

Sequence and structural analysis of FtsZ homologs and comparison of bacterial FTsZ with eukaryotic tubulins

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Abstract

FtsZ, a bacterial cell division protein is a homolog of eukaryotic tubulins. It was postulated that FtsZ of different bacterial species might have specific signature sequences, based on which various FtsZ protein sequences were classified, mined and analyzed. Multiple sequence alignment of the sequences resulted in the highly specific signatures at the C-terminal end of gram-negative bacterial FtsZ. Interestingly, a few organisms belonging to the phylum Bacteroidetes were found to contain 600-650 amino acid-FtsZ sequences having an extra long spacer residues of about 300-350 amino acids. Sequentially, the spacer showed the presence of coils throughout using secondary structure prediction methods. Structurally over a span of 133 residues, a match was found in T7 DNA ligase, suggesting the presence of viral origin of the extra residues. Eukaryotic α -tubulins were also mined and aligned giving rise to high percentage identity even among highly diverged species such as the zebra fish and humans. This suggests the reason behind the high divergence seen between the eukaryotes and the prokaryotes sequentially, though structurally they share 98% similarity. The main focus of the present work was to understand evolution of the bacterial and eukaryotic cytoskeleton proteins.

Keywords: FTsZ, signature sequence, Bacteroidetes, T7 DNA ligase, tubulin, phylogenetic analysis.

Introduction

FtsZ, a major cytoskeleton protein present in bacteria, is a polymer forming GTPase, which drives bacterial cell division. Self-assembled FtsZ forms a highly dynamic ring structure termed the 'Z ring' at the midpoint of the bacterial cell (Errington *et al.*, 2003). The FtsZ gene was first identified as being involved in division of *Escherichia coli* cells (Lutkenhaus *et al.*, 1980). FtsZ contains 4 main domains, as determined by the crystal structure of FtsZ from Archaea *Methanococcus jannaschii* (Lowe & Amos, 1998). They comprise of variable N-terminus, the highly conserved core region, a variable spacer region and the C-terminus region. Secondary structure alignments between alpha/beta tubulin and *M. jannaschii* FtsZ1 (Nogales *et al.*, 1998) reveal an N-terminal extension proximal to the core domain in FtsZ. The function ascribed to the N-terminus is targeting of the protein organelles in eukaryotic organisms. The core region is a stretch of approximately 300 amino acids which contains all of the residues required for GTP binding and hydrolysis. This particular region is highly conserved in all the tubulins and in FtsZ. The spacer region is highly variable in length of about 2-300 amino acids. The C-terminal is not required for assembly, but is essential for the interactions with other membrane proteins FtsA, ZipA, SepF and EzrA (Singh *et al.*, 2007, 2008). Some of these proteins help in the polymerization and stability the Z rings.

FtsZ is a highly conserved protein found in bacteria except for the family Chlamydia and Verrucomicrobia. FtsZ genes are also found in euryarchael branch of Archaea. Although most of the bacteria possess FtsZ, there is a bacterium of genus *Prostheco bacter* belonging

to the phylum Verrucomicrobia, which possess tubulin homologues, BtubA and BtubB (Schlieper *et al.*, 2005). FtsZ homologues are found in a number of eukaryotes. Tubulin, a functional homologue of FtsZ in eukaryotes forms microtubules and enhances cell division (Erickson *et al.*, 1996). FtsZ and the monomers of alpha and beta tubulin show remarkable similarity in their tertiary structures, despite limited primary sequence identity. FtsZ also shows functional similarity to tubulins. FtsZ binds and hydrolyses GTP. GTP binding induces FtsZ self-assembly into protofilaments that consist of a head to tail linear polymers of FtsZ. These protofilaments resemble structures formed by tubulin. Tubulin assembles into microtubules comprising 13 protofilaments arranged around a hollow core. FtsZ instead associate laterally to form bundles and sheets.

