Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 5383–5388, May 1997
Microbiology

Chaperonin filaments: The archaeal cytoskeleton?

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Communicated by Robert Haselkorn, University of Chicago, Chicago, IL, March 10, 1997 (received for review December 31, 1996)

ABSTRACT **Chaperonins are high molecular mass double-ring structures composed of 60-kDa protein subunits. In the hyperthermophilic archaeon *Sulfolobus shibatae* the two chaperonin proteins represent ≈4% of its total protein and have a combined intracellular concentration of >30 mg/ml. At concentrations ≥ 0.5 mg/ml purified chaperonins form filaments in the presence of Mg^{2+} and nucleotides. Filament formation requires nucleotide binding (not hydrolysis), and occurs at physiological temperatures in biologically relevant buffers, including a buffer made from cell extracts. These observations suggest that chaperonin filaments may exist *in vivo* and the estimated 4600 chaperonins per cell suggest that such filaments could form an extensive cytostructure. We observed filamentous structures in unfixed, uranyl-acetate-stained *S. shibatae* cells, which resemble the chaperonin filaments in size and appearance. ImmunoGold (Janssen) labeling using chaperonin antibodies indicated that many chaperonins are associated with insoluble cellular structures and these structures appear to be filamentous in some areas, although they could not be uranyl-acetate-stained. The existence of chaperonin filaments *in vivo* suggests a mechanism whereby their protein-folding activities can be regulated. More generally, the filaments themselves may play a cytoskeletal role in Archaea.**

The chaperonins are high molecular mass complexes that have been identified in a variety of Bacteria, Archaea, and Eukarya by their double-ring appearance in the electron microscope and the sequence similarity of their constituent proteins (1, 2). Depending on the organism from which they are isolated, the double rings may be composed of 14–18 identical or closely related 60-kDa proteins (3, 4). These proteins are sufficiently conserved in all organisms to be identified as a coherent group with two distinct subgroups (5, 6). These two subgroups, (*i*) the Bacteria, mitochondria, and chloroplast group; and (*ii*) the Archaea and eukaryotic cytoplasm group, reflect known or suspected phylogenetic relationships (7). The observation that in Bacteria and Archaea these 60-kDa proteins are heat shock proteins (HSP60s) has been the basis for interpreting their function *in vivo*.

It was proposed over a decade ago that the function of some HSPs during stress is to bind to damaged proteins to prevent them from aggregating and under favorable conditions to release these bound proteins at the expense of ATP hydrolysis, allowing them to refold and regain their normal activities (8). It was later realized that these same HSPs, which are also present in unstressed cells, may function in a similar way to assist in the folding of newly synthesized proteins under normal growth conditions and in this role the HSPs were referred to as "molecular chaperones" (9–12).

The HSP60s are among the HSPs involved in the proposed protein-folding scheme and the supramolecular double-ring structure they form has become known as a "chaperonin" (13). Experiments with purified chaperonins from bacteria (primarily GroEL/S from *Escherichia coli*) established that these chaperonins are able to recognize, bind, and in some cases influence the refolding of unfolded proteins *in vitro* (13–15). Similarities between the bacterial and the archaeal HSP60s and the discovery that the archaeal HSP60s are related to a family of eukaryotic cytoplasmic proteins known as TCP1s (16), has led to the belief that chaperonins may mediate protein folding in all organisms (11, 15). Indeed, although the TCP1s are not HSPs (17), both the archaeal HSP60s and eukaryotic TCP1s form double ring structures and have ATPase activity, like the bacterial chaperonins (16, 18). Unlike the bacterial chaperonins, however, they are not able to fold more than a few specific proteins *in vitro* (19–21). This may be due to technical problems associated with *in vitro* experiments or specialization of these chaperonins for folding specific proteins. Alternatively, it suggests that chaperonins may have other functions *in vivo.*

Current models of chaperonin function focus on the double-ring structure and propose how this structure mediates protein folding (22, 23). They suggest, for example, that protein folding occurs within the central cavity of the double ring; the partially folded protein (molten globule) enters this cavity where it is either protected by the chaperonin from inappropriate interactions with other proteins as it follows its proper folding pathway or it is unfolded by the chaperonin and thereby rescued from nonproductive folding pathways and given additional opportunities to follow its proper pathway. ATP and co-chaperonins influence the folding process by causing structural changes in the chaperonin that affect its interactions with the bound protein (22–25). These models can be applied to understanding protein folding *in vivo*, if we assume that chaperonins exist as double ring structures inside cells. Chaperonin double rings are observed in extracts from lysed cells, but their structure in intact cells is not known.

