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Evolution

# An archaebacterial homologue of the essential eubacterial cell division protein FtsZ

**(Archaea/archaebacteria/cell division/cytoskeleton)**

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**ABSTRACT** **Life falls into three fundamental domains—Archaea, Bacteria, and Eucarya (formerly archaebacteria, eubacteria, and eukaryotes, respectively). Though Archaea lack nuclei and share many morphological features with Bacteria, molecular analyses, principally of the transcription and translation machineries, have suggested that Archaea are more related to Eucarya than to Bacteria. Currently, little is known about the archaeal cell division apparatus. In Bacteria, a crucial component of the cell division machinery is FtsZ, a GTPase that localizes to a ring at the site of septation. Interestingly, FtsZ is distantly related in sequence to eukaryotic tubulins, which also interact with GTP and are components of the eukaryotic cell cytoskeleton. By screening for the ability to bind radiolabeled nucleotides, we have identified a protein of the hyperthermophilic archaeon *Pyrococcus woesei* that interacts tightly and specifically with GTP. Furthermore, through screening an expression library of *P. woesei* genomic DNA, we have cloned the gene encoding this protein. Sequence comparisons reveal that the *P. woesei* GTP-binding protein is strikingly related in sequence to eubacterial FtsZ and is marginally more similar to eukaryotic tubulins than are bacterial FtsZ proteins. Phylogenetic analyses reinforce the notion that there is an evolutionary linkage between FtsZ and tubulins. These findings suggest that the archaeal cell division apparatus may be fundamentally similar to that of Bacteria and lead us to consider the evolutionary relationships between Archaea, Bacteria, and Eucarya.**

By comparing the sequences of highly conserved RNAs and proteins, it has been established that life is divided into three fundamental domains, which have been termed Eucarya (eukaryotes), Bacteria (eubacteria), and Archaea (archaebacteria) (1, 2). This has dispelled the notion that archaebacteria are merely a group of bacteria that are adapted to living in extremes of temperature, salinity, and/or pH. Archaea were not recognized as a distinct phylogenetic entity earlier, because they resemble Bacteria in many ways. For example, eubacterial and archaeal cells are generally similar in morphology, lack a nucleus, and normally have relatively small circular genomes (3, 4). In contrast, eukaryotic cells are generally larger, have bigger genomes that are organized on multiple linear chromosomes, and contain a nucleus and a variety of other organelles.

Recently, much excitement has arisen from the realization that Archaea are, in many ways, related more closely to Eucarya than to Bacteria. For instance, archaeal translation elongation factors and aminoacyl-tRNA synthetases are generally more similar to their eukaryotic equivalents than to their eubacterial counterparts (for example see refs. 5–7). Furthermore, the archaeal RNA polymerase is highly related to RNA polymerases I, II, and III of eukaryotes (4, 8), and Archaea and Eucarya both possess factors such as the TATA binding protein and transcription factor TFIIB/TFB that have no equivalents in Bacteria (for example see refs. 4 and 9–15). In many regards, therefore, eukaryotic and archaeal cells are fundamentally similar in their molecular design. It remains to be determined, however, to what degree this relatedness extends to other cellular components.

A defining feature of the eukaryotic cell is the cytoskeleton, which maintains cell shape and integrity and mediates the partition of cellular constituents during cell division. The cytoskeleton is also required for cell movement and the uptake of materials from the environment by phagocytosis or endocytosis. Significant components of the cytoskeleton are the $\alpha$, $\beta$, and $\gamma$ tubulins which, in the presence of GTP, polymerize to form microtubules. Consistent with their important roles, tubulins are among the most conserved proteins known. Although tubulins are not present in Bacteria, the highly conserved eubacterial cell division protein FtsZ is distantly related to tubulins in sequence and has functional similarities with tubulins (16, 17). Like tubulins, FtsZ binds GTP and is a GTPase (18–20), and it polymerizes to form filaments in the presence of this nucleotide (16, 21). Moreover, FtsZ is required for Bacterial cell division and localizes to the septum that forms between dividing daughter cells (22). Together, these findings raise the possibility that eubacterial FtsZs and eukaryotic tubulins are derived from a common ancestor.