Tubulins are among the most slowly diverging proteins in eukaryotes yet they are highly diverged from their bacterial homolog FtsZ. Bacterial FtsZ's are 40-50% identical in sequence even across very divergent species. Archaeal FtsZ's show a similar levels of identity to one another and to the bacterial FtsZ's (Vaughan *et al.*, 2004). In contrast, alpha tubulins show 75-85% sequence identity across most plants, animals and fungi. The bacterial FtsZ and eukaryotic tubulin diverged so much with evolution (sequentially) and yet retain the function and the structure. The present study helps to find the root where divergence of FtsZ and tubulin in the phylogenetic tree could have taken place. Almost all of the drugs used to treat bacterial infections target one of the four processes: Protein synthesis, nucleic acid synthesis, cell wall synthesis or folate synthesis. Bacterial cell division is one of the essential processes that are not yet targeted

by clinically approved antibacterials. The present work can be useful in developing lead compounds for FtsZ proteins that may be used as anti-bacterial agents. It also focuses on understanding the conservation and differences in FtsZ and tubulin family of proteins.

Materials and methods

BLAST (Basic local alignment search tool)

BLAST is the tool used for the comparison of nucleotide or protein sequences from the same or different organisms. The sequences were obtained from NCBI's map viewer (<http://www.ncbi.nlm.nih.gov/mapviewer>). Gram-positive organisms were collected by blasting against the FtsZ sequence of *Bacillus subtilis* strain (gi 120577) and gram-negative organisms were obtained by blasting with *Escherichia coli* strain (gi 40863).

Multiple sequence alignments

With the aid of multiple sequence alignments, biologists are able to study the sequence patterns conserved through evolution and the ancestral relationships between different organisms. CLUSTAL W (Thompson *et al.*, 1994) individually aligns each sequence to each of the other sequences in a series of pair wise alignment and uses this set to create a guide tree which then helps to form multiple sequence alignment. 30 sequences from gram-negative and 17 sequences from gram-positive were aligned using Clustal X and were checked for the signature sequences. Another set of sequences belonging to the class Gamma-proteobacteria, phylum Firmicutes, Bacteroidetes and Euryarchaeota were collected which lacked the conserved C-terminal end (Vaughan *et al.*, 2004). They were then classified again based on Grams stain and aligned using Clustal X tool.

Secondary structure prediction

Secondary structure prediction methods are used to detect the presence of secondary structure elements from the primary sequence of a protein (Chou & Fasman, 1978). The presence/occurrence of helix, strand or a coil/loop is determined. The sequence of *Robiginitalea biformata* was submitted PSIPRED (<http://insulin.brunel.ac.uk/psiform.html>) and PROF (<http://cubic.bioc.columbia.edu/predictprotein>) web-servers for the secondary structure prediction.

Secondary structure assignment

Secondary structure assignment is a method by which helices, strands and coils are assigned to the residues present in a protein. These assignments are done based on the backbone torsion angles (Φ/Ψ) or the H-bonding between N and O (CO) of a given amino acid. There are several methods such as DSSP (www.cmbi.kun.nl/gv/dssp), STRIDE (www.embl-heidelberg.de/argos/stride/stride_info.html) that helps in the process. Secondary structures were assigned for two PDB structures using STRIDE. One was 1A0I, chain A of ATP-dependent DNA ligase from bacteriophage T7

complex with ATP, and the other was 1FSZ, obtained from *Methanocaldococcus jannaschii*.

Spdb viewer

Swiss-Pdb viewer (<http://www.expasy.org/spdbv/>) is a tool that allows analyzing several proteins at the same time. The proteins can be superimposed in order to deduce structural alignments and compare their active sites or any other relevant parts. SPDBV-viewer allows one to build models from scratch, simply by giving an amino acid sequence.