To investigate the structure of chaperonins *in vivo,* we studied chaperonins from the hyperthermophilic archaeon, *Sulfolobus shibatae.* In this organism, which lives in acidic geothermal hot springs and grows optimally at pH 3.0 and 83°C (26), the chaperonins are composed of its two most abundant proteins (TF55 α and β) (6). We determined the concentration of these proteins in *S. shibatae* grown at 75°C (i.e., under non-heat shock conditions), to establish a basis for *in vitro* experiments with purified chaperonins. In *in vitro* experiments we observed that as the concentration of purified chaperonins increased toward the concentration found in cells, the isolated double-ring structures assembled into ordered filaments. The conditions under which filament formation occurred suggest that chaperonins may form filaments *in vivo,* and considering

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0027-8424/97/945383-6$2.00/0
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Abbreviations: HSP, heat shock protein; AMP-PNP, 5′-adenylylimidodiphosphate; rad, radian; TEM, transmission electron microscopy.

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the abundance of chaperonins *in vivo,* such filaments could form an extensive cytostructure in *S. shibatae.*

MATERIALS AND METHODS

Chaperonin Purification. Chaperonins were purified from cells grown at 75°C in standard yeast extract medium (27), opened by sonication (6 min × 2) in the presence of three volumes of HKM buffer (25 mM Hepes, pH 7.5/10 mM KCl/10 mM $MgCl_2$), treated with DNase (0.25 unit/ml, final), and centrifuged at 30,000 rpm for 30 min in a 50.2 Ti rotor (Beckman) before applying the supernatant to a DEAE-Sepharose column, followed by a Mono-Q column (Pharmacia). Both columns were equilibrated in HKM buffer, and proteins were eluted with a 0 to 1.0 M NaCl gradient as previously described (6). The purified chaperonins were resuspended in 25 mM Hepes buffer (pH 7.5) and protein concentrations were determined by the DC Protein Assay system (Bio-Rad) using BSA as a standard.

PAGE and Spectrophotometry. Purified chaperonin subunits and total *S. shibatae* proteins were analyzed by 10% PAGE. Chaperonin proteins were obtained as described above and total cellular proteins were obtained by adding SDS-sample buffer (final concentration 10% glycerol/100 mM dithiothreitol/2% SDS/50 mM Tris·HCl, pH 6.8/0.1% bromophenol blue) to the cell pellet produced by centrifugation (10,000 rpm at room temperature for 5 min, in a microfuge; Hermle, Germany). Proteins from specific numbers of cells were obtained from cultures in which the concentration of cells was determined using a haemocytometer (Neubauer, Germany). The area and intensity of protein bands stained by Coomassie brilliant blue (R280) were quantified (area × intensity) by digitization using the Powerlook Pro scanner (UMAX program) and the NIH IMAGE program.

Spectrophotometry used a Cary 1G (Varian) with purified chaperonin in a simple buffer (5 mM Hepes, pH 7.0 or 7.5/25 mM $MgCl_2$) or a complex buffer made from cell extracts. Cell extracts were made by washing frozen cells four times in glass distilled water, resuspending the washed pellet in an equal volume of water (ml water = cell wet weight), sonicating for 20 min, centrifuging (30,000 rpm, 30 min at 20°C), boiling the supernatant for 10 min, recentrifuging, and ultrafiltering the supernatant (10 kDa Centricon; Amicon). ATP (Sigma) or 5′-adenylylimidodiphosphate (AMP-PNP, Sigma) were used at final concentrations of 1 mM. Experiments were done at various temperatures regulated by the thermostated cuvette holder in the spectrophotometer.

Electron Microscopy. Purified chaperonin samples were attached to lacy carbon grids with ultrathin Formvar (Ladd Reseach Industries, Burlington, VT), stained with 2% uranyl acetate for 3 min, and air-dried. Cell samples (1 ml) in log phase growth at 75°C were removed from medium by centrifugation (30 s, 12,000 rpm, tabletop centrifuge), washed in water, and resuspended in 50 μl HKM buffer. An 8-μl sample of concentrated cells was placed on Formvar lacy grids treated with 2% Triton X-100 for 3 min, washed in HKM buffer, treated with DNase (final concentration 0.1 unit/ml, Promega) for 10 min, washed again in HKM buffer, stained in 2% uranyl acetate for 3 min, and then air-dried. All solutions were filtered (0.22 μm) and all procedures were done at room temperature.

Samples were viewed in a Philips EM420T or CM30T with LAB6 filaments at 80 to 300 kV or in a JEOL 100CX with tungsten filament at 100 kV. No changes in the microstructure of samples were observed at the working resolution with electron doses of 1–200 electrons/Å^2. Micrographs were taken within this dose range at defocuses of −200 to −800 nm with illumination-convergence angles of about 1 milliradian (mrad) and scattering angle of 5 mrad using a room temperature, double-tilt, beryllium stage. Micrographs were digitized using a flatbed Powerlook Pro scanner (UMAX), and data processing was done using NIH IMAGE and Adobe PHOTOSHOP.

