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Review

Division-cycle in *Mycobacterium tuberculosis*

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ABSTRACT

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Summary

Mycobacterium tuberculosis is a prototrophic and metabolically flexible bacterium. The microorganism kills approximately 2 million people each year and is thought to latently infect one third of the world's population. The success of *M. tuberculosis* as a pathogen is attributed to its extraordinary capacity to adapt to environmental changes throughout the course of infection. Today, based on various in vitro and in vivo models, the researches agreed to consider *M. tuberculosis* as a two-phase microorganism which can appear either in its metabolically active acid fast or in its inactive forms. It is the purpose of this review to discuss and outline the in-depth understanding *of the basic biology of cell division in M. tuberculosis*.

Background

A tremendous amount of work has been devoted to outlines of the basic biology of the *Mycobacterium* species. The early studies dated back to the 1940s and demonstrated evidence of the sequential analysis of the life-cycle in *Mycobacterium avium* [1,2]. In 1945, Brieger and Fell described three forms of slow growth and a "standard" life-cycle [1]. Their description of *M. avium* growing on chicken embryo extract was as follows: "The bacillus elongates during the first 24 h to form a filament several times its original length (2–4 μ); this divides repeatedly to form a bunch of 20–30 filaments which on the second or third day break down into short rods, which continue to multiply slowly by ordinary fission [1,3]. If the bacilli are sub-cultivated in fresh embryo extract, the cycle is repeated but not otherwise. Brieger and Glauert, in 1952, demonstrated similar patterns of cell cycle on a grid with a phase-contrast microscope [4]. Similarly, McCarthy (1971, 1974, 1976, 1978) attributed a diverse life-cycle for *M. avium* [2,5–7]. He described that when a small size of *M. avium* was placed in medium containing palmitic acid [5], it would elongate into long filaments. These filaments then subdivided into many cells of coccobacillary form [5]. The division is accompanied by a burst of synthesis of sulpholipids [6,7]. Thereafter, he proposed a complex relation between bacterial morphology, growth conditions and virulence for animals.

With technical advances in electron microscopy, the intracytoplasmic membrane during cell division were reported in *M. leprea* and *M. lepramurium* and *Mycobacterium* sp. [8–10]. Further studies documented the sequential changes during bacillary division in *M. leprea* [11]. The observed changes included: (1) the development of a slight concavity in the plasma membrane; (2) the formation of two new electron-dense layers between the concavity and the original cell wall; and (3) the invagination or annular growth of the plasma membrane and cell wall until division is complete.

KÖlbel proposed the theory of conjugation for Mycobacteria [12]. However, alternative interpretations of the images, either as accidentally adjacent bacteria or as branched cells, have not been ruled out [13]. Csillage (1962, 1964) described *M. tuberculosis* as dimorphic organisms (having acid fast and nonacid fast forms) in the same sense as are some pathogenic fungi, for instance *Histoplasma capsulatum* [13–16]. Hilson (1965) demonstrated that non-acid fast forms of mycobacteria are derived from contamination *of mycobacteria* with the spores of a Bacillus species [17]. Although Csillage (1970) submitted arguments for the unlikelihood of contamination by isolating mycococci from cultures of *M. Phlei* [13,18], in 1979, Bisset et al. described a strain of *Bacillus Licheniformis,* which apparently are acid fast and went through a life cycle involving L-forms as a source of contamination [19]. From published pictures of other species, it seems that the process of cell division in mycobacteria resembles that of the most gram-positive bacteria. A centripetal invagination of the plasma membrane is followed by formation of a cross wall that splits to form the poles of the two new cells [20,21]. Today with advances in cell-biology techniques, the visualization and understanding of fundamental processes in cell division has become possible. Overall, the *M. tuberculosis* in the exponential phase of growth divides by symmetrical-type of cell division and the cell-cycle includes: (1) DNA replication and segregation; (2) FtsZ ring assembly and maturation (Z-3); septal invagination with constriction of the envelope layer; and (4) septum closure and splitting of daughter cells. Recently, asymmetrical and budding typing of cell division were also reported [22] in highly resistant strains of *M. tuberculosis*. In this review, the first detail of asymmetrical-type cell division will be discussed; thereafter, the asymmetrical and budding type of cell-division will be presented using electron transmission and atomic force microscopy.

