and 1994 (45), respectively.

It is pertinent to note here, as was done above for *hsp70*, that the *hsp40* gene and its protein Hsp40 are named *dnaJ* and DnaJ, respectively, when they are from bacteria. Similarly, the bacterial *hsp23* gene and its protein, Hsp23, are called *grpE* and GrpE, respectively.

We use the terms *hsp40* and Hsp40 when referring to archaea, for the same reasons we use the terms *hsp70* and Hsp70. However, for the *hsp23* gene, we use the terms *grpE* and GrpE, because the alternative *hsp23* and Hsp23 may be confusing since there are several different small heat shock proteins with

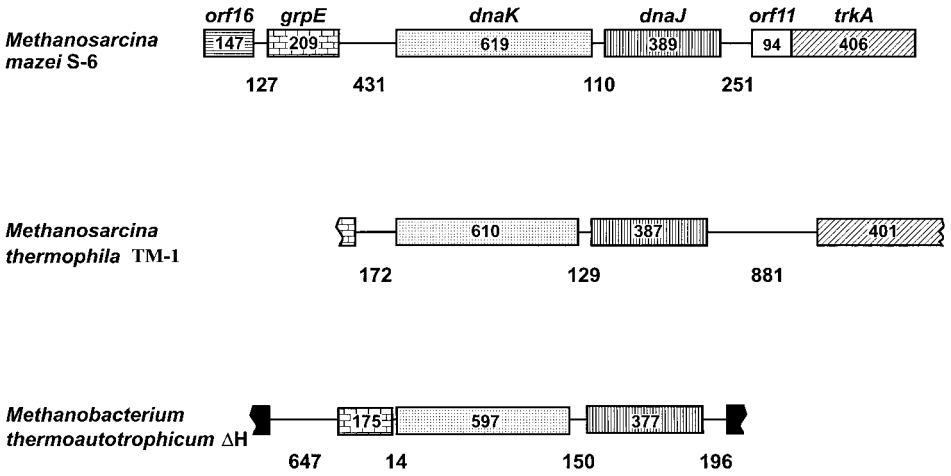

FIG. 1. The *hsp70*(*dnaK*) locus genes of the archaea for which sequences are available, including genes up- and downstream of *hsp70*(*dnaK*). The genes are represented by rectangular boxes from the 5′ to the 3′ end (left to right), with their names above their respective boxes in the locus on top [*dnaK* and *dnaJ* are used instead of *hsp70*(*dnaK*) and *hsp40*(*dnaJ*) for clarity]. The numbers within the boxes indicate the number of amino acids encoded. The lines joining the boxes represent the intergenic regions, with their lengths, in base pairs, shown underneath. The sequences of *M. thermophila* TM-1 *grpE* and *trkA* are still incomplete (what is available would encode 53 and 401 amino acids, respectively). Accession numbers and other details are provided in Tables 2, 5, and 10. Reprinted from references 126 and 179 with permission of the publishers.

a mass close to 23 kDa (76, 78, 156, 206, 227). Also, the archaeal GrpE molecule has a counterpart in bacteria and the eukaryotic organelles of bacterial origin but apparently not in the eukaryotic cytosol. The latter does not seem to have a GrpE protein but has some other alternative to exercise similar functions, although recent findings suggest that *grpE* homologs might also occur in the eukaryotic cytosol (211, 212).

As a result of the sequencing of the *M. mazei* S-6 and *M. thermophila* TM-1 *hsp70* chromosomal regions and the sequencing of the *M. thermoautotrophicum* ΔH genome, there are today three archaeal *hsp70* loci whose structure and organization have been determined (Fig. 1) (31, 126, 179). The gene order 5′-*grpE-hsp70-hsp40*-3′ occurs in the three archaeal loci and is the same as that observed in many bacteria, particularly gram-positive bacteria (Fig. 2) (179). However, there are differences between the three archaeal loci. For example, they differ in the length of the 5′-*grpE-hsp70*-3′ and 5′-*hsp40*-next gene-3′ intergenic regions and in the gene that follows *hsp40* downstream. This gene is the same in *M. mazei* S-6 and *M. thermophila* TM-1 but different in *M. thermoautotrophicum* ΔH.

The length of the intergenic region between *hsp70* and *hsp40* is conserved in the three loci, particularly in comparison with the other intergenic regions.

The meaning of these structural characteristics is not completely understood. They suggest, for example, that *hsp70* and *hsp40* may have evolved together, as a unit. This notion is also supported by the conservation of the homologous gene pair in many bacteria (see, for example, Fig. 2).

As discussed later in this review, there are indications that the *hsp70* gene in archaea was received via lateral transfer from bacteria. Perhaps it was accompanied by *hsp40* and *grpE*, since both are always present whenever *hsp70* is, and the three genes appear next to each other in many bacteria. However, other structural characteristics and experimental results, to be discussed below (see "Occurrence of *hsp70* in nature"), tend to make this notion less credible, at least in its simplest formulation. Analyses of the nucleotide sequences between the protein-coding regions of the genes do not reveal obvious similarities, except for the presence of putative archaea-type promoters and bacterial-type termination signals in the ex-

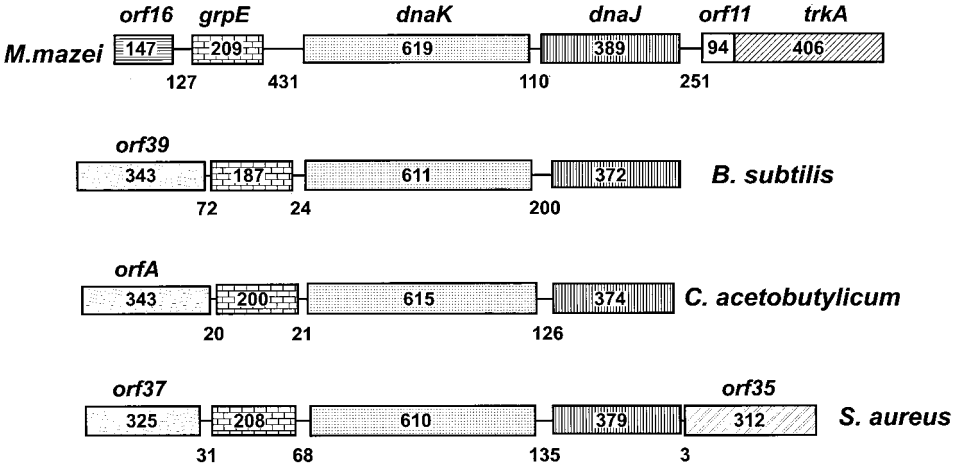

FIG. 2. The *hsp70*(*dnaK*) locus genes of the archaeon *M. mazei* S-6 and three gram-positive bacteria: *B. subtilis* (M84964), *C. acetobutylicum* (M74569), and *S. aureus* (D30690). Symbols are the same as those described in the legend to Fig. 1. Modified from reference 179 with permission of the publisher.

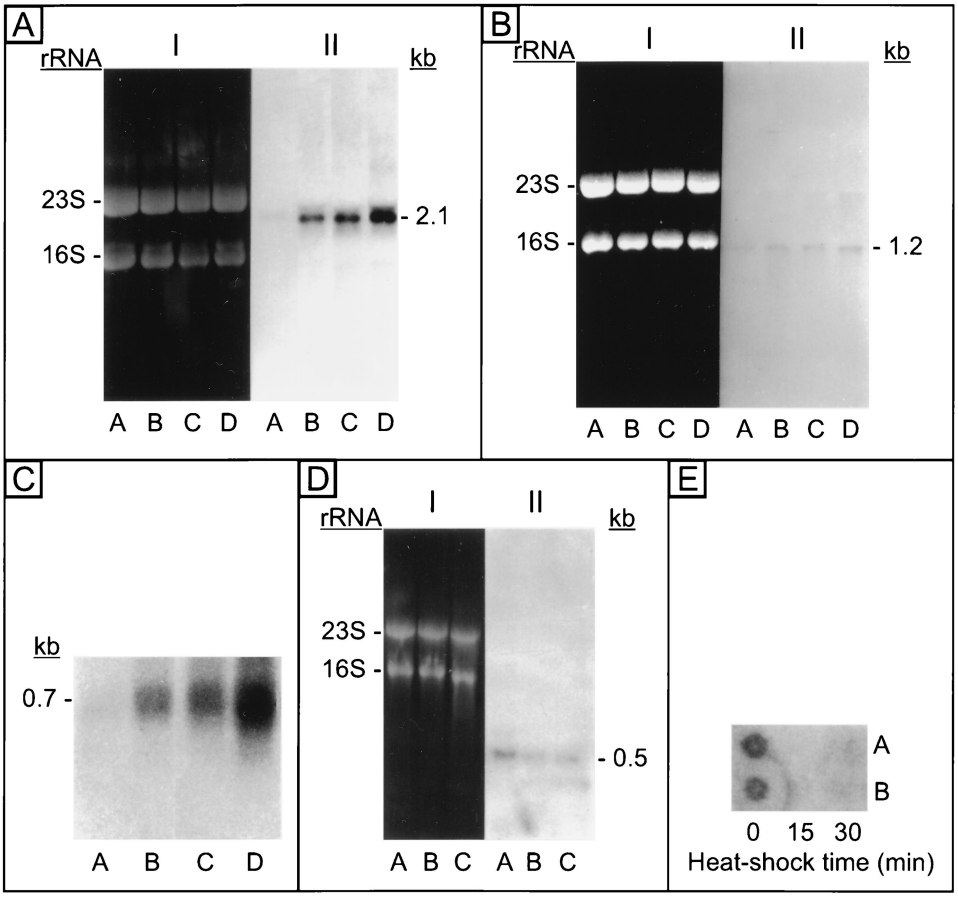

FIG. 3. (A to D) Northern blots with *M. mazei* S-6 total RNA (10 μg/lane) showing an increase in the levels of transcripts of *hsp70*(*dnaK*) (A), *hsp40*(*dnaJ*) (B), and *grpE* (C), and a decrease in the level of the transcript of *orf16* (D) in response to heat shock. (E) Dot blot showing a decrease in the level of the transcript of *orf11-trkA* in response to heat shock. Hybridizations were done in all cases with radiolabelled probes specific for the respective genes. In panels A, B, and D, I is the gel stained with ethidium bromide showing the RNAs, 23S and 16S while II is the corresponding Northern blot. Lanes: A, total RNA from *M. mazei* S-6 cells maintained at the optimal growth temperature of 37°C, i.e., non-heat-shocked cells; B and C or B to D, total RNA from cells heat shocked at 45°C for increasing time periods, from 15 to 60 min. The sizes of the transcripts in panels A to D are indicated in kilobases. Transcripts were detected for all the genes in non-heat-shocked cells. Heat shock caused an increase in the levels of the transcripts of *hsp70*, *hsp40*, and *grpE*. The reverse occurred for *orf16*, and *orf11-trkA*. The latter two genes overlap and are cotranscribed, whereas the other genes are transcribed monocystronically. Reprinted from references 42, 44, 49, and 184, with permission of the publishers.

pected locations with regard to the translation start and stop codons, respectively (44, 181, 182). Other sequence features vary with the intergenic region and the species, but the regions upstream of *hsp70* in two of the methanosarcinas possess a series of repeats and palindromes. They might be *cis*-acting signals, namely, binding sites for regulatory factors (168). In contrast, the region upstream of *hsp70* in *M. thermoautotrophicum* ΔH is very short and lacks anything that might be a promoter or a *cis*-acting site.

No bacterial-type promoter sequences (52, 120, 219, 258, 259) are identifiable in these archaeal intergenic regions, nor are there bacterium-type regulatory elements, such as CIRCE (259, 310, 313) or ROSE (208, 209), that one can detect by sequence comparisons.

If one considers the high degree of conservation of these regulatory sequences among bacteria, it is reasonable to conclude that they do not occur in the three known archaeal loci and that regulation of the *hsp70* locus genes in these organisms is mediated by factors different from those operating in bacteria. Thus, despite the similarities in organization between the archaeal and bacterial *hsp70* loci, their mode of expression and regulatory mechanisms appear to be different.

Also remarkable is that the 5′-*grpE-hsp70*-3′ intergenic region and the distance between *grpE* and the next gene upstream in *M. mazei* S-6 and *M. thermophila* TM-1 are considerably longer than the equivalent regions in bacteria. The latter have their genes closer to one another, in agreement with their polycistronic mode of transcription and their being regulated as a unit, or operon. Instead, the structure of the archaeal loci in the two methanosarcinas shown in Fig. 2 does not suggest the bacterial modes of transcription and regulation but different ones (see also experimental data, given below).

Expression

Functional analyses of the *hsp70* genes have been carried out for *M. mazei* S-6 and to a lesser extent for *M. thermophila* TM-1. No functional information exists for the other four archaeal *hsp70* genes that have been cloned and sequenced thus far.

M. mazei S-6 *hsp70*(*dnaK*), *hsp40*(*dnaJ*), and *grpE* respond to heat shock by an increase in the production of their transcripts (Fig. 3) (42, 44), as one would expect for stress genes. The transcripts are monocistronic as in eukaryotes (302) and in contrast to bacteria (10, 95, 120, 131, 132, 294, 313). Likewise, the peak response in terms of transcript levels is reached after

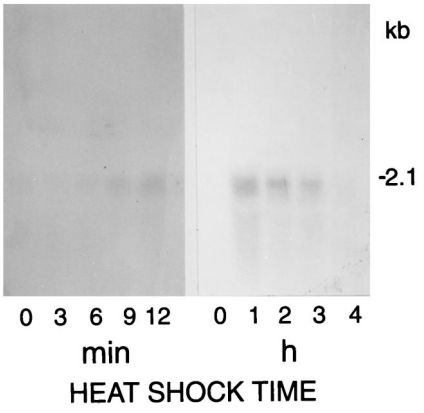

FIG. 4. Response of the *M. mazei* S-6 *hsp70*(*dnaK*) gene to heat shocks of various durations. Northern blots of total RNA (10 μg/lane) extracted from *M. mazei* S-6 cells before heat shock (lane 0 in both panels) or after a heat shock at 45°C for the times indicated in the horizontal axis, in minutes (min) or hours (h). Hybridization was done with a probe for *dnaK*. The size of the hybridization bands in kilobases is indicated to the right. Reprinted from reference 164 with permission of the publisher.

heat shocks longer than those that would induce a peak response in bacteria (Fig. 4) (164), also in agreement with what is observed in eukaryotes. The transcription initiation sites map to positions reminiscent of the eukaryotic initiation sites with respect to the promoter (Fig. 5) (42, 44, 179). Furthermore, the genes respond to temperatures ranging from 45 to 60°C (Fig. 6) (164) and to other stressors such as cadmium (Cd^{2+}) (Fig. 7) (179) and ammonia (165) as expected for heat shock genes. Thus, the data show that the archaeal *hsp70* locus genes are stress or heat shock genes but have mixed bacterial and eucaryal characteristics.

The body of structural and functional data available at present suggests that the mechanism of transcription initiation for the archaeal *hsp70* locus genes differs from those known to operate in bacteria (26, 27, 120, 132, 208–210, 242, 259, 310, 311, 313) and must involve factors which are not of a bacterial type, i.e., different from σ factors (180). These data, as well as the fact that all transcription initiation studies with archaeal systems (albeit none involving heat shock genes and practically all done with hyperthermophilic systems) have demonstrated transcription factors of the eucaryal type (16, 51, 94, 117, 118, 264, 274, 276, 312), force the prediction that initiation for the *M. mazei* S-6 *hsp70*, *hsp40*, and *grpE* genes involves eucaryal-type factors.

TATA-Binding Protein

The archaeal homologs of the eucaryal TATA-binding protein (TBP) and the transcription factor IIB (TFIIB) (aTFB and aTFA, respectively [117, 118]), have been identified and shown to be required for the transcription of archaeal, non-heat shock genes in vitro (57, 94, 117, 118, 276). There is no comparable information for archaeal stress genes, but one may hypothesize, based on the observations described in the previous section, that these genes will also require TBP and TFIIB as basal factors. Moreover, it is likely that other factors would also be necessary to induce the response to stressors and preferentially, or even specifically, start transcription of *hsp70* and its teammates, *hsp40* and *grpE*.

The *tbp* gene of *M. mazei* S-6 has been cloned and sequenced (51). The deduced amino acid sequence of the protein possesses some of the expected archaeal characteristics, but it also shows unique features. For example, like all archaeal TBPs known (reviewed in reference 264), the *M. mazei* S-6 protein is shorter than most eucaryal homologs, amounting to what is the C-terminal domain in eucaryal molecules. Also, the *M. mazei* S-6 protein is acidic, like the other known archaeal proteins, but it differs from them in that its N-terminal third is basic, not acidic. The direct, imperfect repeats found in all TBPs, archaeal and eucaryal, are also present in the *M. mazei* S-6

GENE	PROMOTER SEQUENCE (5' - 3')	DISTANCE (BP) FROM PROMOTER'S CENTER TO: Transcription initiation site	Translation start codon
orf16	****•********** AAAAGTTTATATA	28	51
grpE	** •******* * AACTATTTATAGA	26	69
dnaK	*****•** ** * AAACTTTAATTAA	20	79
dnaJ	*****• * * * AAACCTGTTCACA	19	48
orf11-trkA	****•********** AAACATTTATATA	28	98

FIG. 5. *M. mazei* S-6 promoters for the *hsp70*(*dnaK*) locus genes. Bases with asterisks are identical to those in the consensus sequence for promoters in methanogens, and bases with dots denote positions without base preference (25); underlined bases represent the archaeal box A (reference 312 and references therein). The consensus sequence was derived from comparative analysis of promoters for many non-heat-shock genes (25). There is no information on promoters for archaeal *grpE*, *hsp70*(*dnaK*), or *hsp40*(*dnaJ*), except that shown here and in Tables 2, 5, and 10. Therefore, no consensus sequence is available for these archaeal heat shock genes. Note that while the promoters for the non-heat-shock genes *orf16* and *orf11-trkA* match the consensus 100%, the *grpE*, *hsp70*(*dnaK*), and *hsp40*(*dnaJ*) promoters do not match it to the same extent. Reprinted from reference 179 with permission of the publisher.

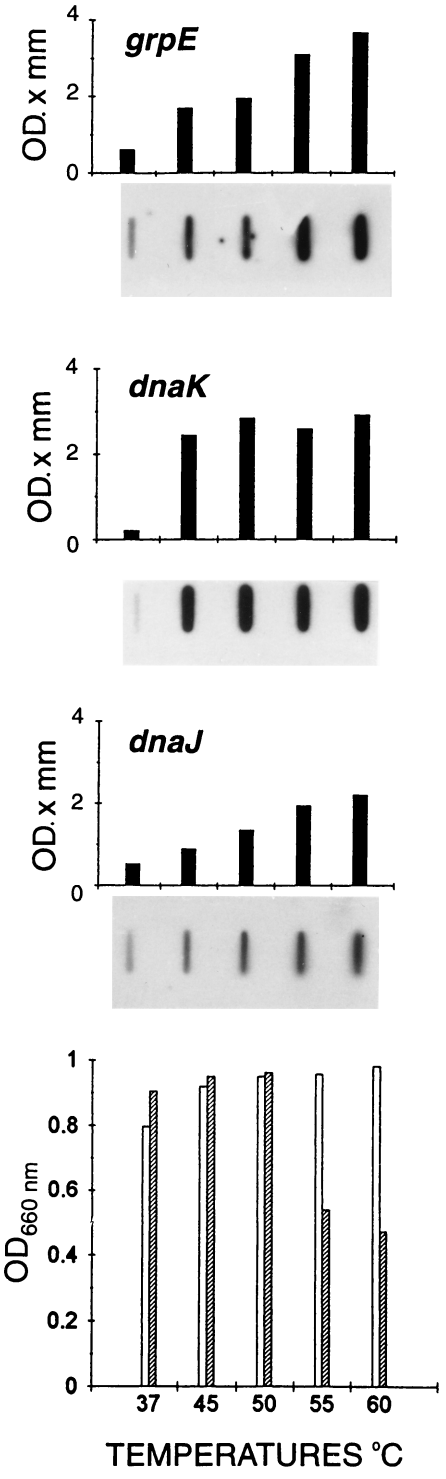

FIG. 6. Response of the *M mazei* S-6 genes *grpE*, *hsp70*(*dnaK*), and *hsp40*(*dnaJ*) to heat shock at various temperatures demonstrated by slot-blotting with *M. mazei* S-6 RNA. The levels of mRNA for *grpE*, *hsp70*(*dnaK), and hsp40*(*dnaJ*) (top three panels) are represented by vertical bars expressed in the optical density (OD) × millimeter units given by the densitometer. The respective slot blots (10 μg of total RNA from S-6 cells per slot) are shown at the foot of the bars, while the heat shock temperatures are indicated in the horizontal axis at the bottom of the figure (°C). Hybridization was done with the respective labelled probes. The culture density is shown in the bottom panel. The OD_{660} was determined at time zero (open bars) and at 30 min (hatched bars) in cultures maintained at 37°C or heat shocked during this 30-min period at the temperatures indicated at the foot of the bars. Reprinted from reference 164 with permission of the publisher.

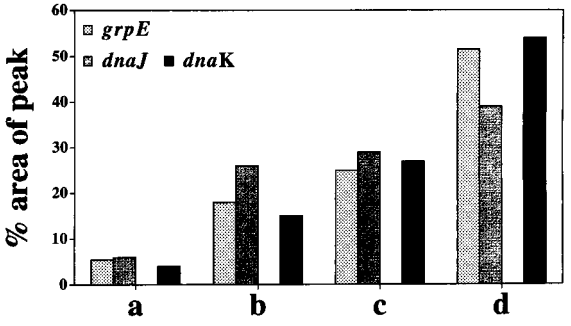

FIG. 7. Response of the *M. mazei* S-6 genes *grpE*, *hsp40*(*dnaJ*), and *hsp70*(*dnaK*) to the stressors cadmium (Cd^{2+}) and heat. The bars represent levels of mRNA determined by slot blotting with probes for the *grpE*, *hsp40*(*dnaJ*), and *hsp70*(*dnaK*) genes. The total RNAs were from cells grown at 37°C (i.e., the optimal temperature for growth of *M. mazei* S-6) in medium without Cd^{2+} (a) and in medium with 5 or 27 mM $CdCl_2$ (b and c, respectively) and from cells grown in medium without Cd^{2+} but heat shocked at 45°C for 30 min (d). Note that the levels of the mRNAs from the three genes increased after heat shock by comparison with the levels before heat shock (constitutive or basal levels; compare a and d). Likewise, the presence of Cd^{2+} in the medium also induced an increase in the levels of the three mRNAs. This effect was more marked with 27 mM than with 5 mM $CdCl_2$ (compare a with b and c; and compare b with c). Reprinted from reference 179 with permission of the publisher.

molecule. Repeats of approximately 42 amino acids separated by a spacing segment of 51 residues on average can be identified (51). The repeats are better conserved in the archaeal than in the eucaryal TBPs, and this is also true for the *M. mazei* S-6 homolog. A few archaeal TBPs have an acidic tail composed of a series of Glu residues in the C-terminal end. This acidic tail is not present in the *M. mazei* S-6 molecule.

The overall and regional characteristics of the *M. mazei* S-6 protein most probably determine its functional properties in what pertains to the binding to DNA at the promoter and to the potential interaction with other transcription factors, such as TFIIB and perhaps stress-specific factors (139a). These structure-function aspects of *M. mazei* S-6 TBP are being investigated at present. Purified TBP binds to the *M. mazei* S-6 *hsp70* promoter, as demonstrated by the electrophoretic mobility shift assay (EMSA) (57a).