ImmunoGold Labeling. ImmunoGold labeling generally followed established procedures (28). Cells were concentrated and attached to transmission electron microscopy (TEM) grids, lysed on grids in HP buffer (50 mM Hepes/4% PEG 4000/50 mM KCl/5 mM MgCl) containing 2.5% Triton X-100 and 0.02 unit/μl DNase (Promega); washed 3 times for 5 min in HP buffer; and fixed in 2% glutaraldehyde, 50 mM Hepes (pH 7.5) for 5 min. After three 5-min washes in PBS containing 0.1 M glycine (PBS-G), cells were treated with PBS containing 0.1% Tween-20, 5% dry milk powder, and 0.1 M glycine for 30 min and then were washed three times for 5 min with PBS-G. Cells were then soaked for 4 h in a humid chamber in 25 μl of primary polyclonal antibody made in rabbits (diluted 1:1000 in PBS), preabsorbed primary antibody (diluted 1:1000 in PBS containing 1.0 mg/ml purified chaperonin), or preimmune antibody (diluted 1:1000 with PBS). Antibody-exposed cells were washed three times in PBS-G, 5 min each; soaked for 1 h in 25 μl of secondary antibody (diluted 1:50), which was goat anti-rabbit IgG conjugated with 5 nm colloidal gold (EY

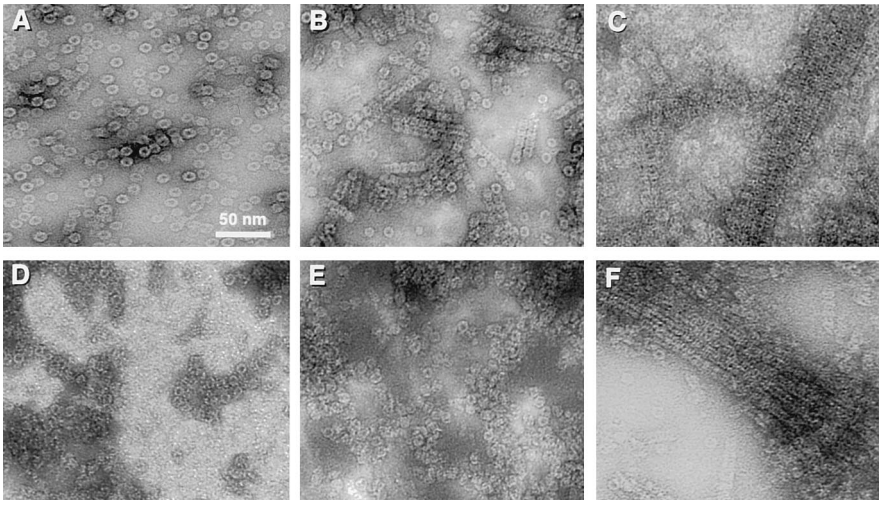

FIG. 1. Above a critical concentration freshly purified *S. shibatae* chaperonins form filaments at room temperature in the presence of Mg^{2+}. (*A*) Purified chaperonin proteins in a HKM buffer appear as double rings at concentrations of 0.1 mg/ml; (*B*) rings and short chains are seen at 0.5 mg/ml; and (*C*) long chains and filaments are present at 1.0 mg/ml. At chaperonin concentrations of 1.0 mg/ml filaments did not form in Hepes buffer alone (*D*), or when 10 mM KCl was added (*E*), but did form when 10 mM $MgCl_2$ was added (*F*).

Laboratories); washed three times with PBS-G, 5 min; fixed as described above; washed for 15 min in filtered (0.22 μm) double-distilled water; and air dried.

RESULTS

The Intracellular Concentration of Chaperonin Proteins. We estimated the amount of the two chaperonin proteins (TF55 α and β) in *S. shibatae* cells by comparing extracts from a determined number of cells with measured amounts of purified chaperonin protein by PAGE. By this procedure, we determined an average of 8.3×10^{-12} mg of TF55 per cell, $\approx$4% of the total cell protein. To determine the concentration of TF55 per cell, we measured cell diameters from calibrated scanning electron micrographs of *S. shibatae* and used the formula for a sphere to calculate an approximate cell volume. Since the average cell diameter was 0.8 μm, the average cell volume was 2.7×10^{-13} ml. Using this value (without correcting for the smaller cytoplasmic volume), we calculated an intracellular concentration for the chaperonin proteins of 31 mg/ml.

