Genes involved in cell division in mycoplasmas

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Abstract

Bacterial cell division has been studied mainly in model systems such as Escherichia coli and Bacillus subtilis, where it is described as a complex process with the participation of a group of proteins which assemble into a multiprotein complex called the septal ring. Mycoplasmas are cell wall-less bacteria presenting a reduced genome. Thus, it was important to compare their genomes to analyze putative genes involved in cell division processes. The division and cell wall (dcw) cluster, which in E. coli and B. subtilis is composed of 16 and 17 genes, respectively, is represented by only three to four genes in mycoplasmas. Even the most conserved protein, FtsZ, is not present in all mycoplasma genomes analyzed so far. A model for the FtsZ protein from Mycoplasma hyopneumoniae and Mycoplasma synoviae has been constructed. The conserved residues, essential for GTP/GDP binding, are present in FtsZ from both species. A strong conservation of hydrophobic amino acid patterns is observed, and is probably necessary for the structural stability of the protein when active. M. synoviae FtsZ presents an extended amino acid sequence at the C-terminal portion of the protein, which may participate in interactions with other still unknown proteins crucial for the cell division process.

Key words: cell division, Mycoplasma spp, genomes.

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Introduction

Cell division in bacteria is a complex process involving the coordinated participation of a group of proteins which assemble at the division site into a multiprotein complex called the septal ring (for reviews see Errington et al., 2003; Weiss, 2004; Goehring and Beckwith, 2005). This process has been well studied in some bacterial model systems, such as Escherichia coli and Bacillus subtilis.

The tubulin-like protein FtsZ, virtually present in all eu­bacteria, several archaeas, chloroplasts of plants and some mitochondria, plays a central role in cell division (for review see Margolin, 2005). This protein polymerizes to form the Z-ring, a structure associated to the cytosolic face of the inner membrane at midcell and essential for recruitment of other proteins to the division site. In E. coli, the following proteins are also components of the septal ring: FtsA, ZipA, ZapA, FtsEX, FtsK, FtsQ, FtsL, FtsB, FtsW, FtsI (PBP3), FtsN, AmiC and EnvC (Weiss, 2004). FtsA, ZipA and ZapA proteins assemble early to the division ring, are mainly cytosolic and are involved in Z-ring stabilization. The remaining proteins assemble later to the ring, have membrane and/or periplasmic localization and except for FtsEX (unknown function) and FtsK (chromosome segregation, see below), participate in the synthesis of septal peptidoglycan.

FtsK is involved in chromosome partitioning during the last steps of DNA segregation, and is highly conserved almost throughout the eu­bacteria. It is a very large protein with an N-terminal transmembrane domain essential for septum formation and a cytoplasmic C-terminal domain which shows ATP dependent DNA translocase activity (Bigot et al., 2004). Recently, it was demonstrated that FtsK interacts with topoisomerase IV, an enzyme responsible for chromosome decatenation (Espeli et al., 2003).

In B. subtilis, besides FtsZ, the proteins FtsA, FtsW, DivIB (FtsQ homolog), DivIC (FtsL-like), and PBP2B (PBP 3 homolog) have been implicated in cell division, and their localization to the division site has been shown to be FtsZ-dependent (Beall and Lutkenhaus, 1992; Katis et al., 1997, 1999; Daniel and Errington, 2000; Daniel et al., 2000; Feucht et al., 2001). In this bacterium, EzrA has been identified as a negative, and ZapA as a likely positive regu-
ator of FtsZ polymerization (Levin et al., 1999; Gueiros-Filho and Losick, 2002).

FtsH and FtsY, even if they were initially identified by mutants that showed a filamentation phenotype, they are in fact involved in general processes that have pleiotropic effects on cell division (Tomoyasu et al., 1993; Seluanov and Bibi, 1997).

Several other proteins are involved in different steps of the cell division process such as site selection (Min proteins, Noc, SlmA) and chromosome segregation (Sme proteins) (for review see Goehring and Beckwith, 2005).

