Chaperones and protein folding in the archaea

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Abstract

A survey of archaeal genomes for the presence of homologues of bacterial and eukaryotic chaperones reveals several interesting features. All archaea contain chaperonins, also known as Hsp60s (where Hsp is heat-shock protein). These are more similar to the type II chaperonins found in the eukaryotic cytosol than to the type I chaperonins found in bacteria, mitochondria and chloroplasts, although some archaea also contain type I chaperonin homologues, presumably acquired by horizontal gene transfer. Most archaea contain several genes for these proteins. Our studies on the type II chaperonins of the genetically tractable archaeon *Haloferax volcanii* have shown that only one of the three genes has to be present for the organisms to grow, but that there is some evidence for functional specialization between the different chaperonin proteins. All archaea also possess genes for prefoldin proteins and for small heat-shock proteins, but they generally lack genes for Hsp90 and Hsp100 homologues. Genes for Hsp70 (DnaK) and Hsp40 (DnaJ) homologues are only found in a subset of archaea. Thus chaperone-assisted protein folding in archaea is likely to display some unique features when compared with that in eukaryotes and bacteria, and there may be important differences in the process between euryarchaea and crenarchaea.

Chaperone-assisted protein folding

The need for molecular chaperones to assist protein folding became apparent when it was realized that this process is under constraints in the cell that do not apply under controlled *in vitro* conditions, where denatured proteins usually refold spontaneously following the removal of a denaturant [1]. However, in the cell, protein aggregation is made more likely in the crowded cellular milieu, and this is a particular problem for proteins being synthesized on ribosomes. Molecular chaperones bind a wide range of proteins and help them to fold to their final active forms. Chaperones act kinetically rather than thermodynamically and mostly are thought to work by binding transiently to regions of polypeptide chains that would otherwise be likely to aggregate or fold incorrectly. The way in which chaperones both bind and mediate subsequent folding depends on the particular molecular chaperone concerned.

Several families of chaperone (classified by sequence similarity) have been identified from a wide range of organisms. These are often called HSPs (heat-shock proteins), although it must be remembered that not all chaperones are HSPs, and vice versa. The major families are Hsp100, Hsp90, Hsp70, Hsp60, Hsp40 and sHSPs (small HSPs). Molecular chaperones vary in size, structure, mechanism, occurrence, role and importance, and it can thus be misleading to generalize about them. Among the families above, for example, are chaperones which are thought to be always essential (Hsp60) or often dispensable (sHSPs), those which exists as large complexes (Hsp60, sHSPs and Hsp100s) or act as monomers or dimers (Hsp70 and Hsp90), those that require ATP binding and hydrolysis (most of them) and those which do not (sHSPs). All of these families have been thoroughly studied in bacteria (particularly *Escherichia coli*) and in many eukaryotes, but studies in archaea have been limited mostly to biochemical studies on purified proteins.

The chaperones of archaea

Surveys of complete genome sequences for chaperone homologues has led to the surprising discovery that the Hsp90 and Hsp100 families are absent in nearly all cases, with the few known being attributed to horizontal gene transfer from bacteria. Moreover, the Hsp70 (DnaK) family which is ubiquitous in bacteria and eukaryotes is unevenly distributed in archaea, being absent from nearly all the thermophilic and hyperthermophilic species [2]. Between them, these chaperone families play a variety of roles in other organisms, including resolubilizing proteins which have become aggregated following heat shock. However, it remains unclear whether archaea use other proteins to do this or whether they lack this ability and instead rely on proteases to remove cellular debris which may accumulate following heat shock. Incidentally, it is important to recall that even organisms that grow at spectacularly high temperatures still show a heat-shock response (see, for example, [3,4]). sHSPs are present in all archaea, and, indeed, the first crystal structure for an sHSP protein complex was an archaeal example [5].

The Hsp60s, also commonly known as chaperonins, are present in all archaea, and, as these are the best-studied of the archaeal chaperones both biochemically and genetically, we now focus on these in more detail.