Chaperonins Form Filaments. To determine the effects of concentration on chaperonin structure, we looked at different concentrations of freshly purified chaperonins in a buffer containing Hepes (pH 7.5), KCl, and $MgCl_2$, using TEM and a standard negative staining procedure. At 0.1 mg/ml, which is a normal working concentration for negative-stained samples, we observed the double-ring structures characteristic of chaperonins (Fig. 1*A*). At 0.5 mg/ml, however, we observed that chaperonins stacked end to end to form short filaments (Fig. 1*B*) and at 1.0 mg/ml, the filaments were longer and frequently aligned side by side or intertwined to form a network of filaments with a distinct periodicity that made them appear striated (Fig. 1*C*). At concentrations >2.0 mg/ml the layer of protein that attached to the sample grids was too thick to be penetrated by the electron beam and appeared black. At chaperonin concentrations of 1.0 mg/ml filaments did not form in a buffer containing Hepes (pH 7.5) alone (Fig. 1*D*) or in Hepes and KCl (Fig. 1*E*), but did form in Hepes and $MgCl_2$ (Fig. 1*F*). This indicates that Mg^{2+} is required for filament formation, which was further supported by the observation that the addition of EDTA dissociated filaments.

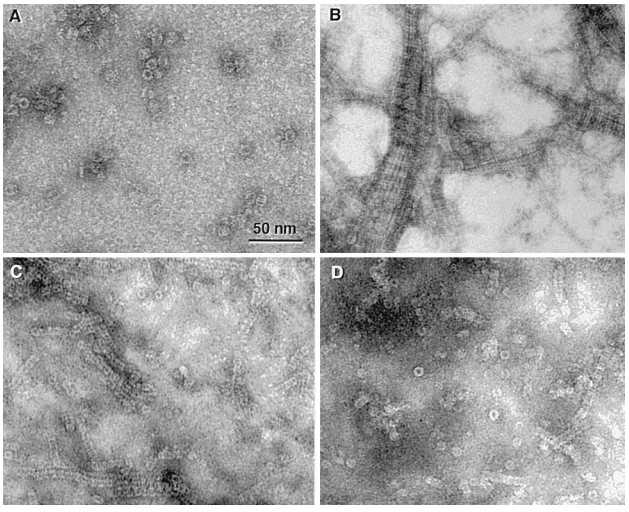

FIG. 2. Chaperonins stored for >48 h require Mg^{2+} and nucleotides to form filaments at a physiological temperature. (*A*) "Aged" chaperonin (1 mg/ml) in Hepes buffer (pH 7.5) and 25 mM $MgCl_2$ after 1 h at 75°C; (*B*) the same chaperonin sample with 1 mM ATP added; (*C*) with 1 mM ADP added; and (*D*) with 1 mM AMP-PNP added.

It should be noted that this Mg^{2+} induced filament formation only occurred with freshly purified chaperonins and did not occur if purified chaperonins were stored for >48 h.

Chaperonin Filament Formation Depends on Nucleotide Binding but Not Hydrolysis. Chaperonins that had lost their ability to form filaments in the presence of Mg^{2+} alone were able to form filaments in the presence of Mg^{2+} and nucleotides at room temperature and physiological temperatures. After 1 h at 75°C, for example, chaperonins stored for >1 week at 4°C did not form filaments in the presence of Mg alone (Fig. 2*A*), whereas they formed an extensive network of filaments in the presence of ATP/Mg (Fig. 2*B*). They formed shorter filaments in the presence of ADP/Mg (Fig. 2*C*), AMP-PNP/Mg (Fig. 2*D*), GTP/Mg and ATPγS/Mg (data not shown). The observation that filaments formed in the presence of the nonhydro-