Mycobacterium chromosome

M. tuberculosis has a single circular chromosome that is not enclosed in a specific membrane-surrounded compartment, but occupies a distinct region in the cytoplasm. It comprises 4,411,529 bp with approximately 3900 genes encoding proteins [23]. The high guanine/cytosine content (G + C; 65.5%)

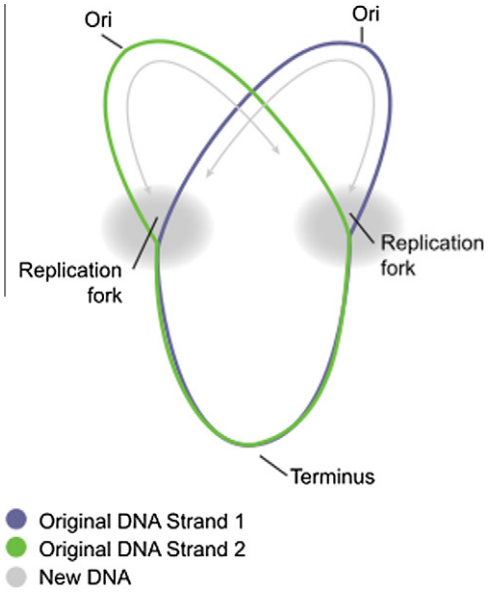

Fig. 1 – DNA replication in circular bacterial chromosome. Proceed bi-directionally (Graphic computer art by Daniel Yuen; From Wikipedia, the free encyclopedia).

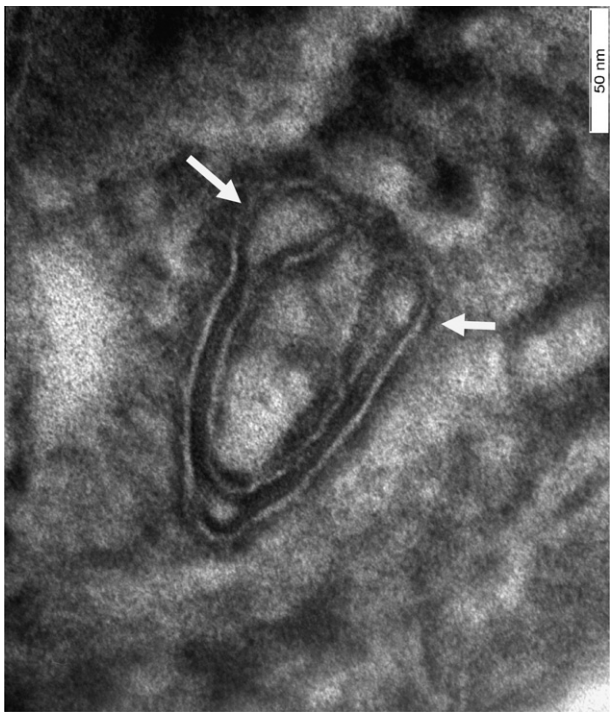

Fig. 2 – Electron transmission microscopy (TEM) shows a circular *Mycobacterium tuberculosis* chromosome during DNA replication. Arrows shows the ori-region.

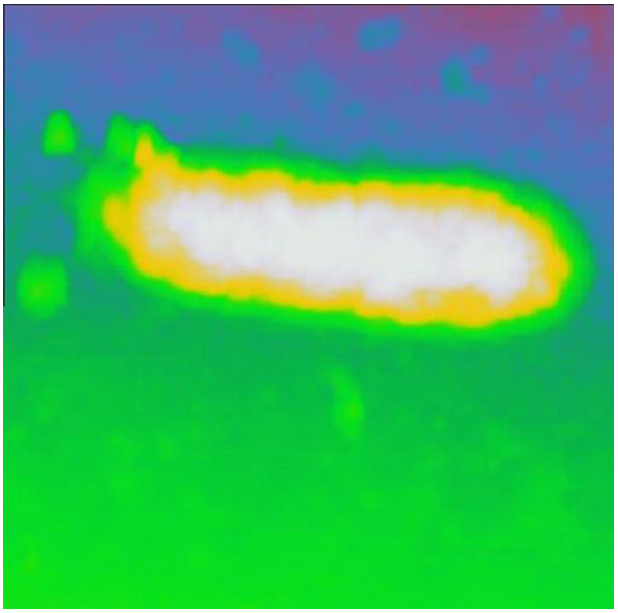

Fig. 3 – Before division, the *M. tuberculosis* mother cell duplicates its chromosome and mass content.

