A C-terminal deletion mutant of *Mycobacterium tuberculosis* FtsZ shows fast polymerization *in vitro*

The FtsZ protein forms a dynamic polymeric ring structure, which functions as the guiding scaffold for septal invagination at the mid-cell site during cytokinesis in bacterial cells (Bi & Lutkenhaus, 1991). FtsZ forms polymers in a GTP-dependent manner *in vivo* and under different cationic conditions *in vitro* (Andreu *et al*., 2002; Beuria *et al*., 2003; Bi & Lutkenhaus, 1991; Bramhill & Thompson, 1994; Caplan & Erickson, 2003; Diaz *et al*., 2001; Erickson *et al*., 1996; Lu *et al*., 1998; Mingorance *et al*., 2001; Mukherjee & Lutkenhaus, 1994, 1999; Rivas *et al*., 2000; Romberg *et al*., 2001; Scheffers *et al*., 2001; Wang *et al*., 1997; White *et al*., 2000; Yu & Margolin, 1997). The rate of polymerization of the FtsZ protein of *Mycobacterium tuberculosis* H37Rv (MtFtsZ) is remarkably slow when compared to that of the *Escherichia coli* FtsZ protein (EcFtsZ) (Bramhill & Thompson, 1994; Mukherjee & Lutkenhaus, 1999; White *et al*., 2000). While polymerization of EcFtsZ reaches steady state in 30 s after the addition of GTP *in vitro* (Bramhill & Thompson, 1994; Mukherjee & Lutkenhaus, 1999), MtFtsZ takes nearly 10 min to reach the same state (White *et al*., 2000). The rate of depolymerization of MtFtsZ was also found to be slow, in conformity with its slow GTPase activity (White *et al*., 2000).

Although such striking differences exist in their polymerization and GTPase activities, MtFtsZ shows notable conservation with EcFtsZ at the N terminus, with the regions...
involved in FtsZ–FtsZ interactions (Wang et al., 1997) being more or less identical. However, the C terminus of MtFtsZ shows remarkable divergence from that of EcFtsZ. This structural variation tempted us to speculate that the differences in the biochemical properties of MtFtsZ and EcFtsZ might be due to the sequence divergence at the C terminus. Structural modelling of protofilaments of Methanococcus jannaschii FtsZ (MjFtsZ) (Lowe & Amos, 1999), based on the three-dimensional structure of MjFtsZ (Lowe & Amos, 1998), and site-directed mutagenesis studies on EcFtsZ (Lu et al., 2001), had indicated that at least four interacting faces, two longitudinal faces and two lateral faces of a FtsZ monomer might be engaged in the formation of protofilaments and polymers (Erickson, 1998; Lu et al., 2001; Romberg et al., 2001). One of these interacting faces maps to the divergent C terminus of the protein, thereby indicating involvement of this region in polymerization. Taking these correlations into consideration, we reasoned that the residues constituting the divergent C terminus of MtFtsZ might be responsible for imposing slow polymerization. If this contention is true, then an MtFtsZ mutant that lacks the divergent C-terminal residues might form polymers in much shorter time than the full-length MtFtsZ would do. In order to verify this premise, we determined the earliest measurable time point at which the deletion mutant MtFtsZ-ΔC169, which lacked 169 residues spanning the divergent C terminus, and full-length MtFtsZ could form equivalent-type long polymers. The time points at which full-length MtFtsZ and MtFtsZ-ΔC169 samples were examined for polymers were 30 s, 1 min, 2 min, 4 min and 10 min after the addition of GTP.

The earliest time at which long polymers of full-length MtFtsZ could be found was 10 min after the addition of GTP (Fig. 1a). Even very short polymers of full-length MtFtsZ could be found only from 2 min after the addition of GTP. By contrast, the MtFtsZ-ΔC169 deletion mutant formed equivalent-type long polymers as early as 30 s after the addition of GTP (Fig. 1b). The polymeric structures of MtFtsZ-ΔC169 protein were stable and could be observed at the end of 10 min incubation as well. Even at 10 min or 30 min, neither full-length

**Fig. 1.** FtsZ polymers were observed under a JEOL JEM-100CX II transmission electron microscope with an accelerating voltage of 80 kV and photographed at appropriate magnifications. The recombinant genes encoding full-length MtFtsZ and the mutant MtFtsZ-ΔC169 were overexpressed from pET20b+ vector in E. coli strain C41 (Miroux & Walker, 1996) as C-terminal hexahistidine-tagged proteins. The overexpressed proteins were purified using Ni²⁺–NTA agarose affinity chromatography under identical denaturing conditions, and refolded in 50 mM HEPES/NaOH (pH 7·2) buffer containing 10% glycerol. Incubation of full-length MtFtsZ and MtFtsZ-ΔC169 with a final concentration of 1 mM DTT was carried out on ice for 5 min prior to polymerization reaction in order to convert dimeric forms of the protein, which might have formed due to Cys–Cys linkage during purification, to monomeric forms. Polymerization reactions were carried out at 5 μM protein concentration as described by Lu & Erickson (1998). The reaction was started with the addition of 1 mM GTP (final concn). After incubation for the required duration, the sample was spotted on formvar and carbon-coated copper grids of mesh size 200. About 20 s elapsed from the end of incubation time to spotting of the sample on the grid. Polymers formed by (a) full-length MtFtsZ in 10 min and (b) mutant MtFtsZ-ΔC169 in 30 s. The pictures of the polymers are representative images of at least 10 independent experiments carried out using different protein preparations. The results were consistent.
MtFtsZ nor MtFtsZ-AC169 mutant formed polymers in the absence of GTP. The polymers formed by MtFtsZ-AC169 were approximately 1.5 times thicker than those formed by full-length MtFtsZ. Taken together, our results showed that MtFtsZ protein, when devoid of C-terminal 169 residues, could form long polymers in dramatically shorter time, as compared to the full-length MtFtsZ protein. This observation indicates that the residues responsible for slow polymerization of MtFtsZ are located in the region spanning the divergent C terminus of the protein. A systematic mutational analysis of the C-terminal region might enable identification of residues that impose slow polymerization on MtFtsZ. Our observations also imply that among the 379 residues of the MtFtsZ protein, a stretch of 210 residues from the N terminus is sufficient for in vitro polymerization of MtFtsZ, which may hold true for FtsZ proteins of other bacteria as well owing to their homology at the N terminus.

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Screening genomes of Gram-positive bacteria for double-glycine-motif-containing peptides

Secreted peptides fulfill major functions in the physiology of eukaryotes as well as prokaryotes. Yet, in many genome-sequencing projects, small peptides either remain un-annotated or are classified as hypothetical open reading frames, without any function associated. Therefore, the identification of signal peptide sequences would help in finding novel peptide genes or in assigning functions to automatically annotated sequences.

In Gram-positive bacteria, the double-glycine (GG) motif plays a key role in many peptide secretion systems involved in quorum sensing and bacteriocin production. Competence-stimulating