Key words: archaeon, chaperone, chaperone containing t-complex polypeptide 1 (CCT), chaperonin, heat-shock protein 60 (Hsp60), thermosome.

Abbreviations used: CCT, chaperone containing TCP-1 (t-complex polypeptide 1); HSP, heat-shock protein; sHSP, small HSP.

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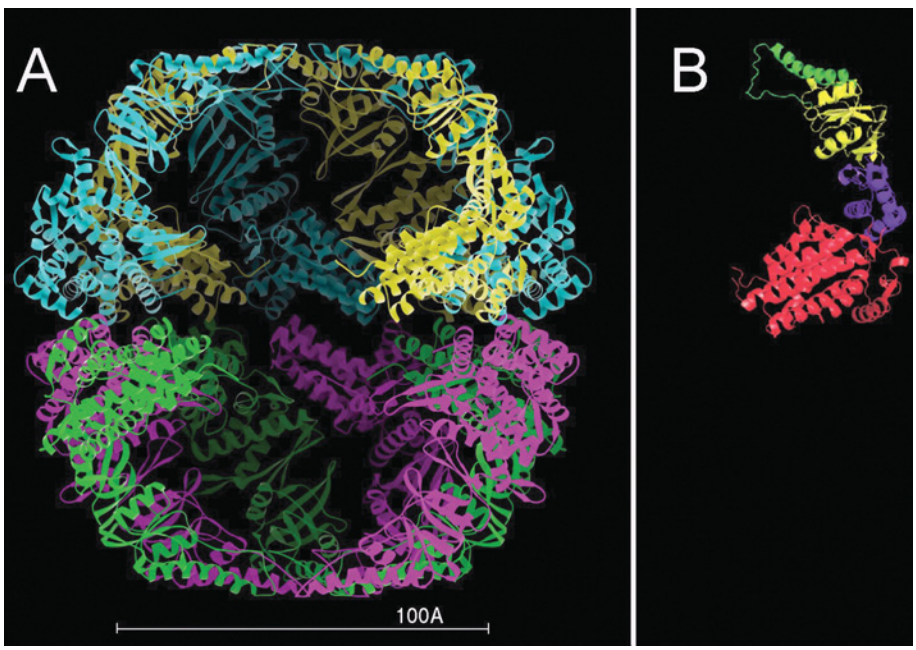
Figure 1 | **Structure of the thermosome of *T. acidophilus* (closed conformation)**

(**A**) In both the top and bottom ring, two subunits have been removed to show the cavity that exists inside the two rings. α and β subunits alternate around both rings. (**B**) Cartoon representation of a single subunit of the thermosome from *T. acidophilum*, colour-coded to show the domain structure of the protein. The equatorial domain is red, the intermediate domain is blue, the apical domain is yellow, and the helical protrusion is green.



The chaperonins

Many studies have shown that chaperonins bind to unfolded or partially folded substrate proteins and assist their folding under conditions where they do not refold spontaneously. This requires binding and usually hydrolysis of ATP. The chaperonins are all large oligomers, with several subunits in two rings, each of which encloses a central cavity (Figure 1).

The mechanism of action of chaperonins has been studied intensively, particularly for the *E. coli* chaperonin GroEL (see [6,7] for recent reviews). In the reaction cycle, the unfolded protein substrate and ATP bind at one end (the *cis* end) of the double ring complex, followed by binding of another protein complex, the co-chaperonin GroES, which caps the cavity in the ring and displaces the substrate into this cavity. The substrate then folds inside the cavity, which means that it does not interact with any other unfolded proteins, a process which could lead to aggregation. Binding of another molecule of substrate to the opposite *trans* ring causes GroES and substrate to be released from the *cis* ring, and the whole process begins again on the *trans* ring of the complex. The hydrolysis of ATP acts as a timer mechanism, as binding of substrate to the *trans* ring cannot occur until the ATP bound to the *cis* ring has been hydrolysed. GroEL could unfold misfolded proteins, enabling them to pass down the folding pathway again. Alternatively (or in addition), it may act by using the cavity in the ring (which becomes a cage when GroES is bound) as the site where protein folding occurs. The cage may function passively by allowing proteins to fold in a protected environment [8] or it may be more active, with confinement favouring the folded state of the substrate protein (e.g. [9]).