Research to determine how transcription initiation starts and proceeds under constitutive (basal) conditions and in response to stress (heat shock) is under way. In experiments with cell lysates from *M. mazei* S-6, it was demonstrated that TBP present in the lysates binds to the *hsp70* promoter (51a). The phenomenon is observed with lysates from both unstressed and stressed cells. In the latter, a protein appears or increases in concentration or in its ability to bind DNA or TBP, which causes an additional shifted band in EMSA. The nature and role of the protein are under investigation. It might be a regulatory factor that binds near the *hsp70* promoter.

ARCHAEAL *hsp70* AND Hsp70

The Gene

The salient characteristics of the archaeal genes sequenced thus far are described in Table 2. The promoters, terminators, and ribosome-binding sequences or sites (RBS) are putative, except for the *M. mazei* S-6 promoter, for which preliminary experimental evidence supports the promoter shown (Fig. 5).

The Protein

The archaeal Hsp70 proteins are quite similar to each other and, most remarkably, equally so to proteins from gram-posi-

TABLE 2. Archaeal *hsp70*(*dnaK*) genes[a]

Organism	Accession no.	Size (bp)/no. of amino acids encoded	Promoter/terminator/RBS	Other structures	Expression	Reference(s)
Halobacterium cutirubrum	L35530	1,887/628	NR/NR/NR[b]	NR	NR	111
Haloarcula marismortui	M84006	1,909/635	NR/NR/NR	NR	NR	110
Methanobacterium thermoautotrophicum ΔH	AE000894	1,791/596	NR/NR/gaggtg (−8)[c]	Downstream repeats; stem-loops	NR	263
Methanosarcina mazei S-6	X60265	1,857/619	aaactttaattaa (−79)/inverted repeats/aggatataa (−5)	Up- and downstream repeats; stem-loops	Heat shock inducible	42, 182
Methanisarcina thermophila TM-1	Y17862	1,833/610	aacttttatcta (−60)/tctttttt (+38)/agtgaggataaa (−7)	Palindrome; distinctive features upstream	Heat shock inducible	126, 164
Thermoplasma acidophilum	L35529	1,785/595	NR/NR/NR	NR	NR	111

[a] See also Tables 3 and 4 and Fig. 10.
[b] NR, not reported.
[c] (−) and (+) refer to the position of the center of the sequence upstream from the translation start codon or downstream from the translation stop codon, respectively.

tive bacteria (Table 3). Among the archaeal proteins, the most similar pairs are those from the two methanosarcinas, the two extreme halophiles, and the two thermophiles (*T. acidophilum* and *M. thermoautotrophicum* ΔH) (see "Hsp70-based phylogenetic trees" below).

The archaeal proteins all have the universal markers for Hsp70 and DnaK and the bacterial markers (Table 4). However, they do not have any of the markers typical of eucaryal molecules. Thus, the archaeal Hsp70 is of bacterial type in sequence and in structural features that reflect its function.

A remarkable feature that appears to be distinctive for the archaeal Hsp70 is the absence of a stretch of 23 to 25 amino acids in the N-terminal quadrant, which became evident when the sequences were aligned with those of proteins from gram-negative bacteria (Fig. 8) (182). This major marker is shared with the DnaK proteins from gram-positive bacteria and is not present in eukaryotic homologs (109–111).

The evolutionary and functional significance of this sequence gap in archaea and gram-positive bacteria has not been elucidated. However, it has given support to a phylogenetic classification that places the archaea closer to gram-positive bacteria than to eukaryotes (105, 106, 109–111), in contrast to the classical 16S-18S rRNA-based tree (11, 299, 300). In the Hsp70-based tree, the extant gram-negative bacteria would have separated from their ancestors, i.e., the ancestors of today's gram-positive bacteria, early in evolution. As this happened, or shortly thereafter, the gram-negative line acquired the 23 to 25 extra amino acids that characterize its Hsp70. Also, within the framework of this hypothesis, the eukaryotic nucleus would have arisen from a fusion of a primitive archaeon with a gram-negative ancestor. The gene that ultimately became established in the eukaryotic line was that which came from the bacterial partner.

These are speculations based on sequence comparisons and other data that are not completely satisfactory in view of all the information available today. Alternative explanations have been put forward and are discussed in some detail in subsequent sections of this review.

ARCHAEAL *hsp40* AND Hsp40

The Gene

The four archaeal *hsp40*(*dnaJ*) genes sequenced thus far are described in Table 5. They are remarkably similar to each other, as are the proteins they encode (see below).

The Protein

The four archaeal Hsp40(DnaJ) proteins known at present are similar to one another and to their bacterial homologs (Table 6). As is the case for the Hsp70, the most similar pair is that of the two proteins from methanosarcinas. The universal motifs and signatures that characterize the Hsp40 molecule, whether from eukaryotes or from bacteria, also occur in the archaeal homologs, except those that are distinctive for the eucaryal molecules (Table 7).

The Gly-rich domain of the *H. cutirubrum* Hsp40 is longer and has a higher percentage of Gly than those of the three molecules from methanogens (Table 8). The *H. cutirubrum* molecule also shows a different pattern of distribution of the CxxCxGxG motif from the molecules from the methanogens (Table 9). Motifs 1 and 2 (counting from the N to the C terminus) are separated by 9 amino acids, motifs 2 and 3 are separated by 18 amino acids, and motifs 3 and 4 are separated by 6 amino acids in the four molecules. However, motif 1

TABLE 3. Comparison of the Hsp70(DnaK) amino-acid sequences from archaea and those most similar from bacteria

Organism name	Accession no.	% Identity or similarity[a]							
		M. mazei S-6	*M. thermophila* TM-1	*C. acetobutylicum*	*B. subtilis*	*M. thermoautotrophicum* ΔH	*H. cutirubrum*	*H. marismortui*	*T. acidophilum*
Methanosarcina mazei S-6	P27094		90.8	67.4	65.4	59.1	57.4	57.6	53.9
Methanosarcina thermophila TM-1	Y17862	94.7		65.6	64.6	59.9	56.0	58.6	55.8
Clostridium acetobutylicum	P30721	76.1	74.8		67.7	59.7	52.9	53.5	55.0
Bacillus subtilis	P17820	75.4	74.4	75.4		58.9	54.6	56.3	55.0
Methanobacterium thermoautotrophicum ΔH	O27351	69.1	69.1	69.3	69.1		54.0	53.5	63.2
Halobacterium cutirubrum	P42372	66.2	64.4	63.6	64.8	64.9		74.6	48.0
Haloarcula marismortui	Q01100	65.9	66.8	63.6	66.8	64.3	82.2		49.5
Thermoplasma acidophilum	P50023	65.1	64.7	64.9	64.9	71.8	59.0	59.9	

[a] Percent identity above and percent similarity (identity plus conservative substitutions) below the diagonal blank space.

begins farther away from the N terminus in the *H. cutirubrum* molecule than in the others. In consequence, motif 4 is the closest to the C terminus in the molecule from the extreme halophile compared with those from the methanogens. The question remains open whether these seemingly unique features of the molecule from the extreme halophile reflect an adaptation to life under high-salinity conditions and/or to cope with salinity changes.

Motif 1 in the *M. thermoautotrophicum* ΔH molecule is, barring a sequencing error, aberrant in that the last residue is Arg (R) instead of Gly (G).

ARCHAEAL *grpE* AND GrpE

The Gene

The two archaeal *grpE* genes whose sequences have been determined are described in Table 10. The genes differ considerably in length; the *M. mazei* S-6 gene encodes a molecule 35 amino acids longer than that encoded in the *M. thermoautotrophicum* ΔH homolog. This disparity confirms the poor degree of conservation of *grpE* and predicts that it will be very difficult to identify homologs in nature on the basis of sequence comparisons alone. The failure to detect GrpE in the eukaryotic-cell cytosol for example, may be due to its diversity. Methods other than structural analyses may be necessary to unveil the true spectrum of this molecule, as suggested by recent work (212) and by the data in Table 10 (see also below).

The Protein

The amino acid sequence of GrpE is not as highly conserved as that of Hsp70 or even Hsp40 (Table 11). However, if discrete regions, for example regions I and II (294), are compared, the similarity increases (Table 12). These regions and the GrpE motifs (45) are shown in Fig. 9. The functions of these structural features have not been determined. It has been suggested that they might be important portions of the molecule, involved in the interaction of GrpE with the other members of the chaperone machine, Hsp70 and Hsp40 (45, 294).

OCCURRENCE OF *hsp70* IN NATURE

The Archaeal Puzzle

The absence of the *hsp70* gene in some archaeal species has been noted since the early 1990s (47) and was also found later, when it could not be detected in the hyperthermophiles *Methanothermus fervidus*, *Sulfolobus* sp., and *M. jannaschii* or in the mesophile *Methanospirillum hungateii* (47, 164). These reports, however, were based on negative results obtained by Northern, Southern, and Western blots with heterologous probes. Consequently, they could not be taken as proof of the absence of the gene.

A definitive confirmation came in 1996, when the sequencing of the *M. jannaschii* genome did indeed reveal that this organism does not contain *hsp70* or the other two genes of the chaperone machine triad, *hsp40* and *grpE* (31). Although this finding helped to give credence to previous negative results obtained by blotting procedures with heterologous nucleic acid and antibody probes and to reaffirm the idea that some organisms may indeed lack *hsp70*, it raised questions about the earlier finding of the gene in *M. mazei* S-6. This organism is a methanogen like *M. jannaschii*. Why is it, then, that the former contains *hsp70* while the latter does not? Was the reported *M. mazei* S-6 gene real or artifactual?

There were additional data confirming the occurrence of *hsp70* in other methanosarcinas, different from *M. mazei* S-6, from before the *M. jannaschii* genome had been sequenced (42). However, once again, these data had been obtained by Northern and Southern blots with a probe for the *M. mazei* S-6 gene, and the possibility of nonspecific hybridizations could not be ruled out.

The situation was finally clarified when the full genome sequence of another methanogen, *M. thermoautotrophicum* ΔH was reported in 1997 (263). Like *M. mazei* S-6, this methanogen contains *hsp70*, as well as *hsp40* and *grpE* (Fig. 1).

As things stand today, it is clear that *hsp70* occurs in some but not all methanogens. It also occurs in extreme halophiles, but it is not known whether there are organisms in this group that lack the gene—this remains to be demonstrated. The gene does not occur in any of the extreme thermoacidophilic archaea investigated up until now. This had been suggested, as mentioned above, by results obtained by blotting procedures (47, 164) and was confirmed for *Archaeoglobus fulgidus* (151) and other species by whole-genome sequencing (Table 13). In addition, a search for the *hsp70*(*dnaK*) relative *hsc66*, found in *Escherichia coli* and other bacteria (260), in the genomes of *A. fulgidus*, *Pyrococcus horikoshii*, *M. jannaschii*, and *M. thermoautotrophicum* did not reveal its presence (180a).

Several important conclusions may be derived from the data available at present: (i) the absence of *hsp70* seems to be a characteristic of archaeal species that live at very high temperatures (hyperthermophiles); (ii) in sharp contrast, no hyperthermophilic bacterium has been found yet that lacks the gene; (iii) *hsp70* is scattered among methanogenic archaea that are either mesophiles or thermophiles, like *M. mazei* S-6 and *M. thermophila* TM-1, but is absent in other methanogens; (iv) whenever *hsp70* was present in a genome, *hsp40* and *grpE* were

TABLE 4. Archaeal Hsp70(DnaK) amino acid sequences deduced from cloned genes: motifs

Motif[a]	Function	Reference(s)	Synonym[a]	Reference	Motif present[c]					
					M. mazei S-6 (182)	*M. thermophila* TM-1 (126)	*M. thermoautotrophicum* ΔH	*T. acidophilum* (111)	*H. cutirubrum* (111)	*H. marismortui* (110)
Phosphate 1	ATPase; nucleotide binding	23	Hsp70 family sig. (ATP α-phosphate)	8	Yes	Yes	Yes	Yes	Yes	Yes
Connect 1		23	Hsp70 family sig. (6 aa)	239	Yes	Yes	Yes	Yes	Yes	Yes
Phosphate 2		23	Hsp70 family sig.	8	Yes	Yes	Yes	Yes	Yes	Yes
Adenosine		23	None	NA	Yes	Yes	Yes	Yes	Yes	Yes
Connect 2		23	None	NA	Yes	Yes	Yes	Yes	Yes	Yes
DnaK loop [N-29,[b] (A,S)-30, E-31, G-32, R-34, E-369]	GrpE binding	28	None	NA	N-29 A-30 E-31 G-32 R-34 E-376	N-29 A-30 E-31 G-32 R-34 E-376	 A-33 E-34 G-35 E-364	 S-30 E-31 G-32 E-362	N-31 E-33 G-34 E-375	N-31 E-33 G-34 R-36 E-375
Residue E-171	Hinge	29	None	NA	E-183 D-171	E-183 D-171	E-170 D-169	E-168 D-174	E-160 D-171	E-162 D-171
T-12, T-13, D-367	Interdomain communication	265, 268	None	NA	T-11 T-12 D-367	T-11 T-12 D-367	T-14 S-15 D-367	T-11 S-12 D-365	T-13 T-14 D-366	T-13 T-14 D-366
Leucine zipper	Oligomerization (?)	180a	None	NA	Yes	Yes	Yes	Yes	Yes	Yes
Hsp70 family sigs. (6 & 7 aa)	?	239	None	NA	Yes	Yes	Yes	Yes	Yes	Yes
EEDKKRRER[b] (archaea, Gram+; not in Gram−)	?	142	Hypercharge run	142	Yes	Yes	Yes	Yes	Yes	Yes
NLS (eukaryotic ct)	Nuclear localization signal	239	None	NA	No	No	No	No	No	No
Eukaryotic (ct; ER) Hsp70 sig.	?	239	None	NA	No	No	No	No	No	No
EEVD[b] (eukaryotic Hsp70 COOH end)	DnaJ binding; regulatory	81	None	NA	E-611 V-612 V-613 D-614	No	No	No	E-624 D-625 V-626 D-627	E-626 D-627 V-628 E-629
GDAWV[b] (mitochondria & alpha, beta, gamma proteobacteria)	?	91	mt sig. Box A	91	No	No	No	No	No	No

[a] Abbreviations: NA, not applicable; sig. or sigs., signature(s); aa, amino acid(s); NLS, nuclear localization signal; ct, cytosol; ER, endoplasmic reticulum; mt, mitochondria.
[b] Amino acid single-letter symbols (followed by position number when pertinent).
[c] References are given in parentheses. Accession numbers are the same as in Table 3.

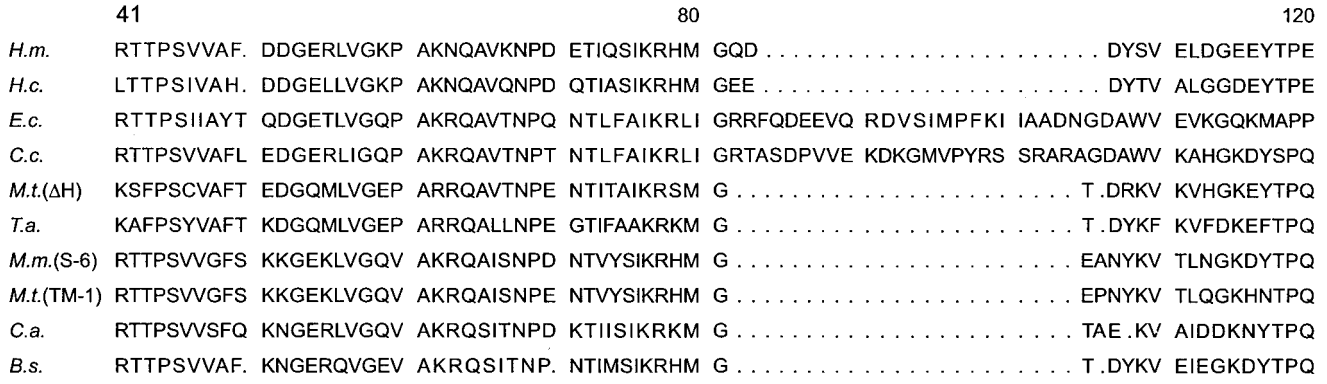

FIG. 8. Amino acid sequences (single-letter symbols) of six archaeal Hsp70(DnaK) proteins and of four bacterial homologs, two from gram-negative bacteria (*E. coli* and *C. crescentus*) and two from gram-positive bacteria (*C. acetobutylicum* and *B. subtilis*) between positions 41 and 120, aligned with the program PileUp (Genetics Computer Group, University of Wisconsin, Madison, Wis.). The absence of 23 residues in the proteins from the archaea and gram-positive bacteria compared with those from gram-negative bacteria is shown by dots. Organisms and accession numbers are as follows: *H.m.*, *Haloarcula* (*Halobacterium*) *marismortui* (Q01100); *H.c.*, *Halobacterium cutirubrum* (P42372); *E.c.*, *Escherichia coli* (P04475); *C.c.*, *Caulobacter crescentus* (P20442); *M.t.* (ΔH), *Methanobacterium thermoautotrophicum* ΔH (O27351); *T.a.*, *Thermoplasma acidophilum* (P50023); *M.m.*, *Methanosarcina mazei* S-6 (P27094); *M.t.* (TM-1), *Methanosarcina thermophila* TM-1 (Y17862); *C.a.*, *Clostridium acetobutylicum* (P30721); *B.s.*, *Bacillus subtilis* (P13343). Modified from references 179 and 182 with permission of the publishers.

also found if enough sequencing was done; (v) conversely, genome sequencing has demonstrated that if the *hsp70* gene is absent, *hsp40* and *grpE* are also absent; and (vi) the gene has been found in two extreme halophiles, but there are no reports of full-genome sequences for this group of organisms, and so it is not possible to be certain about the situation with them. Do they all have *hsp70*, and also *hsp40* and *grpE*? We know that at least one of them, *H. cutirubum*, has *hsp40* in addition to *hsp70* (32). It may be argued that archaea did not have the genes to begin with and that some of them received the genes via lateral gene transfer. However, the observations listed above, particularly (iv) and (v), and other data challenge the lateral-gene-transfer hypothesis, at least in its simplest form.

One must assume that the three genes jumped as a block, or unit, from a bacterium into an archaeon, particularly the pair *hsp70* and *hsp40*. This implies that the unit also carried the intergenic regions. In agreement with the hypothesis, the proteins encoded by the genes, particularly Hsp70 and Hsp40, are of a bacterial type. Against this hypothesis stands the fact that there are no signal sequences of the bacterial type in the intergenic regions. They are typically archaeal. Hence, more data are needed to determine the origin of the archaeal *hsp70* locus genes, i.e., archaeal or bacterial, or prearchaeal or prebacterial; and if the origin is archaeal or prearchaeal, more research is necessary to elucidate the mechanism by which these genes came to be in today's species that have them.

Hsp70-Based Phylogenetic Trees

The Hsp70 molecule lends itself to comparative analyses for making phylogenetic trees. It is widely distributed among organisms of the domains *Bacteria* and *Eucarya*, and it also occurs among the archaea. The molecule is long enough to allow for many mutations to be detected and for useful alignments, since about 500 residues are conserved in a molecule which on average is a little over 600 amino acids in length. Furthermore, Hsp70 has segments that are highly conserved and others that are less so, which allows the detection of variations while maintaining alignable portions and the identification of structural markers that can easily be seen in all members of the family.

Several groups of investigators have used Hsp70 to make phylogenetic trees (22, 99, 105–111, 142, 239). Most of these do not agree with the classical tree based on comparisons of 16-18S rRNA sequences (11, 299, 300). In the rRNA-based tree, archaea and eucarya have a common line up to a point at which they diverge. The archaeal-eucaryal and the bacterial lines are shown to branch off earlier from a primitive, common line. Hsp70-based trees do not support the archaea-eucarya sisterhood or the monophyletic character of archaea suggested by the rRNA-based tree. Some Hsp70 trees suggest a close relationship between archaea and gram-positive bacteria on one side and between eucarya and gram-negative bacteria on the other (105, 106).

The reliability of the trees has been questioned lately. This applies to both rRNA- and protein-based trees (66, 67, 80, 99, 105, 106, 188, 189, 192, 286, 298).

Evidence showing that lateral gene transfer events are more frequent and widespread than was previously realized has been accumulating in the last couple of years (1, 4, 66, 67, 213, 298). Thus, finding that the Hsp70 proteins, for example, of two organisms are very similar does not necessarily mean that the organisms are phylogenetically close. It only means that their Hsp70 molecules are close to each other and have a common ancestor. It does not prove that the ancestor molecule was present in a common ancestor of the two organisms. One of the two organisms may have received its Hsp70 via lateral gene transfer and thus mistakenly appears to be a close relative of the donor's ancestral lineage.

In summary, by studying amino acid sequences, one can follow the natural history of genes and their occurrence in nature, namely, their itinerary, as it were, along the series of organisms in which they are found.

A recent study addresses these points, taking advantage of the fact that considerably more Hsp70 sequences are known now than when previous studies were carried out (99). A systematic search for *hsp70* among archaea was performed, and the gene was cloned from *Aquifex pyrophilus* and *Thermotoga maritima*. These two bacterial species represent the deepest offshoots in the rRNA-based tree. The gene was not found in 8 of the 10 archaea investigated (Table 13). Alignments of 70 Hsp70 sequences, including the 2 new ones from *A. pyrophilus* and *T. maritima*, confirmed the previous observations that the *M. mazei* S-6 protein clusters with the proteins from the low-G+C gram-positive bacteria while the proteins from the extreme halophiles cluster with the proteins from the high-G+C gram-positive bacteria (Fig. 10). Remarkably, the Hsp70 pro-

TABLE 5. Archaeal *hsp40*(*dnaJ*) genes[a]

Organism	Accession no.	Size (bp)/no. of amino acids encoded	Promoter/terminator/RBS	Other structures	Expression	Reference(s)
Halobacterium cutinubrum	U93357	1,167/389	NR/NR/NR[b]	NR	NR	32
Methanobacterium thermoautotrophicum ΔH	AE000894	1,131/376	acatttttttatt (−63)[c]/NR/aggtg (−9)	Up- and downstream repeats; stem-loops	NR	263
Methanosarcina mazei S-6	X60265	1,167/389	aaacctgttcaca (−100)/t-rich region/aacagggaatctg (−8)	Up- and downstream repeats; stem-loops	Heat shock inducible	42, 181
Methanosarcina thermophila TM-1	AJ010152	1,167/388	aaacctgcact (−55)/tcttttt (+30), t-rich region/atgacagggaa (−11)	Inverted repeat upstream; t-rich region downstream	NR	126

[a] See Tables 6 to 9.
[b] NR, not reported.
[c] (−) and (+) refer to the position of the center of the sequence upstream from the translation start codon or downstream from the translation stop codon, respectively.

teins from *T. acidophilum* and *M. thermoautotrophicum* ΔH clustered together (Table 3), along with those from the *Aquifexales*, *Thermotogales*, and green nonsulfur bacteria (Fig. 10). The two archaeal Hsp70 proteins in this group appeared to have an ancestor in common with *T. maritima*. In brief, the Hsp70 from the archaeal species *T. acidophilum* and *M. thermoautotrophicum* ΔH did not cluster with the proteins from gram-positive species, as suggested by others, but with bacteria unrelated to the gram positive ones. Another unexpected finding was that the Hsp70 from *T. maritima* did not have the 23-amino-acid insert characteristic, it was believed, of gram-negative bacteria. Thus, *T. maritima* Hsp70 possesses a structural feature (i.e., a 23-amino-acid gap in its N-terminal quadrant [Fig. 8]) that is assumed to be distinctive for gram-positive bacteria and archaea, despite the fact that this organism is not a gram-positive bacterium or an archaeon.