Results and discussion

Recognition of FtsZ signature sequences

Gram-positive organisms obtained by blasting against the FtsZ sequence of *Bacillus subtilis* strain (gi 120577) and gram-negative organisms obtained by blasting with *E. coli* strain (gi 40863) were taken. 30 sequences were taken from gram-negative and aligned using Clustal X. 17 sequences were taken from gram-positive and aligned using the same tool. Signature sequences are contiguous patterns of amino acids 10-50 residues long that are associated with a particular structure or function in proteins. A specific signature was found at the C-terminal domain in gram-negative organisms, which is conserved as ³⁶⁹[D/E]IP[A/S]FLR[K/R][Q/R][A/S]D³⁷⁹. Higher divergence however was observed in the C-tail residues of gram-positive bacterial FtsZ. The sequence for these was observed to be [D/E][I/V]PXF[I/L/M/F][R/K].

Another set of sequences belonging to the class Gamma-proteobacteria, phylum Firmicutes, Bacteroidetes and Euryarchaeota were collected as they lacked the conserved C-terminal end (Vaughan *et al.*, 2004). They were then classified again based on Grams stain and aligned using Clustal X tool. It was observed that a particular group of sequences which belonged exclusively to phylum Bacteroidetes (gram-negative), showed high conservation at the C-termini, though the conserved sequence motif was completely different from the observed signature sequence. The conserved sequence ⁶⁵⁸NNSFLHDNVN⁶⁶⁷ was found in *Kordia algicida*, *Gramella forsetti*, *Robiginitalea biformata*, *Polaribacter irgensii*, *Capnocytophaga sputigena*, *Flavobacteria bacterium*. These FtsZ proteins had longer spacer arm with an extra set of 200-300 amino acids approximately.

Sequence analysis of FtsZ

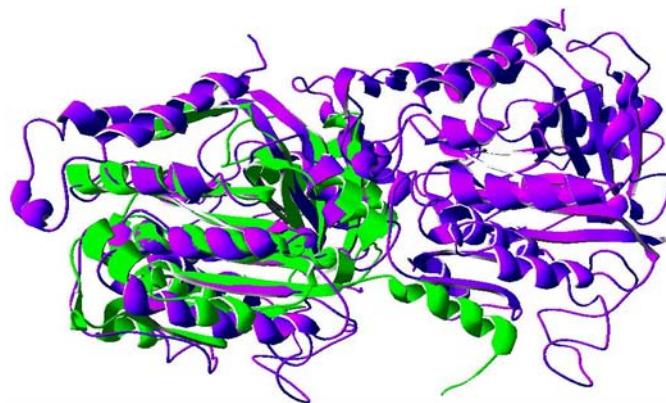
To understand the functional role and origin of these spacer sequences, blastp was performed with the spacer as the query sequence. The hits obtained had a sequence identity of 35-60% among 300-400 amino acid residues. Most of the FtsZ proteins have less than 400 residues. The particular set of sequences that were found from the multiple sequence alignment, having an extra large spacer arm, belong to the phylum Bacteroidetes. These FtsZ proteins typically showed a sequence length of ~590 residues. Interestingly, the sequence of its N-terminal and core domain matches very well with other

FtsZ orthologs. However, these proteins showed extra residues (spacer) of about 250-300 residues. A Fasta search using EBI server was done to identify proteins showing similarity with the extra residues found in the spacer arm of FtsZ belonging to phylum Bacteroidetes. Residues 323- 642 of *Flavobacteria bacterium* FtsZ (gi 126663239) was used as the query sequence (shares the common C-terminal domain with *Robiginitalea biformata*). It was seen that this sequence showed higher percentage of similarity with *Bartonella bacilliformis*, which had 591 residues. *Rhizobium meliloti* shares 91% sequence identity with *B. bacilliformis* at the N-termini (1-320 residues) and 46% identity at the C-terminal over 256 amino acids (Padmalayam *et al.*, 1997). From the sequence alignment, it can be concluded that these form a sub-family of FtsZ proteins. Interestingly, these proteins also possess a conserved C-terminal tail with the sequence motif of NN[K/R/S]F[F/L][T/H]D[K/N][T/N]D. The significance of the conserved C-tail residues in the sub-family is not understood. The C-terminal of *R. meliloti* contained high portions of proline and glutamine repeats, which were predicted to form a stacked beta sheet (Margolin *et al.*, 1991). *B. bacilliformis* has a high content of serine and glutamine residues, thus making it hydrophilic at the C-tail. Theoretical predictions based on flexibility, hydrophilicity and surface probability showed the presence of antigenic sites (Padmalayam *et al.*, 1997). As the spacer does share a homology with the T7 DNA ligase, it might be functional in different aspects. However, it shares only 26% homology that falls into the twilight zone, along with the mismatch in structure prediction, one cannot say for sure.

