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Isolation of an *ftsZ* Homolog from the Archaebacterium *Halobacterium salinarium*: Implications for the Evolution of FtsZ and Tubulin

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We have isolated a homolog of the cell division gene *ftsZ* from the extremely halophilic archaebacterium *Halobacterium salinarium*. The predicted protein of 39 kDa is divergent relative to eubacterial homologs, with 32% identity to *Escherichia coli* FtsZ. No other eubacterial cell division gene homologs were found adjacent to *H. salinarium ftsZ*. Expression of the *ftsZ* gene region in *H. salinarium* induced significant morphological changes leading to the loss of rod shape. Phylogenetic analysis demonstrated that the *H. salinarium* FtsZ protein is more related to tubulins than are the FtsZ proteins of eubacteria, supporting the hypothesis that FtsZ may have evolved into eukaryotic tubulin.

FtsZ is an essential cell division protein in *Escherichia coli* and *Bacillus subtilis* (3, 9) and appears to be conserved among eubacteria (8). It polymerizes to form a circumferential ring at the division site, constricting at the leading edge of the invaginating septum that will eventually separate the two daughter cells (6, 26). FtsZ both shares biochemical properties with and has structural similarities to eukaryotic tubulins. Both bind and hydrolyze GTP, polymerize to form tubules in a GTP-dependent manner, and are involved in cytoskeletal processes in the cell during cell division (7, 10, 31, 32, 37). All identified FtsZ proteins from eubacteria contain a conserved N terminus that carries the tubulin-like GTP-binding motif and a hydrophilic, variable C terminus.

Although the role of FtsZ in cytokinesis differs from the actin-mediated cytokinesis in eukaryotic cells, the biochemical and amino acid sequence data have led to the proposal that FtsZ is a prokaryotic version of tubulin (14). Since the origins of the eukaryotic cytoskeleton are unknown, an intriguing hypothesis is that FtsZ evolved into tubulin. Since it is believed that the archaea and eucarya diverged after the divergence of archaea from bacteria, then identification of an FtsZ protein in an archaebacterial species might shed light on this evolutionary question (18, 21). Because archaebacteria in general have a prokaryotic cellular organization similar to that of eubacteria, it is reasonable to propose that their cell division requires an FtsZ homolog derived from the common ancestor of all living cells (44).

To gain insight into FtsZ evolution and diversity, we have isolated and determined the nucleotide sequence of an *ftsZ* gene from the extremely halophilic archaebacterium *Halobacterium salinarium*, which grows as long, rod-shaped cells. We show that *H. salinarium ftsZ* is remarkably similar to the eubacterial versions, particularly in the region most similar to tubulins. An alignment of the *H. salinarium* FtsZ sequence with those of eubacterial FtsZ proteins and tubulins suggests that *H. salinarium* FtsZ is more related to tubulin than are the eubacterial FtsZs, supporting the idea that tubulin may have evolved from FtsZ. We also show that *H. salinarium ftsZ* is not flanked by genes normally found adjacent to several known eubacterial *ftsZ* genes. Finally, we show that expression of a segment of DNA containing *ftsZ* from a multicopy plasmid in *H. salinarium* causes significant changes in cell morphology.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. *E. coli* JM109 and *H. salinarium* Pho81Wr$^-$, a restriction-deficient Pho81 strain (40, 45), were used as hosts for all experiments. Plasmid pKJ305 (20), a shortened derivative of pVJY1 (45), is a shuttle vector with a multiple cloning site that can replicate in *E. coli* and in *H. salinarium* and can express both ampicillin resistance in *E. coli* and mevinolin (MEV) resistance in *H. salinarium* (24). *H. salinarium* was cultured as described previously (23) in CM supplemented with MEV at 4 μg/ml on agar plates, or 1 μg/ml in liquid, at 37°C. *E. coli* was cultured at 37°C in Luria-Bertani broth or agar (38) supplemented with ampicillin at 50 μg/ml.

Chemicals and enzymes. Most restriction enzymes, T4 DNA ligase, and *Taq* polymerase were purchased from Promega. Vent DNA polymerase and the remainder of restriction enzymes were purchased from New England Biolabs.

Molecular cloning techniques. Plasmid isolation, restriction digestion, ligation, transformation, Southern blotting, and library screening were performed essentially as described by Sambrook et al. (38). DNA fragments were isolated from agarose gels by the freeze-squeeze technique (42) or by direct isolation from low-melting-point agarose. Total genomic DNA from *H. salinarium* Pho81 was obtained from E. Spudich and K. Olson.