Mycoplasmas belong to the class Mollicutes, a group of bacteria characterized by the absence of a cell wall. These bacteria present AT-rich genomes with sizes ranging from 580,070 bp in Mycoplasma genitalium to 1,358,633 bp in Mycoplasma penetrans. Most of them are parasites of a wide group of organisms, including mammals, birds, reptiles, arthropods, fish, and plants (Razin et al., 1998). Only a few studies on Mollicutes have been dedicated to genes involved in cell division, particularly the gene ftsZ (Wang and Luktenhaus, 1996; Kukekova et al., 1999; Momynalieva et al., 2002; Benders et al., 2005). This gene is present in all mycoplasma genomes analyzed so far, with the exception of Ureaplasma urealyticum serovar 3 and Mycoplasma mobile (Glass et al., 2000, Jaffe et al., 2004). Mycoplasmas present genomes with reduced sizes, and the M. genitalium genome is considered to be the smallest for a self-replicating organism (Fraser et al., 1995).

Presently, there are twelve completely sequenced mycoplasma genomes. Since these bacteria do not present a cell wall and present reduced genome sizes, it was important to compare their genomes to analyze putative genes involved in cell division processes. This work presents the results of such analysis and shows that a reduction in size of mycoplasma genomes leads to the loss of most genes involved in cell division. We also show that ftsZ is present in most of the mycoplasma genomes, and that its sequence and structure do not present regions involved in known interactions with other cell division proteins.

Material and Methods

DNA sequence analysis

The data on the complete genomes were downloaded from the Entrez genome except for the Mycoplasma synoviae and Mycoplasma hyopneumoniae strain J and Mycoplasma hyopneumoniae strain 7448, which were analyzed within the Brazilian National Genome Sequencing Consortium and the Southern Genome Investigation Program (PIGS). The comparison of CDS among the studied genomes was done through the SABIA (System for Automated Bacterial Integrated (genome) Annotation) software (Almeida et al., 2004). Genes possibly involved in cell division in mycoplasmas were identified by blastp analysis using cell division genes from E. coli and B. subtilis.

Sequences presenting hits with E value of E-05 or less and a coverage higher than 30% were selected.

Protein homology modeling

Multiple sequence alignment for modeling was prepared using Mycoplasma sp. sequences taken from SWISSPROT (ODonovan et al., 2002) and then aligned using ClustalW (Thompson et al., 1994). Theoretical structural framework models for FtsZ proteins from M. synoviae and M. hyopneumoniae 7448 were constructed using the atomic coordinates of FtsZ proteins from other bacterial species (1FSZ and 1W5B from Methanococcus jannaschii) (Lowe and Amos, 1998; Oliva et al., 2004), 1OFU from Pseudomonas aeruginosa (Cordell et al., 2003), and 1RLU from Mycobacterium tuberculosis (Leung et al., 2004) and is available with these codes at RCSB website (Berman et al., 2000). After the construction of a structural sequence alignment between FtsZ templates, models and their parts were calculated using the program MODELLER 8v1 (Sali and Blundell, 1993; Marti-Renom et al., 2000) by the previous calculation of pairwise and multiple alignments between the template structures/sequences and the target sequences (FtsZ sequences from M. synoviae and M. hyopneumoniae 7448). Models that matched the lowest objective function score, as well as those which satisfied visual and stereochemical inspection, were chosen to produce the final models used in this work. Stereochemical validation was done using the package PROCHECK (Morris et al., 1992, Laskowski et al., 1993).

Results and Discussion

Putative genes involved in cell division in mycoplasmas

The genes that may be involved in cell division in ten species of mycoplasma are shown in Table 1. The gene encoding FtsZ is present in all except U. urealyticum serovar 3 (Glass et al., 2000) and M. mobile (Jaffe et al., 2004). FtsK, a protein involved in chromosome partitioning, is present only in Mycoplasma gallisepticum and U. urealyticum serovar 3. Species of the clade Pneumoniae (M. genitalium, M. gallisepticum, Mycoplasma pneumoniae and U. urealyticum) seem to have conserved more genes involved in cell division than members of the clade Hominis (M. hyopneumoniae, M. synoviae, Mycoplasma pulmonis and M. mobile). Orthologs of the structural maintenance of chromosomes (SMC) proteins, involved in chromosome segregation, are present in all genomes. The gene encoding ParA, a protein involved in chromosome partitioning, is present in M. genitalium, M. pneumoniae, M. penetrans, and M. gallisepticum. M. penetrans, M. pulmonis and U. urealyticum serovar 3 present genes encoding recombinases (xerD/xerC family) involved in resolution of the chromosomes. EzrA, a protein identified as a negative regulator of FtsZ polymerization,
was observed only in the *M. gallisepticum* genome. The genes encoding FtsH and FtsY are present in all species analyzed, and although they may affect cell division, they are not directly involved in this process.