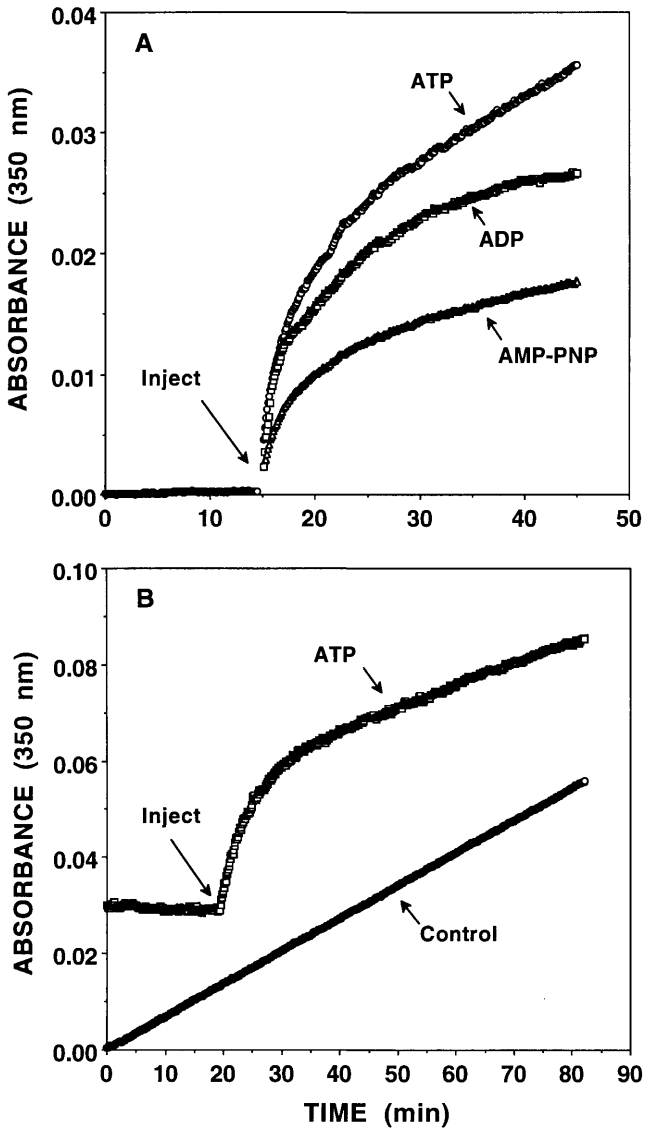

FIG. 3. Chaperonin filament formation at 75°C in the presence of Mg^{2+} and nucleotides in different buffers measured by light scattering. (*A*) Changes in absorption (350 nm) of purified chaperonin (1.0 mg/ml) in Hepes buffer after the addition (inject) of 25 mM $MgCl_2$ and 1.0 mM ATP, ADP, or AMP-PNP; (*B*) changes in absorption of purified chaperonin in a complex buffer made from cell extract (see *Results*) after the addition of $MgCl_2$ and ATP (inject); the cell extract buffer itself (control) increased in absorption due to aggregation, which was suppressed by the addition of chaperonins but not after the chaperonins formed filaments. TEM analysis of samples before and after the addition of nucleotides indicate that the increased absorption in chaperonin samples is correlated with the presence of filaments.

lyzable nucleotide, AMP-PNP, indicates that the process depends on nucleotide binding rather than hydrolysis. The variation in filament formation with different nucleotides suggests differences in binding affinities.

We monitored the kinetics of chaperonin filament formation by a spectrophotometric procedure similar to the one used to monitor the polymerization of α and β tubulin (29). After establishing that increases in light scattering (absorption) at 350 nm correlated with chaperonin filament formation by TEM, we compared the rates of filament formation at 75°C in the presence of ATP/Mg, ADP/Mg and the nonhydrolyzable AMP-PNP/Mg (Fig. 3*A*). In all cases filaments formed rapidly after injecting nucleotides and continued to form more slowly for long periods of time. As expected from TEM observations (Fig. 2), the effects of ATP/Mg on filament formation were more pronounced than the effects of the other two nucleotides.

Since we are interested in determining the structure of chaperonins *in vivo* (i.e., if they form filaments in *S. shibatae* cells), we repeated the spectrophotometric experiments using a buffer made from cell extracts. Cell-extract buffer was prepared by boiling extracts from lysed cells, pelleting the precipitates, and ultrafiltering the supernatant (see *Materials and Methods*). This effectively removed or destroyed most macromolecules (PAGE indicated only small peptides remained), leaving primarily salts and thermostable solutes. The extract was adjusted to pH 7.0, which is within the intracellular range reported for *Sulfolobus* spp. (30). At 75°C the buffer itself showed a gradual increase in light scattering presumably due to precipitation of remaining components (Fig. 3*B*, "control"). This precipitation was not apparent when chaperonins were present (1.0 mg/ml, final concentration) and did not influence their filament forming ability, which occurred immediately after the injection of ATP/Mg (Fig. 3*B*, ATP). Here too we verified that the increase in light scattering correlated with the presence of filaments by TEM.

Chaperonins Could Form an Extensive Cytostructure. The potential distribution of chaperonin filaments in cells can be calculated from the number of chaperonins per cell and their sizes. From the amount of chaperonin protein in *S. shibatae* we calculated that there are as many as 4635 chaperonins per cell. From the reported size of *Sulfolobus* chaperonins, 17.5 nm in length (31), we calculated a potential filament length of ≈80 μm, assuming all 4635 chaperonins are associated with the filament. Considering the average diameter of *S. shibatae* cells (0.8 μm), chaperonin filaments could therefore traverse the diameter of a cell ≈100 times. We reasoned that if such an extensive cytostructure exists, it should be visible in the electron microscope, unless it is destroyed during sample preparation.