There are two clear phylogenetic groups of chaperonins, usually referred to as group I and group II. Group I chaperonins are found in bacteria, chloroplasts [10] and mitochondria [11]. Group II chaperonins are found in the eukaryotic cytosol and in archaea. The similarity between group I and group II chaperonins leads to significant similarity in structure. Both form double-ring structures, each ring being made up of seven (in group I), eight or, in a few cases in archaea only, nine subunits (in group II). X-ray structures are known for *E. coli* GroEL protein [12] and for the group II chaperonins from *Thermoplasma acidophilum* (see Figure 1A) [13] and *Thermococcus* strain KS1 [14]. Cryo-electron microscopy images show that the eukaryotic group II chaperonin {also called CCT [chaperone containing TCP-1 (t-complex polypeptide 1)] or TriC} has the same basic structure as archaeal group II proteins [15]. The major difference between group I and group II chaperonins is that in the group II proteins, a helical protrusion of approx. 30 residues (coloured green in Figure 1B) forms a lid to the cavity which is thought to open and close during the course of the reaction

cycle. This replaces the lid formed by the co-chaperonin GroES and its homologues in the group I chaperonins.

Structural and biochemical properties of archaeal chaperonins

The archaeal chaperonins (which are often referred to as the thermosome, or as CCT in mesophilic organisms) have 8- or sometimes 9-fold symmetry. The structure has been determined at a resolution of 2.6 Å (1 Å = 0.1 nm) for the thermosome of *T. acidophilum* both without and with nucleotide [13]. It consists of two eight-membered rings of alternating α and β subunits, with a spherical shape; each ring has a central cavity. Crystal structures of the *Thermococcus* KS1 thermosome are also available [14], showing a similar structure to that of the *T. acidophilum* thermosome. In both cases, the structure is closed, with the helical protrusion occluding the central cavity, but it is thought that the protrusion will open under physiological conditions during the course of the reaction cycle, allowing the substrate to enter and exit the cavity. Cryo-electron microscopy evidence for open structures comes from studies of *T. acidophilum* and *Sulfolobus shibatae* thermosomes [16–18], and SAXS (small-angle X-ray scattering) studies also show the chaperonin in both the open and closed states, with the signal for closure apparently being ATP binding rather than hydrolysis [19,20].

All chaperonins have ATPase activity, which has been determined for thermosomes from several different archaea. Most of these activities are magnesium-dependent and often also require the presence of K^+ ions; other ionic requirements are known in specialized cases. In group I chaperonins, there are two allosteric transitions induced by ATP at different ATP concentrations. The first of these is caused by positive co-operativity of ATP binding within a single ring, and the second by negative co-operativity between the rings. The situation may be different for the archaeal group II chaperonins. For example, the thermosome from *T. acidophilum* lacks inter-ring-positive co-operativity [21], although it is seen in the thermosomes from *Methanococcus maripaludis* [22] and *Pyrococcus furiosus* [23]. However, like the group I chaperonins, the group II chaperonins show intra-ring-negative co-operativity. It is thought that the allosteric nature of the ATPase activity in chaperonins enables the two rings to act in succession to each other in binding and folding proteins, and this appears to be a universal property of chaperonins.

Studies on protein binding and folding by archaeal chaperonins

There are various methods to determine chaperone activity, of which the most complete is to show ATP-dependent protein refolding under conditions where folding does not measurably occur without a chaperone present. Other assays used include preventing thermal aggregation of a substrate protein and binding of unfolded protein; these are useful assays, but do not show complete chaperone activity. A summary of the protein folding abilities of thermosomes is shown in Table 1, and it can be seen that there is clear evidence for *in vitro* chaperone activity in at least some of the archaeal chaperonins, although *in vivo* substrates remain to be determined.