Currently, very little is known about the mechanism and control of cell division in Archaea. Furthermore, although there is some evidence for cytoskeletal systems in certain Archaea (23, 24), no components of an archaeal cytoskeleton have so far been reported. Here, we take advantage of the tight binding of FtsZ and tubulins to GTP to probe for archaeal homologues of these molecules.

## MATERIALS AND METHODS

**λgt11 Expression Library Construction and Screening.** *Pyrococcus woesei* genomic DNA (10 μg) was sheared by sonication and incubated with T4 DNA polymerase in the presence of 100 μM dNTPs to generate blunt ends. After chromatography on Sepharose CL-2B, fractions containing DNA fragments of 0.5–3 kb were pooled. DNA was then ligated to *Eco*RI adaptors and cloned into λgt11. For screening, the library was grown at 42°C for 2.5–3.5 hr, then isopropyl β-D-thiogalactoside (IPTG)-impregnated nitrocellulose filters were placed on the plates at 37°C for 3.5 hr (first lifts) or 4 hr (second lifts). Filters were blocked in TBST (Tris·HCl, pH 8.0/150 mM NaCl/0.05% Tween-20) containing 5% milk powder for 1 hr and transferred into NBB (10 mM Hepes, pH

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Data deposition: The sequences reported in this paper have been deposited in the GenBank data base (accession nos. P06138, P45498, P17865, U37584, U56247, X06748, M21414, M20405, U02069, U39877, K02842, M10347, M63447, X01703, J00314, M61764, M14643, M20419, and M76765).

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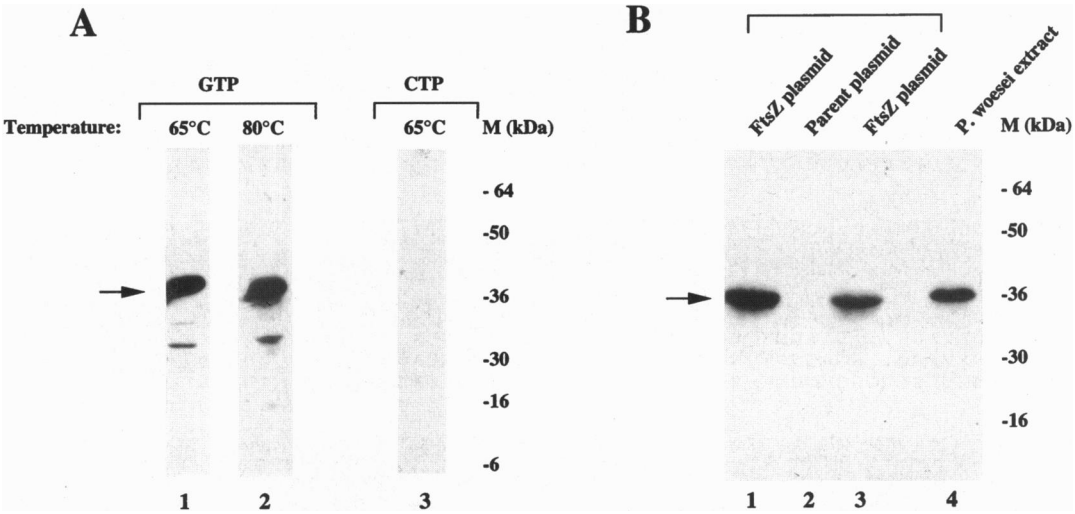


FIG. 1. (*A*) Detection of GTP-binding proteins in extracts of *P. woesei* cells. Crude extract of *P. woesei* cells (50 μg) was electrophoresed on an SDS/12% polyacrylamide gel and then proteins were transferred eletrophoretically to a nitrocellulose membrane, and this membrane was probed with [α-$^{32}$P]GTP (lanes 1 and 2) or [α-$^{32}$P]CTP (lane 3). After incubation at 65°C (lanes 1 and 3) or 80°C (lane 2) and washing at the same temperature as the incubation, bound GTP was detected by autoradiography. The major *P. woesei* GTP-binding protein is indicated by an arrow. The electrophoretic positions of protein markers (M) are indicated to the right. (*B*) GTP-binding properties of *P. woesei* FtsZ expressed in *E. coli*. Extract of *P. woesei* cells (50 μg of total protein; lane 4), *E. coli* cells containing plasmids bearing the PwFtsZ gene (100 μg of total *E. coli* protein; lanes 1 and 3), or *E. coli* containing the parental plasmid vector (100 μg of total *E. coli* protein; lane 2) was treated as in *A* and probed with [α-$^{32}$P]GTP at 65°C. The GTP-binding polypeptide expressed in *E. coli* from the plasmid containing the PwFtsZ gene is indicated by an arrow.