To make the *ftsZ* gene probe, a 0.6-kb *Nsi*I-*Mlu*I fragment was isolated from pJC06 (28) and labeled by random priming with a Prime-a Gene kit (Promega) and [α-^{32}P]dATP (Amersham). The probe was then hybridized to a blot containing *H. salinarium* genomic DNA digested with *Pst*I, *Bam*HI, *Not*I, and *Xho*I. The *Bam*HI-digested DNA was chosen because the hybridizing fragment was smaller than those for the other restriction endonucleases and probably easier to clone into a plasmid. A subgenomic plasmid library was constructed by gel isolation of the 4-kb region of *Bam*HI-digested *H. salinarium* genomic DNA and ligation to *Bam*HI-digested pBluescript SK+ (Stratagene). Positive colonies were isolated and cultured, and their plasmids were digested with *Bam*HI to confirm the presence of a 4-kb insert. The location of the *ftsZ* homology within the 4-kb insert was then determined by reprobing a Southern blot of plasmid DNA from the clones. One clone, pMK24, was chosen to continue the subcloning.

To clone *ftsZ* into the shuttle vector pKJ305, a 2.5-kb *Xba*I-*Kpn*I fragment from pRW11 (see below) containing the intact *H. salinarium ftsZ* gene and its putative native promoter was isolated. This fragment contained the 2.4-kb *Hin*dIII-*Pvu*II fragment insert in pRW11 plus additional pBluescript SK+ polylinker sites between *Hin*cII and *Kpn*I and *Hin*dIII and *Xba*I. The fragment was ligated to pKJ305 digested with *Xba*I and *Kpn*I, which are unique sites in this plasmid. The ligation was transformed into JM109, and correct clones were confirmed. Plasmid DNA from one of these transformants (pRW12) was transformed into *H. salinarium* Pho81Wr$^-$ as described previously (23). Cells were plated on CM

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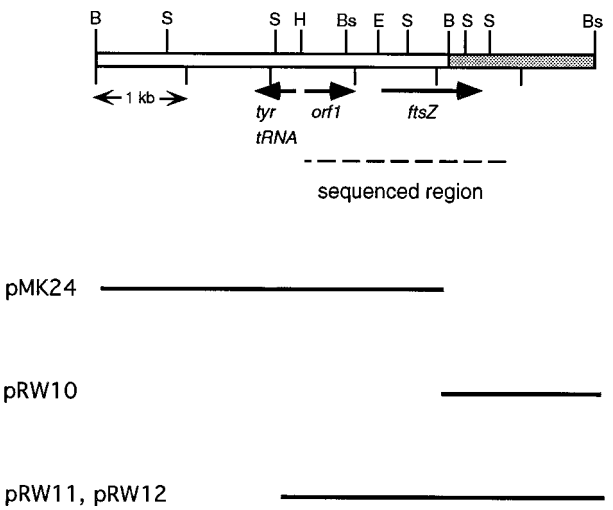


FIG. 1. Map of cloned *H. salinarium* DNA containing *ftsZ*. DNA cloned from the genomic 4-kb *Bam*HI fragment (open bar) and DNA cloned by inverse PCR (stippled bar) are indicated. Restriction site positions are indicated as follows: B, *Bam*HI; S, *Sal*I; H, *Hin*dIII; Bs, *Bsi*WI; and E, *Eco*RV. ORFs and their direction (large arrows), 1-kb intervals (hatch marks below the boxes), the region of DNA sequenced on both strands (dashed line), and the insert DNA present in the plasmids listed (lines) are also shown.

plus MEV and incubated at 37°C for 5 to 10 days. Isolates were cultured in CM plus MEV; plasmids were prepared by alkaline lysis (38) and transformed into JM109 for amplification. Plasmid pRW13 carries a deletion or rearrangement in the pRW12 insert and was isolated from *H. salinarium* cells that grew faster than those carrying pRW12.

Inverse PCR and cloning of the intact gene. Since the entire *ftsZ* gene was not present on the clone isolated from the plasmid library, inverse PCR (33) was used to amplify DNA flanking the cloned region to ultimately obtain the entire gene. The template for inverse PCR was *H. salinarium* genomic DNA cleaved with *Eco*RV, which digests just upstream of *ftsZ* and approximately 2 kb downstream. This digested DNA was self-ligated into circles, so that upon annealing, normally divergent primers become convergent. The two primers were HAL2 (5′GAGAACGCCTTCCCGATCG3′) and HAL3 (5′CTGTTGGACGTCGAT TACC3′). PCR mixtures of 20 μl contained 100 pmol of primers, 1× Thermo buffer for *Taq* polymerase supplemented with 1.5 mM $MgSO_4$, 0.1 to 1 μg of template DNA, 0.5 mM deoxynucleoside triphosphates, 2.5 U of *Taq* polymerase, and 0.2 U of Vent polymerase. PCR was performed on a Perkin-Elmer 2400 Thermal cycler with an initial hot start of 94°C for 5 min followed by 25 cycles at 94°C (30 s), 60°C (1 min), and 72°C (2 min) and a final polymerization step of 72°C for 7 min. The inverse PCR product was isolated from an agarose gel and digested with *Bam*HI and *Bsi*WI, which cleaves about 2 kb to the right of *Bam*HI. The digested DNA was cloned into pBluescript SK+ cleaved with *Bam*HI and *Acc*65I to create pRW10. The entire *H. salinarium ftsZ* gene was then reconstructed and placed under the control of the *lac* promoter of pBluescript SK+ by joining the 1.85-kb *Hin*dIII-*Bam*HI fragment of pMK24 to the 0.5-kb *Bam*HI-*Pvu*II fragment of pRW10 and ligating it to *Hin*dIII-*Hin*cII-cut pBluescript SK+ to make pRW11.