The position of the substrate-binding sites is still not completely clear for the archaeal group II chaperonins. Regions of the protein-binding (apical) domain of the thermosome appear to have been homogenized by repeated gene conversions, and these may include the substrate-binding domains, but the resolution of this study was not high enough to determine the contributions of individual residues [37]. Substrate proteins are mostly thought to bind to the archaeal chaperonins by hydrophobic interactions [38], and two hydrophobic patches in the *T. acidophilum* thermosome [39,40] (one in the helical protrusion and one in the domain below the helical protrusion) are good candidates for substrate-binding sites. Both of these patches are predicted to be exposed in the open structure, but are buried in the closed structure [16,41]. A possible mechanism is that substrate is bound, followed by its release into the cavity for folding as the chaperonin goes from the open to the closed form. However, direct experimental proof for this mechanism is not yet available.

Eukaryotes and archaea also possess prefoldin (or GimC) which is a hexameric complex, and, in the case of most archaea, is encoded by two genes usually called *pfdα* and *pfdβ* [42]. Prefoldins are able to bind newly synthesized or unfolded proteins and to deliver them to CCT or the thermosome for folding [43,44]. There are, as yet, no genetic data on the importance of prefoldin activity in archaea.

Genetic analysis of archaeal chaperonins

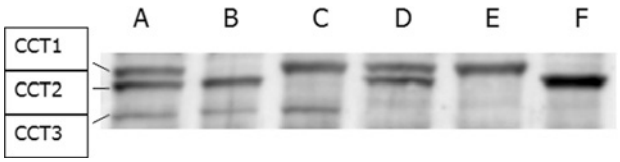
Genetic analysis of chaperonin function has only been carried out in one archaeon to date: the genetically tractable halophile *Haloferax volcanii* [25]. *H. volcanii* has three *cct* genes: *cct1–cct3*), each of which can be deleted, but either *cct1* or *cct2* must be present for survival (Figure 2). There is evidence for the co-regulation of *cct1* and *cct2*, as loss of one gene results in a compensatory increased expression of the other. Recent phylogenetic studies have raised the possibility that some divergence and specialization of function of the different thermosome subunits may occur in archaea with three or more genes [45]. Interestingly, the limited *in vivo* data currently available do support this: although two of the three *H. volcanii cct* genes can support growth when expressed on their own, the third cannot [25]; also a *H. volcanii* strain has been constructed in which the only copy of a *cct* gene is under the control of a regulatable promoter [46], where the native gene can be switched off. Also, of the four *cct* genes encoded by the related halophile *Haloarcula marismortui*, only two could be expressed in *H. volcanii* and only one of these could functionally replace loss of all the *H. volcanii cct* genes. The precise nature of this divergence of function, and its adaptive significance, remains unknown.

Table 1 | **Comparison of chaperone activities of thermosomes from various archaea**

A, archaeal; B, bacterial; E, eukaryotic; N, native; R, recombinant

Organism	Protein used in assay	Substrate used	Chaperone activity found	Reference
Aeropyrum pernix	R	Rhodanese (E), alcohol dehydrogenase (E)	Prevention of thermal aggregation	[24]
Haloferax volcanii	N	–	Not detected	[25]
Methanococcus jannaschii	N	Luciferase (B)	Inhibits refolding	[26]
Methanococcus maripaludis	R	Rhodanese (M)	Nucleotide-dependent folding	[27]
		Citrate synthase (E)	Prevention of thermal aggregation	
Methanococcus thermonlithotrophicus	R	Citrate synthase (A)	Nucleotide-dependent folding	[28]
		Glucose dehydrogenase (A)	Nucleotide-dependent folding	
Methanopyrus kandleri	N/R	–	Not reported	[29]
Methanosarcina mazei	R (reconstituted complexes)	–	Not detected	[30]
Pyrococcus horikoshii	R	Citrate synthase (E)	Prevention of thermal aggregation	[31]
		Isopropylmalate dehydrogenase (B)	Prevention of thermal aggregation	
		Green fluorescent protein (B)	Nucleotide-dependent folding	
Pyrococcus furiosus	R	Malate dehydrogenase (B)	Prevention of thermal aggregation, nucleotide-dependent folding	[32]
Pyrococcus furiosus	R	Lysozyme (E)	Prevention of thermal aggregation	[23]
Pyrodictium occultum	N/R	Citrate synthase (E), yeast alcohol dehydrogenase (E)	Prevention of aggregation, but forms dead-end complexes	[33]
Sulfolobus shibatae	N	Dihydrofolate reductase fusion protein (E)	Binds denatured protein	[34]
Sulfolobus solfataricus	N	Malic enzyme, alcohol dehydrognease, glutamate dehydrogenase (all from *Sulfolobus solfataricus*)	Inhibits refolding and nucleotide-dependent folding (all cases)	[35]
Thermoplasma acidophilum	N	Lactate dehydrogenase (B)	Nucleotide-dependent folding	[21]
Thermococcus KS-1 α homo-oligomer	R	Green fluorescent protein (B)	Nucleotide-dependent folding	[36]
Thermococcus KS-1 β homo-oligomer	R	Green fluorescent protein (B)	Nucleotide-dependent folding	
		Citrate synthase (A)	Nucleotide-dependent folding	