7.7/40 mM KCl/0.1 mM EDTA/1 mM $MgCl_2$/0.025% Nonidet P-40/1% milk powder) containing 50 μCi of [α-$^{32}$P]GTP (3000 Ci/mmol). After incubation for 4 hr at room temperature, filters were washed in NBB, air-dried, and exposed to x-ray film. Following secondary screens, DNA inserts from purified positive bacteriophage were amplified by PCR using flanking λ primers and cloned into pBluescript (Stratagene).

**Nucleotide Binding Assays.** *Escherichia coli* strain XL-1 Blue, containing plasmids harboring the putative *P. woesei* FtsZ gene, was resuspended in R buffer (20 mM Tris·HCl, pH 7.5/100 mM KCl/0.5 mM EDTA/0.5 mM DTT/10% glycerol), lysed by sonication, and mixed with SDS protein sample buffer. This material, or *P. woesei* cell extract, was subjected to SDS/PAGE, and proteins were transferred electrophoretically to nitrocellulose filters. After blocking for 1 hr in TBST containing 5% milk powder, filters were transferred into NBB containing 50 μCi of [α-$^{32}$P]GTP or other ribonucleotide (3000 Ci/mmol). Filters were then incubated for 1 hr at 22°C, 65°C, or 80°C, washed in NBB (prewarmed to the appropriate temperature), dried, and exposed to x-ray film. *P. woesei* extracts were prepared as follows at 4°C. Cells (wet weight, 4 g) were resuspended in 16 ml of Buffer E (50 mM Tris·HCl, pH 8.0/250 mM KCl/15 mM $MgCl_2$/20 μM $ZnSO_4$/1 mM DTT/1 mM EDTA) and lysed by the addition of Triton X-100 to 0.03%. The protein extract was then subjected to centrifugation at 100,000 × *g* for 30 min, and the resulting supernatant was stored at −70°C.

**DNA Sequencing and Sequence Analyses.** Sequencing was by Sequenase version 2.0 (United States Biochemical). Data base searches were performed by using the FASTA program (25), and alignments were performed using the PILEUP and LINEUP programs of the GCG package (University of Wisconsin; ref. 26). Phylogenetic comparisons were conducted using CLUSTAL version 1.4 (27).

## RESULTS

To identify archaeal GTP-binding proteins, we electrophoresed *P. woesei* cell extract on an SDS/polyacrylamide gel, transferred the proteins onto a nitrocellulose membrane, and then probed this membrane with radiolabeled GTP (Fig. 1*A*). Notably, strong binding of GTP to a protein of ≈40 kDa was detected, along with weaker binding to one or two smaller polypeptides. Importantly, no labeling was observed when other nucleotides were employed (Fig. 1*A* and data not shown), indicating that binding is specific for GTP. Consistent with the hyperthermophilic nature of *P. woesei*, GTP binding was high when incubations were performed at 65°C or 80°C (Fig. 1*A*), whereas lower levels of binding were obtained at ambient temperatures (data not shown).