### The *ftsZ* gene cluster

In bacteria, several genes involved in cell division and synthesis of the cell wall precursors are organized in the *dcw* (division and cell wall) cluster, a highly conserved group of about 16 genes, including *ftsZ* (Margolin, 2000; Mingorance *et al.*, 2004). The functions of the first two genes in the cluster (*mraZ* and *mraW*) are unknown. The *mraW* gene encodes an S-adenosyl-methionine-dependent methyltransferase, and although the structure has been resolved, no function has been defined for this protein in cell division. Six *fts* genes (*ftsL, ftsI, ftsW, ftsQ, ftsA* and *ftsZ*) are also present in this cluster, and the products of these genes are involved in the formation of the division ring. Several genes involved in synthesis of murein precursors also belong to this cluster: genes *murBCDEFG, mraY* and

### Table 1 - Putative genes involved in cell division in mycoplasmas. Genomes of ten different Mycoplasma species have been compared. Data from three different strains of *Mycoplasma hyopneumoniae* are presented.

<table>
<thead>
<tr>
<th>Species</th>
<th>Length</th>
<th>Gene*</th>
<th>Product*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. genitalium</em></td>
<td>612</td>
<td>smcC</td>
<td>P115 protein</td>
</tr>
<tr>
<td></td>
<td>982</td>
<td>soj</td>
<td>soj protein</td>
</tr>
<tr>
<td></td>
<td>326</td>
<td><em>ftsZ</em></td>
<td>cell division protein</td>
</tr>
<tr>
<td><em>M. pneumoniae</em></td>
<td>270</td>
<td>soj</td>
<td>ParA family of ATPases involved in chromosome partition</td>
</tr>
<tr>
<td></td>
<td>982</td>
<td>smc</td>
<td>SMC family, chromosome/DNA binding/protecting functions</td>
</tr>
<tr>
<td></td>
<td>380</td>
<td><em>ftsZ</em></td>
<td>cell division protein</td>
</tr>
<tr>
<td><em>M. penetrans</em></td>
<td>275</td>
<td>soj</td>
<td>Soj protein</td>
</tr>
<tr>
<td></td>
<td>984</td>
<td>smc</td>
<td>structural maintenance of chromosomes SMC superfamily proteins</td>
</tr>
<tr>
<td></td>
<td>253</td>
<td>MYPE2900</td>
<td>recombining (xerD/xerC family)</td>
</tr>
<tr>
<td></td>
<td>476</td>
<td><em>ftsZ</em></td>
<td>cell division protein FtsZ</td>
</tr>
<tr>
<td><em>M. gallisepticum</em></td>
<td>263</td>
<td>parA</td>
<td>ParA/Soj</td>
</tr>
<tr>
<td></td>
<td>983</td>
<td>smc-like</td>
<td>Smc-like</td>
</tr>
<tr>
<td></td>
<td>1931</td>
<td>Ezra</td>
<td>conserved hypothetical</td>
</tr>
<tr>
<td></td>
<td>462</td>
<td><em>ftsZ</em></td>
<td>FtsZ</td>
</tr>
<tr>
<td></td>
<td>655</td>
<td><em>ftsK</em></td>
<td>FtsK</td>
</tr>
<tr>
<td><em>U. urealyticum</em> serovar 3</td>
<td>981</td>
<td>p115 (smc)</td>
<td>p115 protein</td>
</tr>
<tr>
<td></td>
<td>251</td>
<td>codV</td>
<td>integrase-recombining protein</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>xerC</td>
<td>integrase-recombining protein (xerD/xerC family)</td>
</tr>
<tr>
<td></td>
<td>351</td>
<td><em>ftsY</em></td>
<td>SRP family of GTP-binding proteins - cell division protein</td>
</tr>
<tr>
<td><em>M. hyopneumoniae</em> 252</td>
<td>979</td>
<td>p115 (smc)</td>
<td>p115 protein</td>
</tr>
<tr>
<td></td>
<td>327</td>
<td><em>ftsZ</em></td>
<td>cell division protein</td>
</tr>
<tr>
<td><em>M. hyopneumoniae</em> 7448</td>
<td>980</td>
<td>smc</td>
<td>ABC transporter ATP-binding protein P115-like</td>
</tr>
<tr>
<td></td>
<td>322</td>
<td><em>ftsZ</em></td>
<td>cell division protein</td>
</tr>
<tr>
<td><em>M. hyopneumoniae</em> 1</td>
<td>980</td>
<td>smc</td>
<td>ABC transporter ATP-binding protein P115-like</td>
</tr>
<tr>
<td></td>
<td>322</td>
<td><em>ftsZ</em></td>
<td>cell division protein</td>
</tr>
<tr>
<td><em>M. pulmonis</em></td>
<td>979</td>
<td>smc</td>
<td>P115-Like - ABC transporter ATP-binding protein integrase-recombining (xerD/xerC family)</td>
</tr>
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<td></td>
<td>390</td>
<td>MYPE5310</td>
<td>cell division protein</td>
</tr>
<tr>
<td><em>M. synoviae</em></td>
<td>981</td>
<td>smc</td>
<td>ABC transporter ATP-binding protein P115-like</td>
</tr>
<tr>
<td></td>
<td>543</td>
<td><em>ftsZ</em></td>
<td>cell division protein</td>
</tr>
<tr>
<td><em>M. mobile</em></td>
<td>974</td>
<td>smc</td>
<td>segregation of chromosomes protein</td>
</tr>
<tr>
<td><em>M. mycoides</em></td>
<td>472</td>
<td>smc</td>
<td>conserved hypothetical protein</td>
</tr>
</tbody>
</table>