PCR was used to verify that the reconstructed gene was the same as the genomic gene. First, a PCR primer corresponding to the downstream portion of *ftsZ* obtained by inverse PCR was synthesized. Then this primer and HAL3 (sense orientation, upstream portion) were used to successfully amplify a strong PCR product of the expected size from *H. salinarium* genomic DNA.

DNA sequencing and analysis. Initial templates for DNA sequencing included pMK24 and pRW10 sequenced from the insert ends with T7 or T3 polymerase primers or with custom oligonucleotide primers purchased from Bioserve, Inc. (Laurel, Md.). Subsequent sequencing templates included partial deletions of the initial templates. All double-stranded DNA templates were prepared with a Prep-a Gene kit (Bio-Rad) and sequenced on an Applied Biosystems automated DNA sequencer (Microbiology and Molecular Genetics Core Facility, University of Texas, Houston). Most DNA sequence data were obtained from both strands. The DNA and predicted protein sequences were analyzed with University of Wisconsin Genetics Computer Group (GCG) software (11). Database searches for similarity with other proteins were performed with BLASTP and FASTA. The FtsZ protein alignment was assembled for publication with the SeqVu 1.0.1 program (Garvan Institute).

Phylogenetic analysis. Amino acid sequences of 10 FtsZ proteins and 9 tubulins were aligned manually with Lineup (GCG) after the alignment of Mukherjee and Lutkenhaus (32) was first optimized by adding more amino acid residues at the C terminus. The alignment was further optimized with the Pileup program (GCG). To verify tree topologies, the orders of the sequence sets were jumbled. For neighbor-joining analysis, a Jukes-Cantor distance matrix was made of the alignment by using Distances (GCG), and a tree was generated with Growtree (GCG). The PAUP 3.1 program (41) was used to make minimum-length trees. The same alignment made in Lineup was imported into PAUP 3.1 as a Nexus file. Heuristic searches using branch-swapping techniques were done with various taxa as out-groups. In addition, subsets of the alignment were also analyzed by heuristic searches, and the branch-and-bound method was used to more rigorously calculate the optimum tree from the complete alignment. Bootstrap analysis was performed on heuristic searches with 100 replicates.

Nucleotide sequence accession number. The DNA sequence data from this study were submitted to GenBank and have been assigned accession no. U32860.

RESULTS AND DISCUSSION

Cloning of the *H. salinarium ftsZ* gene. Since the G+C content of *H. salinarium* DNA is relatively high, we probed *H. salinarium* genomic DNA with an *ftsZ* gene from another high-G+C-content bacterium, *Rhizobium meliloti*. To increase specificity, we used a restriction fragment containing a highly conserved N-terminal portion of the *R. meliloti ftsZ1* gene (28). Probing of a genomic Southern blot of various *H. salinarium* DNA restriction digestions revealed a single band in all lanes, including a 4-kb *Bam*HI fragment (data not shown). The single band suggested that only one copy of this locus exists in *H. salinarium*. The 4-kb *Bam*HI fragment was cloned (see Materials and Methods), and after preliminary DNA sequencing it was found that the C terminus of the gene was not present on this clone. Since several attempts to clone overlapping fragments that would contain the rest of the gene were unsuccessful, we used inverse PCR to clone the downstream flanking DNA and subsequently reconstructed the intact cloned gene (see Materials and Methods). A diagram of this clone, representing the insert in plasmid pMK24, is shown in Fig. 1.

Nucleotide sequence of *H. salinarium ftsZ*. The DNA sequence of the 2.5-kb region between the *Hin*dIII site and past the *Bam*HI site revealed two major open reading frames (ORFs) (Fig. 2). The upstream ORF, ORF1, encodes a predicted protein of 259 residues and above-average bias for commonly used *H. salinarium* codons. It exhibited no homology with other eubacterial cell division genes, such as *ftsQ* and *ftsA*, which are conserved in their location upstream of *ftsZ* in many bacterial species (1, 16). One other known exception to this conserved arrangement is the *Anabaena* species *ftsZ* gene, which contains an ORF of unknown function upstream (13). A search of the database revealed no sequence similarity between the *Anabaena* sp. ORF and ORF1 from our sequence.

The second ORF encodes a predicted protein of 375 amino acids that is similar to eubacterial FtsZ proteins (Fig. 2). The ORF is probably expressed in *H. salinarium*, since it contains a high percentage of typical *H. salinarium* codons, and has only six rare codons as analyzed by the GCG Codonpreference program with a rare-codon threshold of 0.1. Another indication that this ORF is expressed in *H. salinarium* is the presence of a consensus halobacterial promoter box A sequence (15, 36) which would place the transcript initiation site just several base pairs upstream of the putative ribosome binding site for the ORF (Fig. 2).