There are several possibilities to explain these observations. For example, if one assumes that the eucarya and archaea had a common ancestor that contained *hsp70*, it is possible that both received the gene but some archaea lost it afterwards. A second possibility is that there was no *hsp70* in the common ancestor and the gene was acquired after the three lineages separated, with some archaea being excluded. A third possibility is that there was a common ancestor which contained *hsp70* and that the three lineages received the gene vertically but the archaeal lineage lost it very early (the species that have the gene today received it via lateral transfer). Finally, if one disregards the common-ancestor idea, another possibility is that the archaea never had the gene while the bacteria and eucarya had it from the beginning. Here, also, archaea that have the gene today must have acquired it by lateral transfer.

The above possibilities and others one might easily think of are more or less improbable depending on (i) how one interprets available data from other studies; (ii) what molecule(s) and criteria were used to generate these data; (iii) what methods were applied to obtain, study, and statistically validate the data; and (iv) what classification scheme was adopted as a master scaffolding to compare with the Hsp70-based tree.

In any case, all the possibilities mentioned share an important characteristic: they stimulate research in this fascinating area of biology and evolution. Molecular phylogeny and detailed analyses of proteins and other macromolecules have already demonstrated their enormous value as tools for research. They are instrumental in uncovering relationships between organisms, the origins of the eukaryotic cell components, the functional meaning of structural motifs, and the role of domains in large proteins of eukaryotes whose ancestors are smaller proteins in more primitive organisms.

FUNCTIONS OF ARCHAEAL MOLECULAR CHAPERONES

Biochemistry

There is little information on the functions of the archaeal Hsp70, Hsp40, and GrpE molecules, as assessed in vitro or in vivo. Since they are so similar in sequence and/or structural features to the homologs from bacteria (Tables 4 and 7; Fig. 9), it may be assumed that both groups of proteins have the same functions and participate in the same mechanisms as molecular chaperones and regulators of their own synthesis.

For example, the bacterial Hsp70(DnaK) is an ATPase and binds ATP and unfolded polypeptides (substrate) (191, 214, 236). Hsp70(DnaK)-ATP has a lower affinity for substrate than Hsp70(DnaK)-ADP. Thus, ATP hydrolysis, which is enhanced by Hsp40(DnaJ) via interaction of its J domain with at least

TABLE 6. Comparison of the Hsp40(DnaJ) amino-acid sequences from archaea and those most similar from bacteria

Organism	Accession no.	% Identity or similarity[a]					
		M. mazei S-6	*M. thermophila* TM-1	*B. subtilis*	*C. acetobutylicum*	*M. thermoautotrophicum* ΔH	*H. cutirubrum*
Methanosarcina mazei S-6	P35515		80.1	49.6	50.4	49.0	41.3
Methanosarcina thermophila TM-1	AJ010152	84.7		46.7	48.9	50.7	39.2
Bacillus subtilis	P17631	59.9	56.8		50.5	49.0	41.5
Clostridium acetobutylicum	P30725	59.7	59.6	59.3		48.3	40.8
Methanobacterium thermoautotrophicum ΔH	O27352	56.4	57.5	59.7	57.7		43.7
Halobacterium cutirubrum	U93357	47.0	45.2	49.2	49.7	50.7	

[a] Percent identity above and percent similarity (identity plus conservative substitutions) below the diagonal blank space.

two sites on the Hsp70(DnaK) molecule (87, 270), promotes substrate binding, and the polypeptide is maintained in an extended form, avoiding aggregation. Interaction with GrpE, or nucleotide exchange factor, regenerates the Hsp70(DnaK)-ATP complex, lowers the affinity for the substrate, and releases it (the polypeptide may then be taken by the chaperonin system for final folding (see "Chaperonins" below). Hsp40(DnaJ) is thought to also bind the substrate, before Hsp70(DnaK) does, and to tag the polypeptide so that the Hsp70(DnaK)-ATP complex "sees" it and binds it (176, 191, 236, 272). Also, *E. coli* DnaK interacts with ribosome-bound trigger factor (62). There is no experimental information on whether the archaeal Hsp70 system operates like that of bacteria and, if so, to what extent the details described above are similar or dissimilar. This is an area that requires investigation and deserves to be explored. It has the potential for revealing how a bacterial-like molecular machine works in a cell whose genome bears eucaryal-like features and probably encodes accessory (regulatory, auxiliary) factors of the eucaryal type while lacking the complementary Hsp60 system of the bacterial type.

Regulation: More Archaeal Puzzles

It would be of particular interest to explore whether a self-regulating circuit similar to that described for *E. coli* (references 6, 20, 90, and 259 and references therein), or some variation of it, also operates in archaea. Hsp70(DnaK) in some bacteria participates along with Hsp40(DnaJ) and perhaps also GrpE in the degradation of σ^{32} as a way to down-regulate *hsp70*(*dnaK*) transcription. How much of this mechanism operates in archaea? We know that archaea do not have σ factors, and so regulatory circuits for Hsp70(DnaK) synthesis cannot include this factor. Does it include another?

We also know that in eukaryotes, the Hsp70 protein intervenes to prevent trimerization of the heat shock factor (HSF) and thus precludes induction of the *hsp70* gene (205, 253). Archaea do not have an identifiable HSF, or heat shock element (75), in the *hsp70* promoter region (180a). How, then, is the archaeal *hsp70* gene regulated? Does Hsp70 participate in this process? If so, how? Does it interact with an archaeal

TABLE 7. Archaeal Hsp40(DnaJ) amino acid sequences deduced from cloned genes: motifs[a]

Motif	Function	Reference(s)	Synonym	Reference(s)	Motif present[c]			
					M. mazei S-6 (181)	*M. thermophila* TM-1 (126)	*M. thermoautotrophicum* ΔH	*M. cutirubrum* (32)
J-domain	Regulates Hsp70 ATPase; acts as competitive inhibitor of DnaJ in protein refolding	176, 272, 284	N terminus	37	Yes	Yes	Yes	Yes
G-rich domain	Flexible spacer between domains; aids J-domain in stimulating Hsp70 ATPase; competitive inhibitor of DnaJ in protein refolding	176, 272, 284	None	NA	Yes	Yes	Yes	Yes
HPD[b]	Stimulates Hsp70 ATPase	284	None	NA	Yes	Yes	Yes	Yes
Zn finger (CXXCXGXG)[b]	Interacts with unfolded polypeptides and with denatured proteins	176, 272	None	NA	Yes	Yes	Yes	Yes
C terminus	Polypeptide (substrate) binding; possibly aids Zn fingers interact with protein substrates	176, 272	None	NA	Yes	Yes	Yes	Yes
HDEL[b]	ER retention signal	34, 107	None	NA	No	No	No	No
CAAX[b]	Prenylation	34, 35	CaaX box	34, 35	No	No	No	No

[a] Abbreviations: NA, not applicable; ER, endoplasmic reticulum.
[b] Amino acid single-letter symbols (X, any amino acid).
[c] References are given in parentheses. Accession numbers are the same as in Table 6.

TABLE 8. Archaeal Hsp40(DnaJ) amino acid sequences deduced from cloned genes: glycine-rich domain[a]

Organism (reference)	Amino acid position		Total no. of:		% Gly
	First	Last	Amino acids	Gly residues	
M. mazei S-6 (181)	70	114	45	12	26.67
M. thermophila TM-1 (126)	70	113	44	11	25.00
M. thermoautotrophicum ΔH	69	118	50	12	24.00
H. cutirubrum (32)	68	135	68	32	47.06

[a] Accession numbers are the same as in Table 6.

equivalent of the eucaryal HSF or with another kind of regulator?

These are but some of the fascinating questions posed by recent research with the archaeal *hsp70* locus genes. The answers to these questions will shed light on the details of the transcription initiation machinery for stress-inducible genes in archaea and will help us to understand the evolution and principles of the transcription mechanisms in the three phylogenetic domains, not just in the *Archaea*.

CHAPERONINS

Chaperonin Systems I and II

One of the most striking features of archaea is that although they are prokaryotes, they do not have a chaperonin (Hsp60) system like that of the other prokaryotes, the bacteria, but instead have a eukaryotic type of chaperonin complex. No exception to this rule has yet been reported; all archaea investigated have a chaperonin system which resembles that of the eukaryotic cytosol.

The bacteria have the "bacterial" type (group) I chaperonins, i.e., the genes/proteins *groEL*/GroEL and *groES*/GroES (191, 214, 235, 247). A three-dimensional (3-D) view of the barrel-like GroEL/S complex is shown in Fig. 11. What makes the lack of this chaperonin system in archaea very intriguing is that these organisms, at least some of them, have an Hsp70(DnaK) molecular chaperone machine very similar to the bacterial homolog, as described in previous sections. The absence of type I chaperonins and the presence of the Hsp70(DnaK) chaperone machine in a single organism is perplexing if one considers that in bacteria the two systems coexist and seemingly evolved together to interact with each other. The Hsp70(DnaK) system acts early in protein biogenesis to avoid aggregation of nascent polypeptides during translation or immediately thereafter. Subsequently, the polypeptide reaches the GroEL/S system for final folding (191, 214, 235, 247). Now we know that only a minority of polypeptides require the GroEL/S machinery for correct folding (214), but there is no doubt that the coordinated action between the Hsp70(DnaK) and GroEL/S systems is an important physiologic characteristic of the bacterial cell.

In contrast, archaea, even those that have the *hsp70* locus genes [i.e., those that possess the components of the molecular chaperone machine Hsp70(DnaK), Hsp40(DnaJ), and GrpE], do not have the chaperonin type I but the type (group) II system. An example of an archaeal chaperonin complex is shown in Fig. 12. It has also a cylindrical shape like the GroEL/S bacterial complex, but both ends are flat; there is no equivalent of GroES in archaea. In this regard, the archaeal complex resembles that of the eukaryotic cytosol, which is also called TCP-1 (tailless complex polypeptide 1), CCT (chaperonin-containing TCP-1), or TRiC (TCP-1 ring complex) (245, 295). A 3-D reconstruction of ATP-bound CCT is shown in Fig. 13, in stereoview for 3-D visualization with appropriate glasses. In this figure, the X-ray structure of an archaeal chaperonin has been superposed on CCT to demonstrate how the two complexes match with each other.

It should be noted here that the organelles of the eukaryotic cell, e.g., mitochondria and chloroplasts (which are the descendants of endosymbiotic bacteria) (1, 73, 91, 146, 229, 243, 244, 256, 296), do have the type I chaperonins. Notably, however, the genes coding for the organellar chaperonins are located in the nucleus—to where, it is believed, they migrated from the genome of the primitive bacterium after the endosymbiotic event (1, 19, 296). In fact, comparative analyses of the amino acid sequences of chaperonins and chaperones have helped considerably in establishing the origins of the components of the eukaryotic cell (discussed in other sections of this review).

Structure-Function

The chaperonin systems of the members of the *Bacteria*, *Archaea*, and *Eucarya* are relatively large multimeric rings that form barrel-like structures visible under the electron microscope (Fig. 11 to 13). As a consequence, they have been characterized morphologically and functionally by combining two or more of the following procedures: electron microscopy, molecular genetics, biochemistry, and crystallography. A sample of the results of structural studies of archaea is given in Table 14.

The bacterial type (or group) I chaperonin system consists of two protein components, GroEL and GroES, of approximately 60 and 10 kDa, respectively (this system is classified within the Hsp60 family because of the size of GroEL) (235, 247). The two proteins form homoheptameric rings. GroEL builds a barrel-like complex or cage, with two stacked rings (14 subunits in all). Each subunit has three domains, equatorial, intermediate,

TABLE 9. Archaeal Hsp40(DnaJ) amino acid sequences deduced from cloned genes: CXXCXGXG motif

Motif no.[a]	Organism (reference)[d]	Amino acid position		Sequence quality[b]
		First	Last	
1	*M. mazei* S-6 (181)	144	151	C—C-G-G (4/4)
	M. thermophila TM-1 (126)	143	150	C—C-G-G (4/4)
	M. thermoautotrophicum ΔH	148	155	C—C-G-R[c] (3/4)
	H. cutirubrum (32)	165	172	C—C-G-G (4/4)
2	*M. mazei* S-6 (181)	161	168	C—C-G-G (4/4)
	M. thermophila TM-1 (126)	160	167	C—C-G-G (4/4)
	M. thermoautotrophicum ΔH	165	172	C—C-G-G (4/4)
	H. cutirubrum (32)	182	189	C—C-G-G (4/4)
3	*M. mazei* S-6 (181)	187	194	C—C-G-G (4/4)
	M. thermophila TM-1 (126)	186	194	C—C-G-G (4/4)
	M. thermoautotrophicum ΔH	191	198	C—C-G-G (4/4)
	H. cutirubrum (32)	208	215	C—C-G-G (4/4)
4	*M. mazei* S-6 (181)	201	208	C—C-G-G (4/4)
	M. thermophila TM-1 (126)	200	207	C—C-G-G (4/4)
	M. thermoautotrophicum ΔH	205	212	C—C-G-G (4/4)
	H. cutirubrum (32)	222	229	C—C-G-G (4/4)

[a] For all the organisms, motifs 1 and 2, 2 and 3, and 3 and 4 are separated by 9, 18, and 6 amino acids, respectively.

[b] -, one amino acid and —, two amino acids. Numbers in parentheses indicate the number of amino acids matching the four consensus residues.

[c] Deviation from the norm, R (Arg) instead of G (Gly).

[d] Accession numbers are the same as in Table 6.

TABLE 10. Archaeal *grpE* genes[a]

Organism	Accession no.	Size (bp)/no. of amino acids encoded	Promoter/terminator/RBS	Other structures	Expression	Reference(s)
Methanobacterium thermoautotrophicum ΔH	AE000894	525/174	aaatttttatata (−87)[b]/NR[c]/aggtg (−7)	Upstream repeats; stem-loops	NR	263
Methanosarcina mazei S-6	X74353	630/209	aactatttataga (−69)/inverted repeat (+76)/atggg (−11)	Up- and downstream repeats; stem-loops	Heat shock inducible	44, 45

[a] See Tables 11 and 12 and Fig. 1 and 5.
[b] (−) and (+) refer to the position of the center of the sequence upstream from the translation start codon or downstream from the translation stop codon, respectively.
[c] NR, not reported.

and apical. The equatorial domain is at the base of the ring, the apical domain forms the contour of the ring toward the base of the barrel, and the intermediate domain connects the other two. Since the intermediate domain is thinner than the other domains it leaves open spaces or windows that connect the outside with the inner cavity of the barrel (Fig. 11). GroES, instead, is a single ring (seven subunits) and completes the functional chaperonin by serving as a lid to occlude one of the two ends of the barrel formed by the GroEL rings (Fig. 11).

CCT, the chaperonin of the eukaryotic cytosol, is the paradigm of the type (or group) II system. It has a structure similar to that of the GroEL/S complex but is built with eight different subunits in the 50- to 68-kDa range per ring (heteropolymeric ring) (170, 171, 235, 245, 246, 273, 295). In addition to the morphologic differences, type I and II chaperonins differ in functional aspects. For example, while GroEL binds a number of different polypeptide substrates (although it may not actively fold all the substrates it can bind), CCT may be more selective. It is able to bind and mediate the ATP-dependent folding of actin and tubulin, but more data are needed to determine the range of substrates that CCT can bind and fold (it might turn out to be wider than is currently believed).

The archaeal chaperonin system (152) has been studied mostly in thermophiles and extreme thermophiles but there is also some information on the homolog of one extreme halophilic species (Tables 14, 15, and 16 and references therein). It consists of either one or two subunits, depending on the species, although recent findings suggest the existence of a third subunit (see "Evolution" below). The subunits are similar to each other in organisms that have more than one subunit and have been given different names (e.g., α and β, a and b, or 1 and 2). They form complexes with the same general design in all species. Detailed features of the archaeal chaperonin complex as seen by electron microscopy were first reported in 1991 (230). The structure was described as a "large cylindrical complex" present in relatively large quantities in the cytoplasm of the extreme thermophilic archaeon *Pyrodictium occultum*. Salient characteristics were its thermostable ATPase activity and its accumulation after heat shock. The component protein, TF55, of a chaperonin complex from *Sulfolobus shibatae* was also purified, and its encoding gene was cloned and sequenced in 1991 (281). TF55 formed oligomeric complexes of two stacked rings similar to the bacterial GroEL complex. However, the amino acid sequence of TF55 was found to be more similar to that of the eukaryotic cytosolic TCP-1 (now known to be a component of CCT, as mentioned above) than to that of GroEL. This finding helped to assign possible functions to TCP-1 and boosted research on the eukaryotic cytosolic chaperonin.

The archaeal chaperonin subunits form homo- or heteropolymeric rings (Table 14) and two rings stacked end to end build a barrel (called a thermosome in some species) that is assumed to be a peptide-folding cage (2, 3, 153, 216, 217, 308, 309). The archaeal barrel does not have the detachable lid characteristic of the bacterial folding chamber (Fig. 11), because archaea lack a homolog of the bacterial small-subunit GroES. Instead, archaea possess one or two large subunits, depending on the species, of approximately 60 kDa—hence the assignment of this system to the Hsp60 family. The subunit(s) is equivalent to the eukaryotic TCP-1 molecule. Recent results (5) indicate that a third gene homolog exists in some organisms and that its expression product would be a third chaperonin subunit. If this protein were in fact present in the cytoplasm, it could account for the ninefold symmetry of the chaperonin complexes observed in the *Sulfolobus* species that have the third gene (Table 14) (see "Evolution" below).

One of the best-studied archaeal chaperonin complexes is the thermosome from *T. acidophilum* (65). It is composed of two rings, each with two alternating, different subunits (named α and β) of ~58 kDa. Electron microscopy and crystallography showed a spheroid or short cylinder with dome-shaped ends instead of the bacterium-like barrel that has one open end and one closed end. The peculiar appearance of the archaeal complex might have been caused by the crystallization conditions. Also, the structure might have been "fixed" while the folding cage was closed, as one would expect it to be during peptide

TABLE 11. Comparison of the GrpE amino acid sequences from archaea and those most similar from bacteria

Organism	Accession no.	% Identity or similarity[a]				
		M. mazei S-6	*C. acetobutylicum*	*B. burgdorferi*	*B. subtilis*	*M. thermoautotrophicum* ΔH
Methanosarcina mazei S-6	P42367		34.0	31.4	28.6	27.0
Clostridium acetobutylicum	P30726	45.7		35.2	40.1	32.7
Borrelia burgdorferi	P28609	41.6	44.7		27.9	30.1
Bacillus subtilis	P15874	40.5	49.5	39.9		33.1
Methanobacterium thermoautotrophicum ΔH	O27350	39.1	47.4	42.2	50.6	

[a] Percent identity above and percent similarity (identity plus conservative substitutions) below the diagonal blank space.

TABLE 12. Comparison of the GrpE amino acid sequences from *M. mazei* S-6 and *M. thermoautotrophicum* ΔH: entire molecule, and regions I and II

Organism (reference)[c]	% Identity and similarity[a]					
	Entire sequence		Region 1[b]		Region II[b]	
	M. mazei S-6	*M. thermoautotrophicum* ΔH	*M. mazei* S-6	*M. thermoautotrophicum* ΔH	*M. mazei* S-6	*M. thermoautotrophicum* ΔH
M. mazei S-6 (45)		27.0		35.3		33.3
M. thermoautophicum ΔH	39.1		49.0		53.3	

[a] Percent identity above and percent similarity (identity plus conservative substitutions) below the diagonal blank space.
[b] Reference 294.
[c] Accession numbers are the same as in Table 11.

folding, according to what is known for the bacterial GroEL/S system (235, 247, 251). In the bacterial system, the barrel would be alternatively open (acceptor stage, when the polypeptide needing assistance for folding enters the barrel) or closed (folding stage). In the latter stage, the polypeptide would encounter a hydrophilic environment inside the barrel and would bury its hydrophobic residues to stay in solution. The closed stage occurs when the GroES ring attaches to one of the ends of the barrel and covers it as if it were a lid. At this point, the chaperonin complex viewed from the side looks like a bullet, with one end flat and the other convex with the GroES ring (Fig. 11). Instead, the *T. acidophilum* thermosome appeared more as a sphere than as a barrel (65). The two ends of the double-ringed barrel were not flat. In this archaeal thermosome, protruding structures from the inner surface of the rings themselves might act as a built-in lid.

The functional inferences from these and other structural and morphodynamic analyses (235, 246) are that while the GroEL barrel is closed, the inside of its wall changes from hydrophobic to hydrophilic, owing to conformational changes induced by the binding of ATP and the GroES ring to the GroEL barrel (Fig. 11). Thus, the prisoner polypeptide finds no partner to aggregate with but does find conditions conducive to correct folding. The next move would be the opening of the barrel by nucleotide-driven release of the GroES ring and the exit of the folded polypeptide. If folding is incomplete, the same polypeptide would reenter the barrel and another cycle would begin. Recycling would continue until folding was complete.