To clone the gene encoding the major *P. woesei* GTP-binding protein, we generated a bacteriophage λ expression library of *P. woesei* genomic DNA and screened this with [α-$^{32}$P]GTP. Five of 20,000 plaques hybridized strongly, and analysis showed that they contained overlapping regions of *P. woesei* DNA. Determining the sequence of one of these clones revealed the presence of a single long open reading frame (ORF), which is capable of encoding a protein of 366 aa, corresponding to a predicted molecular mass of 39.5 kDa. To verify that this gene encodes a *P. woesei* GTP-binding protein, the DNA fragment was subcloned into a plasmid vector and extracts of *E. coli* cells harboring this vector were tested for GTP-binding activity using the nitrocellulose blotting assay. As shown in Fig. 1*B*, a major GTP-binding protein of ≈40 kDa is observed (lanes 1 and 3). Several lines of evidence indicate that this protein corresponds to the product of the *P. woesei* ORF. First, labeling is not observed using extracts of *E. coli* cells that contain the parental plasmid vector (lane 2). Second, the size of the polypeptide is consistent with length of the ORF and the size of the major GTP-binding protein present in *P. woesei* cell extracts (lane 4). Third, the recombinant protein binds specifically to GTP, as no labeling is observed with CTP or ATP (data not shown). Finally, strong binding occurs at 65°C and 80°C, in accordance with the thermophilic nature of *P. woesei*.

Strikingly, sequence comparisons (Fig. 2) reveal that the product of the *P. woesei* ORF is highly related to the sequence of bacterial FtsZ proteins, the FtsZ protein of the *Arabidopsis thaliana* chloroplast (28), and the product of a recently identified ORF of the archaeon *H. volcanii* (see below). The *P. woesei* protein is also related, albeit to a lesser degree, to eukaryotic α, β, and γ tubulins (Fig. 2). Although *P. woesei* FtsZ (PwFtsZ) aligns with eubacterial FtsZs essentially