GC content is found to be variable with different organisms, the process of which is envisaged to be contributed to by variation in selection, mutational bias and biased recombination-associated DNA repair (Birdsell, 2002). G+C content of the nucleotide sequence was taken as another means to check whether the spacer residues are intrinsic to the phylum Bacteroidetes or have resulted due to fusion of some mobile islands of other species, like viruses. The completed genomes were obtained from NCBI or from genome sequencing databases. They also provide information about the GC content of the organisms. The complete genome of *E. coli* and *R. biformata* was taken from TIGR and NCBI, which had the required FtsZ coding sequence in them. The nucleotides were then checked for the coding regions of FtsZ using in silico translation. The percentage GC of the query sequence was compared with original FtsZ sequence using Bioedit suit. The complete genomes of *E. coli* and *R. biformata* consisted of 51.55% and 56.62% GC content, respectively. It was seen that FtsZ belonging to *Escherichia coli* had a G+C content of 53.82% and *R. biformata* FtsZ had a G+C content of 58.42%. Clearly, the two FtsZ sequences could be distinguished on the basis of the GC content of the species. The GC content of the spacer residues of *R. biformata* was also checked for and

was found to be 58.92%, which matches the native FtsZ sequence. The analysis thus did not distinguish the spacer sequence as of mobile origin. Interestingly, the amino acid composition for the spacer arm was also found to be normal with a higher number of glutamine residues.

Fig. 1. Superimposed images of *Pseudomonas aeruginosa* (1ofu-pink, 2vaw-green) obtained from swiss model & EsyPred3D servers using *R. biformata* as the query sequence.



Structural analysis of FtsZ

The sequence of *R. biformata* FtsZ was submitted in Swiss-Model and EsyPred3D to find out about the structure of the protein. In Swiss-Model, 15-312 residues of *P. aeruginosa* (PDB code, 1ofu) were matching with the core region of our query. The EsyPred3D server used 2vaw as a template and modeled the structure corresponding to the core region. The structures were superimposed in SPDB-viewer. The structural comparison revealed high similarity in the two models with RMSD of 0.67 Å for 284 atoms (only alpha carbon atoms) and a RMSD of 1.51Å with 2081 atoms involved (all atoms). The low RMSD also suggested accuracy of the partial structural model of *R. biformata* FtsZ for residues 13-320 constructed by homology modeling approaches (Fig. 1).

The data mining methods also showed some match between the additional spacer residues of *R. biformata* FtsZ and the sequence of PDB entry 1A0i, that corresponds to DNA ligase of T7 bacteriophage (Fig. 2). The structural model of this region was however not constructed as the alignment indicated 26% sequence identity between the two proteins over a span of 133 residues. We thus attempted to predict the secondary structure of the spacer residues, which itself could provide clues about the orthologs.

Prediction of the secondary structure of extra spacer residues

The sequence of *R. biformata* submitted to PSIPRED and PROF web-servers predicted coil structure mainly for the spacer residues. The secondary structure was assigned for the two PDB structures (Chain A of ATP-dependent DNA ligase from bacteriophage T7 complex with ATP, and *Methanocaldococcus jannaschii*). Since

Fig. 2. A Blastp performed against the PDB entries, with the spacer arm of 350 residues belonging to *R. biformata* as the query sequence. The alignment shown here is between the query sequence with 1a0i (T7 bacteriophage) as the subject. The alignment is seen over a span of 133 residues, where the match is seen in the spacer residues of the query sequence.