These functional inferences may not apply entirely to the archaeal chaperonin complex. As pointed out above, its structure is different from that of its bacterial counterpart. Also, the latter may receive polypeptides from the Hsp70(DnaK) chaperone machine and seems prepared for this interaction.

```
1                                                                    50
MKKSRKKENM DSKERNQKEA ERSEARNSES PAEKAGETKV SPENEPSSPE
~~~~~~~~~~ ~~~~~~~~~~ ~~~MCEDKKT DSRSQQECQK ELEELRERLK

                                D     R
51                             AExxNx KxR                       100
AEKNPEEACR EENEILKDQL FRLAADFDNF RKRTARQMEE NRKSVLEQVL
DLENEIKKKE EEVREYTSHL QRLQADFDNY KKQMEKQELE IIKNANERLI

       D    R
101    Exx xKA                                                   150
LDFVEVTDNF DRAIKSARTA EDMGPIVSGI EQLSKQFFSI LEKYGLERVK
LKLLDVYEDL ERAIENQDSS MD......GL EVIYRKFRDT LTKEGLSEIP

      FD
151   LNPx xHxAx                                                200
CEKAGEFDPH RHEAIHHIET SEVPDNTIVE IYKEGYALNE KVVRPALVSV
AE.GEKFDPF LHEAVMVEDH DGYEDGIIIE ELSRGYRLND RIIKHSIVKV