Figure 2 | Western blot analysis of *H. volcanii cct*-knockout strains
(**A**) Wild-type (H26) with three CCTs expressed. (**B**) Δ*cct1*. (**C**) Δ*cct2*. (**D**) Δ*cct3*. (**E**) Δ*cct2*/Δ*cct3*. (**F**) Δ*cct1*/Δ*cct3*. Reprinted from [25] with permission. © 2006 Blackwell Publishing.



Archaeal Hsp70 (DnaK)

The Hsp70 (DnaK) family is ubiquitous in bacteria and eukaryotes and is usually associated with the co-chaperones GrpE and Hsp40 (DnaJ). These three proteins are found in many archaea, but are absent from many thermophilic and hyperthermophilic species, especially members of the Crenarcheota [2,47]. Hsp70 may have been acquired by lateral gene transfer from bacteria, although this is far from certain [47,48]. Several archaeal DnaK proteins, including one from a halophile, can complement *E. coli* Δ*dnaK* mutants [49]. Using the genetically tractable *H. volcanii*, we have recently found that DnaK is not essential in this organism, and the Δ*dnaK* mutant shows no obvious stress phenotype. Expression of DnaK is surprisingly not heat-induced (M.D. Goldberg and P.A. Lund, unpublished work) unlike in other archaea (e.g. [50]).

Conclusion

The archaeal chaperones are not yet as well studied as their relatives in eukaryotes and bacteria, but such a study is likely to reap dividends, both in providing a more in-depth understanding of their more complex homologues in eukaryotes, and also in gaining further insights into protein folding in the extreme conditions in which many archaea grow. The advent of improved genetic systems for several archaea promises that rapid progress may now be made in linking *in vivo* and *in vitro* observations, including an understanding of how the archaea are able to grow well in the absence of several chaperone families that are highly conserved in bacteria and eukaryotes.