A Filamentous Cytostructure Exists in *S. shibatae*. We looked for filaments in *S. shibatae* grown at normal temperatures by rapidly concentrating cells by centrifugation, allowing them to attach to TEM sample grids, treating them with a nonionic detergent (Triton X-100) to remove their protein surface layer, DNase to remove DNA, and lightly staining them with uranyl acetate. (Glutaraldehyde and formaldehyde fixation were omitted to avoid producing cross-linking artifacts and because we had observed these fixatives to obscure the striation pattern of *in vitro* chaperonin filaments). After this procedure most cells were so heavily stained they appeared completely black in the TEM. There were some cells, however, in which areas of the cell were lightly stained and in these areas filamentous structures were seen (Fig. 4). The thickness of the cells and the inconsistency of staining made it difficult to obtain high resolution images of these filaments, but we did find cells in which filaments were visible at both low and high magnifications (Fig. 4 *A* and *A′*). The distribution and interweaving of these filaments suggest that they extend throughout the cytoplasm (Fig. 4 *A′–E*) and stereomicrographs confirmed that they were distributed throughout the cell volume (not shown). The width of the intracellular filaments (where it could be measured) was similar between cells; ≈11 ± 1 nm for individual filaments. In comparison, the width of the chaperonin filaments formed *in vitro* measured 10.7 ± 0.6 nm.

ImmunoGold Labeling Reveals that Chaperonins Are Associated with Insoluble Cell Components that Look Filamen-

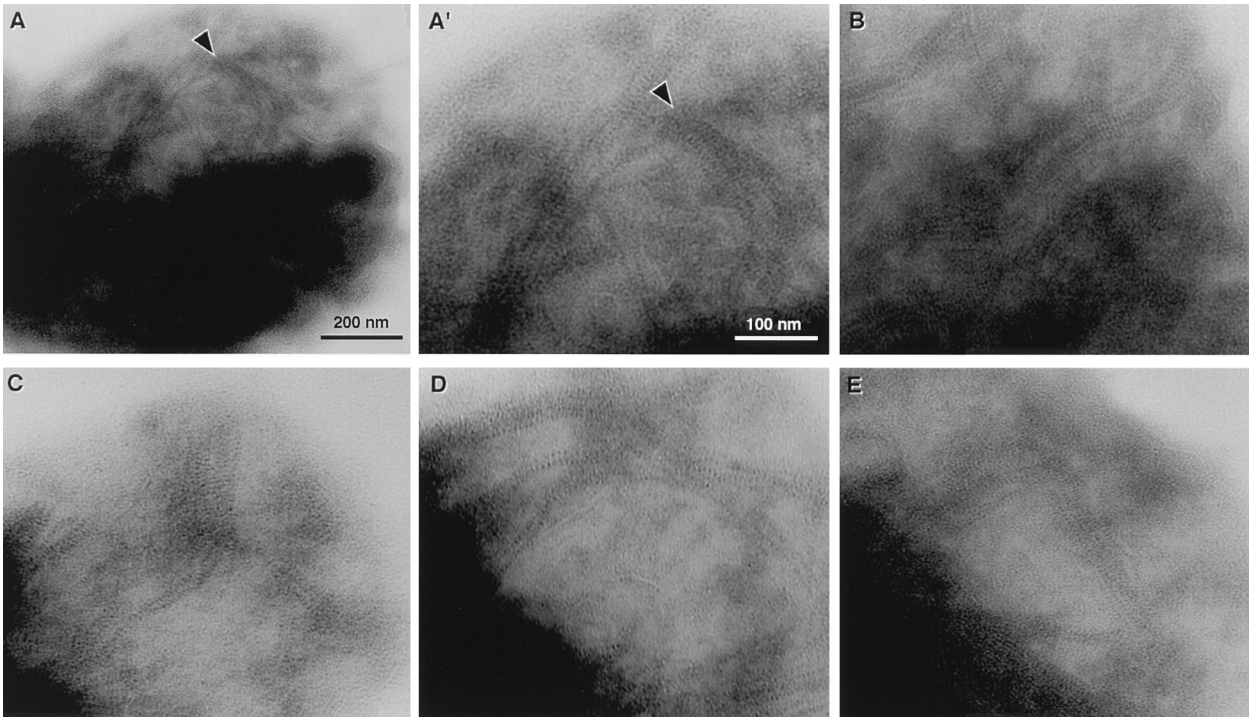

FIG. 4. Micrographs of intracellular filaments in detergent-treated, unfixed *S. shibatae* cells lightly stained with uranyl acetate, taken using intermediate voltage TEM (100–300 kV). A low (*A*) and high (*A′*) magnification of the same cell shows the distribution and distinct periodic structure of the intracellular filaments (arrowheads). Similar filaments were seen in many cells and four representative micrographs are shown (*B–E*).