Limited DNA sequence analysis upstream and downstream of the region shown in Fig. 2 demonstrated no homology to any protein in the database. However, a 77-nucleotide region with 83% identity to the *Haloferax volcanii* tyrosine tRNA was found just to the left of the *Hin*dIII site, oriented away from ORF1 and *ftsZ* (data not shown). This finding is further evidence for the lack of conservation between the upstream DNA of *H. salinarium ftsZ* and those of eubacteria, since tRNA genes are not located near eubacterial *ftsZ* genes. Whether the

FIG. 2. Nucleotide and predicted amino acid sequences of the cloned region. Shown is 2.4 kb of DNA sequence, with single-letter amino acid residues of ORF1 and *ftsZ*. Stop codons (asterisks), the consensus halobacterial promoter region (Box A), and the predicted startpoint of the transcript (double dashed arrow below the sequence) are indicated.

genetic organizations in the related *H. salinarium* and *H. volcanii* are similar remains to be confirmed by further DNA sequence comparison in this region.

Comparison of the *H. salinarium* FtsZ protein sequence with eubacterial FtsZ protein sequences. The deduced amino acid sequence of *H. salinarium* FtsZ revealed a 39.3-kDa protein with obvious similarity to eubacterial FtsZ proteins but with a high degree of divergence expected from the evolutionary distance. The *H. salinarium* FtsZ protein is 32 and 38% identical to the *E. coli* and *B. subtilis* FtsZ proteins, respectively. This compares with 47% identity between the *E. coli* and *B. subtilis* FtsZ proteins. An alignment of a number of eubacterial FtsZ protein sequences with the *H. salinarium* sequence exhibits conservation of overall length, with by far the greatest degree of sequence conservation in and around the tubulin signature sequence implicated in GTP binding. Hydropathy analysis, estimated isoelectric points, and secondary structure predictions (data not shown) suggest that the *H. salinarium* protein is similar to the eubacterial homologs. This similarity includes the divergent C termini found in FtsZ proteins, which despite the lack of primary sequence conservation share highly hydrophilic residues that are likely to be at the exposed surface of the protein. To date, several FtsZ proteins, including those from *Haemophilus influenzae*, *R. meliloti* (FtsZ2), *Arabidopsis thaliana* chloroplasts, and *H. salinarium*, appear to lack a small, otherwise-conserved domain at the extreme C terminus, D I/V P X F/Y L (Fig. 3).

It is notable that *H. salinarium* FtsZ contains no more acidic residues than, for example, the FtsZ protein of *Streptomyces griseus*, which has a similarly high G+C coding bias. This is surprising, considering that in halophilic proteins acidic residues are often substituted for basic ones found in homologs of nonhalophiles. However, FtsZ proteins in general are acidic, and their function even in a high-salt environment may be constrained by factors other than charge.

Morphological changes induced by *H. salinarium ftsZ* expression. Expression of *H. salinarium ftsZ* from pMK24 in *E. coli* cells (strain JM109) did not lead to a cell division defect