201
ARSPEEAEK  M.m  S-6
CKKS~~~~~  M.t. ΔH
```

FIG. 9. Amino acid sequences (single-letter symbols) of the archaeal GrpE proteins from *M. mazei* S-6 (*M.m. S-6*; P42367) and *M. thermoautotrophicum* ΔH (*M.t.* ΔH; O27350) aligned with the program PileUp. Regions I and II (294), in that order from the N terminus, are underlined. Motifs 1, 2, and 3 (45), also from the N to the C terminus, are shaded, with their respective consensus sequences on top (light and dark shades show hydrophobic and hydrophilic residues, respectively). The *M. thermoautotrophicum* ΔH molecule is shorter than the *M. mazei* S-6 protein, with amino acids missing at the beginning and the end (tildes) and inside (dots).

TABLE 13. Occurrence, or lack thereof, of the *hsp70*(*dnaK*) gene among archaea and representatives of thermophilic and hyperthermophilic bacteria

Organism	OTG (°C)	*hsp70* (*dnaK*) present	Genome size (Mb)	Demonstrated by[a]:	Reference(s)
Archaea					
Methanosarcina mazei S-6	37	Yes	2.8	S, N, W, seq.	42, 44, 182
Methanosarcina mazei JC3	37	Yes	ND[b]	N	42
Methanosarcina mazei LYC	37	Yes	ND	N	42
Methanosarcina sp. strain JVC	37	Yes	ND	N	42
Methanosarcina acetivorans C2A	37	Yes	2.7	N	42
Methanosarcina barkeri	37	Yes	2.7	S	10
Methanosarcina thermophila TM-1	50	Yes	2.7	S, N, seq.	126, 164
Methanospirillum hungateii	37	No	ND	S	164
Methanobacterium thermoautotrophicum ΔH	65	Yes	1.7	seq.	263
Methanococcus voltae	37	No	ND	S, W	119
Methanococcus vannielii	37	No	ND	S, P	99
Methanococcus jannaschii	85	No	1.7	S, seq.	31, 164
Methanothermus fervidus	85	No	ND	S, P	99, 164
Methanopyrus kandleri	100	No	ND	S, P	99
Haloarcula marismortui	45	Yes	ND	seq.	110
Halobacterium cutirubrum	45	Yes	ND	seq.	111
Halobacterium halobium	45	Yes	ND	S, P	99
Thermoplasma acidophilum	55	Yes	1.7	seq., P	99, 111
Sulfolobus solfataricus	70	No	3.1	S, P	99
Sulfolobus sp.	70	No	ND	S	164
Archaeoglobus fulgidus	83	No	2.2	seq., P	99, 151
Desulfurococcus mobilis	85	No	ND	S, P	99
Thermococcus tenax	88	No	ND	S, P	99
Pyrococcus furiosus	100	No	2.0	seq.	293a
Pyrococcus horikoshii	100	No	1.7	seq.	144, 145
Pyrococcus woesei	100	No	ND	S, P	99
Pyrococcus abyssi	100	No	1.8	seq.	*P. abyssi*; website
Pyrobaculum aerophilum	100	No	2.2	seq.	79, 79a
Aeropyrum pernix K1	100	No	1.7	seq.	143
Bacteria					
Thermus thermophilus	70	Yes	ND	seq.	220
Thermomicrobium roseum	70	Yes	ND	seq.	108
Thermotoga maritima	80	Yes	ND	seq.	99, 213
Aquifex aeolicus	83	Yes	ND	seq.	58
Aquifex pyrophilus	83	Yes	ND	seq.	99

[a] S, N, and W, Southern, Northern, and Western blotting, respectively; P, PCR; seq., sequencing of gene or genome.
[b] ND, not determined.

However, archaea that have the Hsp70(DnaK) protein, like *T. acidophilum* [it is not yet known if this organism also has Hsp40(DnaJ) and GrpE], have a eucarya-like chaperonin system, which may require a different mechanism for interaction with the chaperone machine. In fact, the eukaryotic CCT does not seem to interact with the Hsp70 system and also differs in other aspects from the bacterial GroEL/S complex (152, 170, 171, 245, 273, 295). It remains to be seen how the two systems interact, if at all, in the archaea, and how they work. This is one area in which research with archaeal systems may provide crucial insights into protein folding and refolding mechanisms pertinent to all species, not just the archaea.

The chaperone system of the extreme halophile *Haloferax volcanii* consists of at least two, perhaps three, components (162, 163, 277). Two genes have been sequenced, and a third has been inferred from a partial sequence that encompasses a putative promoter region (Table 15). These genes have been named *cct1*, *cct2*, and *cct3*, which emphasizes their similarity to the eukaryotic CCT system. However, in contrast to the eukaryotic *cct* genes, which are not heat shock inducible, the

TABLE 14. Examples of multimeric complexes formed by some archaeal chaperonins and related proteins[a]

Organism	Protein or complex[b]	Method[b]	Details[b]	Reference(s)
Methanococcus jannaschii	sHsp	Expression in *E. coli*, X-ray crystallography	Oligomeric complexes, 24-mer, octahedron, V_m 22 Å^3/Da	148, 149
Thermoplasma acidophilum	Thermosome	Overexpression in *E. coli*, crystallization, transmission EM, cryo-EM, electron tomography	Two stacked, 8-membered rings of alternating α and β subunits, α-α and β-β pairs, spherical; OD = 164 by 158; lid domain blocks central cavity; 75% of surface area of α and β are solvent exposed; ID = 54–86; chamber volumes = 325,000 Å^3/assembled complex and 130,000 Å^3/ring; hydrophobic, access through side windows; nonintact proteins only	65, 216, 289, 290
Methanopyrus kandleri	Thermosome	Transmission EM	Two stacked, 8-membered rings; homo-oligomers; OD = 145 by 136, ID = 43	3
Methanococcus thermolithotrophicus	MTTS	EM	Barrel-like structure; forms filaments in vitro	84
Sulfolobus sp. strain 7	Chaperonin	EM	Barrel-like structure	207
Sulfolobus shibatae	Chaperonin (TF55)	EM, circular dicroism spectroscopy, PAGE, spectrophotometry	Two stacked, 9-membered rings; 9-fold symmetry; 2 subunits; open and closed complexes; forms filaments	234, 280, 281, 307
Sulfolobus solfataricus	Chaperonin (TF55)	EM	Two stacked rings; 9-fold symmetry; 2 subunits; OD = 160 by 175, ID: = 45; crystallization	71, 154, 187
Pyrodictium occultum	Chaperonin (thermosome)	EM	Two stacked, 8-membered hetero-oligomeric rings; OD = 160 by 165, ID = 155; crystals	230, 231
Pyrodictium brockii	Chaperonin (thermosome)	EM	Barrel-like structure; OD = 160 by 155, ID = 165	231
Thermococcus strain KS-1	Chaperonin	EM	Forms homo-oligomeric rings in vitro	308

[a] See also Fig. 15.
[b] Abbreviations: V_m, volume to mass ratio; EM, electron microscopy; OD, outer diameter (angstroms); ID, inner diameter (angstroms); PAGE, polyacrylamide gel electrophoresis; MTTS, *Methanococcus thermolithotrophicus* chaperonin.

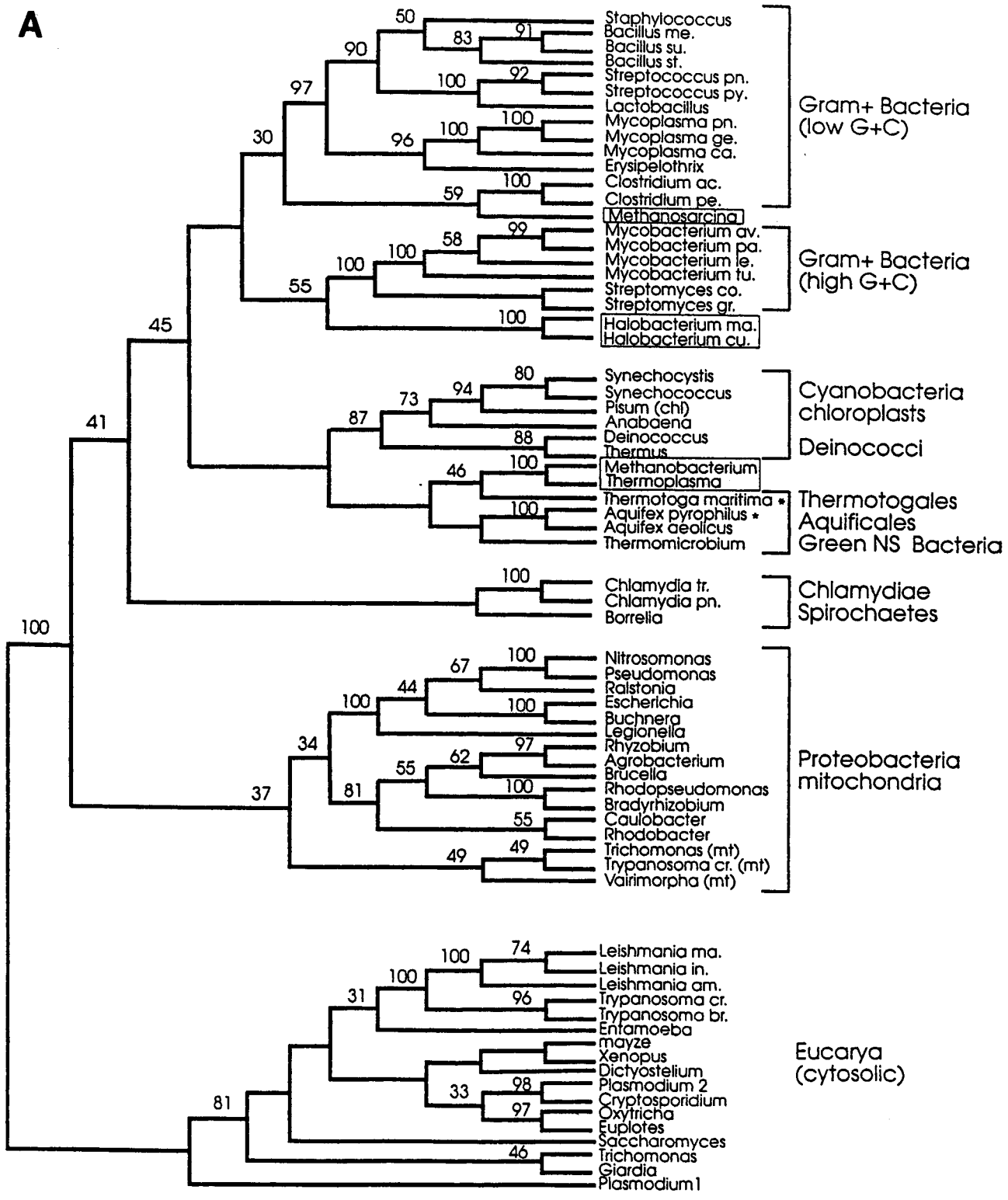

FIG. 10. Maximum-parsimony (A) and evolutionary-distance (B) phylogenetic trees based on Hsp70(DnaK) sequences. Both trees show essentially the same clustering of the archaeal molecules with those from gram-positive bacteria and a group formed by the *Aquifexales*, *Thermotogales*, and green nonsulfur bacteria. Numbers represent bootstrap confidence levels calculated from 100 bootstraps (only those that were 30% or higher are shown). Asterisks indicate the newly described genes-proteins (see reference 99). Abbreviations: ac, *acetobutylicum*; am, *amazonensis*; av, *avium*; br, *brucei*; ca, *capricolum*; chl, chloroplasts; co, *coelicolor*; cr, *cruzis*; cu, *cutirubrum*; ge, *genitalium*; gr, *griseus*; in, *infantum*; le, *leprae*; ma, *marismortui* or *major* (*Leishmania*); me, *megaterium*; mt, *mitochondria*; NS, nonsulfur; pa, *paratuberculosis*; pe, *perfringens*; pn, *pneumonia*; py, *pyogenes*; st, *stearothermophilus*; su, *subtilis*; tr, *trachomatis*; and tu, *tuberculosis*. Reprinted from reference 99 with permission of the American Society for Microbiology.

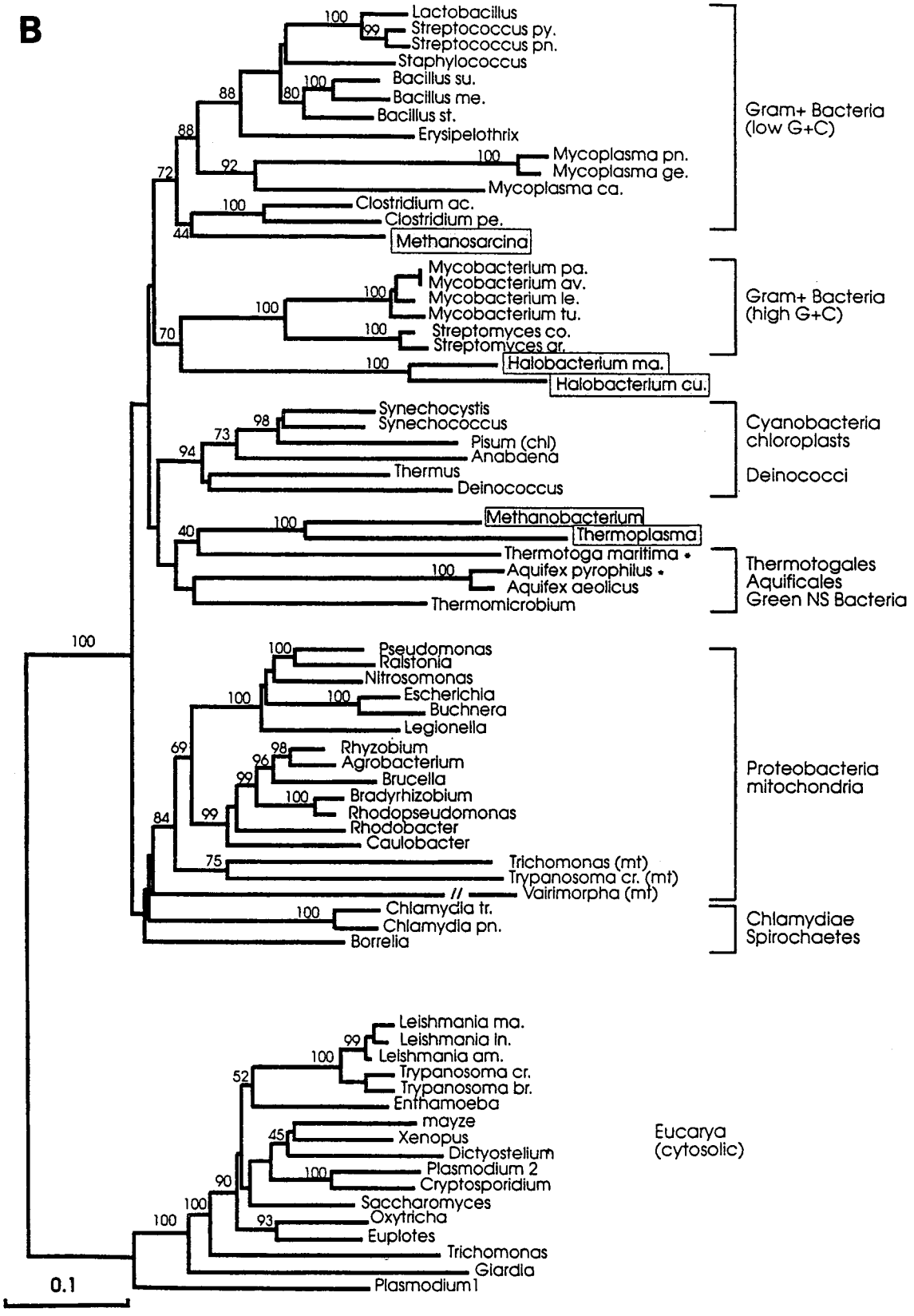

FIG. 10—*Continued.*

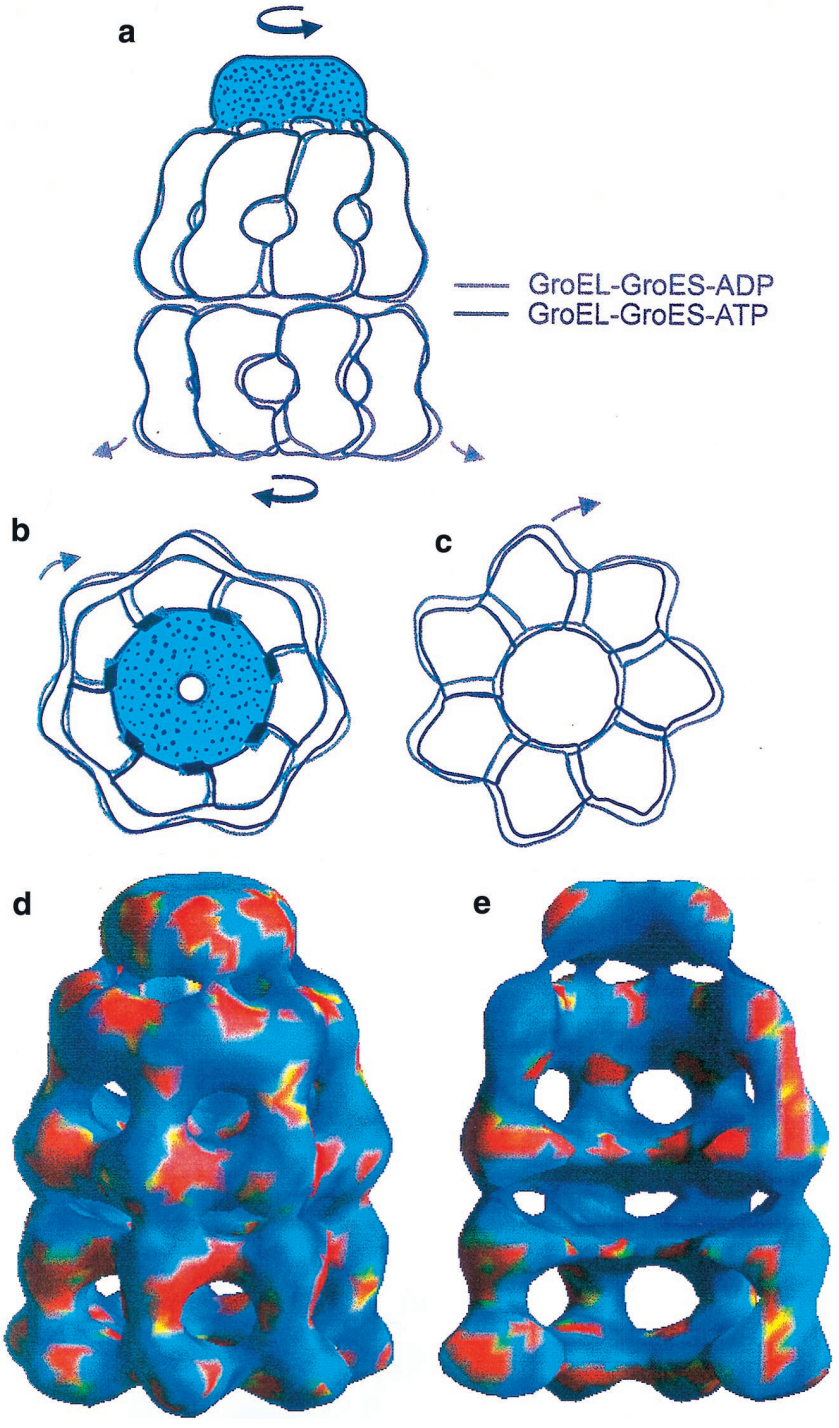

FIG. 11. The bacterial chaperonin complex GroEL/S, and its allosteric changes upon interaction with nucleotide phosphate, which is a major player in chaperonin action. The barrel shape of the complex is apparent, with one base flat and the other convex due to GroES (dotted shading in panels a and b). Also apparent are the two stacked GroEL rings that form the barrel, the subunits of the rings, the domains of the subunits, and the windows between the intermediate domains. GroES looks like a lid, occluding one of the bases of the barrel. The figure also shows the morphologic changes that the complex undergoes when it passes from the ADP to the ATP-bound stages. The structural differences between GroEL-GroES complexes in ATP and ADP were determined by cryoelectron microscopy and computer-assisted image reconstruction. The upper part of the figure (a to c) illustrates domain movements between GroEL/S-ADP and GroEL/S-ATP complexes (gray and black outlines, respectively). The complexes are viewed from the side (a; GroES is dotted-shaded), from above (b; *cis* apical and equatorial domains surrounding the dotted-shaded GroES), and from below (c; transapical domains). The comparison showed small twists of the subunit domains, particularly in the apical domains of the lower ring. The lower part of the figure represents the GroEL/S-ATP complex as a whole viewed from the side (d) and the same complex cut open to expose the inner cavity (e). The complex is color coded to display the significance map of the differences between the ADP- and ATP-bound stages; i.e., the different colors show significance differences from *t* tests between the two structures. Regions with significant change ($P \ll 0.0005$) are red, and regions with no significant change are blue. The comparison demonstrated that there were domain movements throughout the complex. The main regions of differences (red) observed in GroEL were the ends of the apical and equatorial domains and the hinge regions. There was a localized region of significance at the interring contact 2 (between the equatorial massess, on the outside surface of the structure). The pinwheel pattern of variation in GroES suggested that its subunits were being twisted by the change in the orientation of the apical domain of GroEL. Reprinted from reference 246 with permission of the publisher.

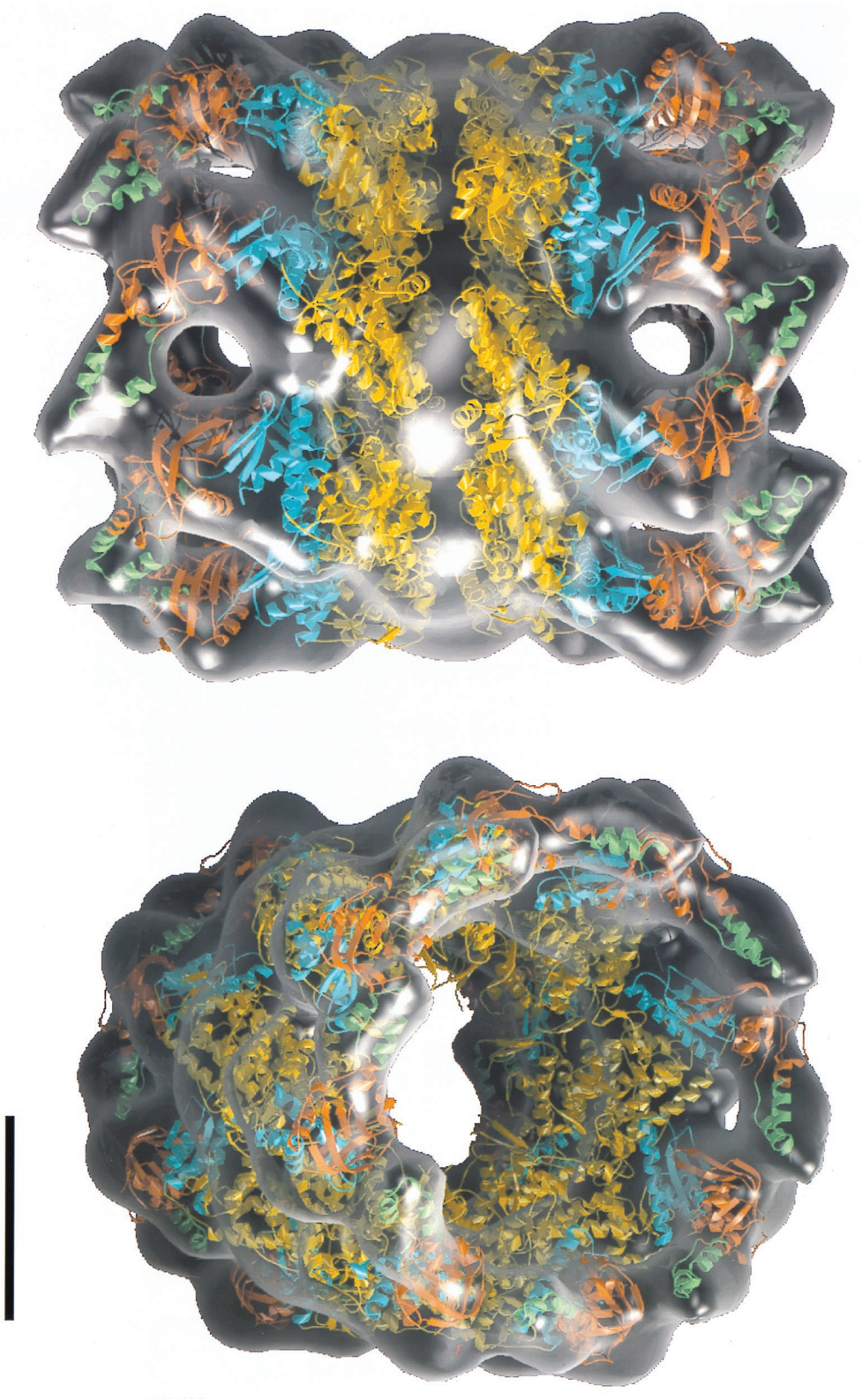

FIG. 12. An example of archaeal chaperonin complex. The cylindrical, barrel-like shape is apparent in the top panel, but in contrast to the bacterial GroEL/S complex (Fig. 11), both bases are flat (there is no GroES homolog here). The figure is a semitransparent surface representation of the electron-tomographic 3-D reconstruction of the α-only thermosome showing the complex in an open conformation with a composite atomic model fitted into it. The atomic model was derived from the crystal structures of the intermediate (blue) and equatorial (yellow) domains of the *cis*-ring of GroEL/S and the apical domain (orange and light green) of the thermosomal α subunit. The complex is viewed from the side (top) and at 60° with respect to the *x-y* plane (bottom). The black scale bar (bottom left) corresponds to 5 nm. Reprinted from reference 217 with permission of the publisher.

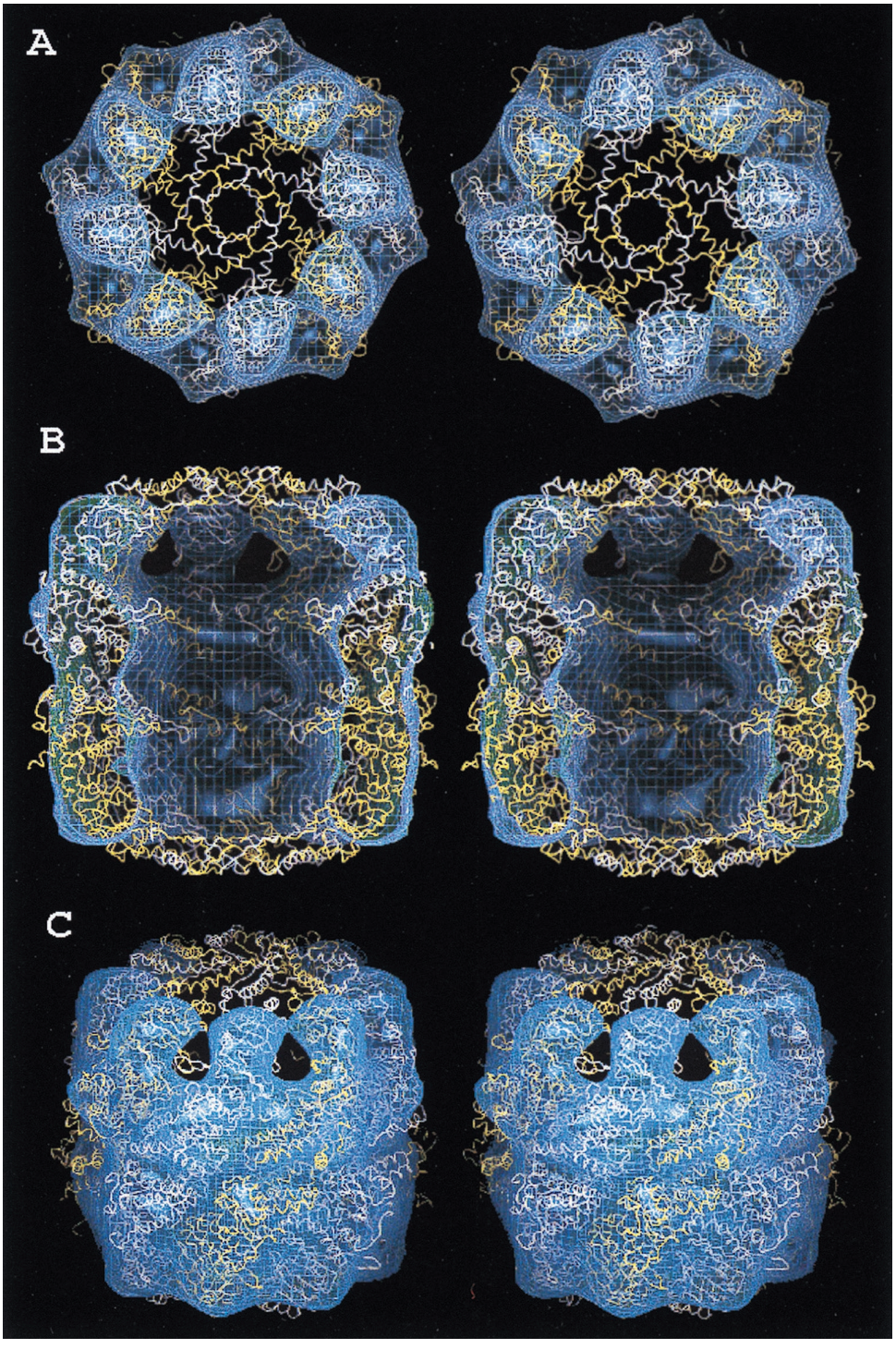

FIG. 13. The archaeal and eukaryotic chaperonin complexes resemble each other; both look like a barrel with flat bases. The structure of the archaeal complex (thermosome) from *Thermoplasma acidophilum* as determined by X-ray crystallography (yellow ribbon) is shown superposed on the 3-D reconstruction of CCT bound to ATP generated by cryoelectron microscopy and computer-assisted image processing (blue). The stereoview pairs (which produce single three-dimensional images when viewed with appropriate glasses) are as follows: (A) a base, or end, of the chaperonin complex seen along the longitudinal axis; i.e., the barrel-like chaperonin complex may be imagined to be standing upright on one of its bases and viewed from above to see the inner cylindrical cavity; (B) the complex split open in half; i.e., the barrel has been cut through the sagittal plane, and the half-cut structure is seen from a line of view perpendicular to the longitudinal axis of the barrel, into the inner cavity; and (C) the whole barrel seen from the side as if it were standing on one of its bases, slightly tilted against the longitudinal axis toward the observer to expose the other base. Note that the fitting is between an asymmetric (CCT-ATP) and a symmetric (thermosome) complex. The fitting in the CCT-ATP complex is excellent for the ATP ring but not as good in the apo-ring. The slight mismatch of the apo-rings is consistent with the fact that the apical domains in the apo-ring of CCT do not point toward the cavity but contact each other around the circumference of the ring whereas the apical domains of the thermosome protrude toward the central cavity. Reprinted from reference 170 with permission of the publisher.

archaeal *cct* homologs are induced by heat and hypotonic shocks (the latter osmotic shock is a stressor for *H. volcanii*, since this organism is adapted to live in hypersaline environments).

Evolution

The evolution of Hsp70(DnaK) in archaea is puzzling, as discussed elsewhere in this review, and challenges the imagination of evolutionary and molecular biologists and taxonomists. Likewise, the evolution of the archaeal chaperonin subunits presents a rather interesting panorama in which gene duplication is a salient feature (5). Archaeal chaperonin subunits in a given organism tend to be more similar to each other than to their homologs from other archaea. For example, the α subunit of *Thermoplasma acidophilum* is more closely related to the β subunit of this organism than to the chaperonin subunits of other archaea (Fig. 14). Also intriguing is the variation in the number of subunits among archaeal species, which may affect the structural configuration (and perhaps functionality) of their chaperonin complexes (Fig. 15). A few archaeal species have only one subunit, others have two, and still others may have three. Indeed, beside the genes encoding the two well-known chaperonins, some archaeal species have another gene, which encodes a third subunit named γ (5). In this work, seven new archaeal genes encoding chaperonin subunits were identified by cloning and sequencing in the archaeotae *Sulfolobus solfataricus*, *Sulfolobus acidocaldarius*, *Sulfolobus shibatae*, and *Desulfurococcus mobilis*. The relationship of these genes to each other and to those from other archaea are shown in Fig. 14 and 15. In the latter figure, known and predicted subunit genes are listed along with the known and inferred symmetries of the respective chaperonin complexes. Type (or group) II chaperonin systems with multiple subunit species appear to have arisen many times independently during archaeal evolution. It remains to be determined how such differences in the number of subunits in a given organism might affect the function and/or mechanism of action of the chaperonin complex.

Expression

Expression of genes encoding archaeal chaperonin subunits has been studied in extreme thermophiles to determine the basal levels in unstressed cells and to measure the effect of heat shock (Tables 15 and 16). For example, *Sulfolobus shibatae* (OTG, 70°C) was heat shocked at 85 or 90°C, and the levels of mRNA for the α and β subunit genes were assessed (141). The levels of the two mRNAs increased in the heat-shocked cells. Extremely stressful conditions caused by exposing the cells to 90°C or higher temperatures stopped all detectable synthesis of all cellular proteins, except that of the two chaperonin subunits. The role that these subunits and the assembled chaperonin complex play in cell survival or in protein biogenesis in general in stressed and unstressed cells has not yet been fully elucidated. Studies in this area may help in the development of methods to protect cells from extreme environments, and they will have practical implications for the design of strategies to explore and exploit such environments. However, these studies must await the development of usable vectors, transformation protocols, mutants, and other tools necessary to carry out the required experiments.

Studies of the expression of the two fully sequenced *hsp60* genes, *cct1* and *cct2*, in the extreme halophile *H. volcanii* have shown that they are inducible by heat and hypotonic shocks (163, 277). The amount of mRNA detected by Northern blotting increased when the cells were heat shocked at 60°C (the OTG for *H. volcanii* is 45°C) for different periods ranging from 5 to 75 min each. The first clear increase in mRNA was observed after a heat shock of 30 min, and the peak response occurred after the longest heat shock tested, i.e., 75 min. In this regard, the response of *H. volcanii hsp60* genes is similar to that of the *hsp70* locus genes in the mesophilic methanogen *M. mazei* S-6 (Fig. 4 and 6). A common feature of these archaeal stress genes is that the duration of the heat shock that induces the highest increase in the amounts of gene products is considerably longer than that which causes a peak response in bacteria. Likewise, the response was detectable in *H. volcanii* when the cells were heat shocked for 45 min at various temperatures, from 45 to 65°C. This pattern is also similar to that observed for the *hsp70* locus genes in *M. mazei* S-6 (Fig. 6).

Thus, in terms of the duration of heat shock that induces a peak response and of the magnitude of temperature upshift that induces a measurable response, the two archaeal species follow a similar pattern, which differs from that of most bacteria. The latter reach a peak response with a heat shock of about 15 min, and longer heat exposures inhibit the response (10, 95, 120, 259, 292). Similarly, heat shocks at temperatures that would cause a clear response in archaea would not have this effect in bacteria or would kill the cells, even if the OTG for the bacteria and archaeal species under comparison were the same.

Regulation

There is limited information on the molecular mechanisms of transcription initiation and regulation of the chaperone genes in hyperthermophilic archaea, except for some structural and culture data (Tables 15 and 16).

Some information is available for the *cct* genes in the extreme halophile *H. volcanii* (222, 277). As mentioned above, this organism responds to heat shock by an increase in the production of mRNA for *cct1* and *cct2*. A study focused on *cct1* showed that the heat shock response depends on signals located in a DNA region of about 200 bp upstream of the gene. In this DNA segment, a putative promoter was identified with the sequence 5′-TTTATA-3′ centered 25 bp upstream of the transcription initiation site. Deletion mutagenesis indicated that basal and heat-induced transcriptions required sequence elements very close to the promoter and on both sides of it.

Interestingly, three TBP (*tbp*) and six TFIIB (*tfIIb*) genes were found in *H. volcanii* (222). One of these genes, named *tfb2*, seems to be induced by heat (278). These observations are noteworthy. Only a single copy of the *tbp* gene has been found among the archaea studied thus far; however, these did not include an extreme halophile, and so it is not yet clear whether a multiplicity of *tbp* and *tfIIb* is a characteristic of all extreme halophiles or is unique to *H. volcanii*. Extreme halophiles harbor large extrachromosomal elements (172), which may account for the gene multiplicity as suggested by the *H. volcanii* case, since in this organism some of the *tbp* and *tfIIb* genes were indeed located in extrachromosomal DNA.

It has been suggested that in *H. volcanii* a specific TBP-TFIIB pair might be involved in the selective induction of the *cct1* gene in response to heat shock (222). This hypothesis remains to be proven. However, the information available at present indicates that transcription initiation and regulation of the *hsp60* genes in *H. volcanii* must be mediated by a mechanism different from that operating in *M. mazei* S-6 for the *hsp70* locus genes. Moreover, these two gene groups are evolutionarily quite diverse: the *hsp60* system is of the eukaryotic (archaeal) type, whereas the *hsp70* locus genes are of the bacterial type, very close to the homologs from gram-positive bacteria. While the former may be aboriginal to the archaea

TABLE 15. A sample of archaeal *hsp60* (chaperonin) genes[a]

Organism	Chaperonin (accession no.)	Size (bp)	Promoter
Archaeoglobus fulgidus	thsA/Cpn-α (AE000950)	1,635	tatata (−49)[b], tgcaa (−19)
	thsB/Cpn-β (AF035826)	1,635	cttata (−49), tgcaa (−19)
Desulforococcus strain SY	HHSP (S79557)	1,635	aggagg (−15)
Haloferax volcanii	cct1 (AF010470)	1,683	tttata (−26), cgaa (−34)
	cct2 (AF010469)	1,674	tttata (−26), cgaa (−34)
	cct3 (AF029873)	Partial seq.	tttata (−26), cgaa (−36)
Methanobacterium thermoautotrophicum ΔH	Chaperonin (α-subunit) (MT0794)	1,617	NR
	Chaperonin (MT0218)	1,659	NR
Methanococcus jannaschii	Chaperonin (U67542)	1,626	NR
Methanococcus thermolithotrophicus	MTTS (AB015435)	1,632	tttatata (−75)
Methanopyrus kandleri	Thermosome (Z50745)	1,635	tttaaata (−60), atgc (−42)
Pyrobaculum aerophilum	TF55 (PA306[e])	NR	NR
	TF56 (PA307[e])	NR	NR
	TF55 (PA27[e])	NR	NR
Pyrococcus kodakaraensis KOD1	*cpkA* (AB018432)	1,647	NR
	cpkB (D29672)	1,641	NR
Pyrococcus abyssi	α-Subunit-thsA (PAB2410)	1,650	NR
Sulfolobus shibatae	TF55-β (X63834)	1,659	tttata (−40)
	TF56-α (L34691)	1,680	tttata (−40)
Sulfolobus sp. strain 7	α-Subunit (AB001085)	1,680	ttttatata (−37) and tgc (−18)
	β-Subunit (AB001086)	1,659	ttttatata (−37) and tgc (−18)
Thermococcus strain KS-1	α-Subunit (AB001080)	1,638	NR
	β-Subunit (AB001081)	1,647	NR
Thermoplasma acidophilum	α-Subunit (Z46649)	1,638	tttata (−40)
	β-Subunit (Z46650)	1,632	ttatata (−33), tttatttttta (−21)
Aeropyrum pernix K1	Thermosome subunit (AEP0907)	1,671	NR