was found to be uniform along most of the genome, confirming the hypothesis that horizontal gene transfer events are virtually absent in modern *M. tuberculosis* [24,25]. The highest GC content belongs to *M. avium* ssp. paratuberculosis (69.3%) and lower content found (57.7%) in *M. leprae* [25–27].

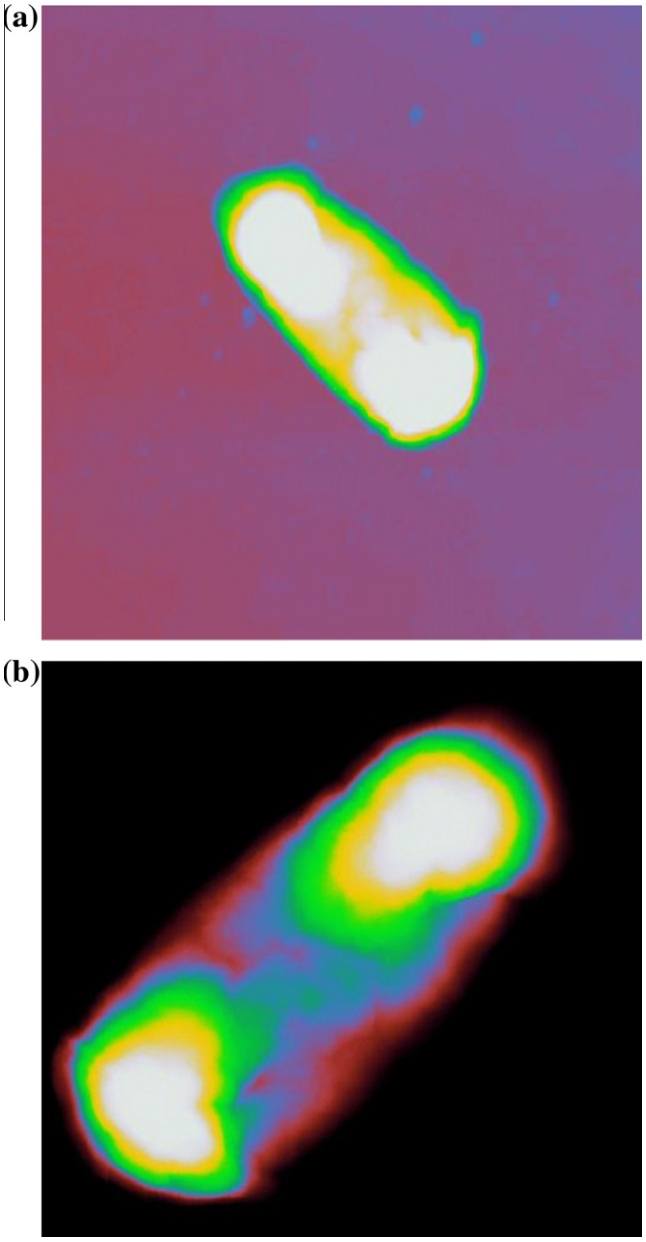

Fig. 4 – (a and b). The replicated chromosome and duplicated mass moves to the polar side of the cells (starting FtsZ ring assembly).

The three dimensional arrangement of the bacterial chromosome inside the cell using site-specific DNA labeling showed that in various bacteria, the origin (*oriC*)and terminus (ter) of replication present at a very reproducible pattern of localization during the cell cycle (Figs. 1 and 2). The *oriC* region (origin region of the chromosome) is generally AT nucleotide rich, containing repeats of AT-rich nucleotide sequences varying in length from 13 to 16, together with several 9-nucleotide DnaA protein recognition sequences called DnaA boxes [28]. Deletions in the *oriC* region abolish *oriC* activity, and point mutations in the DnaA box severely decreased *oriC* activity, indicating the importance of both the integrity of *oriC* and the sequence of the DnaA boxes in mycobacterial replication initiation [28–30].