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>pdb|1A0I|A Chain A, Atp-Dependent Dna Ligase From Bacteriophage T7
Complex With Atp
Length=348
Score = 31.2 bits (69), Expect = 0.65, Method: Compositional matrix
adjust.
Identities = 35/133 (26%), Positives = 61/133 (45%), Gaps = 20/133 (15%)

Query 167 QISLHFDMLDQTQEDSEESAENVITFDLDEVDVRDMEVREHVE-ITPVL-EYKKEGETR-- 222
          I L+ +PL E E D DV + ++EHV+ + P+L EY E E +
Sbjct 144 HIKLYAILPLHIVESGE-----DCDVMTLMLQEHVKNMLPLLQYFPEIEWQAA 192

Query 223 --YSLDYMELEQQLTGATSKAEKYEKIVEDEL-VFEKKTVEGSGSESPTPDADPTDRP 279
          Y + D +EL+Q +AE +E IV+D + +++ G P +AD +
Sbjct 193 ESYEVYDMVELQQLY--EQKRAEGHEGLIVKDPMCYKRGKKSQWMMKPENEADGIIQG 250

Query 280 ISEILRERADERR 292
          + + A+E +
Sbjct 251 LVWGTKGLANEGK 263
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PDB did not provide the entire genome sequence for *M. jannaschii*, the FASTA sequence of the completed genome was taken from Uniprot (Q57816). From 1A0I, only 133 residues were taken that matched with the extra spacer arm found in *R. biformata* (Blast results). The other hit found from blast (1OQY) was not taken because it had less percentage of sequence identity (only 45% over 46 residues). The assigned structures were put together in a FASTA file along with FtsZ sequence and the PROF predicted secondary structure. The assigned files were put together with the prediction files and the alignment was viewed in Bioedit (sequence alignment editor). It was seen that the predicted results of PSIPRED and PROF matched with each other. They were also matching with the assigned structures of 1FSZ (marked as stride1) throughout the entire stretch of N-terminal and core residues. This observation confirms that, our query sequence (sequence of interest) indeed belongs to the FtsZ family. Since the secondary structures are similar to each other, it is suggested that the topology and fold of the protein may also be similar. As structurally they are similar, chances of the function being the same is high. In addition, sequentially the core domain matches along with GTP binding site, indicating that our sequence (gi 8806891) shares the same function (Fig. 3).

Interestingly it was seen that the extra 250-300 residues found in the spacer arm of *R. biformata* formed a coiled coil throughout in PSIPRED results. PROF predicted the presence of very few random strands and helices. Strikingly, the presence of coils in such a long stretch of amino acids without any stabilizing disulphide bridges/bonds (absence of cysteine residues) raises questions/doubts about the stability of the protein. If the protein is unstable, then chances of it being destroyed in cell's machinery are high. The 133 residues (144-263) of 1A0I belonging to T7 ligase was mapped with the spacer arm of *R. biformata* and showed the presence of helices and strands. These secondary structures, assigned by

STRIDE, were found to match weakly with the PROF results. The match between the experimental secondary structure of 1A0I and the predicted secondary structure of FtsZ for the corresponding residues authenticated the most likely origin of the spacer residues.

As a part of the spacer arm matches with the T7 ligase (of viral origin), it can be assumed that these extra residues might have come from the viruses. It is rather intriguing that only a particular set of organisms seems to have inherited the "longer" spacer arm residues from the viral species. This observation suggests that, the chances of such an occurrence taking place might be due to horizontal gene transfer or gene shuffling. Since the spacer is not showing a definitive structure, prediction of its function

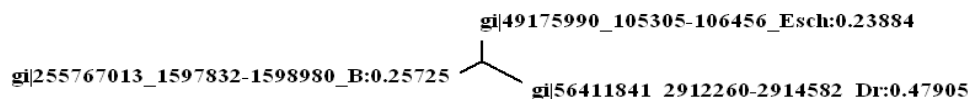
becomes a difficulty. The function of spacer region in other bacterium is also not well understood.