	Thermosome subunit (AEP2072)	1,665	NR

[a] See also Fig. 14 and 15.
[b] (−) and (+) refer to position of center of sequence upstream from translation start codon or downstream from translation stop codon, respectively.
[c] NR, not reported.
[d] Conserved sequences as an extension of Box A and between Box A and Box B.
[e] Identification number used in genome project.

and is perhaps the ancestor of the eukaryotic cytosolic CCT, the *hsp70* system of *M. mazei* S-6 and other archaea may not be genuinely archaeal but, rather, may be foreign.

ORIGINS: VERTICAL VERSUS LATERAL

Studies of archaeal stress genes and proteins have provided insights into many areas of biology beyond microbiology. One recent example is the demonstration that while some archaea have the *hsp70* gene, others do not, and those that have it probably received it via lateral transfer (99, 164). This finding highlighted the role of lateral gene transfer in determining the genome contents of the cells in nature and thus their behavior and evolution.

Along the same lines, comparative analyses of the amino acid sequences of archaeal chaperones and chaperonins among themselves and with homologs from bacteria and the various components of the eukaryotic cell enhanced our understanding of the origins of the mitochondria, plastids, and other eukaryotic-cell organelles such as the hydrogenosome (73, 91, 107, 146, 167, 229, 243, 244, 256, 296). The impact of these contributions is reflected, for example, in the way in which phylogenetic trees based on Hsp70 and other proteins are interpreted (32, 99, 103–111, 142, 229, 239). Other illustrations of this effect are the classification schemes of cells and organisms we see today in the literature, which display not only the gram-positive bacteria, gram-negative bacteria, cyanobacteria, archaea, etc., but also the mitochondria, chloroplasts, and cytosol as life units with a history of their own (Fig. 10). This notion originated well before the archaea entered the scene, but received considerable input from studies of stress genes and proteins since 1991, when the first archaeal *hsp70* gene and the first archaeal chaperonin were sequenced (152, 182). Subsequent comparative analyses of Hsp70(DnaK), Hsp40(DnaJ),

TABLE 15—*Continued*

Terminator	RBS	Remarks	Reference(s)
NR[c]	aggtg (−9)	Enhanced Box A element[d]	74, 151
NR	aggtg (−9)	Enhanced Box A element	
tttga–t-rich-9–tcaaa (+18)	NR		140
NR	NR	Induced by heat and hypotonic shocks; enhanced Box A element	163, 277
NR	NR	Induced by heat and hypotonic shocks; enhanced Box A element	
NR	NR	Induced by heat shock	
NR	NR		263
NR	NR		
NR	NR		31
t-rich region (+20)	NR	Recombinant	84
c-rich region	aggtgat (+18)		3
NR	NR		79
NR	NR		
NR	NR		
NR	NR		137, 305
NR	NR		
NR	NR		*P. abyssi* website
ttttttt (+7)	aggtg (−8)	Enhanced Box A element	141, 281
ttttttt (+6)	tgaggt (−4), ggtg (−12)	Enhanced Box A element	
NR	ggtg (−6)		207
NR	ggtg (−9)		
NR	NR	Recombinant	308
NR	NR	Recombinant	
t-rich region (+6 to +38)	atgcg (−22), aggtgat (−10), caggttcc (+16), tccat (−12)		288
ttttt and aaaaa (+14), attta-9-taaat (+35)	tccat (−12)		
NR	NR		143
NR	NR		

GrpE, and Hsp60 types I and II from organisms belonging to the domains *Archaea*, *Bacteria*, and *Eucarya* have revealed the distribution and the origins of these proteins in nature (Table 17).

The main features revealed by the data are as follows: (i) archaea have chaperonin II as the eukaryotic cystosol and Hsp70(DnaK) like that of the gram-positive bacteria; (ii) the eukaryotic organelles and gram-negative bacteria have the same Hsp70(DnaK); (iii) the type I chaperonin system is present in bacteria and organelles; (iv) hyperthermophilic archaea have the type II chaperonin system but no Hsp70(DnaK), Hsp40(DnaJ), or GrpE; and (v) GrpE is present in bacteria and in some archaeal species but has not yet been identified in eukaryotes outside the mitochondria, although recent work suggests otherwise (211, 212).

OTHER ARCHAEAL STRESS GENES AND PROTEINS

A number of genes and proteins have been found, in a variety of archaeal species, that may be considered pertinent to the stress response because of one or more of the following observations: (i) the amount of protein and/or the amount of mRNA increased during or after stress; (ii) the protein was present at higher concentrations than usual during a period of stress tolerance; (iii) the protein assisted polypeptide folding in vitro; and (iv) the sequence was similar to that of a known stress gene or protein. An illustrative list of these proteins is shown in Table 18. An interesting group comprises the small heat shock proteins (sHsp) (76, 78, 177, 206, 227), with a molecular mass around or below 30 kDa (Table 1). A subgroup is composed of proteins with peptidyl-prolyl *cis-trans* isomerase (PPIase) activity, which catalyze the isomerization of the peptidyl-prolyl bond and thus play an important role in protein biogenesis in bacteria and eukaryotes (78). PPIases can be distinguished from one another because they bind to and are inhibited by different immunosuppressive agents (this is the reason why PPIases are sometimes called immunophilins). Some PPIases bind cyclosporin and are termed cyclophilins, while others bind FK506. Examples of both types have been found in the archaea. A cyclophilin homolog was identified in *H. cutirubrum* (135), but most of the other PPIase gene homologs found in the archaea encode proteins of the FK506-

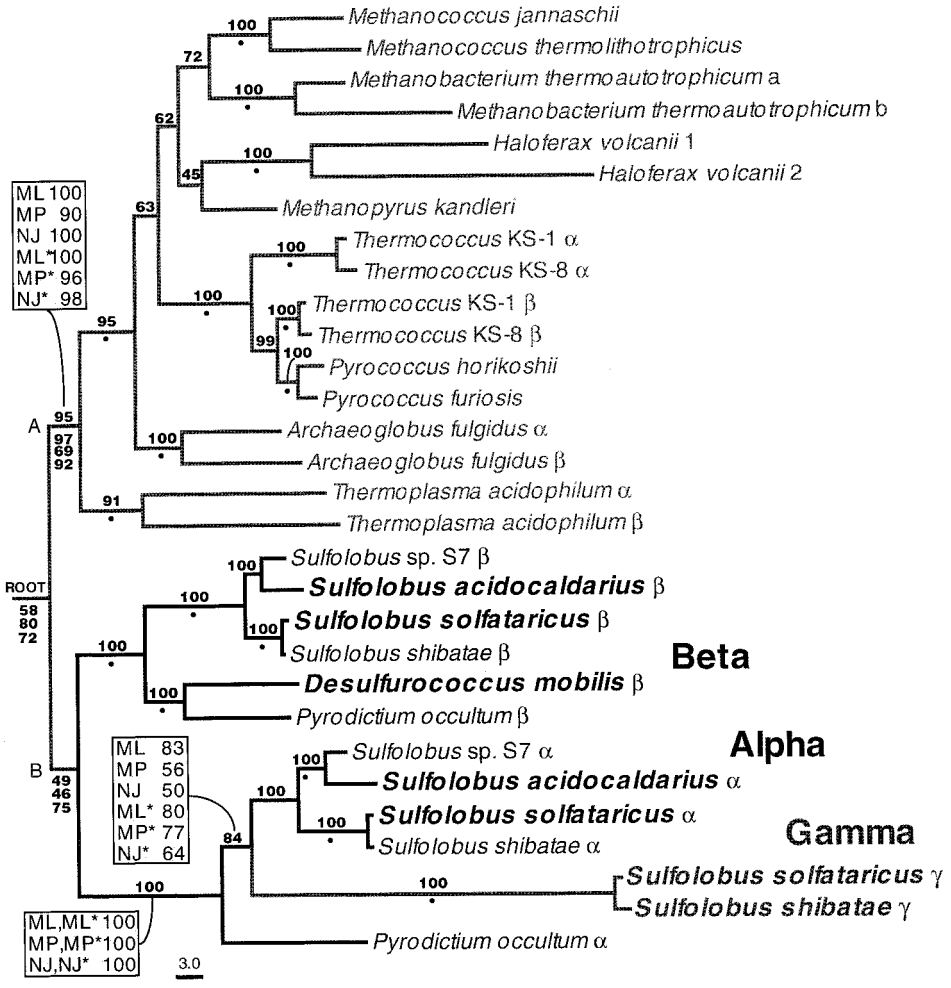

FIG. 14. Phylogeny of archaeal chaperonins as illustrated by a maximum-likelihood tree constructed with the chaperonin amino acid sequences. The two major branches, euryarchaeota (top half; light lines) and crenarchaeota (bottom half; dark lines), are shown. Percent support values are given above each node. Inset boxes indicate support for nodes of particular interest—values were derived from various tree reconstruction methods: maximum-likelihood (ML), maximum-parsimony (MP), and neighboring-joining distance (NJ). The influence of site-by-site rate variation on the support for these nodes was also tested; support values from analyses in which fastest-evolving sites were removed are labeled with an asterisk. The position of the eukaryote outgroup root (ROOT) was determined from additional phylogenetic analyses. Support values for nodes A, B, and ROOT are given in the order ML, MP, and NJ from top to bottom. The names of the species from which new chaperonin genes (not listed in Table 15) were cloned and sequenced are shown in boldface. The scale bar indicates 3.0 substitutions per 100 amino acid sites. The data suggest that among euryarchaeota, linage-specific gene duplications occurred in *M. thermoautotrophicum*, *H. volcanii*, *A. fulgidus*, *T. acidophilum*, and the *Pyrococcus*/*Thermococcus* clade, and that among crenarchaeota, the α and β genes arose from a duplication pre-dating the separation of *Sulfolobus* and *Pyrodictium* but the α and γ paralogs of *Sulfolobus* resulted from a duplication subsequent to that separation. Reprinted from reference 5 (in which sources of sequences, methods, and other details are given) with permission of the publisher.

binding type (83, 136). The functions and mechanisms of action of the archaeal PPIase homologs remain to be determined.

Recently, a disulfide oxidoreductase that contains two thioredoxin fold units from the hyperthermophilic archaeon has been characterized (238). This protein falls within a large group of enzymes that share the sequence motif CXXC and comprises various subgroups such as the protein disulfide isomerase (PDIase), thioredoxin, glutaredoxin, and DsbA families (76, 238). The archaeal enzyme had the capacity to catalyze dithiol oxidation and reduced disulfide bridges. The crystal structure revealed similarities to the eukaryotic PDIase.

Other putative stress genes have been identified by computer analysis in sequenced archaeal genomes; a sample of these is presented in Table 19. These genes would encode proteins with sequences similar to known stress or stress related proteins. As in other fields of biology, the availability of whole-genome sequences has helped archaeal research and will continue to do so. We have already discussed how critical were the contributions made by the sequencing of the *M. jannaschii* and *M. thermoautotrophicum* ΔH genomes to the clarification of the distribution of *hsp70* among archaea and its possible origin. This is just one example from a long list. The future is very promising. For example, an important area that will benefit from the availability of genome sequences is the study of the factors involved in the regulation of the archaeal stress genes. Also, it would be interesting to identify the whole set of genes that are active during stress and to determine which ones are essential for protein biogenesis under physiological conditions in the hyperthermophiles that lack the Hsp70(DnaK) chaperone machine.

STRESSORS

As pointed out above, cell stress has become known, for historical reasons, as the heat shock response (reference 177

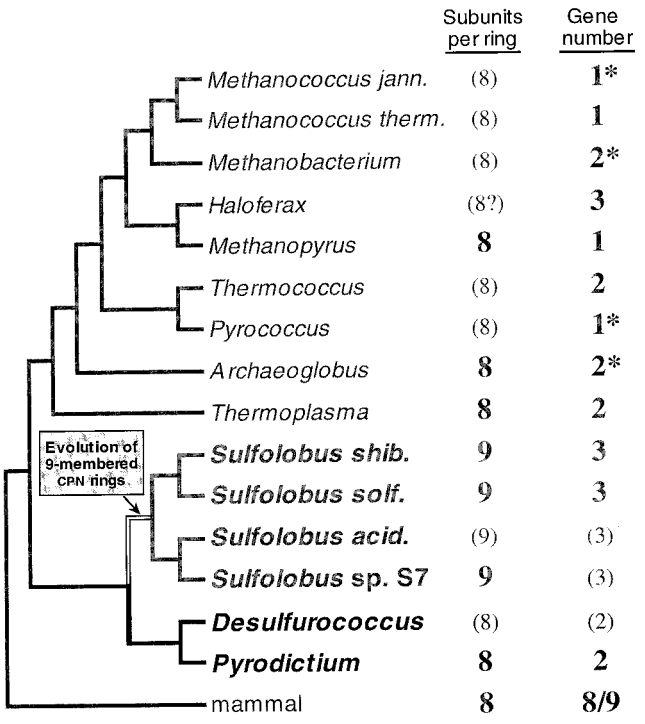

FIG. 15. A proposal for the evolution of chaperonin gene number and chaperonin complex symmetry. The cladogram on the left displays the archaeal relationships based on the phylogenetic analysis of the chaperonin amino acid sequences shown in Fig. 14. The number of known or predicted subunits per chaperonin ring and of known or predicted chaperonin genes in each archaeal species are listed in the two columns on the right. Subunits per ring: boldface values indicate known subunit stoichiometry from electron microscopic studies (see also Table 14); values within parentheses are predicted. Gene number: boldface values indicate known gene numbers from sequence data; asterisks indicate total gene numbers confirmed by complete genome sequence; values within parentheses are predicted. The data show that the eight-membered chaperonin ring is widespread among euryarchaeota (see also Table 14), as is the case in eukaryotes (295), but that the situation may be different in *Sulfolobus*. It would appear from gene sequence data that a transition from eight- to nine-membered chaperonin (CPN) rings (inset) occurred in an ancestor of the extant *Sulfolobus* during crenarchaeal evolution. This suggestion awaits confirmation from experiments that would demonstrate the existence of a third, distinct protein that is functional in vivo along with the other two subunits. Reprinted from reference 5 (in which source of sequences, methods, and other details are given) with permission of the publisher.

and references therein). While this name acknowledges the pioneers who discovered the reaction of a cell to a temperature elevation, it does not reflect the entire scope of the field.

It is true that the archetypal stressor used by most investigators to test genes and measure the stress response of a cell is heat. Nonetheless, there are many other stressors, at least as important as heat from the ecological, biotechnological, and medical viewpoints (177, 250, 252, 292). Certain stressors are specific to some organisms that live in ecosystems very different from those inhabited by terrestrial mammals such as humans. Some archaea do, indeed, thrive in extreme environments, under conditions of temperature, pressure, pH, or salt concentration which would stress or even kill human cells. "Extremophilic" archaea offer a unique opportunity to study how a cell copes with agents and conditions that would not be tolerated by other cells. The information may one day prove useful, for example, to design means of improving the resistance of human or plant cells to stressors that cause disease or impair crops.

Research in this particular area has included the testing of a range of stressors that are mostly pertinent, in each case, to the environment in which the organism lives, as illustrated by the examples listed in Table 20.

STRATEGIES AND METHODS USED TO STUDY THE STRESS RESPONSE, GENES, AND PROTEINS IN THE ARCHAEA

Now that we have become acquainted with the archaeal organisms and the stress genes, proteins, and stressors pertinent to these organisms, we can summarize the methods that were applied as a practical guide for future research. The strategies and procedures were varied, as shown in Table 21. They included most of the methods routinely applied to the study of bacterial and eukaryotic cells but with the modifications necessary to deal with the special problems posed by some archaeal organisms. Examples of these special problems are growth at very high temperature and pressure, anaerobiosis required to preserve enzymatic activity, high resistance to cell disruption with the resulting difficulty in obtaining good yields of macromolecules relatively undamaged or unfragmented, and other equally serious technical obstacles that make research with some archaea quite challenging.

STRESS TOLERANCE

Questions

It is difficult to think of a cell or organism that will not be exposed to stressors and suffer stress many times during its life. Does a cell learn anything from stress? Does it learn how to better cope with a second aggression by the same stressor or a similar one? Can a cell develop acquired resistance to a stressor or groups of similar stressors after a first aggression? How about archaea? Are they different from the better-studied bacteria and eucarya?

Research to answer these questions is important because it will not only uncover mechanisms of cell resistance but it will also provide the basis for practical applications aimed at the fortification of cells to enhance survival and recovery. The implications for the biotechnology industry, medicine, planetary exploration, and other endeavors of interest to mankind are obvious and need not be mentioned here.

Cells are equipped to deal with a first encounter with stressors as explained in preceding sections. In addition, there is experimental evidence indicating that cells do acquire higher levels of resistance to a second encounter, although this enhanced resistance (stress tolerance) is of relatively short duration (47, 114, 128–130, 203, 250, 261, 279, 282).

Typically, stress tolerance is defined by measuring cell survival after each of two consecutive stresses. The first is induced by a nonlethal dose of stressor, but the cells are challenged the second time with a dose that would be lethal for nonpretreated cells. If the cells survive the lethal dose, it is concluded that they have developed stress tolerance.

What makes the cell more resistant to stressors? What is the mechanism of stress tolerance?

We mentioned at the beginning of this review that a key component of stress is protein denaturation, followed by aggregation and ultimately cell death. Therefore, the central point of defense for the cell is to prevent protein denaturation and aggregation or to reverse these processes while they are still reversible. The cell has several means of achieving these goals, i.e., stress (heat shock) proteins, molecular chaperones, and chaperonins, as explained throughout this review. Moreover, cells have other molecules and mechanisms to deal with stress. These are thermoprotectants, formation of multicellular

TABLE 16. Examples of archaeal Hsp60 proteins (chaperonins) that have been studied experimentally

Organism	Protein or structure[d]	Length (amino acids)/ mass (kDa)[a]	Function/structure	Remarks	Reference(s)
Archaeoglobus fulgidus	ThsA/Cpn-α ThsB/Cpn-β	545/59.0 545/59.7	NR[b] NR	Heat shock-induced increase	74
Desulforococcus strain SY	HHSP		NR		140
Haloferax volcanii	Cct1 Cct2	560/59.0 557/59.0	Two ATP-binding sites, including GDGTT[c] Two ATP-binding sites, including GDGTT		163
Methanococcus jannaschii	Chaperonin	NR/60.0	ATP-binding site (GDGTT)	Refolding activity	161
Methanopyrus kandleri	Thermosome	545/59.5	ATP-binding site (GDGTT)	NH_4^+-dependent ATPase activity	2, 3
Methanococcus thermolithotrophicus	MTTS	544/NR	ATP-binding site (GDGTT); unique GGX (M,D,S)[c] repeats in C terminus	ATPase activity; refolding activity; forms filaments; specific nucleotide requirements	84
Pyrococcus kodakaraensis KOD1	CpkA CpkB	549/59.2 546/59.1	ATP-binding site (GDGTT) ATP-binding site (GDGTT)	Enhances solubility of peptide Prevents thermal denaturation; enhances thermostability	137, 305
Pyrodictium brockii	ATPase complex	NR/56 and 59	NR	Heat shock-induced increase	230
Pyrodictium occultum	TF55/ATPase complex	NR/56 and 59	NR	Heat shock-induced increase; ATPase activity	202, 230
Sulfolobus shibatae	TF55-β TF56-α	552/59.7 560/59.9	No conserved ATP-binding site ATP-binding site (GDGTT)	Heat-shock induced increase; ATPase activity; binds unfolded protein; conformational changes; forms filaments	141, 234, 280, 307
Sulfolobus solfataricus	α subunit β subunit	NR/NR NR/NR	NR NR	Associated with aminopeptidase activity; RNA binding; involved in rRNA processing; specific endonuclease activity; refolding activity	43, 101, 154, 187, 249
Sulfolobus sp. strain 7	α subunit β subunit	559/60.2 552/59.9	NR NR	No ATPase activity; involved in refolding; able to form homo-oligomeric complexes in vitro	207, 309
Thermococcus strain KS-1	α subunit β subunit	545/59.1 548/59.2	ATP-binding site (GDGTT), G-M[c] motif in C-terminus ATP-binding site (GDGTT)	ATPase activity; able to form homo-oligomeric complexes in vitro; involved in refolding	308
Thermoplasma acidophilum	α subunit β subunit	545/58.2 543/58.5	ATP-binding site (GDGTT) ATP-binding site (GDGTT)	Alternating α and β units; binds unfolded protein; ATPase activity	65, 153, 216, 289, 290

[a] Calculated from the deduced amino acid sequence.
[b] NR, not reported.
[c] Amino acid single-letter symbols.
[d] HHSP, hyperthermophilic heat shock protein; MTTS, *Methanococcus thermolithotrophicus* thermosome.

TABLE 17. Molecular chaperones and chaperonins in the three phylogenetic domains

Protein in bacteria[a]	Equivalent protein in:	
	Archaea[a]	Eucarya[a]
GroEL (Hsp60)	No[b]	mt, chl: Hsp60 (Rubisco subunit binding protein); ct, ER: No
GroES (Hsp10)	No	mt, chl: Yes; ct, ER: No
No	TF55; thermosome subunits	ct: TRiC (CCT; TCP-1) subunits; mt, chl, ER: No
G+, DnaK(Hsp70)	Hsp70(DnaK)[c]; No-hyp.	No
G−, DnaK(Hsp70)	No	mt, chl: Hsp70; ct, ER: Hsp70 para.[f]
DnaJ(Hsp40)	Hsp40(DnaJ)[c]; No-hyp.	ct, mt, chl, ER: Yes
GrpE	GrpE; No-hyp.	mt, chl: Yes; ct, ER: No
G−, HptG	No	ct, ER: Hsp90 para.; mt, chl: No

[a] Abbreviations: mt, mitochondria; chl, chloroplast; ct, cytosol; ER, endoplasmic reticulum; G+ and G−, gram-positive and -negative type of DnaK, respectively. No-hyp., not yet investigated, or investigated but not yet found in hyperthermophiles; para., paralogous.

[b] No, not yet investigated or not well characterized, or investigated but not yet found.

[c] Protein similar to homologs in gram-positive bacteria but transcription initiation mechanism similar to that of eucarya.

structures, and other factors such as intrinsic thermoresistance of the proteins themselves.

Thermoprotectants

A variety of nonprotein molecules also play a role in protecting the cell from the effects of stressors, and some of these molecules may play a role akin to that of the chaperone proteins (41, 122, 128, 190).

Two thermoprotectants have been found in hyperthermophilic archaea: di-*myo*-inositol phosphate (DIP) and cyclic diphosphoglycerate (cDPG) (128). High concentrations of both DIP and cDPG have been measured in *Methanothermus fervidus* and *Methanopyrus kandleri* (122, 128). DIP has been found in *A. fulgidus*, *Pyrodictium occultum*, *Methanoccus igneus*, *Pyrococcus woesei*, and *Pyrococcus furiosus* (40, 193, 257).

cDPG and DIP were tested in vitro and found to stabilize certain proteins, but not others, to make them resistant to high temperatures (128). These compounds are believed to be important for thermotolerance in organisms living at high temperatures and for their survival under stress. It is assumed that the enzymes needed to synthesize DIP, cDPG, and other molecules necessary to withstand stress must also be active during heat shock, perhaps due to built-in thermoresistant features (36, 56, 215, 254). These features are not yet completely understood, although clues that might explain why some proteins are more resistant to heat than others are beginning to emerge (115, 156). However, these enzymes may be considered stress proteins, since they would fall within the broad definition of these molecules that includes the characteristics of being present or increased, and active, during and immediately after stress.

Trehalose also plays a role in protein folding and in stress tolerance, at least in some organisms (reference 261 and references therein). The disaccharide has been found in a few archaeal species, although its functions have not been elucidated (61, 194). Likewise, osmolytes that seem to play a role in osmotic adaptation and in the response to osmotic stress have been identified in archaeal cells (54, 68, 122, 190, 233, 266).

Multicellular Structures

