Investigating the Impact of FtsQ on FtsZ Filament Curvature in the *Mycobacterium tuberculosis* Divisome

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Abstract

In this study, we aim to characterize the effects of FtsQ NT on FtsZ polymers. Specifically, we hypothesized that FtsQ NT induces curvature in FtsZ filaments due to clashes between FtsQ and FtsZ protomers in straight filaments. Using negative-stain transmission electron microscopy (TEM), we revealed that FtsQ NT altered the shape and curvature of FtsZ filaments. Further investigations may support the QZ interface as a new target site for cell-division inhibitors.

Introduction

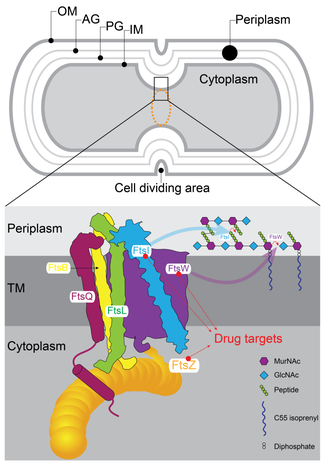

Figure 1. Schematic representation of the key proteins in the *M. tuberculosis* divisome. Not depicted divisome proteins include ChiZ, CrgA, and CswA.

- *Mycobacterium tuberculosis* is the causative agent of tuberculosis (TB), a disease that affects a quarter of the world population and caused 1.6 million deaths in 2021 alone (WHO, 2023).
- The rise in multi-drug and extensive drug-resistant TB cases underscores the urgent need for novel therapeutic strategies.
- Understanding the intricate cellular processes of *M. tuberculosis* division is critical for identifying potential drug targets.
- The cell division process is initiated by FtsZ, which forms the Z-ring at the division site through GTP-dependent polymerization and interacts with various other proteins, including FtsQ.
- Previous studies have shown that an amphipathic helix in the otherwise disordered N-terminal (NT) of FtsQ directly interacts with FtsZ monomers at the GTPase domain, suggesting that FtsQ may influence the polymerization of FtsZ (Smrt et al., 2023).

Methods

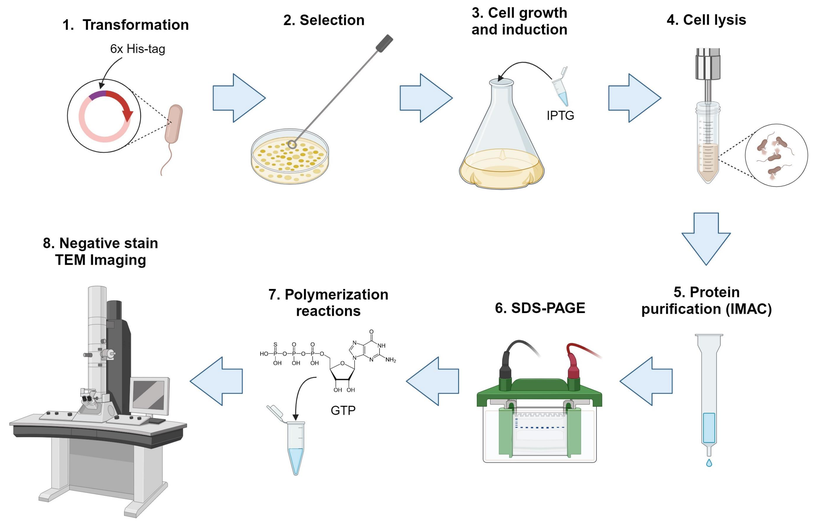

Figure 2. Overview of experimental procedures.

Results

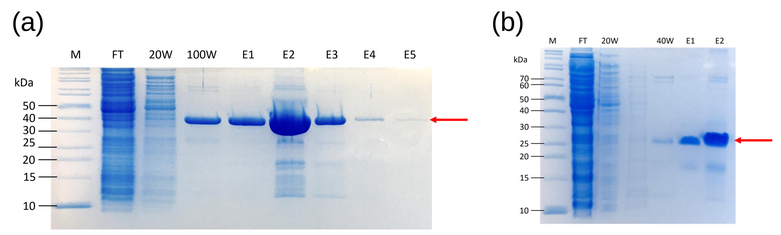

Figure 3. SDS-PAGE the products of protein purification for (a) FtsZ 1-312 and (b) FtsQ 1-99. The respective proteins are indicated by the arrow.