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References

1 Anfinsen, C.B. (1973) Principles that govern the folding of protein chains. Science **181**, 223–230
2 Macario, A.J. and de Macario, E.C. (1999) The archaeal molecular chaperone machine: peculiarities and paradoxes. Genetics. **152**, 1277–1283
3 Rohlin, L., Trent, J.D., Salmon, K., Kim, U., Gunsalus, R.P. and Liao, J.C. (2005) Heat shock response of *Archaeoglobus fulgidus*. J. Bacteriol. **187**, 6046–6057
4 Shockley, K.R., Ward, D.E., Chhabra, S.R., Conners, S.B., Montero, C.I. and Kelly, R.M. (2003) Heat shock response by the hyperthermophilic archaeon *Pyrococcus furiosus*. Appl. Environ. Microbiol. **69**, 2365–2371
5 Kim, K.K., Kim, R. and Kim, S.H. (1998) Crystal structure of a small heat-shock protein. Nature **394**, 595–599
6 Horwich, A.L., Fenton, W.A., Chapman, E. and Farr, G.W. (2007) Two families of chaperonin: physiology and mechanism. Annu. Rev. Cell Dev. Biol. **23**, 115–145
7 Lin, Z. and Rye, H.S. (2006) GroEL-mediated protein folding: making the impossible, possible. Crit. Rev. Biochem. Mol. Biol. **41**, 211–239
8 Ellis, R.J.(2003) Protein folding: importance of the Anfinsen cage. Curr. Biol. **13**, 881–883
9 Sharma, S., Chakraborty, K., Müller, B.K., Astola, N., Tang, Y.C., Lamb, D.C., Hayer-Hartl, M. and Hartl, F.U. (2008) Monitoring protein conformation along the pathway of chaperonin-assisted folding. Cell **133**, 142–153
10 Ellis, R.J. (1990) Molecular chaperones: the plant connection. Science **250**, 954–959
11 Reading, D.S., Hallberg, R.L. and Myers, A.M. (1989) Characterization of the yeast *HSP60* gene coding for a mitochondrial assembly factor. Nature **337**, 655–659
12 Braig, K., Otwinowski, Z., Hegde, R., Boisvert, D.C., Joachimiak, A., Horwich, A.L. and Sigler, P.B. (1994) The crystal structure of the bacterial chaperonin GroEL at 2.8 Å. Nature **371**, 578–586
13 Ditzel, L., Löwe, J., Stock, D., Stetter, K.O., Huber, H., Huber, R. and Steinbacher, S. (1998) Crystal structure of the thermosome, the archaeal chaperonin and homolog of CCT. Cell **93**, 125–138
14 Shomura, Y., Yoshida, T., Iizuka, R., Maruyama, T., Yohda, M. and Miki, K. (2004) Crystal structures of the group II chaperonin from *Thermococcus* strain KS-1: steric hindrance by the substituted amino acid, and inter-subunit rearrangement between two crystal forms. J. Mol. Biol. **335**, 1265–1278
15 Llorca, O., Smyth, M.G., Carrascosa, J.L., Willison, K.R., Radermacher, M., Steinbacher, S. and Valpuesta, J.M. (1999) 3D reconstruction of the ATP-bound form of CCT reveals the asymmetric folding conformation of a type II chaperonin. Nat. Struct. Biol. **6**, 639–642