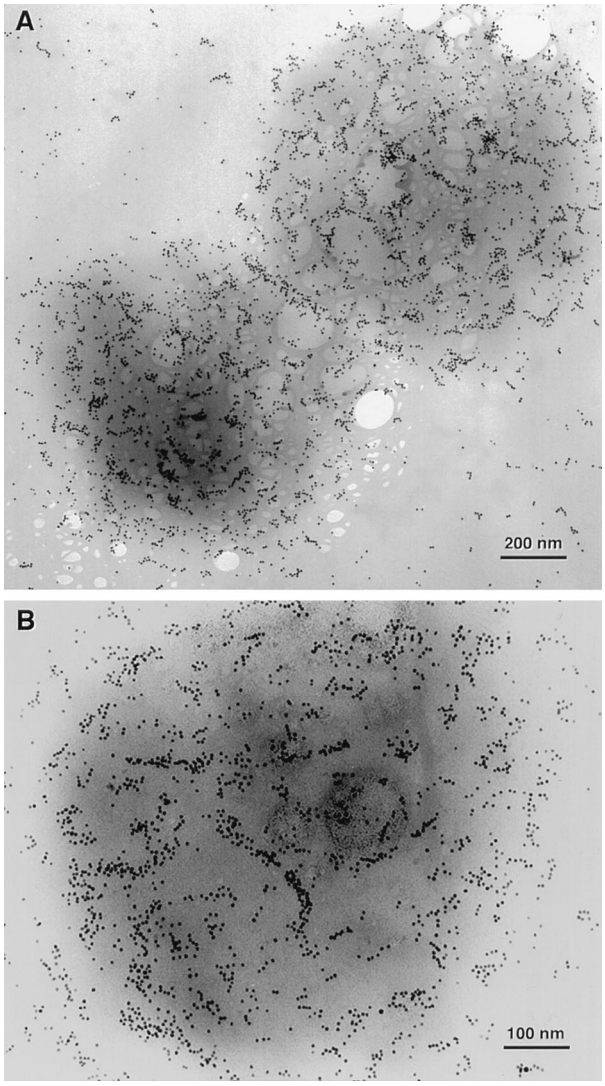

FIG. 5. ImmunoGold labeling of detergent-treated *S. shibatae* cells with polyclonal antibodies against the chaperonin seen at low (*A*) and high (*B*) magnification. The 5-nm gold particles (visible as black spots) distributed on the support grid around the cells suggests that chaperonins were released during sample preparation, but the abundance of gold particles remaining associated with cells indicates that a large number of chaperonins remained associated with an insoluble matrix. The pattern of gold particles in some regions is suggestive of filaments. Uranyl acetate-stained filaments, such as those shown in Fig. 4, were not seen in samples prepared for ImmunoGold labeling, therefore uranyl acetate staining was omitted to visualize better the distribution of gold particles.

tous in Areas. Using polyclonal antibodies against chaperonins and ImmunoGold techniques, we attempted to establish a link between the intracellular filaments and chaperonin filaments (Fig. 5). Unfortunately, after processing cells for ImmunoGold labeling we were unable to see filaments like those shown in Fig. 4, so a direct link was not established. However, this technique did provide indirect evidence that chaperonin filaments exist *in vivo.*

Since the ImmunoGold procedure we used would be expected to remove most soluble proteins from cells, the number of gold particles found associated with cells indicates that a considerable number of chaperonins is present in detergent-resistant (i.e., insoluble) structures (Fig. 5*A*). Furthermore, the distribution of gold particles suggests that these structures are filamentous in some areas (Fig. 5*B*).

To insure the specificity of the labeling for chaperonins, we used a preabsorption control, in which purified chaperonin was added to the antiserum before using it on cells, and a preimmune control, in which serum from an uninoculated animal was used. Both of these controls gave minimal background labeling, indicating that there were no other antigens in the cells responsible for the labeling shown in Fig. 5. In addition, immunoblots of total *S. shibatae* proteins indicated that the antibodies were specific for TF55 α/β (not shown). Overall, while ImmunoGold labeling did not establish a definitive link between the intracellular and chaperonin filaments, it did provide additional evidence that chaperonins may be part of an intracellular structure.