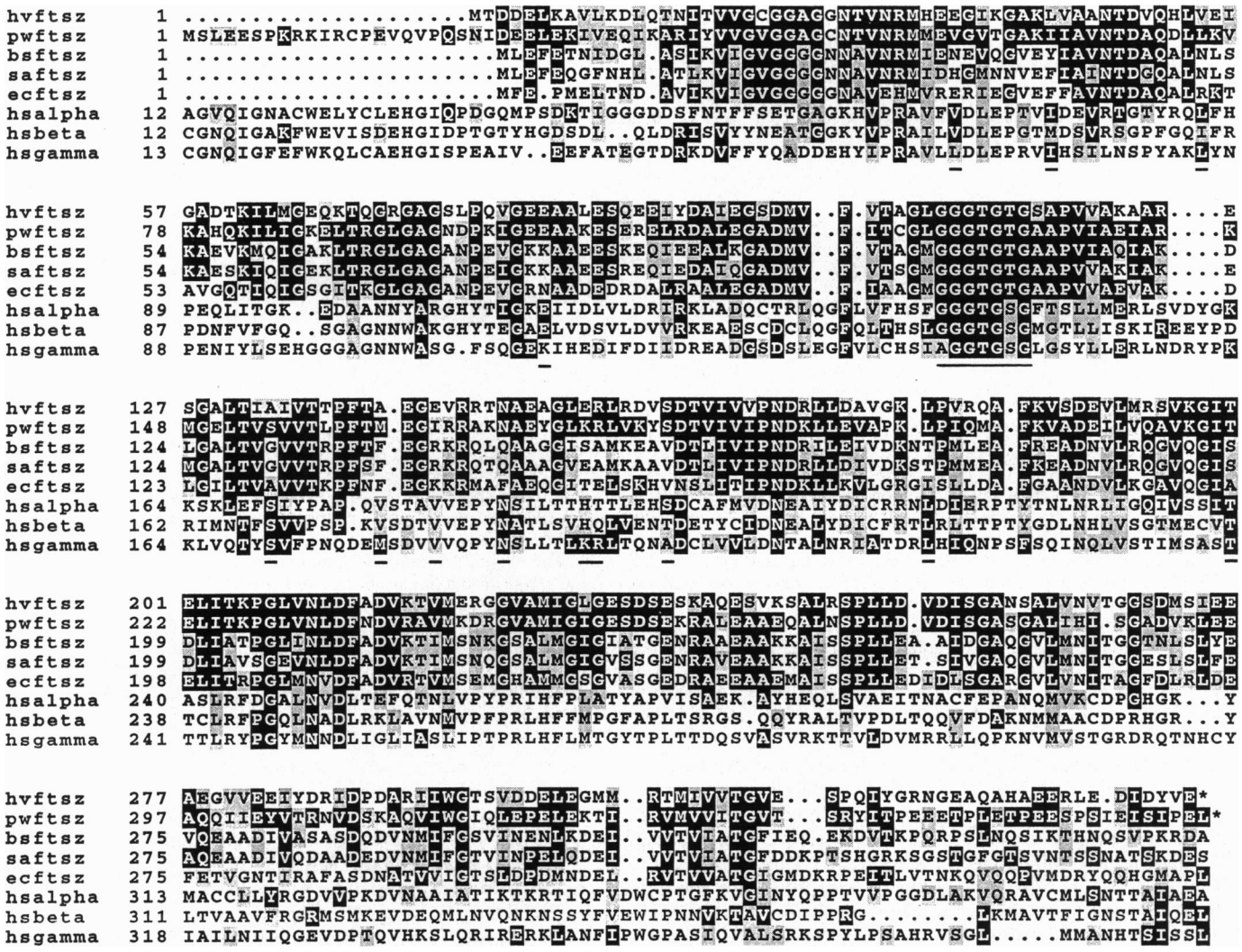

FIG. 2. Comparisons of PwFtsZ with FtsZ proteins of *H. volcanii* Bacteria, and eukaryotic tubulins. Residues that are identical to the PwFtsZ sequence are highlighted by reverse shading and residues that are similar are shaded in grey. The location of the tubulin "signature" motif is underlined. Also underlined are residues that are more conserved from PwFtsZ to tubulins than from PwFtsZ to eubacterial FtsZ proteins. hvftsz, *Haloferax volcanii* FtsZ; pwftsz, *P. woesei* FtsZ; bsftsz, *Bacillus subtilis* ftsZ; saftsz, *Staphylococcus aureus* FtsZ; ecftsz, *E. coli* FtsZ; hsalpha, *Homo sapiens* α-tubulin; hsbeta, *Homo sapiens* β-tubulin; and hsgamma, *Homo sapiens* γ-tubulin. The accession numbers for the sequences used are given in the legend to Fig. 3.

throughout its length, it lacks the C-terminal extension that is present in all other FtsZ proteins. This is in line with the fact that archaeal proteins are frequently shorter than their eubacterial and eukaryotic counterparts (for example see refs. 11, 12, and 14). Consistent with its nucleotide binding characteristics, PwFtsZ is particularly strongly related to tubulins and FtsZs within the region that contains the tubulin "signature" motif (underlined)—in tubulins and FtsZ proteins, this motif is thought to mediate interactions with GTP. Significantly, certain residues of PwFtsZ (Fig. 2; indicated by underlining) are more similar to the corresponding residues of tubulins than to those of eubacterial FtsZs. Indeed, because of these residues, the alignment shown in Fig. 2 is significantly different than that reported previously for tubulins and eubacterial FtsZs (16). Specifically, the inclusion of PwFtsZ strengthens the bridge between the tubulin and FtsZ groupings and allows the construction of an alignment that contains fewer gaps than previous alignments.

To assess the relationships between the various tubulins and FtsZ molecules more precisely, sequence alignments were used to construct a phylogenetic tree (Fig. 3). This analysis leads us to the following conclusions. First, PwFtsZ is most similar to the product of the *H. volcanii* ORF (HvFtsZ)—indeed, these two FtsZ-related proteins form a statistically significant branch of the dendrogram. Second, PwFtsZ and HvFtsZ are much more similar to bacterial FtsZs than to tubulins. Third, despite the above, the archaeal FtsZs fall outside the close grouping of eubacterial FtsZs and are marginally more similar than eubacterial FtsZs to eukaryotic tubulins. Fourth, the α, β, and γ families of tubulins are all approximately equidistant from the FtsZ grouping.