16 Nitsch, M., Walz, J., Typke, D., Klumpp, M., Essen, L.O. and Baumeister, W. (1998) Group II chaperonin in an open conformation examined by electron tomography. Nat. Struct. Biol. **5**, 855–857
17 Schoehn, G., Quaite-Randall, E., Jiménez, J.L., Joachimiak, A. and Saibil, H.R. (2000) Three conformations of an archaeal chaperonin, TF55 from *Sulfolobus shibatae*. J. Mol. Biol. **296**, 813–819
18 Schoehn, G., Hayes, M., Cliff, M., Clarke, A.R. and Saibil, H.R. (2000) Domain rotations between open, closed and bullet-shaped forms of the thermosome, an archaeal chaperonin. J. Mol. Biol. **301**, 323–332
19 Iizuka, R., So, S., Inobe, T., Yoshida, T., Zako, T., Kuwajima, K. and Yohda, M. (2004) Role of the helical protrusion in the conformational change and molecular chaperone activity of the archaeal group II chaperonin. J. Biol. Chem. **279**, 18834–18839
20 Iizuka, R., Yoshida, T., Ishii, N., Zako, T., Takahashi, K., Maki, K., Inobe, T., Kuwajima, K. and Yohda, M. (2005) Characterization of archaeal group II chaperonin–ADP–metal fluoride complexes: implications that group II chaperonins operate as a "two-stroke engine". J. Biol. Chem. **280**, 40375–40383
21 Bigotti, M.G. and Clarke, A.R. (2005) Cooperativity in the thermosome. J. Mol. Biol. **348**, 13–26
22 Kusmierczyk, A.R. and Martin, J. (2003) Nested cooperativity and salt dependence of the ATPase activity of the archaeal chaperonin Mm-cpn. FEBS Lett. **547**, 201–204
23 Chen, H.Y., Chu, Z.M., Ma, Y.H., Zhang, Y. and Yang, S.L. (2007) Expression and characterization of the chaperonin molecular machine from the hyperthermophilic archaeon *Pyrococcus furiosus*. J. Basic Microbiol. **47**, 132–137
24 Son, H.J., Shin, E.J., Nam, S.W., Kim, D.E. and Jeon, S.J. (2007) Properties of the α subunit of a chaperonin from the hyperthermophilic Crenarchaeon *Aeropyrum pernix* K1. FEMS Microbiol. Lett. **266**, 103–109
25 Kapatai, G., Large, A., Benesch, J.L.P., Robinson, C.V., Carrascosa, J.L., Valpuesta, J.M., Gowrinathan, P. and Lund, P.A. (2006) All three chaperonin (*CCT*) genes in the archaeon *Haloferax volcanii* are individually dispensable. Mol. Microbiol. **61**, 1583–1597
26 Kowalski, J.M., Kelly, R.M., Konisky, J., Clark, D.S. and Wittrup, K.D. (1998) Purification and functional characterization of a chaperone from *Methanococcus jannaschii*. Syst. Appl. Microbiol. **21**, 173–178
27 Kusmierczyk, A.R. and Martin, J. (2003) Nucleotide-dependent protein folding in the type II chaperonin from the mesophilic archaeon *Methanococcus maripaludis*. Biochem. J. **371**, 669–673
28 Furutani, M., Iida, T., Yoshida, T. and Maruyama, T. (1998) Group II chaperonin in a thermophilic methanogen, *Methanococcus thermolithotrophicus*: chaperone activity and filament-forming ability. J. Biol. Chem. **273**, 28399–28407