DISCUSSION

The observations that the chaperonin protein concentration in *S. shibatae* is >30 mg/ml and that purified chaperonins at concentrations ≥0.5 mg/ml form filaments at physiological temperatures and in biologically relevant buffers led us to the hypothesis that chaperonin filaments exist *in vivo*. It should be emphasized that it is not only the observation that the *S. shibatae* chaperonins are able to form filaments *in vitro* that led to this hypothesis; a number of proteins, such as glutamine synthetase, RNA polymerase, and RecA, which are not believed to form filaments *in vivo*, can be induced to form filaments *in vitro* under conditions different from those in cells (32). Unlike these proteins, however, the chaperonins formed filaments under conditions that approximate those found in *S. shibatae* cells. The TEM observations of filaments in *S. shibatae* that resemble chaperonin filaments in size and appearance support this hypothesis, although a direct link between the intracellular and chaperonin filaments has yet to be established. ImmunoGold labeling did establish that a large number of chaperonins are associated with cellular structures that resist detergent extraction and that these structures have filament-like features (see Fig. 5).

It is possible that the intracellular filaments we observed are remnants of chromatin not destroyed by DNase digestion, fragments of the cell's surface layer not removed by Triton X-100 treatment, or actin or tubulin filaments not previously observed in *S. shibatae*. These possibilities are not as convincing as chaperonin filaments, however. Published micrographs of chromatin from Archaea (33, 34) and surface layers from *Sulfolobus* spp. (35) do not resemble the filaments we observed, and while actin and tubulin filaments do resemble these filaments, there is no convincing evidence that either actin or tubulin is present in *Sulfolobus* (36). Considering the abundance and concentration of chaperonin proteins in *S. shibatae*, the conditions under which they form filaments *in vitro*, and the similarity between these filaments and those seen *in vivo*, we believe that our suggestion that the intracellular filaments are composed of chaperonins is the most plausible.

The existence of chaperonin filaments in cells has important implications for understanding chaperonin function *in vivo*. For example, in light of current models about chaperonin-mediated protein folding, chaperonin filaments may serve a regulatory function. This is suggested by the fact that protein folding is believed to occur within the chaperonin's central cavity (22, 37) and access to this cavity is expected to be blocked in filaments. Cells may therefore sequester chaperonins in filaments to limit their activities or dissociate filaments to access these activities rapidly, without the need for synthesizing chaperonin proteins. This may be important in responding to rapidly changing environmental conditions such as heat shock or chemical stresses and may be controlled by intracellular levels of nucleotides, Mg^{2+} or other divalent cations.

A more intriguing possibility is that the chaperonin filaments themselves form a cytoplasmic structure in Archaea that is functionally similar to the cytoskeleton in eukaryotes. It has been suggested that *Sulfolobus,* and other species of Archaea that lack rigid cell walls, must have an internal "cytoskeleton"

based on their ability to maintain an irregular shape in solution, change their shape when attached to surfaces, and the gelling properties of their cytoplasm (36, 38). The composition of this putative cytoskeleton has remained obscure, perhaps because efforts have been directed at finding Archaeal homologs of eukaryotic cytoskeleton proteins (lamins, actin, tubulin, calmodulin, giardin, and myosin) (36). Based on the results reported here, we propose that chaperonins are the building blocks for such an Archaeal cytoskeleton. This idea is supported by their filament-forming ability and abundance in *S. shibatae,* and their abundance in other Archaea (6, 39), as well as recent studies of the eukaryotic chaperonin known as TCP1 (40–44).

The TCP1 chaperonins consist of a family of 60 kDa proteins that share >35% amino acid identity with the Archaeal chaperonins (2, 6, 16). *In vitro* experiments suggest that as chaperonins, TCP1 chaperonins are specialized in folding actin and tubulin (19, 20). *In vivo* mutational analyses indicate that TCP1 plays an essential role in cytoskeleton organization (i.e., mutants show irregular distributions of actin and microtubules and are unable to segregate their nuclei properly) (40, 41, 43). In addition, overexpression of TCP1 partially suppresses actin mutations (41, 43). In HeLa cells, TCP1 is found associated with the centrosome and appears to play a critical role in microtubule assembly (44). In medulla cells, TCP1 (referred to as chromobindin A) is found associated with chromaffin granules and plays a role in exocytosis (42). These observations indicate that TCP1 function is associated with the eukaryotic cytoskeleton and it is not limited to protein folding. Thus the closely related archaeal chaperonins may also have cytoskeleton-related functions.

We thank S. J. Trent, M. Ellis, F. Stevens, and D. Nadziejka for editorial comments. This work was supported by the U.S. Department of Energy, Office of Health and Environmental Research, and the Office of Basic Energy Sciences under Contract W-31-109-Eng-38.

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