## DISCUSSION

We have shown that the major GTP-binding protein of the archaeon *P. woesei* is related strongly in sequence to FtsZ proteins of Bacteria. PwFtsZ can be aligned to eubacterial FtsZs essentially throughout its length; this suggests that it will function in an analogous way to its eubacterial counterparts. Thus, in addition to binding GTP, it is likely that PwFtsZ will function as a GTPase and will polymerize in the presence of this nucleotide. Also, in light of FtsZ's functions in eubacterial systems, our results suggest that PwFtsZ may play a key role in mediating and controlling cell division in *P. woesei*. In Bacteria, FtsZ is targeted by regulators of cell division and interfaces with other cell division components, such as the actin-related

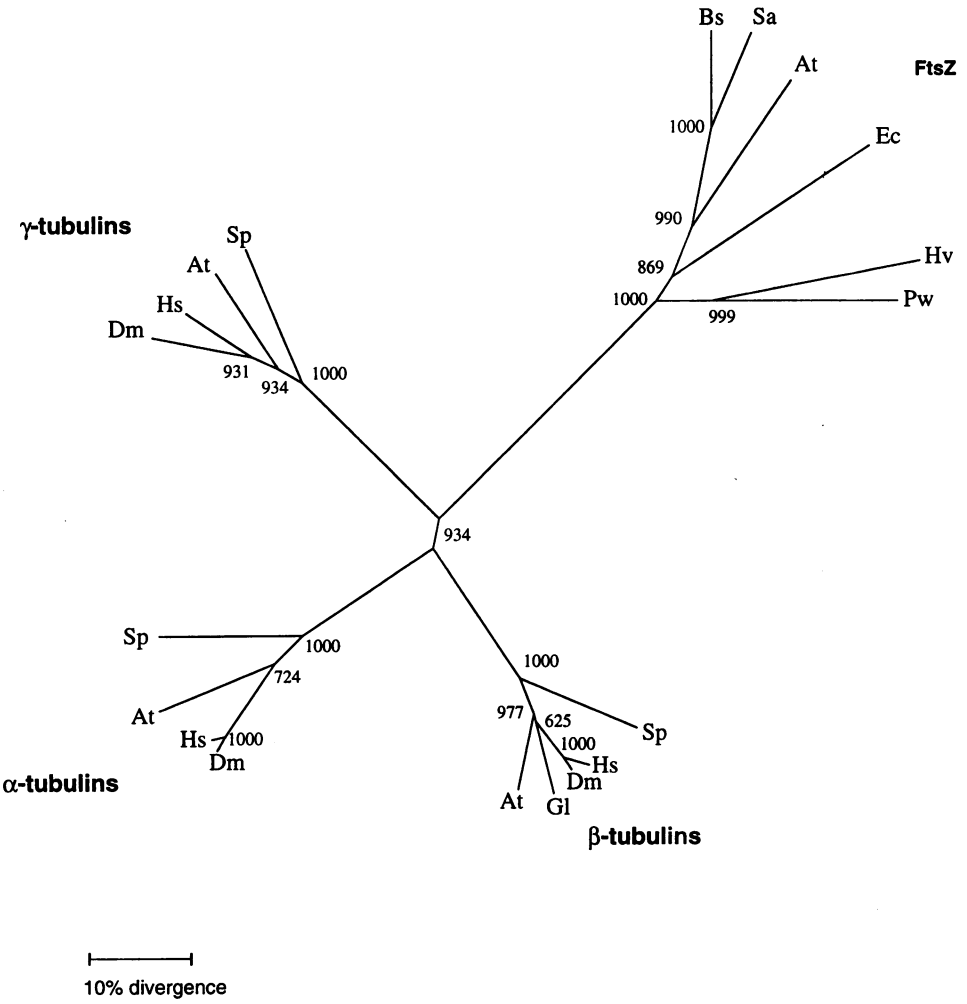


FIG. 3. Phylogenetic tree derived from an alignment of PwFtsZ with various eubacterial FtsZ proteins and eukaryotic tubulins. The number next to the node refers to the number of bootstrap trials (out of 1000) that support the branching pattern at that node. The scale bar indicates the length of a branch that corresponds to 10% divergence. Dm, *Drosophila melanogaster*; Hs, *Homo sapiens*; Sp, *Schizosaccharomyces pombe*; Gl, *Giardia lamblia*; At, *A. thaliana*; Ec, *E. coli*; Sa, *Staphylococcus aureus*; Bs, *B. subtilis*; Pw *P. woesei*; and Hv, *H. volcanii*. Accession numbers for the sequences are as follows: Ec FtsZ, P06138; Sa FtsZ, P45498; Bs FtsZ, P17865; HvFtsZ, U37584; PwFtsZ, U56247; Gl β-tubulin, X06748; At α-1-tubulin, M21414; At β-1-tubulin, M20405; At Columbia ecotype γ-1-tubulin, U02069; At chloroplast FtsZ, U39877; Sp α-tubulin 2, K02842; Sp β-tubulin, M10347; Sp γ-tubulin, M63447; Hs α-tubulin, X01703; Hs β-tubulin, J00314; Hs γ-tubulin, M61764; Dm α-tubulin, M14643; Dm β-1 tubulin, M20419; and Dm γ-tubulin, M76765.

protein FtsA (29–33). It will thus be interesting to ascertain whether homologues of these factors exist in Archaea and whether the cell division apparatus and its control mechanisms are fundamentally conserved between Archaea and Bacteria. If this were so, it would be in line with previous studies, which indicate that archaeal and eubacterial cells tend to resemble one another morphologically and which indicate similarities between the process of septation and cytokinesis in Archaea and eubacteria (for example see ref. 34).

Because certain residues of PwFtsZ are more similar to the equivalent residues of tubulins than are the corresponding residues of eubacterial FtsZs, we have been able to refine the FtsZ/tubulin alignments reported previously so that fewer gaps are employed. Our data, therefore, support the view that FtsZ and tubulins are evolutionarily related. Nevertheless, the alignments clearly reveal a much higher degree of relatedness between the archaeal and eubacterial factors than between the archaeal and eukaryotic proteins. This contrasts