Another line of defense of some archaea against mechanical, physical, and chemical stressors is the cell wall (18, 50, 100, 157, 158, 228, 262, 267), which may be supplemented by the formation of supracellular structures. These may be flat or globular and are named biofilm and packet or granule, respectively (48, 134, 197, 306). They may be composed of a single archaeal species, as illustrated in Fig. 16 (197), or of several different organisms, including archaeal and bacterial species (43, 134, 185). An example of the latter mixed microbial structure or consortium, taken from a bioreactor processing organic substrates, is shown in Fig. 17 (180a). Methanosarcinas and other methanogens are found in these granular consortia. In addition, other archaea, for example *A. fulgidus*, *M. jannaschii*, and *Thermococcus litoralis*, can build up biofilms (166, 241). *A. fulgidus* maintained at the OTG (83°C) does not form a biofilm, but it does so when it is exposed to higher temperatures.

M. mazei S-6 packets are considerably more resistant than single cells to mechanical and chemical stressors (180a). Also, induction of a detectable heat shock response in packets, assessed in terms of an increase in the level of the mRNA from the *hsp70* locus genes, requires higher temperatures and longer heat shocks than does induction of the response in single cells. The results suggest that the cells inside the packets are protected from environmental changes by comparison with single cells, as illustrated in Fig. 18 (44).

The archaeal multicellular structures have an intercellular connective material made of acidic polysaccharides and proteins (86, 157, 158). There is some information about the biochemistry of these components, but more research is needed to elucidate their role, thermoresistance, insulating properties, and other characteristics that might achieve cell protection without interfering with the influx of nutrients and the efflux of catabolites. The bioreactor granules, for example, have a net of microtubes crisscrossing the interior and the cortex, in which the connective material is abundant, as shown in Fig. 19 (185). These tubes may provide a circulation network to ensure that the cells in the interior of the granules, even those in the deepest zones, receive nutrients and find a way to discharge what they produce. It is likely that the granules have evolved because of the frequent modifications in temperature, pH, and other conditions that occur in the ecosystems in which they are found and are better equipped than the solitary cells to withstand environmental changes.

Other Factors

The cell surely possesses other means in addition to all those already discussed to protect itself and withstand environmental changes. For example, there must be several genes and proteins that are required to produce the known chaperones and chaperonins, the thermoprotectants, and the materials to form a biofilm or granule, which must be active during stress. Some of these proteins must be enzymes and other cofactors of various types, with built-in structural features that must make them more stable than the rest under stress conditions (13, 36, 56, 102, 115, 156, 215, 224, 254). Among this group of intrinsically stress-resistant molecules are probably the chaperones and chaperonins themselves.

It has been reported recently that *Sulfolobus acidocaldarius*, a thermoacidophilic archaeon, changes its motility according to the ambient temperature (169). The findings suggested that motility changes were a response to heat stress and were a mechanism for escaping lethal temperatures. This is an interesting working hypothesis that ought to be tested by comparing

TABLE 18. Other examples of archaeal stress or stress-related genes and proteins

Protein	Mass (kDa)	Organism	Presumed function	Inducer	Reference(s)
Superoxide dismutase	20	*Halobacterium halobium*	Scavenger of oxygen free radicals	Heat	17
Crx protein trio	40.8, 42.3, and 42.9	*Methanobacterium bryantii*	Copper or general resistance	Copper	147
Betaine transporter	NR[a]	*Methanosarcina thermophila* TM-1	Maintenance of internal ionic balance	Osmotic stress	233
Inositol compounds	NR	*Methanococcus igneus*, *Pyrococcus woesei*, *Pyrococcus furiosus*	Maintenance of internal ionic balance	Osmotic stress	40, 193, 257
TrkA	44.1	*Methanosarcina mazei* S-6	Maintenance of internal K^+ balance	Ammonia	164
DNA repair system	NR	*Pyrococcus* AL585	DNA repair	UV light	24
Prefoldin	14–23[b]	*Methanococcus jannaschii*	Protein folding	NR	287
sHsp	NR	*Methanococcus jannaschii*	RNA stabilization, thermotolerance	NR	148
ClpB	NR	*Methanosarcina acetivorans*	Growth and survival at high temperatures; involved in proteolysis	NR	98
Bacterioruberin	NR	*Haloferax mediterranei*	Maintenance of internal ionic balance	Osmotic stress	68
Several, unidentified	22, 25, 40, 70, 55, and 90	*Thermococcus peptonophilus*	General stress protection	Heat and pressure	33
PPIase	19.4–31[c] 16 or 42[c] NR 17.6	*Halobacterium cutirubrum* *Methanococcus thermolithotrophicus* *Methanococcus jannaschii* *Thermococcus* sp. KS-1	Acceleration of rate-limiting step in protein folding	NR	31, 83, 135, 136
Two, unidentified	90 and 150	*Pyrococcus* ES4	General stress protection	Cold shock	129
Several, unidentified	20, 22, 29, 30, 31, 32, 36, 42, and 88	*Sulfolobus acidocaldarius*	General stress protection	P starvation	221
17, unidentified	Mainly in the groups 21–28, 44–45, and 75–105	*Haloferax volcanii*	General stress protection	Hyposalinity	55
Proteasome	24 and 22[d] 25.8 and 22.3[d] NR	*Methanosarcina thermophila* TM-1 *Thermoplasma acidophilum* *Methanobacterium thermoautotrophicum* ΔH	Protein degradation	NR	195, 196, 248, 263

[a] NR, not reported.
[b] Six subunits within the indicated size range.
[c] Depends on the method used.
[d] α- and β-subunits, respectively.

archaeal species from various habitats with different temperatures.

Lastly, some conditions in the environment may increase stress tolerance in organisms that live in extreme ecosystems, for example deep down on the ocean floor. It has been reported that hydrostatic pressure enhances thermotolerance and protein stability (121, 128–130, 138, 159, 200, 201, 232). For instance, *Pyrococcus* strain ES4 (OTG, 99°C) acquired thermotolerance to a heat shock at 105°C (a lethal temperature for ES4) after exposure to 102°C for 90 min (130). Cells grown at low pressure (3 MPa) also acquired thermotolerance when they were transferred to a culture at 22 MPa. This thermotolerance lasted for a considerable time after the cells were returned to 3 MPa, suggesting that hyperpressure had set in motion events that were meant to persist.

PERSPECTIVES, OPEN QUESTIONS, AREAS FOR EXPLORATION, AND APPLICATIONS

Biochemistry and Function of Archaeal Chaperones and Chaperonins

The functions of the archaeal Hsp70, Hsp40, and GrpE (the Hsp70 machine) proteins have not been studied in vitro or in vivo to any significant extent; they are certainly much less extensively studied than their homologs from the bacteria and eucarya. It is not known to what extent the archaeal proteins resemble those from the other two domains in their interaction with each other and with substrates, nucleotides, ions, cochaperones, auxiliary factors, and regulatory molecules.

There are several aspects that are unique to the archaea and whose elucidation will enhance our understanding of protein

TABLE 19. Some stress related gene and protein homologs identified in sequenced archaeal genomes

Organism	Protein	Identification no.	Reference(s)
Methanococcus jannaschii	Heat shock protein X	MJ1682	31
	Heat shock protein 31	MJ0285	
	DNA repair protein 45	MJ0869	
	DNA repair protein RAD51	MJ0254	
	DNA repair protein RAD2	MJ1444	
	PPIase	MJ0278	
	PPIase	MJ0825	
	Proteasome α-subunit	MJ0591	
	Proteasome β-subunit	MJ1237	
	Survival protein	MJ0559	
Methanobacterium thermoautotrophicum ΔH	Heat shock protein X	MTH569	263
	Heat shock-related protein X	MTH1817	
	Heat shock protein class I	MTH859	
	DNA repair protein rad2	MTH1633	
	DNA repair protein rad51	MTH1693	
	DNA repair protein radA	MTH541	
	DNA repair protein rad32	MTH1383	
	PPIase	MTH1125	
	PPIase B	MTH1338	
	Proteasome, α subunit	MTH686	
	Proteasome, β subunit	MTH1202	
	Survival protein (SurE)	MTH1435	
Archaeoglobus fulgidus	Heat shock protein (htpx)	AF0235	151
	Small heat shock protein (hsp20-1)	AF1296	
	sHsp (hsp20-2)	AF1971	
	Proteasome, α subunit	AF0490	
	Proteasome, β subunit	AF0481	
Pyrobaculum aerophilum	Heat shock protein	PA95	79
	Heat shock protein	PA305	
	DNA repair protein rad2	PA289	
	DNA repair protein (XRCC1)	PA290	
	β-Type proteasome	PA403	
	Proteasome β	PA408	
Pyrococcus horikoshii OT3	Thermophilic factor	PH0017	144, 145
	DNA damage-inducible protein	PH1807	
	DNA repair protein	PH0263	
	DNA repair protein, putative	PH1704	
	Proteasome, β subunit, putative	PH0245	
	Proteasome, β-subunit precursor	PH1402	
	Proteasome, α subunit, putative	PH1553	
Pyrococcus abyssi	Heat shock protein (htpX)	PAB0758	*P. abyssi* website
	Heat shock protein (htpX)	PAB1974	
	sHsp (hsp20)	PAB2072	
Aeropyrum pernix K1	Heat shock protein	APE0754	143
	Heat shock protein (htpX)	APE1045	

biogenesis and molecular chaperoning in general. For example, is prefoldin (89, 287) critical for protein folding in archaea, and does it require another complementary chaperonin system? Does the archaeal Hsp70 machine interact with chaperonins that have yet to be identified? Does the Hsp70 machine interact with the type (or group) II chaperonins in archaea? If so, how? What is known about interactions between the Hsp70 machine and the chaperonins has been learned mostly from studies in bacteria. These have type I chaperonins which differ from those of type II. Along the same line, another question is pertinent: What, if anything, replaces the Hsp70 machine in the archaeal species that lack it? Is there an archaeal equivalent of the Hsc66 protein in some bacterial species in addition to Hsp70(DnaK) (260), which performs at least some of the functions typical of the latter? The answers to these questions will most probably shed light on the protein-folding problem in general. If a substitute for Hsp70(DnaK) were found in archaea, it would probably be the ancestor of a eucaryal homolog, whose functions could then be better understood by studying both in parallel.

All the archaeal species that lack the Hsp70 machine and that have been examined to identify chaperonins have been found to possess the type II system. This raises the following question: does the archaeal chaperonin system (thermosome) require interaction with a second system that would play a role similar to that of the Hsp70 machine? Data from eukaryotic models suggest that the cytosolic chaperonin system (type II) does not interact with the Hsp70 machine. It is not safe, however, to conclude that the same is true for the archaea, or at least for all archaea. Although the archaeal and eukaryotic chaperonin complexes (e.g., CCT and the thermosome), with their respective subunits, are similar and are both classified as type II, they do show structural differences (Fig. 13). Hence, differences in the function and mechanism of action between the thermosome and CCT are to be expected. What are their respective, preferred substrates? Do they both interact with prefoldin?

It would also be of interest, for example, to determine the substrates for the archaeal Hsp70 machine and how this machine recognizes the substrates. Differences by comparison with the bacterial machine will probably be found, since differences in binding properties between the cytosolic and mitochondrial Hsp70 molecules, for example, have been demonstrated (7).

Elucidation of the mechanism of polypeptide folding in vitro by the archaeal chaperonin complex should be pursued. For example, additional data ought to be obtained on the open stage of the thermosome, polypeptide capture, and release.

TABLE 20. Examples of stressors, other than heat, tested with archaeal cells

Stressor	Organism	Reference(s)
Hyperosmolarity	*Pyrococcus furiosus*, *Methanosarcina thermophila* TM-1, *Methanosarcina mazei* S-6, *Haloferax volcanii*, *Methanococcus igneus, Methanococcus thermolithotrophicus*	40, 41, 77, 165a, 193, 204, 233
Hypoosmolarity	*Haloferax mediterranei*, *Haloferax volcanii*	68, 77, 163, 204
Pressure	*Pyrococcus* strain ES4, *Pyrococcus* strain ES1, *Methanococcus thermolithotrophicus*, *Methanococcus jannaschii*, *Thermococcus peptonophilus*	33, 130, 138, 200, 232
Ethanol	*Methanococcus voltae*	119
UV light	*Sulfolobus acidocaldarius*, *Pyrococcus furiosus*	64, 301
Copper	*Methanobacterium bryantii*	147
Heavy metals	*Methanosarcina mazei* S-6	179
H_2O_2	*Methanococcus voltae*	119
Casamino Acid/Fe^{2+} starvation	*Metallosphaera sedula*	114, 225
pH	*Pyrococcus furiosus*	36
Ammonia	*Methanosarcina mazei* S-6, *Methanosarcina thermophila* TM-1	165, 165a
P starvation	*Sulfolobus acidocaldarius*	221

TABLE 21. Strategies and methods used to study the stress response, genes, and proteins in archaea

Method	Stressor/conditions[a]	Gene or protein and organism[b]	Reference(s)
Culture			
Viability, acquired stress tolerance	Heat	PES4, SSH, MSE	33, 64, 68, 114, 129, 130, 201, 204, 221, 225, 233, 279, 281, 282
	Pressure	MJA, PES4, PES1, TPE	
	Osmotic stress	MTP, HXV	
	Irradiation	PFU	
	P starvation	SAC	
Growth	Continuous culture[c]	MSE	114, 255
Morphology	Heat/starvation	MSE (membrane potential effects)	165a, 255
	Ammonia	MMA, MTP (cell shape)	
DNA			
Southern blot as only identifier		*hsp70*: MAC, MBA, MHU, HHA *pho*: SAC	10, 42, 99, 164, 221
Sequencing of gene		*hsp60*: AFU, DSY, HXV, MTH, MJA, MTL, MKA, PAE, PKOD, SSH, SSP7, TKS, TAC *hsp70*: HCU, HMA, MTH, MMA, MTP, TAC HCU (*hsp40*), MMA (*grpE*, *hsp40*, *trkA*), MTP (*hsp40*), PAL585 (*dinF*), MBR (*crx*) Other[d]: MJA, AFU, PAE, PHO, MTH	3, 24, 31, 32, 45, 79, 84, 105, 110, 111, 126, 140, 141, 143, 144, 147, 151, 163, 181–183, 207, 263, 281, 288, 305, 308
Stability of DNA	Heat	PFU	224
DNA repair	UV light	SAC, PFU	64, 301
DNA (plasmid) topology	Heat and cold	*Sulfolobus* species	173, 174
RNA			
Stress related increase in transcript (Northern/slot blot)	Heat	*hsp60*: SSH, HXV	42, 44, 126, 141, 147, 163, 165, 277
	Heat, ammonia, metal	*hsp70*: MMA, MTP[d]	
	Heat and ammonia	Others: MMA (*hsp40*, *grpE*, *trkA*)	
	Copper	MBR (*crx*)	
Transcriptionally active regions of genome	Heat and osmotic stress	HXV	77, 283
Protein			
1-D electrophoresis (unidentified proteins)	Osmotic stress	HME	33, 68, 114, 129, 130, 141, 147, 225, 230
	Pressure	TPE, PES4	
	Heat shock	POC, TPE, MSE, SSH	
	Copper	MBR	
	Starvation	MSE	
	Cold	PES4	
1-D electrophoresis, radiolabelled	Osmotic stress	HXV (several), HME, MTP (betaine)	55, 68, 110, 119, 139, 141, 204, 233, 234, 279, 282
	Heat	SSH (Hsp60[e]), MVO (11[f]), HMA (4), SAC (4)	
	Oxidative stress	HXV	
	Ethanol	MVO	
		MVO (11[f]), SAC (none)	
2-D electrophoresis (unidentified proteins)	Heat	HXV, MSE	114, 204, 221
	P starvation	SAC	
2-D electrophoresis, radiolabelled	Pressure	MTL	55, 138, 204, 221
	Osmotic stress	HXV	
	P starvation	SAC	
	Heat	HVO	
Isoelectric focusing		SSO(Hsp60)	154
Stress-accumulation determined by NMR	Osmotic stress	PFU, MIG, PWO (inositol compounds), PFU, MIG (inositol)	40, 41, 193, 257
	Heat		
Cross reaction with antibodies for stress proteins		Hsp60: ABR, TTE, POC, SMA, MSE, SAC, PAB, HBU, DAM, AFU, TAC	114, 141, 225, 230, 290
Peptide analysis/sequencing		Hsp60: SSO, TAC, SSP7, MKA Crx: MBR	3, 147, 154, 207, 289
Purification of protein		Hsp60: SSH, TAC, MKA, PKOD, SSO, POC, SSP7, MJA, MTL, TKS sHsp: MJA Crx: MBR	3, 65, 84, 101, 147, 148, 154, 161, 187, 207, 216, 230, 234, 281, 289, 290, 305, 308

Continued on following page

TABLE 21—*Continued*

Method	Stressor/conditions[a]	Gene or protein and organism[b]	Reference(s)
Structure	See Table 14	Hsp60: SSH, TAC, MKA, MTL, POC, SSP7, PBR, SSO, TKS sHsp: MJA	3, 65, 84, 148–150, 154, 187, 207, 216, 230, 231, 234, 279, 280, 281, 289, 290, 307, 308
Conformational changes	Heat or ATP	Hsp60: SSH, SSO	101, 154, 170, 171, 234, 246, 307
Stress accumulation (Western blotting/activity gels)		Hsp60: POC, MSE, AFU Hsp70: MMA Superoxide dismutase: HHA Crx: MBR	17, 42, 74, 114, 147, 230
Analytical gel filtration and ultrafiltration; spectral analysis	Recombinant proteins tested in vitro	Hsp60 (α and β subunits)	202
Refolding experiments		Hsp60: SSP7, SSH, SSO, MTL, PKOD, TKS, MJA	84, 101, 161, 207, 234, 305, 308
ATP-binding site in sequence		Hsp60: TAC, MKA, MJA, SSH, PKOD, HXV, MTL, TKS	3, 65, 84, 141, 161, 163, 289, 305, 308
ATPase activity as indicator of chaperonin action		Hsp 60: PKOD, SSO, SSH, SSP7, POC, TAC, MKA (negative result), MJA, MTL, TKS	3, 65, 84, 101, 154, 161, 207, 230, 234, 281, 289, 305, 308
Stabilization of proteins	Pressure Heat	MIG, MJA (protease) POC, MJA (protease), PKOD, MJA (Hsp60)	121, 161, 200, 230, 305
Recombinant expression of stress proteins in *E. coli*		TAC, MJA, PKOD, PAL585 (*dinF*), HXV, MTL, TKS, AFU	24, 74, 84, 148, 163, 216, 290, 305, 308
Binding of denatured proteins		SSH, TAC, SSO, PKOD, MJA	101, 161, 234, 289, 305
Proteasome function Thermostability of protein		TAC MJA, POC	248 200, 230
Reassembly		Hsp60: SSO, SSH, TAC	141, 154, 234, 290
Filament formation		Hsp60: MTL, SSH	84, 280, 307
RNA binding and processing		Hsp60: SSO	249
Protein modification	P starvation	SAC (phosphorylation) SSO (methylation)	13, 221

[a] Blanks in this column indicate absence of information.

[b] Organisms (in capitals) are: ABR, *Acidianus brierleyi*; AFU, *Archaeoglobus fulgidus*; DAM, *Desulfurolobus ambivalens*; DMO, *Desulfurococcus mobilis*; DSY, *Desulfurococcus* strain SY; HBU, *Hyperthermus butyricus*; HCU, *Halobacterium cutirubrum*; HHA, *Halobacterium halobium*; HMA, *Haloarcula marismortui*; HME, *Haloferax mediterranei*; HXV, *Haloferax volcanii*; MAC, *Methanosarcina acetivorans*; MBA, *Methanosarcina barkeri*; MBR, *Methanobacterium bryantii*; MFE, *Methanothermus fervidus*; MHU; *Methanospirillum hungateii*; MIG, *Methanococcus igneus*; MJA, *Methanococcus jannaschii*; MMA, *Methanosarcina mazei* S-6; MKA, *Methanopyrus kandleri*; MSE, *Metallosphaera sedula*; MTH, *Methanobacterium thermoautotrophicum* ΔH; MTL, *Methanococcus thermolithotrophicus*; MTP, *Methanosarcina thermophila* TM-1; MVO, *Methanococcus voltae*; PAB, *Pyrodictium abyssi*; PAE, *Pyrobaculum aerophilum*; PAL585, *Pyrococcus* strain IFREMER585; PBR, *Pyrodictium brockii*; PES1, *Pyrococcus* strain ES1; PES4, *Pyrococcus* strain ES4; PFU, *Pyrococcus furiosus*; PHO, *Pyrococcus horikoshii* OT3; POC, *Pyrodictium occultum*; PKOD, *Pyrococcus* st. KOD; PWO, *Pyrococcus woesei*; SAC, *Sulfolobus acidocaldarius*; SMA, *Staphylothermus marimus*; SSP7, *Sulfolobus* Sp. strain 7; SSH, *Sulfolobus shibatae*; SSO, *Sulfolobus solfataricus*; TAC, *Thermoplasma acidophilum*; TPE, *Thermococcus peptonophilus*; TKS, *Thermococcus* strain KS-1; TTE, *Thermoproteus tenax*. DNA (genes) and RNA (transcripts) are shown in italics.

[c] All others are batch experiments.

[d] Part of whole-genome projects.

[e] Protein (first letter in capital and rest in lower case); Hsp60/*hsp60*, heat shock protein 60/gene belonging to the chaperonins; Hsp70/*hsp70*, heat shock protein 70/gene belonging to the Hsp70(DnaK) chaperone system; Crx/*crx*, copper-responsive protein/gene.

[f] Number of induced proteins within parentheses.

Cryoelectron tomography of a thermosome formed only by recombinant α subunits expressed in *E. coli* revealed the 3-D structure of the open state (Fig. 12) (217). This experimental model of a homopolymeric complex was about 18 nm long and had a diameter of approximately 15 nm. The central channel was calculated to have a volume probably too large to influence the selection of which substrate would enter the cavity solely based on size. It seems unlikely that such a large cavity would have the plasticity to discriminate between substrates that differ in size, particularly when the differences are not pronounced and when the substrates are very large or very small. In contrast, the estimated volume of the closed chamber was small and would accommodate substrates (polypeptides) of up to 50 kDa at most, which is about the same as that suggested for the bacterial chaperonin complex (251). Several questions remain unanswered. For instance, is prefoldin involved in substrate selection and/or presentation to the thermosome and in the closing of the folding chamber? Experiments ought to be done to elucidate how a folding cavity without a removable lid passes from an acceptor to a folding phase. Moreover, a comparison between the chaperonins of hyperthermophiles and those of mesophiles and psychrophiles should provide insights into the evolution of the system and its modifications to cope with different environments.

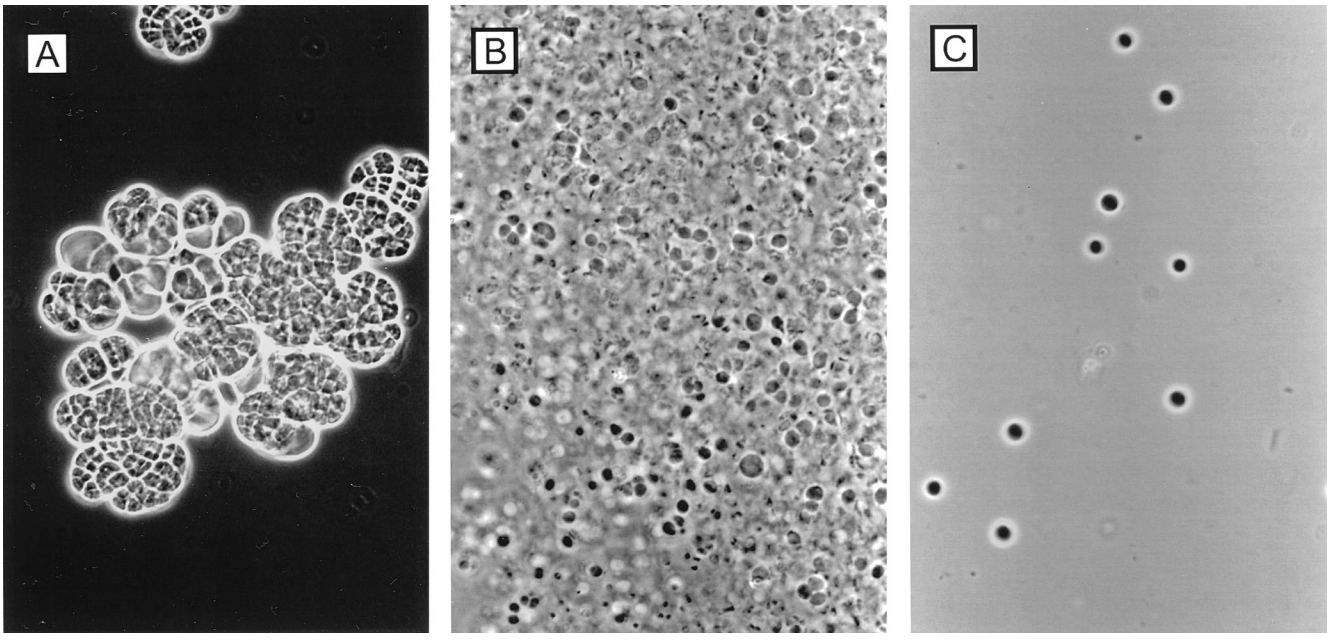

FIG. 16. Archaeal multicellular structures. *M. mazei* S-6 packets (A) and lamina (B) are displayed, along with the single-cell morphotype (C) for comparison (197). The diameter of the single cells is ~3 μm, and the magnification factor is the same for the three panels. The photographs were taken with phase-contrast optics of wet samples from live cultures between the glass slide and coverslip (180a).