Chromosome replication and segregation

The chromosome replication in *M. tuberculosis* proceeds in initiation, elongation and termination stages [30,31](Figs. 3 and 4a and b). These stages ensured signal chromosomal replication in each cell cycle. The *oriC* region of *M. tuberculosis* has 13 putative DnaA boxes [31,32]. The consensus sequence for *Mycobacterium* DnaA box is TTG/CTCCACA [29,30,32]. Generally, histone-like proteins [HU] unwind DnaA protein near the left side of *oriC*. Then, DnaA recruits the replicative helicase, DnaB, from the DnaB–DnaC complex to the unwound region to form the pre-priming complex [33–35]. After translocation of DnaB, the helicase unwinds parental DNA and interacts momentarily with primase (DnaG). The replication begins with uploading DNA polymerase III holoenzyme into the DNA. DNA is synthesized bi-directionally. Replication forks meet approximately half way around the chromosome within the "terminus region" where replication is terminated by a specific mechanism [29,32,36]. Segregation and replication starts in parallel, immediately after *oriC* regions are separated and move to conserved positions in the incipient daughter cells. Subsequently, as each new locus is being replicated, the two new copies are condensed and incorporated into the nucleoid in formation. In this way, the DNA loci are organized accordingly to the order of replication, which assures the maintenance of the original ordered arrangement of the chromosome.

FtsZ-ring assembly

When the chromosomes start to partition, the divisome begins assembling at the middle of the cell. The divisome is responsible for directing the synthesis of new cytoplasmic membrane and new peptidoglycan to form the division septum [36]. It consists of a set of 10–15 proteins (FtsA, FtsB, FtsE, FtsI, FtsK, FtsL, FtsN, FtsQ, FtsW, FtsX, FtsZ, Zip A, AmiC and EnvC) encoded in different bacterial genomes [36–39]. The FtsZ is one of the first proteins that assemble at mid-cell [40]. The formation of a ring under plasma membrane gives the assembled divisome the name Z ring (Fig. 5). This sub cellular organelle, a functional analog of the contractile ring used in cytokinesis of many eukaryotic cells, may act as a scaffold for recruitment of the other key cell division proteins. Recently, it has been shown that small changes in the concentration of divisome proteins might disrupt cell growth in *M. tuberculosis* [36,41,42].

Place of septum formation

To prevent unequal partitioning of DNA and chromosomes, several regulatory systems are working together [36]. The FtsZ regulatory system is named min system [43,44]. The min-system directs division at the center of the cell. In the gram-negative bacteria *Escherichia coli*, the min-system consists of the

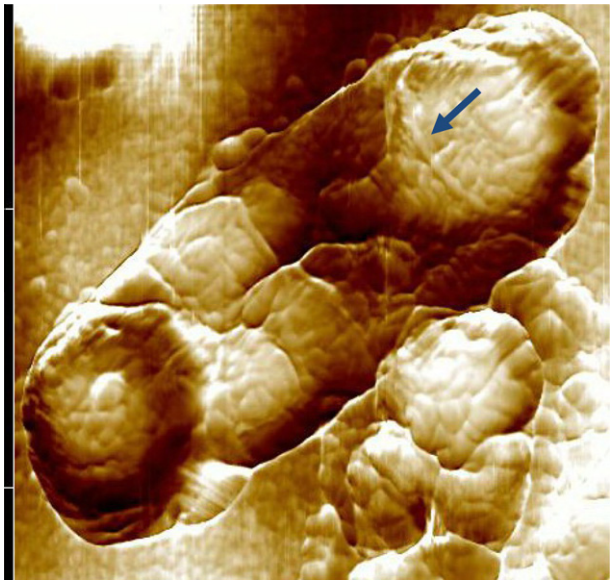

Fig. 5 – Atomic force microscopy shows the FtsZ ring beneath plasma-membrane (arrow).