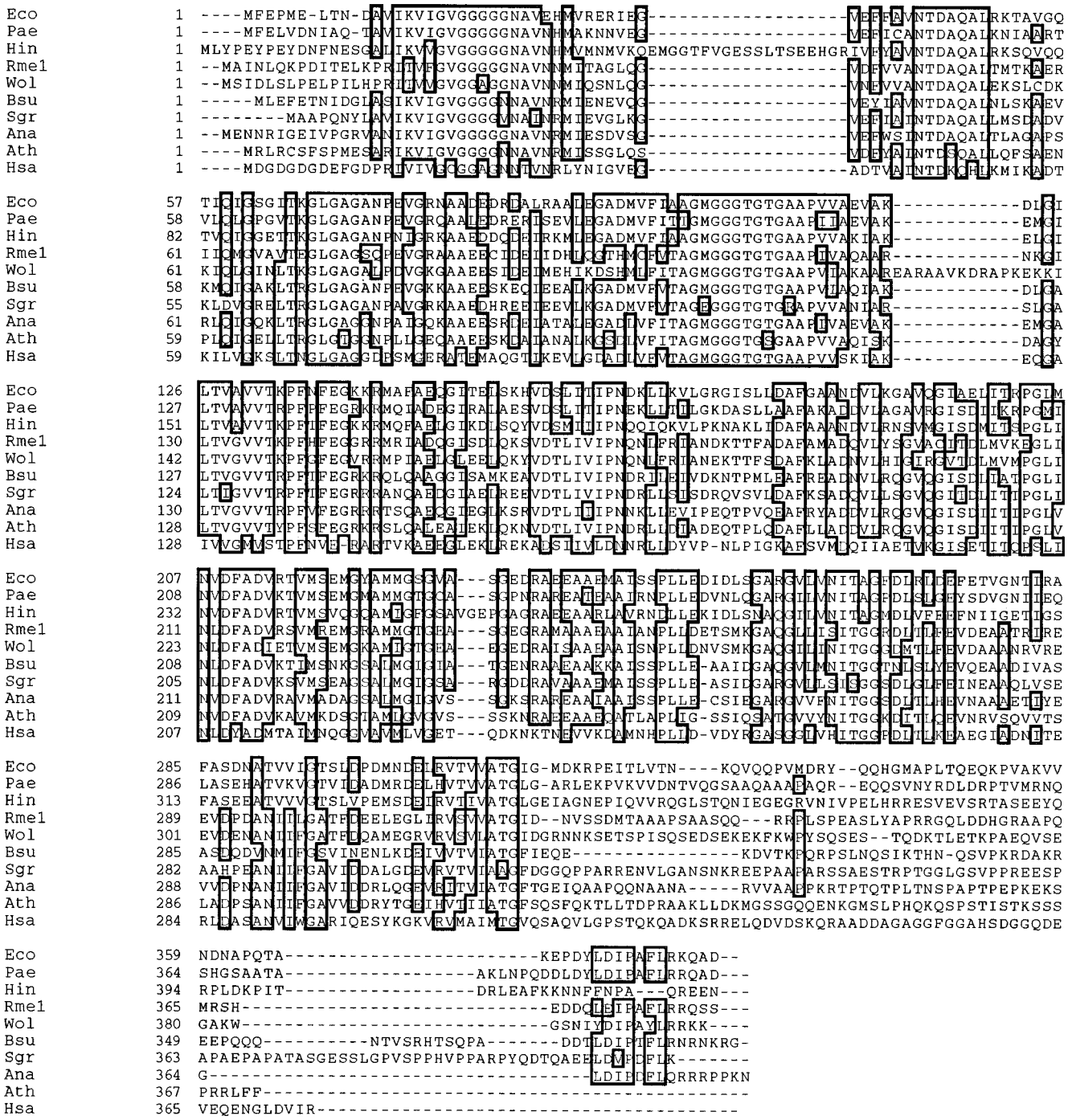


FIG. 3. Alignment of *H. salinarium* FtsZ with other selected FtsZ proteins. Single-letter amino acid codes are shown. Positions with identical residues for at least 6 of the 10 sequences (boxes) and gaps (dashes) are indicated. The FtsZ sequences are as follows: Eco, *E. coli* (5, 46); Pae, *Pseudomonas aeruginosa*, GenBank accession no. U19797; Hin, *H. influenzae*, GenBank accession no. L45779; Rme1, FtsZ1 of *R. meliloti* (28) from which the central nonconserved domain has been removed (residues 320 to 526); Wol, a *Wolbachia* species (17); Bsu, *B. subtilis* (2); Sgr, *S. griseus* (12); Ana, an *Anabaena* sp., residues 50 to 428 (13); Ath, chloroplast FtsZ from *A. thaliana*, residues 62 to 433 (35); and Hsa, *H. salinarium* FtsZ. The tubulin signature sequence is GGGTGTG (residues 105 to 111 of *E. coli* FtsZ).

that could be observed microscopically, probably because FtsZ protein requires the extremely high salt concentrations found inside *H. salinarium* cells in order to be active. To obtain a measure of its biological activity, the gene was expressed in *H. salinarium* from a shuttle plasmid, pRW12, able to stably replicate in *H. salinarium* under selection for MEV resistance. A few large colonies and many small colonies which continued to grow at wild-type rates or at about half the wild-type rates, respectively, were obtained (data not shown). Plasmids from these strains were transformed back into *E. coli* and analyzed. Plasmids from the small-colony strains had the same restriction digestion pattern as the wild type, while those from the large-