* The information about the genes or gene products is based on the original annotation of the genomes.
In mycoplasmas, which lack a cell wall, the number of genes in this cluster is much lower (Benders et al., 2005). The *ftsL, ftsI, ftsW* and *ftsQ* gene products are all transmembrane proteins with the bulk of the proteins extended to the periplasm, and are involved in peptidoglycan synthesis during division (Weiss, 2004). The absence of a cell wall in mycoplasmas might explain why these genes are absent in their genomes. The same reasoning could be extended to the genes *murBCDEF*, *mraY* and *ddlB*. The comparison of the *dcw* cluster in mycoplasmas shows that the organization of the genes in this group of bacteria is conserved (Figure 1). The *M. genitalium, M. pneumoniae* and *M. gallisepticum* *dcw* cluster is composed of four genes, *mraZ, mraW*, a conserved hypothetical CDS, and *ftsZ*. A similar organization was observed in *M. pulmonis* and *M. synoviae*, but the conserved hypothetical CDSs are not related to each other. In *M. hyopneumoniae*, the cluster is composed of three CDSs: *mraZ, mraW* and *ftsZ*. The *mraW* gene is present in *M. penetrans*, but in another position of the genome (424743..425681). No sequence related to the *mraZ* gene was observed in the *M. penetrans* genome, and the three CDSs located close to the *ftsZ* gene are conserved.

*Figure 1* - Comparison of the organization of genes of the *dcw* cluster in mycoplasmas. The organization of the genes in *E. coli* and *B. subtilis* is also presented for comparison.

*Figure 2* - Sequence alignment of FtsZ proteins from different species of mycoplasmas. The extended amino acid sequence concentrated at the C-terminal portion of the *M. synoviae* FtsZ protein is indicated by 

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*Figure 2* - Sequence alignment of FtsZ proteins from different species of mycoplasmas. The extended amino acid sequence concentrated at the C-terminal portion of the *M. synoviae* FtsZ protein is indicated by 

hypothetical sequences with low similarity to other sequences in the nr database.