An area in need of development pertains to in vivo studies of chaperone and chaperonin function and mechanism of action. In this regard, the most rewarding work will be the standardization of transformation vectors and protocols and the identification and generation of mutants. For some archaeal groups, like the methanogens, the methods necessary for molecular genetic manipulations are still scarce (46, 199, 285).

Very little is known also about archaeal stress proteins that are not members of the Hsp70 and Hsp60 families and that may or may not be chaperones but still play a role in the stress response or in stress tolerance. Small heat shock proteins (sHsp) (149, 150) are good candidates for study. Also, experiments with bacterial models are revealing novel genes that respond to environmental stressors (21, 112). Do archaea have similar genes? Comparative genomics will help initiate experimental research, which will probably unveil antistress mechanisms that are as important as those attributed to the better-known chaperones and chaperonins and should help our understanding of stress resistance in eukaryotes. It is also possible that these novel mechanisms will be important during physiologic differentiation and during the development of supracellular structures in response to stress.

Regulation

Regulation of stress genes in the archaea is an open field and a very intriguing one. In brief, the *hsp70* locus genes produce proteins with bacterial characteristics, betraying their origin, but they have to function within cells containing a genome in which the transcription machinery is of the archaeal (eucarya-like) type. This suggests a hybrid heritage. It remains to be established when, and how, the *hsp70* locus genes came to be located in the archaea that have them and how are they regulated. It is known that the transcription initiation factors in archaea are homologs of some of the eucaryal transcription factors (16, 264, 276). However, it is unclear whether the regulatory signals and factors are of bacterial or eucaryal type or whether they have novel, archaeal, characteristics (180). These points remain to be elucidated and are relevant to basic and applied sciences. For example, stress proteins are expressed as a reaction to pollutants (21, 112). Moreover, heat shock gene promoters have been used, along with reporter genes, to detect stressors of relevance to public health (15, 69, 70, 269). Can archaeal heat shock gene promoters be used similarly? Will these archaeal promoters function inside eukaryotic cells?

Likewise, very little is known about posttranscriptional regulation. The information available comes from genome analyses, but experimental data are scarce. For instance, there are very few data on transcription termination of archaeal stress genes. Examination of sequences and computer analyses suggest that the formation of an mRNA hairpin may be the device used for termination by some genes (42, 44, 45, 49, 50, 179, 181–184). However, a recent computer analysis of available archaeal genomes suggested that hairpin formation was not the mechanism used by most genes (291).

Cell Differentiation, Development, and Adaptation

Heat shock genes in many organisms are expressed under normal, physiological conditions. This basal or constitutive expression is low level by comparison with that which occurs in response to stressors. Furthermore, many heat shock genes respond to normal, physiological signals related to cell differentiation and tissue and organism development (95, 205, 240, 253). This aspect of stress gene regulation has not been studied in the archaea to any significant extent. Preliminary data suggest that the *hsp70*(*dnaK*) locus genes in *M. mazei* S-6 are constitutively expressed at levels that vary with the stage of the developmental cycle, which includes the formation of packets, single cells, and lamina (180a).

The lamina morphotype is a flat structure, typically with a thickness of one cell (although "old" lamina may be thicker and encompass two or three cells, vertically) and abundant intercellular connective material. It resembles a biofilm formed

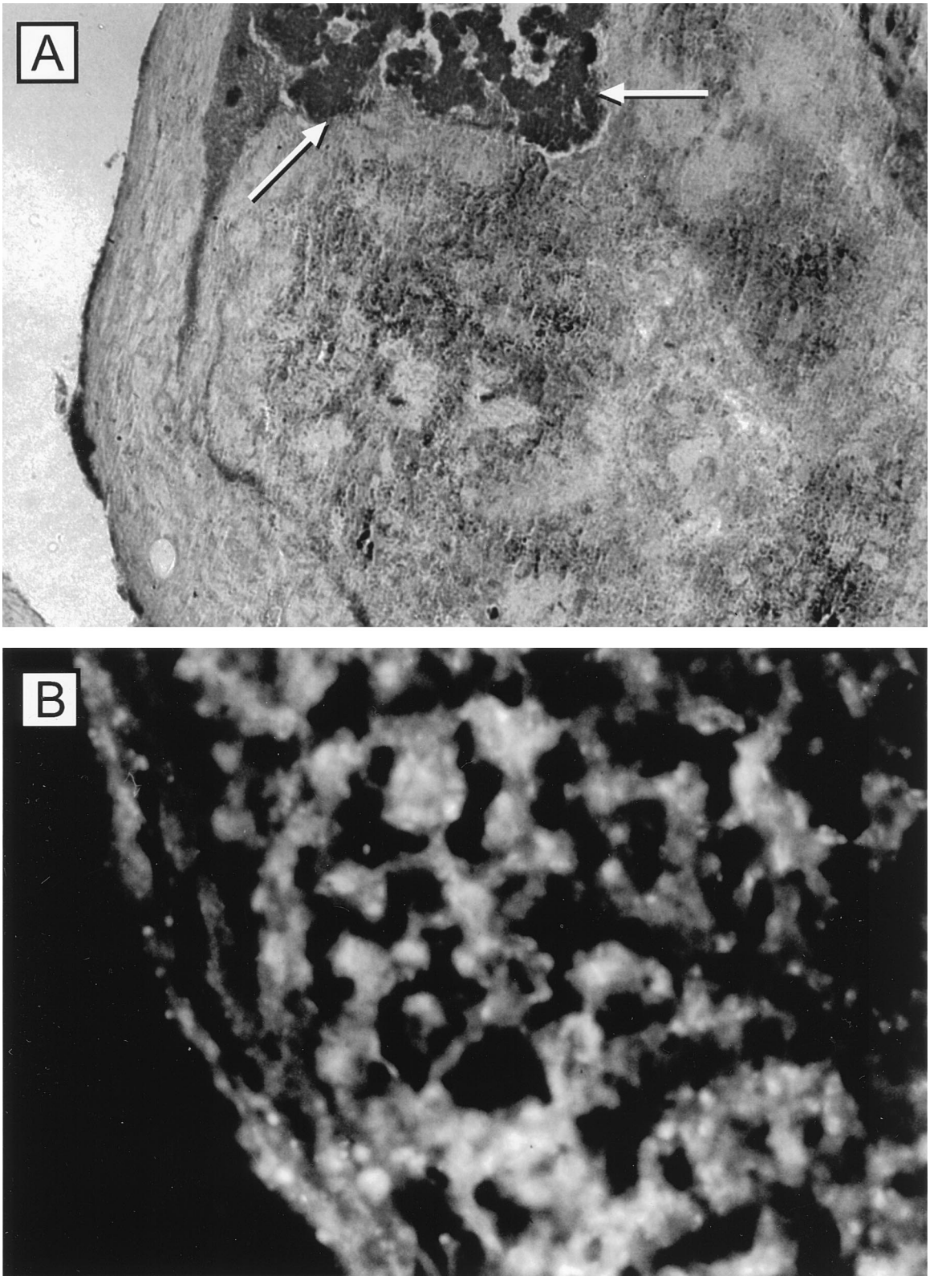

FIG. 17. Archaea-bacterial multicellular structures shown in thin histological sections. (A) Cross section of a granule (multicellular consortium) from a thermophilic (50°C), anaerobic, methanogenic bioreactor. Visible are the cortex and medulla (48, 134) and a large island of methanosarcina cells and packets (arrows) (180a). Hematoxylin-eosin stain. Magnification, ×736. (B) Another section of the same granule in which the presence of *Methanosarcina thermophila* TM-1 (optimal temperature for growth, 50°C) is demonstrated with a antibody probe for TM-1 by immunofluorescence (180a). Magnification, ×3,680.

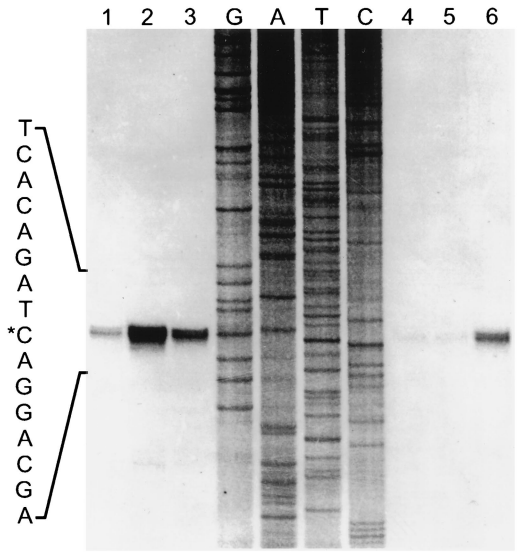

FIG. 18. Heat resistance of archaeal multicellular structures compared with the single-cell phenotype. Primer extension mapping of the transcription initiation site for *M. mazei* S-6 *grpE* was performed. A radiolabelled oligonucleotide primer complementary to bases 57 through 77 within the *grpE* coding region was used with 10 μg of total RNA from single cells (lanes 1 to 3) or packets (lanes 4 to 6) per test. Single cells and packets were grown at 37°C (lanes 1 and 4) or heat shocked at 45°C for 30 (lanes 2 and 5) or 60 (lanes 3 and 6) min. The primer-extended products were electrophoresed in a 6% acrylamide sequencing gel in parallel with the products of a sequencing reaction that was done with the same primer and the dideoxy chain termination method (lanes G, A, T, and C). These lanes show the complementary (antisense) strand sequence. The coding (sense) strand sequence and the initiation site (asterisk) are shown on the left. Reprinted from reference 44 with permission of the American Society for Microbiology.

by many bacteria and some archaea, as discussed above (see "Multicellular structures"). However, the lamina morphotype has peculiar characteristics that distinguish it from other biofilms. For instance, a lamina does not adhere to glass or plastic surfaces. It sits on the bottom of the culture flask, or floats when the culture is stirred or when there is methane trapped within it that provides buoyancy. Packets have a different anatomy. They are compact globular structures, with the cells present inside a layer of intercellular material, and may reach a diameter equivalent to 10 cells or more. These globular masses are very resistant to disruption by chemical and mechanical means and do not allow antibiotics to reach the cells inside (180a; see also reference 40 for possible applications of packet formation). Why is it that *M. mazei* S-6 has evolved the capacity to form laminas and packets? Do these structures represent developmental stages different from the single-cell phenotype? Did the capacity to form packets (the most resistant of the three phenotypes to stressors) evolve as a survival mechanism to cope with harmful environmental changes? Research addressing these questions will provide information useful to our understanding of the evolution of histogenesis and its advantages for survival.

Voids To Be Filled: Proteases and Auxiliary Factors

There are many molecules and molecular families directly or indirectly related to stress and/or molecular chaperoning and protein management in the bacteria and eucarya that have not yet been identified or well characterized in archaea. A few examples follow.

The Clp system occurs in bacteria and consists of a family of ATP-dependent proteases that are relatively well studied in *E. coli* (96–98) and *Bacillus subtilis* (92). Proteases do, indeed, play a critical role in maintaining the physiological array of proteins and their optimal levels by, among other mechanisms, eliminating those that are damaged and whose accumulation inside the cell would be deleterious (60, 96–98, 123, 124). It is not known whether the archaea harbor a system resembling the bacterium-type Clp, or any other that might take its place, but with unique, archaeal characteristics. A suggestion in this direction is provided by a report of positive hybridization obtained with a probe for Clp and DNA from *Methanosarcina acetivorans* (98).

The proteasome is another protease system (14, 39, 60, 167, 314). It is a large structure with a central cavity or chamber, formed by stacked rings constituted of several protein subunits. Superficially, the proteasome resembles the thermosome, but it differs from the latter in several important aspects and has different functions. The proteasome digests polypeptides inside its central chamber rather than promoting their correct folding as the thermosome is thought to do. Although a proteasome has been identified in a couple of archaeal species (167, 195, 196), little is known about its function, mechanism of action, and diversity according to ecosystem or phylogenetic branch. Experiments done with *T. acidophilum* have demonstrated that proteasome inhibition does not affect cell viability under physiologic conditions but causes growth arrest in heat-shocked cultures (248). The data indicated that the proteasome plays a role in the survival of *T. acidophilum* under stress.

The group of sHsp is widespread, diverse, and important for bacteria and eukaryotes (76, 78, 177, 206, 227). Some information is beginning to emerge about sHsp in the archaea. For example, an sHsp of 16.5 kDa from *M. jannaschii* has been purified and crystallized (148, 149). The folding unit was found to consist of nine β-strands in two sheets, two short helices, and one short β-strand, with one of the β-strands coming from an adjacent subunit. Twenty-four monomers formed a large (~400-kDa) spherical complex with octahedral symmetry (Fig. 20). The sphere appeared to enclose a cavity with a volume of 140,000 $Å^3$, i.e., about half that of the estimated volume of the inner space in the GroEL barrel. In vitro studies showed that the *M. jannaschii* sHsp protected other proteins from heat denaturation and prevented their aggregation (150). Thus, this archaeal protein seems to play a role similar to that of the sHsp homologs in organisms of the other two phylogenetic domains.

Among the sHsp of *E. coli*, one has been identified very recently that binds nucleic acids (160). Although its role in vivo was not elucidated, it was deemed important because of its abundance. Do archaea have homologs that also bind nucleic acids (and assist them in some way) rather than polypeptides?

On the other side of the spectrum, the occurrence and role of high-molecular-weight (90 kDa or higher) (Table 1) (30, 53, 93, 175, 177, 218, 236, 293) stress proteins and chaperones in the archaea have not been determined. This is another area of archaeal research open for exploration that will benefit from genomics and that in turn will help the study of chaperone machines different from the Hsp70(DnaK) and chaperonin systems.

Cooperation between chaperones and chaperonins seem to be an important physiological characteristic of the cell, as mentioned in previous sections of this review. This idea is being extended to encompass cooperation with regulatory or auxiliary factors, or cofactors (38, 62, 82, 88, 127). Examples of the latter are Hip (for "Hsc-70 interacting protein") and Hop (for "Hsp70/Hsp90-organizing protein"); the latter is also named p60, IEF-SSP-3521, extendin, or Sti1 (38, 127). These factors occur in eukaryotes; hence, it is justified to suspect that they

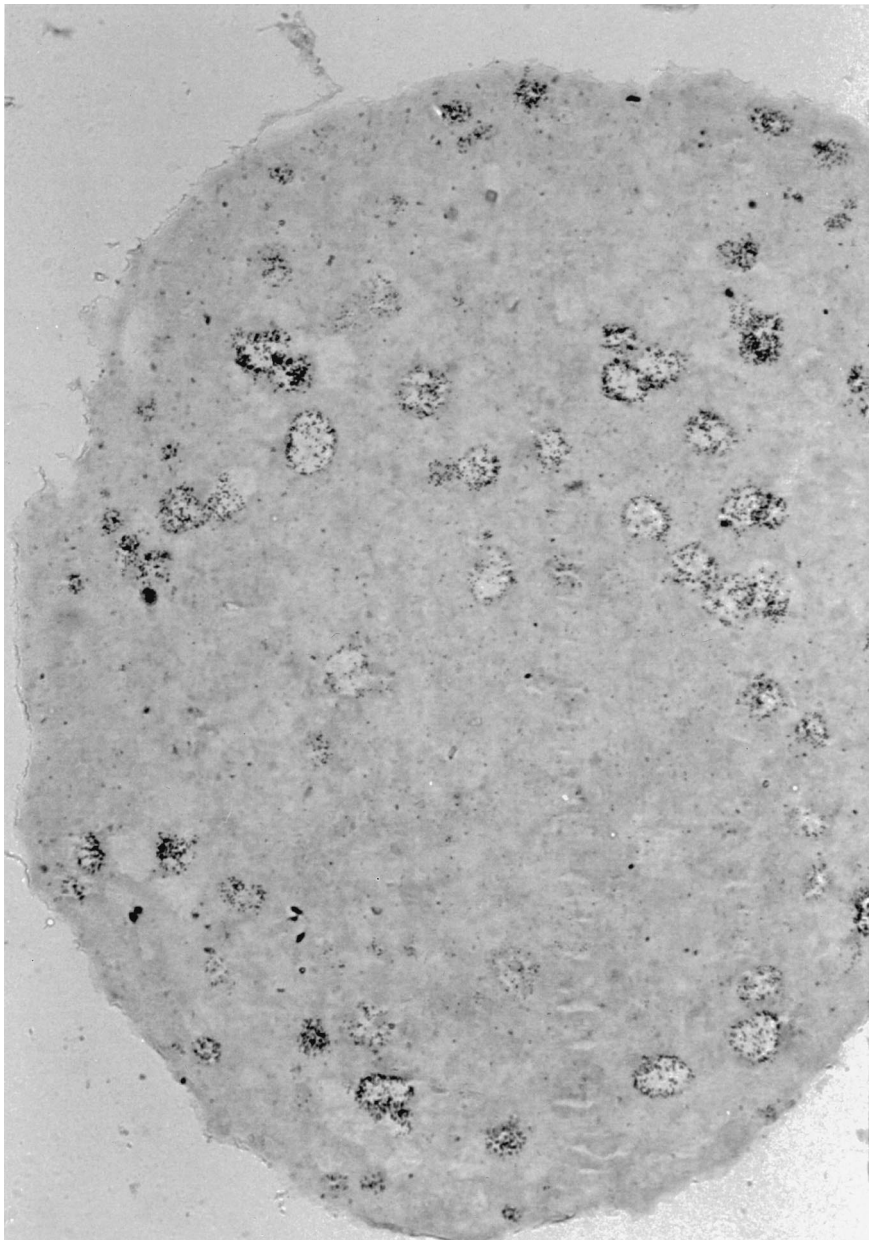

FIG. 19. Superficial, histological thin section of a granule like that shown in Fig. 17, passing through the cortex. Visible are circular openings which represent cross sections of the tubes that crisscross the cortex and enable communication between different zones of the granule (180a, 185). Hematoxylin-eosin stain. Magnification, ×800.

(or their primitive ancestors) might also be present in the archaea, or at least in some of them.

Beyond Chaperones and Chaperonins

There are other important areas for investigation that extend beyond chaperones and chaperonins. One pertains to all the other stress genes and proteins alluded to in previous sections. A second topic that also deserves investigation and promises practical dividends is stress resistance. Archaea are particularly interesting in this regard because they encompass an array of species that inhabit a wide variety of ecosystems, some very similar to the optimal conditions for human cells and some that are extreme by comparison, with all gradations in between (9, 11, 12, 59, 178, 186, 232, 297). To what extent do protein biogenesis under physiological conditions and molecular chaperoning under stress differ in species that live in disparate ecosystems? How different must two ecosystems be in terms of temperature, pH, salinity, or pressure to require differences in the chaperoning mechanisms of the organisms that thrive in them? What are the sensors for temperature stress, for example, in psychrophilic compared with thermophilic species? All these questions point to specific aspects of the stress response in archaea that merit investigation. Again, genome analyses are helping our knowledge to progress. Some experimental data on the thermostability of DNA and some proteins are already available (13, 36, 56, 101, 102, 173, 174, 215, 224, 254), and they should also enhance the implementation of projects to clarify more completely how life is sustained in extreme environments.

Similarly, thermoprotectants are being identified (128), as

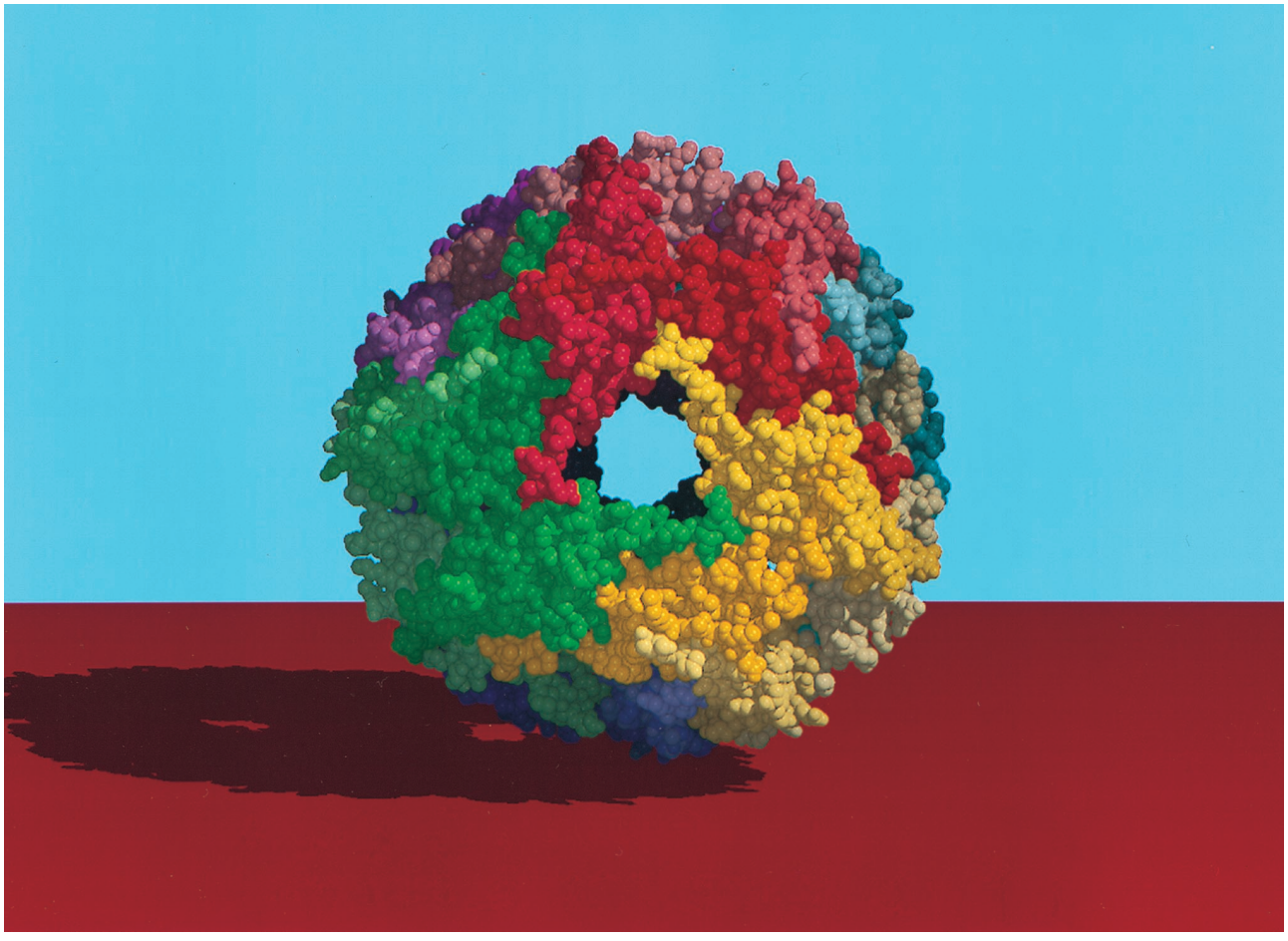

FIG. 20. Space-filling model of the multimeric complex formed by an sHsp from the hyperthermophilic methanogenic archaeaon *M. jannaschii*. The model was derived from crystallographic analysis at 2.9-Å resolution, and each atom is represented by a small sphere. The whole structure consists of 24 identical protein (the sHsp) subunits (16.5 kDa each) arranged in regular octahedral symmetry with a total molecular mass of about 400 kDa. Twelve dimers are represented by different colors, with monomers in each dimer shown by different shading of the same color. The complex is a hollow sphere with eight triangular and six square openings or windows that connect the inner cavity with the outside. The view in the figure is along the axis that aligns two triangular windows, one in front, closest to the observer, and the other on the back of the sphere, farthest from the observer. Reprinted from reference 149 with permission of the publisher.

are molecules that may be seen as insulants, like histones and small, nonhistone DNA-binding proteins (226, 237). Progress in this area should improve our understanding of the mechanisms used by the archaea, particularly those that live in extreme environments, to pack DNA and to unpack it to allow gene transcription.

Studies of the thermoprotectants made by the archaea followed by laboratory synthesis of similar compounds with desired properties should provide a means of increasing stress resistance to a variety of stressors in useful organisms. These improved organisms could then be used in a variety of activities such as decontamination of toxic wastes and planetary exploration.

Stress resistance and survival depend to a great extent on the properties of the cell envelope. Some archaea have an S-layer with protective functions, allowing the passage of water and other molecules (50, 158, 262 [and other articles in the same issue], 267). The characteristics of the S-layer proteins of *M. mazei* S-6 seem to have evolved to provide rapid adaptation to environmental changes and to promote the formation of multicellular structures when necessary (50, 262). This field, encompassing stress resistance mechanisms mediated by cell surface molecules and intercellular connective material, will probably undergo major advances in the near future if enough attention is directed to it. Firstly, it will be necessary to understand the mechanism responsible for the formation of multicellular structures in response to environmental signals. Second, the proteins and other molecules involved will have to be characterized and their genes will have to be studied to determine what induces them and how they are regulated. Finally, manipulation of these genes ought to provide means of engineering multicellular structures as needed, for instance, in bioreactors with violent flow and drastic, unpredictable oscillations in temperature or pH. Moreover, learning about the formation of multicellular structures in the archaea will help in the development of methods to prevent the formation of or to destroy undesirable biofilms, such as those involved in biofouling and in disease.

Along these lines, the prospects are promising for multicellular structures with several archaeal and bacterial species in a food web, like the granular consortia of methanogenic bioreactors. How are these consortia formed at the molecular level? What brings the various species together, and what keeps them close to each other? What is the composition of the extracellular connective material? When and how do the genes respon-

sible for the synthesis of the extracellular material become activated?

If one considers the complex architecture of the multicellular structures of *M. mazei* S-6 (197) and even more so that of the granular consortia (48, 185), it is easy to imagine that the building process must involve a complicated mechanism with stress sensors, genes, and molecules of various kinds. The process must include not only the synthesis and export of the building blocks for the intercellular connective material but also the construction of the microtubes that crisscross the granules, with the participation, most probably of extracellular proteases.

CONCLUSION

Inferences from the data and hypotheses have been enunciated throughout the text, mostly at the end of each subsection. In brief, there is considerable information on the archaeal Hsp70 family of chaperones in what pertains to their genes but very little in what pertains to their function. Virtually no experimental data are available that will elucidate what these molecules do and how, when, and where they will do it. Since the proteins are very similar to bacterial homologs whose function have been extensively studied in vitro and in vivo, one may be tempted to extrapolate the data and assume that the archaeal molecules do the same things in the same way as the bacterial chaperones. This may be true but only to a degree. The intracellular environment and the batteries of cochaperones and auxiliary molecules that interact with the Hsp70 team may be different in archaea from in bacteria. This is certainly an area of biochemistry that deserves investigation; it promises to unveil details of molecular interactions that studies with bacteria cannot reveal and that may be useful to our understanding of comparable interactions in the eukaryotic cell.

In contrast to Hsp70, the functions of the archaeal Hsp60 molecules have been studied more extensively than their genes. Actually, initial studies of archaeal chaperonin molecules helped research on the chaperonins of the eukaryotic-cell cytosol. However, the bulk of our information comes from in vitro studies. We must wait for in vivo experiments to show the real-life functions of the archaeal chaperonins and their mechanisms of action inside a living cell.

sHsp are beginning to emerge as important players in cell physiology and stress resistance. A few have been identified in archaea that are PPIases, and another has been crystallized. Studies with archaeal sHsp should enhance our understanding of the chaperone functions of these proteins, their interaction and cooperation with other chaperone and chaperonin systems in the cell, and their role in the assembly of the cytoskeleton. Archaea do not possess a cytoskeleton, in contrast to eukaryotes, but several reports (43, 84, 280, 307) suggest that they might have a primitive version of it. This hypothesis merits further investigation. The fact that archaea do have sHsp and chaperonins known to associate with tubulin and actin in eukaryotes is another incentive to search for cytoskeletal ancestors in the archaea.

The study of archaeal and mixed (archaeal-bacterial) supracellular structures has been rewarding so far, to the extent that it has revealed complex formations with regional heterogeneity and hints of organization as if they were primitive tissues and has shown that these structures are more resistant to stressors than are the single-celled morphotypes. These findings ought to serve as the starting point for investigations of the molecular and genetic mechanisms involved in this primeval type of histogenesis and their role in stress resistance.

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