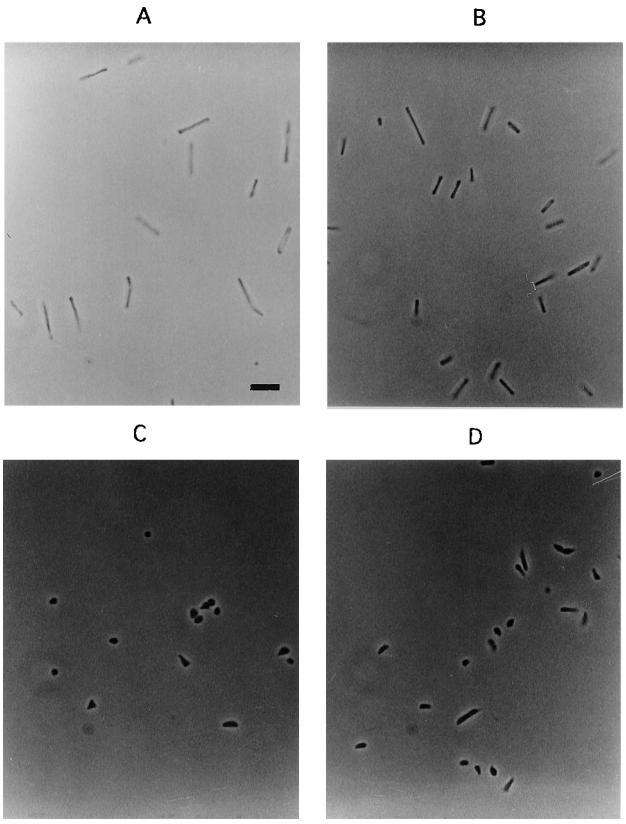


FIG. 4. Morphology of *H. salinarium* Pho81Wr$^-$ cells with and without the *ftsZ* expression plasmid pRW12. Phase-contrast micrographs were taken of cells in mid- to late-logarithmic-phase liquid cultures containing no plasmid (without MEV) (A) or pRW13, containing a rearranged *ftsZ* gene (B), and two different small-colony isolates containing the *ftsZ* plasmid pRW12 (C and D). Bar = 1.5 μm.

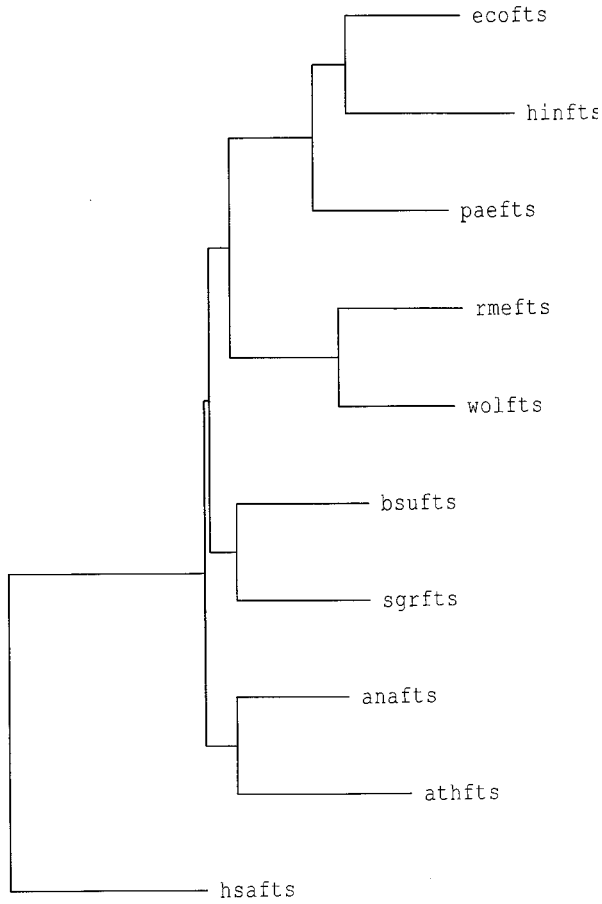


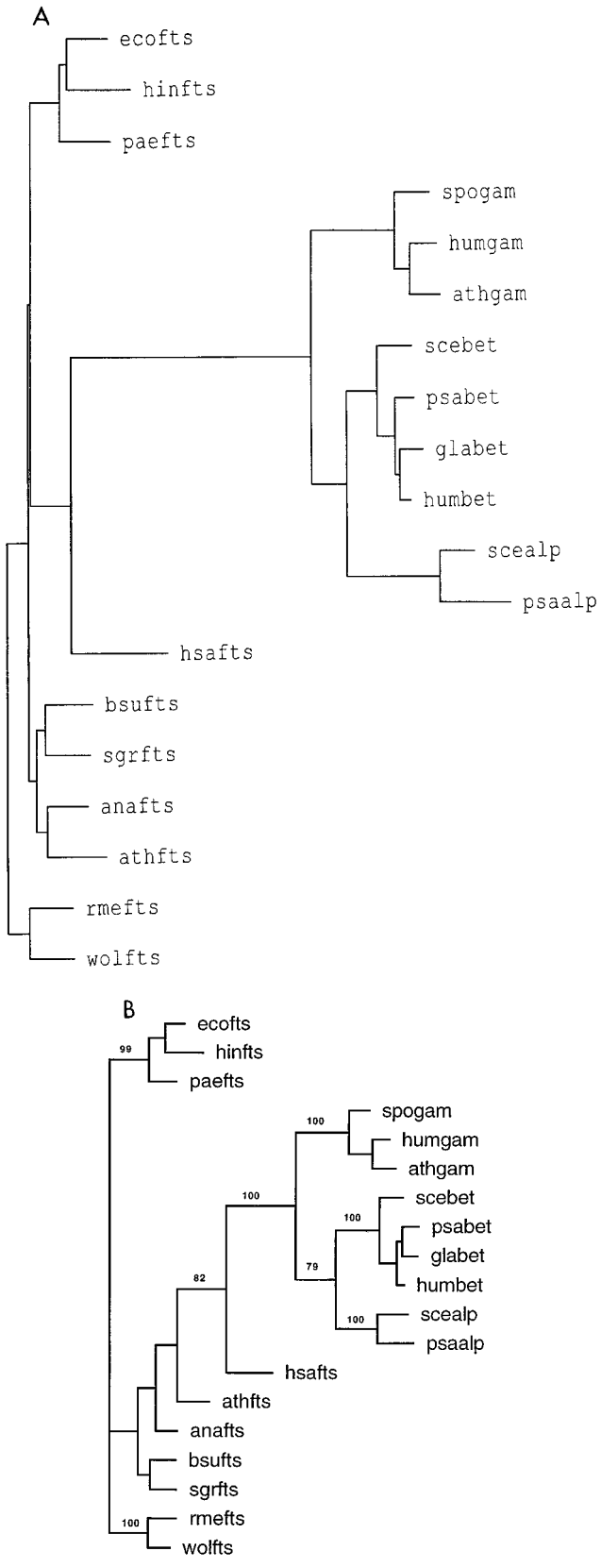
FIG. 5. Phylogenetic tree derived from a set of FtsZ proteins. A Jukes-Cantor distance matrix was used to calculate a phylogenetic tree based on the neighbor-joining algorithm (see Materials and Methods). Abbreviations for FtsZ proteins are the same as in the legend to Fig. 3, except for the suffix "fts." Rmefts is Rme1.