Sequence and structural comparison of bacterial FtsZ with eukaryotic tubulin

Bacterial FtsZ is a functional homolog of eukaryotic tubulin proteins. Several forms of tubulin have been reported. However, the present comparison has been restricted to the two major α and β forms of tubulin, which constitute a functional dimer of the microtubules. The basic objective of the present study was to elucidate structure-sequence similarity of tubulins with FtsZ from gram-negative or gram-positive bacterium. Attempts were also made to understand evolution of bacterial tubulins, which interestingly, are expected to show better relatedness with eukaryotic tubulins rather than the bacterial FtsZ proteins. Sequences of α -tubulins were mined from NCBI protein database using BlastP. The 25 sequences were aligned using Clustal X to find the conserved motifs in α -tubulins. Interestingly, the alignment showed very high identity even for sequences belonging to species which are known to have diverged more than 500 M years ago, for instance, 98% identity was observed between human and *Danio rerio* (zebra fish) α -tubulins. In comparison, bacterial FtsZ orthologs showed much rapid/higher divergence.

The α -tubulins were then aligned both with gram-positive and gram-negative sequences to check for sequence identity. It was found that there was less than 30% sequence identity. Gram negative organisms showed 14% sequence identity over a span of 515 residues and gram positive organisms showed 15% sequence identity over 504 residues. Tubulins were also aligned with the sequences belonging to the class Bacteroidetes to check for sequence identity. They showed a better sequence identity (20% over 797 residues) than the previous alignment sharing more

Fig. 4. A phylogram showing the distance length between FtsZ (gram positive *Bacillus subtilis* & gram negative *Escherichia coli*) & eukaryotic tubulin (*Drosophila melanogaster*).



structures wherein the helices and the strands are perfectly aligned. Comparison of the FtsZ structures with the tubulins shows both of them are structurally similar.

Conclusion

The specific signature sequences found in gram-positive and gram-negative organisms had slight variations seen in them suggesting that evolutionary changes might have occurred among the bacterial species. These changes must have further lead to the development of tubulin sequences. Yet they have maintained their functions throughout indicating the presence of selection pressure among the species. The structures are also retained along with the functions of the proteins. The structural alignments confirm that they are true homologues. It can be assumed that, after FtsZ arose in the common ancestor of bacteria and archaea, it evolved a mechanism that was quite efficient for cytokinesis. As FtsZ later evolved in the diverging species, it preserved this mechanism largely (unchanged). It must have evolved as a functional protein before the genetic code was complete because a mechanism for cell division would be required even in the earliest stage of development of cellular life form. The divergence must have arisen when a redundant FtsZ in early eukaryotes lost the constraints to do cell division and began evolving a completely different function to form microtubules. The redundant FtsZ could have come from gene duplication or after actin replaced FtsZ from cytokinesis.

It is of widely accepted opinion that bacteria and archaea evolved first and from them eukaryotes have emerged. Hence we can assume that tubulins have come from bacterial cell division proteins. It is still a mystery as to how this phenomenon took place. The extra set of sequences that were found in the FtsZ family with a longer spacer arm, has a viral connection, indicating that the evolution process has taken quite a few turns. Since the spacer arm does not present with any specific function, the addition of extra residues has not hampered the function of these sequences, thus showing the conservation in GTP binding. In addition to this, these sequences are also classified under gram negative, thus gaining a place in the phylogenetic tree. The only question that remains unanswered is the exact place or position where tubulins have diverged from FtsZ.

Phylogenetic analysis also showed the presence of FtsZ in few archaeal species. Such conservation even among divergent species emphasizes the importance of the cell division proteins. Since the protein prediction methods did not give a clear picture of the structure of *R. biformata*, the function could not be predicted. In addition, most of these proteins belong to class Proteobacter, which are known to interact closely with the eukaryotic cells. So chances of these sequences helping in the process of evolution can be considered.

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