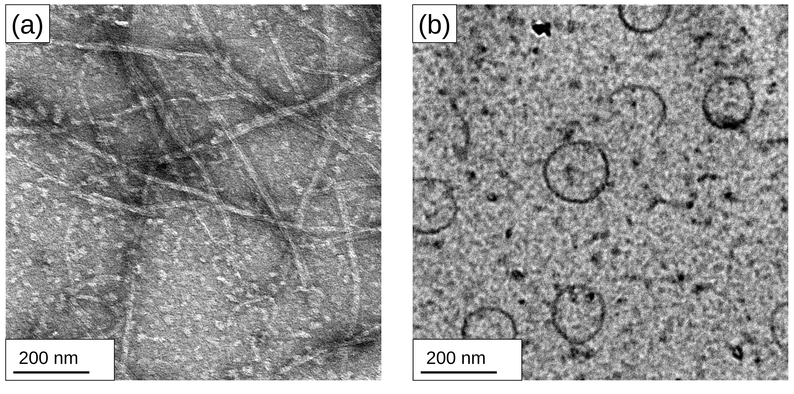

Figure 4. Negative-stain TEM images of FtsZ 1-312 (a) without and (b) with FtsQ 1-99 at a molar ratio of FtsZ/FtsQ = 3. FtsZ 1-312 by itself appeared as double-stranded linear filaments ~500-1000 nm in length. With the addition of FtsQ, the filaments became arcs and circles ~100 nm in diameter.

Conclusion

- FtsZ 1-312 alone formed linear, double-stranded filaments. Upon the addition of FtsQ 1-99, the filaments became shorter and curved.
- This discovery supports the hypothesis that FtsQ NT induces curvature in FtsZ filaments to avoid clashes in straight filaments as shown in Figure 5.
- This finding implicates that FtsQ may play a role in anchoring the Z-ring to the membrane during cell division in *M. tuberculosis*.

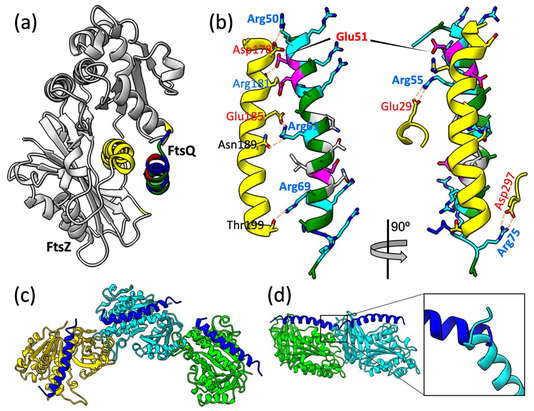

Figure 5. (a) The FtsQ helix docked to the GTPase domain of FtsZ (PDB entry 1RQ2). (b) Salt bridges and hydrogen bonds between the FtsQ helix and the inter-subdomain cleft of the FtsZ GTPase domain. (c) The FtsQ helix docked to a GTPase domain in a curved FtsZ polymer (PDB entry 4KWE), free of clashes with neighboring GTPase domains. (d) The FtsQ helix docked to a GTPase domain in a straight FtsZ polymer (PDB entry 1W5A), clashing with neighboring GTPase domains.

Future directions:

- Determine the structure of the FtsQ NT complexed with FtsZ polymers at high resolution using cryo-EM.
- Examine the impact of the QZ interaction on cell physiology and division using in vitro and in vivo biochemical experiments.

References

- Smrt, S. T., Escobar, C. A., Dey, S., Cross, T. A., & Zhou, H.-X. (2023). An Arg/Ala-rich helix in the N-terminal region of M. tuberculosis FtsQ is a potential membrane anchor of the Z-ring. *Communications Biology*, *6*(1), Article 1. https://doi.org/10.1038/s42003-023-04686-5
- WHO. (2023, April 21). *Tuberculosis*. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/tuberculosis