colony strain were rearranged in their insert portion so that several diagnostic *Sal*I fragments internal to *ftsZ* were missing (data not shown). This strongly suggests that the large-colony strain carries a plasmid, designated pRW13, with an inactivated *ftsZ* gene. The plating efficiency on CM plus MEV of the small-colony strains was at least 100-fold lower than that of the large-colony strain, further indicating that the expression from pRW12 was deleterious.

Cells from the large colonies were morphologically wild type (Fig. 4A and B), consistent with the idea that the *ftsZ* gene on the plasmid had been inactivated to allow faster growth. However, cells from several more slowly growing transformants containing the unchanged input plasmid were pleomorphic. The cells were often spherical and sometimes triangular or trapezoidal, and there were also some rod-shaped cells (Fig. 4C and D). The triangular and spherical forms were reminiscent of the cell shapes of this species induced by lowering the NaCl concentration of the medium (30). In addition, the treatment of *H. salinarium* cells with bacitracin, which blocks glycosylation of the surface layer (S-layer) glycoprotein, causes similar changes from rod to spherical shape (29). It is likely that the expression of *ftsZ* from its putative promoter on this plasmid was directly causing this phenotype. However, we cannot at present rule out the possibility that the morphological changes were also due to the expression of ORF1, which is also present on pRW12 and could be expressed from a vector promoter.

One possible explanation for the FtsZ-dependent morphological alterations is that the small, spherical cells were minicells, which in *E. coli* are formed by the enhanced activity of FtsZ at cell poles in addition to midcell sites. However, in *E. coli*, minicells are usually about 10-fold shorter than normal cells and quite uniform in size, while the *H. salinarium ftsZ*-overexpressing small cells are on average 20 to 40% as long as the nonoverexpressing cells and nonuniform in size. Also, classic minicells are spherical and do not contain apices. Finally, minicell strains also contain cells of relatively normal length from which minicells emerge. In the case of *H. salinarium ftsZ* overexpression, many cultures contain only misshapen cells, and some of these cells can be observed to be dividing at the cell midpoint (Fig. 4D). Therefore, it is more likely that the altered cell morphology is due to a widespread and deleterious change in the cell surface glycoprotein or cytoskeleton and not merely an effect of increased cell division causing minicells.

To confirm that the pRW12 plasmid containing *ftsZ* was required for the observed phenotype, the overexpressing strains were grown in the absence of MEV in order to obtain plasmid-free segregants. These cell populations quickly regained the wild-type rod shape and normal plating efficiencies (data not shown), suggesting that the plasmid was easily lost without selection and that the unusual morphological changes were due to increased expression of *ftsZ*.



Phylogenetic relationship between FtsZ proteins and tubulins. We first performed phylogenetic analysis on a set of 10 FtsZ proteins, including eight eubacterial FtsZ proteins, a recently described FtsZ homolog from *A. thaliana* chloroplasts (35), and *H. salinarium* FtsZ. The neighbor-joining tree shown in Fig. 5 is consistent with known phylogeny of the prokaryotic species tested based on genes coding for rRNA (34) and with the suspected relatedness of the *Anabaena* sp. and chloroplast proteins. This analysis suggests that, as might be predicted from known taxonomic relationships, *H. salinarium* FtsZ is the most divergent. In addition, it shares the highest degree of similarity with the *Anabaena* sp. and chloroplast proteins.