**FtsZ protein in mycoplasmas**

In mycoplasmas, the gene encoding FtsZ was initially characterized in *M. pulmonis*. The FtsZ protein of this organism was able to bind GTP (Wang and Lutkenhaus, 1996). It was also shown that its extreme carboxy tail, which is highly conserved between the *E. coli* and *B. subtilis* FtsZ proteins, is not as well conserved in *M. pulmonis* FtsZ (Wang and Lutkenhaus, 1996). We have extended the FtsZ comparative analysis to other mycoplasma

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**Figure 3** - Structural alignment of FtsZ proteins. FtsZ templates (pdb codes) *M. jannaschii*; *P. aeruginosa*; *M. tuberculosis* used to construct structural models of FtsZ proteins for *M. synoviae* and *M. hypopneumoniae*. Helices (h) and strands (s) in the template structures are represented along their amino acid sequences. The sequence alignment of mycoplasma amino acid sequences is represented above the template structural alignment.
species. The alignment of mycoplasma FtsZ sequences shows that the conserved C-terminal portion, which appears in several eubacteria species, including *E. coli* and *B. subtilis*, is absent in this group of bacteria (Figure 2). Despite the low similarity of *M. synoviae* and *M. hyopneumoniae* 7448 FtsZ amino acid sequences with the FtsZ templates used in this work (around 20-30% of sequence identity), it was possible to suggest reasonable carbon alpha amino acid framework models for *M. synoviae* and *M. hyopneumoniae* 7448 FtsZ proteins, based on identities and similarities present in the structural alignment of templates (Figures 3 and 4). According to the models, no structural differences were observed for the main domains of FtsZ and its relationships. The conserved residues essential for GTP/GDP binding, typical for the FtsZ family, are present in proteins from both species. There is strong conservation of hydrophobic amino acid patterns along the sequences, which are probably necessary for the structural stability of the protein when active (Figures 3 and 4).

Despite the suggested structural uniformity for mycoplasma FtsZ proteins compared with the template’s structures used during modeling, the *Mycoplasma* sp. amino acid sequences show some particularities. *M. synoviae* FtsZ presents an extended amino acid sequence (more than 200 amino acids) concentrated at the C-terminal portion of the protein (Figure 2). In several species this region is essential for interaction with other proteins, but not for the protofilament formation, since the central core of the protein (GTP binding site and neighbour sites) is involved in these processes. Since Mycoplasma genomes lack other important proteins involved in cell division processes and Z-ring formation (like ZipA and FtsA, if compared with other eubacteria), this amino acid tail is probably involved with other still unknown proteins crucial for cell division processes. It is well known that ZipA and FtsA help in the anchoring of FtsZ to the membrane during Z-ring formation (Hale and de Boer, 1997; Ma et al., 1997). These proteins interact with FtsZ mainly through the C-terminal peptide. As *M. synoviae* does not present these proteins or similar ones, a possible explanation for the function of the long tail located at the C-terminus of FtsZ could be its ability to fold in a domain that could interact directly with the inner bacterial membrane or with a transmembrane protein inserted into it (allowing the attachment necessary for the Z-ring formation). Actually, the deletion of C-terminal segments of FtsZ proteins blocks FtsZ functions, probably by preventing its interaction with other proteins important to the stability of the Z-ring (Ma and Margolin, 1999; Redick et al., 2005). It was not possible to suggest a structural model for this C-terminal region as there is no solved structure similar enough to be used as a template. Both *M. synoviae* and *M. hyopneumoniae* 7448 amino acid sequences show a smaller amount of amino acids between strand03 and helix03, when compared with the template sequences (Figure 3). The region is spatially close to the GTP binding site, in some way contributing to the chemical environment and stability that directly affects the GTP molecule. As this region is smaller in mycoplasma sequences, it is reasonable to assume that the GTP molecule will be more exposed than normally. This situation could contribute positively or negatively to the GTP hydrolysis and/or protofilament formation, since both processes are highly dependent on GTP stability/accessibility or GTP binding site protection (Margolin, 2005).

Conversely, mycoplasma amino acid sequences were larger than usual between helix11 and strand10 (Figure 3). The extra amino acids are located on the protein surface and are highly exposed. There are no data to suggest that these amino acids could play an important role in the function of the protein. However, as several typical proteins involved in cell division processes are not observed in mycoplasmas, the importance of these fragments in the interaction with other proteins cannot be discarded.

The cell division process in mycoplasma involves a lower number of proteins, compared to the machinery described for *E. coli* and *B. subtilis*. However, the possible involvement of other proteins, with different amino acid sequences, cannot be ruled out. The molecular mechanisms of the cell division process in mycoplasma remain unclear.

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