29 Andrä, S., Frey, G., Jaenicke, R. and Stetter, K.O. (1998) The thermosome from *Methanopyrus kandleri* possesses an NH_4^+-dependent ATPase activity. Eur. J. Biochem. **255**, 93–99
30 Klunker, D., Haas, B., Hirtreiter, A., Figueiredo, L., Naylor, D.J., Pfeifer, G., Müller, V., Deppenmeier, U., Gottschalk, G., Hartl, F.U. and Hayer-Hartl, M. (2003) Coexistence of group I and group II chaperonins in the archaeon *Methanosarcina mazei*. J. Biol. Chem. **278**, 33256–33267
31 Okochi, M., Matsuzaki, H., Nomura, T., Ishii, N. and Yohda, M. (2005) Molecular characterization of the group II chaperonin from the hyperthermophilic archaeum *Pyrococcus horikoshii* OT3. Extremophiles **9**, 127–134
32 Hongo, K., Hirai, H., Uemura, C., Ono, S., Tsunemi, J., Higurashi, T., Mizobata, T. and Kawata, Y. (2006) A novel ATP/ADP hydrolysis activity of hyperthermostable group II chaperonin in the presence of cobalt or manganese ions. FEBS Lett. **580**, 34–40
33 Minuth, T., Frey, G., Lindner, P., Rachel, R., Stetter, K.O. and Jaenicke, R. (1998) Recombinant homo- and hetero-oligomers of an ultrastable chaperonin from the archaeon *Pyrodictium occultum* show chaperone activity *in vitro*. Eur. J. Biochem. **258**, 837–845
34 Trent, J.D., Nimmesgern, E., Wall, J.S., Hartl, F.U. and Horwich, A.L. (1991) A molecular chaperone from a thermophilic archaebacterium is related to the eukaryotic protein t-complex polypeptide-1. Nature **354**, 490–493
35 Guagliardi, A., Cerchia, L., Bartolucci, S. and Rossi, M. (1994) The chaperonin from the archaeon *Sulfolobus solfataricus* promotes correct refolding and prevents thermal denaturation *in vitro*. Protein Sci. **3**, 1436–1443
36 Yoshida, T., Kawaguchi, R., Taguchi, H., Yoshida, M., Yasunaga, T., Wakabayashi, T., Yohda, M. and Maruyama, T. (2002) Archaeal group II chaperonin mediates protein folding in the *cis*-cavity without a detachable GroES-like co-chaperonin. J. Mol. Biol. **315**, 73–85
37 Archibald, J.M. and Roger, A.J. (2002) Gene duplication and gene conversion shape the evolution of archaeal chaperonins. J. Mol. Biol. **316**, 1041–1050
38 Guagliardi, A., Cerchia, L. and Rossi, M. (1995) Prevention of *in vitro* protein thermal aggregation by the *Sulfolobus solfataricus* chaperonin: evidence for nonequivalent binding surfaces on the chaperonin molecule. J. Biol. Chem. **270**, 28126–28132
39 Klumpp, M., Baumeister, W. and Essen, L.O. (1997) Structure of the substrate binding domain of the thermosome, an archaeal group II chaperonin. Cell **91**, 263–270
40 Bosch, G., Baumeister, W. and Essen, L.O. (2000) Crystal structure of the β-apical domain of the thermosome reveals structural plasticity in the protrusion region. J. Mol. Biol. **301**, 19–25
41 Gómez-Puertas, P., Martín-Benito, J., Carrascosa, J.L., Willison, K.R. and Valpuesta, J.M. (2004) The substrate recognition mechanisms in chaperonins. J. Mol. Recognit. **17**, 85–94
42 Leroux, M.R., Fändrich, M., Klunker, D., Siegers, K., Lupas, A.N., Brown, J.R., Schiebel, E., Dobson, C.M. and Hartl, F.U. (1999) MtGimC, a novel archaeal chaperone related to the eukaryotic chaperonin cofactor GimC/prefoldin. EMBO J. **18**, 6730–6743
43 Martín-Benito, J., Boskovic, J., Gómez-Puertas, P., Carrascosa, J.L., Simons, C.T., Lewis, S.A., Bartolini, F., Cowan, N.J. and Valpuesta, J.M. (2002) Structure of eukaryotic prefoldin and of its complexes with unfolded actin and the cytosolic chaperonin CCT. EMBO J. **21**, 6377–6386
44 Okochi, M., Yoshida, T., Maruyama, T., Kawarabayasi, Y., Kikuchi, H. and Yohda, M. (2002) *Pyrococcus* prefoldin stabilizes protein-folding intermediates and transfers them to chaperonins for correct folding. Biochem. Biophys. Res. Commun. **291**, 769–774
45 Ruano-Rubio, V. and Fares, M.A. (2007) Testing the neutral fixation of hetero-oligomerism in the archaeal chaperonin CCT. Mol. Biol. Evol. **24**, 1384–1396
46 Large, A., Stamme, C., Lange, C., Duan, Z., Allers, T., Soppa, J. and Lund, P.A. (2007) Characterization of a tightly controlled promoter of the halophilic archaeon *Haloferax volcanii* and its use in the analysis of the essential *cct1* gene. Mol. Micro. **66**, 1092–1106
47 Gribaldo, S., Lumia, V., Creti, R., de Macario, E.C., Sanangelantoni, A. and Cammarano, P. (1999) Discontinuous occurrence of the hsp70 (dnaK) gene among Archaea and sequence features of HSP70 suggest a novel outlook on phylogenies inferred from this protein. J. Bacteriol. **181**, 434–443
48 Macario, A.J., Brocchieri, L., Shenoy, A.R. and de Macario, E.C. (2006) Evolution of a protein-folding machine: genomic and evolutionary analyses reveal three lineages of the archaeal hsp70 (dnaK) gene. J. Mol. Evol. **63**, 74–86
49 Zhang, H., Lin, L., Zeng, C., Shen, P. and Huang, Y.P. (2007) Cloning and characterization of a haloarchaeal heat shock protein 70 functionally expressed in *Escherichia coli*. FEMS Microbiol. Lett. **275**, 168–174
50 Zhang, W., Culley, D.E., Nie, L. and Brockman, F.J. (2006) DNA microarray analysis of anaerobic *Methanosarcina barkeri* reveals responses to heat shock and air exposure. J. Ind. Microbiol. Biotechnol. **33**, 784–790

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