Phylogenetic analysis was then applied to two α-, four β-, and three γ-tubulins in addition to the set of FtsZ proteins. The highly divergent C termini of FtsZ proteins and tubulins were omitted from this analysis, as were the extreme N-terminal residues, leaving a total of 380 characters in the alignment (including gaps), which spanned sequences corresponding to residues 14 and 316 of *E. coli* FtsZ (KVIG--TGIG) and residues 4 and 354 of human β-tubulin (IVHL--TAVC). Two unrooted phylogenetic trees were generated by different algorithms, one using neighbor-joining consensus (Fig. 6A) and the other using protein parsimony (Fig. 6B). The parsimony tree was verified by multiple bootstrapping, with confidence levels shown. A tree with topology identical to that in Fig. 6B was produced by a rigorous branch-and-bound search (data not shown). Although the distances between the FtsZ and tubulin homologs are large and the tree algorithms are different, the topologies of the two trees are remarkably similar and generally agree with known phylogenetic relationships based on analysis of genes coding for rRNA.

What was particularly notable was that *H. salinarium* FtsZ was consistently placed closer to tubulins relative to the eubacterial FtsZ proteins. This placement was also tested with different subsequences from the alignment. All sequence groups showed similar topology provided that they contained residues C terminal to position 110 of *E. coli* FtsZ, which include the tubulin-like GTP-binding motif present in all FtsZ proteins. When only the N-terminal 110 amino acids of the alignment were used to generate phylogenetic trees, *S. griseus* FtsZ was most closely related to the tubulins, and the β-tubulins were the most closely related to the FtsZ proteins. The different results obtained with the N terminus suggest that this region of the protein may have evolved in a different manner, perhaps by recombination or horizontal transfer.

This analysis suggests several interesting possibilities. One is that the archaebacterial FtsZ protein represents an evolutionary midpoint between eubacterial FtsZ and eukaryotic tubulin. Another is that when the entire alignment was used, γ-tubulin appeared to be more related to the prokaryotic FtsZ proteins than was β- or α-tubulin. The function of γ-tubulin is to nucleate microtubule formation (19). Further progress in under-

FIG. 6. Phylogenetic trees derived from an alignment of FtsZ and tubulin sequences. Proteins were aligned as described in Materials and Methods. (A) Tree calculated as for Fig. 5. (B) One of two trees resulting from a heuristic search using maximum parsimony in PAUP. For this particular tree, *E. coli*, *H. influenzae*, and *P. aeruginosa* FtsZ proteins were made into a monophyletic in-group. Numbers on selected branches refer to the percentage of bootstrap trees yielding the topology shown. Abbreviations for FtsZ are the same as for Fig. 5. Abbreviations and Swissprot accession numbers for tubulins are as follows: Spogam, *Schizosaccharomyces pombe* γ-tubulin, P25295; humgam, human γ-tubulin, P23258; athgam, *A. thaliana* γ-tubulin, P38557; scebet, *Saccharomyces cerevisiae* β-tubulin, P02557; psabet, *Pisum sativum* (garden pea) β-tubulin, P29500; glabet, *Giardia lamblia* β-tubulin, P05304; humbet, human β-tubulin, P05217; and scealp, *S. cerevisiae* α-tubulin, P09733. psaalp, *P. sativum* α-tubulin, GenBank accession no. U12589.

standing the functions of FtsZ and γ-tubulins may shed light on this potential evolutionary link.

Physiological and evolutionary implications. Haloarchaea, unlike other bacteria, do not appear to maintain their sacculus with turgor pressure (43), presumably because of the extreme ionic strength of both their intracellular and extracellular environments. Therefore, these cells do not have obvious turgor-based shape constraints (22) and instead use their S-layer glycoprotein array (25) (for a review, see reference 4) and possibly a cytoskeleton to define cell shape. Since overproducing FtsZ in *H. salinarium* mimics the loss of rod shape observed with lowered external salt concentration, it is reasonable to propose that altered FtsZ levels, and hence altered interactions between the FtsZ division polymer and the cell wall, could perturb the shape-determining features of the S-layer without causing lysis. This idea also is consistent with the presence of a tubulin-like FtsZ cytoskeleton in these cells, the structure of which is changed because of altered FtsZ levels. Alteration of FtsZ levels also has been shown to have global effects on the morphology of certain eubacteria, such as *R. meliloti* (27). Understanding the mechanism by which cell division is coupled to cell shape in *H. salinarium*, and perhaps other archaebacteria, awaits further study.

A major question that faces evolutionary biologists concerns how and when the eukaryotic cytoskeleton was first constructed. The phylogenetic analysis presented here suggests that archaebacteria may contain the missing link between FtsZ and tubulin, both of which form GTP-dependent polymers that act structurally to control the cell cycle. Since haloarchaea are adapted to extremely high intracellular salt concentrations, another species of archaebacteria, such as the presumed eocyte progenitor of eukaryotic cells, might have contained an FtsZ protein much more similar to tubulin. Also, a tubulin-like homolog may exist in archaebacteria, since *H. salinarium* has been reported to be sensitive to certain antitubulin drugs (39). There may be a protein in *H. salinarium* and other archaebacteria that is more related to tubulin whose gene failed to hybridize to *ftsZ* DNA in our experiments. It will be necessary to isolate additional homologs of archaebacterial FtsZ proteins and to further understand prokaryotic cell division in order to gain more insight into these fundamental evolutionary and mechanistic questions.

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