markedly with evolutionary trees based on the sequences of components of the transcriptional and translation systems, which place Archaea and Eucarya as sister groups. There are several explanations for this apparent paradox. One, unlikely in our view, is that FtsZ proteins exist in Eucarya but have not yet been detected, despite intensive biochemical and genetic analyses of eukaryotic systems. Another is that FtsZ was present in the last common ancestor of Archaea and Eucarya but that this protein was subsequently lost from the eukaryotic lineage or evolved rapidly into the tubulins. It is difficult, however, to explain how FtsZ, which interacts with many other proteins, could have broken free from the intense constraints on its sequence that are observed in eubacterial systems. A third possibility is that the archaeal FtsZs were acquired from Bacteria by horizontal gene transfer. Arguing against this, however, we have identified an FtsZ gene in the related but distinct archaeal species *Pyrococcus furiosus* and have detected a GTP-binding protein of a similar size to PwFtsZ in extracts of the archaeon *Sulfolobus shibatae*, which is evolutionarily highly diverged from *P. woesei*. Furthermore, of the sequences that exist in the databases, PwFtsZ is most related to the putative product of an ORF of the archaeon *H. volcanii* (see Fig. 2). Indeed, although diverged substantially from one another, phylogenetic analyses reveal that PwFtsZ and HvFtsZ form a statistically significant grouping. This is consistent with previous studies, which have suggested that *P. woesei* and *H. volcanii* reside on two divergent branches of a monophyletic archaeal kingdom. Taken together, these results suggest that FtsZ may

be universal in the evolutionarily diverse archaeal kingdom (35) and imply that FtsZ is likely to have been present in the last common ancestor of all Archaea.

If the above model is correct, a repercussion is that the evolutionary history of the eukaryotic cytoskeletal and cell division systems might be distinct from that of several other cellular components. Thus, whereas the eukaryotic transcription and translation machineries appear to be akin to those of Archaea, the available data suggest that the eukaryotic cytoskeletal and cell division systems have no equivalents in the eubacterial or archaeal cells and may, therefore, have been acquired from another, as yet uncharacterized, lineage. Our data tally with speculations made previously in light of the seemingly unique aspects of the eukaryotic cytoskeleton (for example see refs. 36 and 37). Biochemical, molecular, and genetic approaches directed toward identifying other components of the archaeal cell division apparatus, together with genome sequencing projects currently underway, will doubtless provide insights onto these important evolutionary issues in the near future.

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