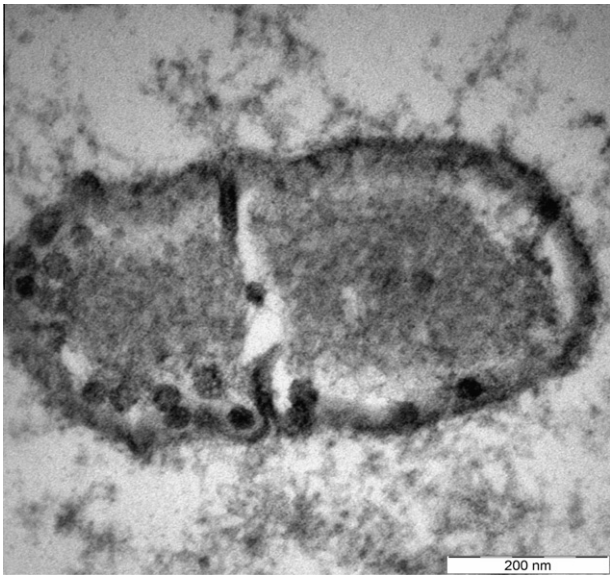

Fig. 6 – TEM shows separation of septal cell wall material.

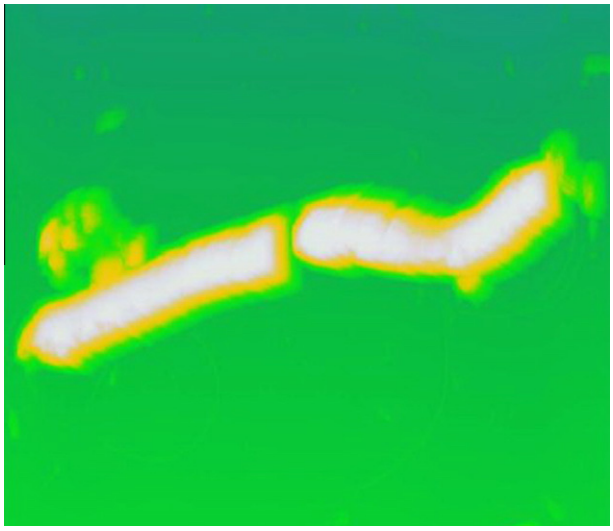

Fig. 7 – *M. tuberculosis* cells starting their separation.

three proteins MinC, MinD and MinE, while in Gram-positive bacteria *Bacillus subtilis*, the min-system consist of MinC, MinD and DivIVA. MinC and MinD form a complex that directly inhibits Z-ring formation, whereas the role of MinE and DivIVA is for topological specificity [36]. In addition, some other regulator of FtsZ, i.e., nucleoid occlusion (Noc), inhibits the Z-ring assembly by binding to nucleoid. Nucleoid occlusion and the Z-ring regulator MipZ have not been identified in the mycobacterial genome; often these systems co-exist and are thought to cooperate in directing the location of Z-ring formation to mid-cell for completion of DNA replication and segregation [36,44–46].

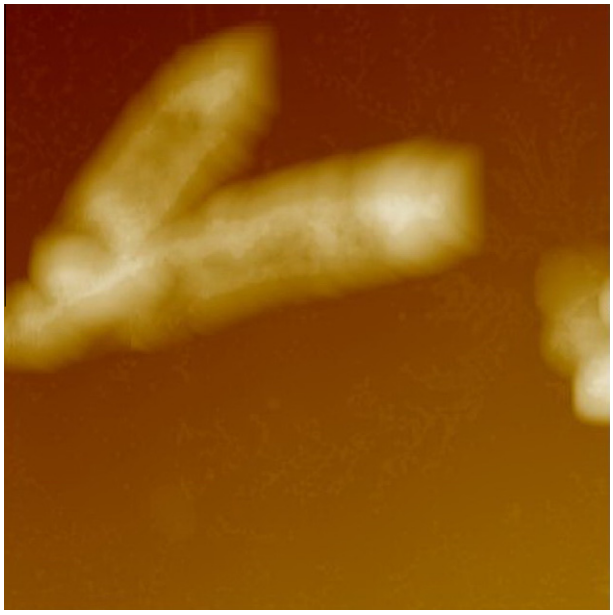

Fig. 8 – Snapping or V-shape cells occurred in the last stage of cell-division.

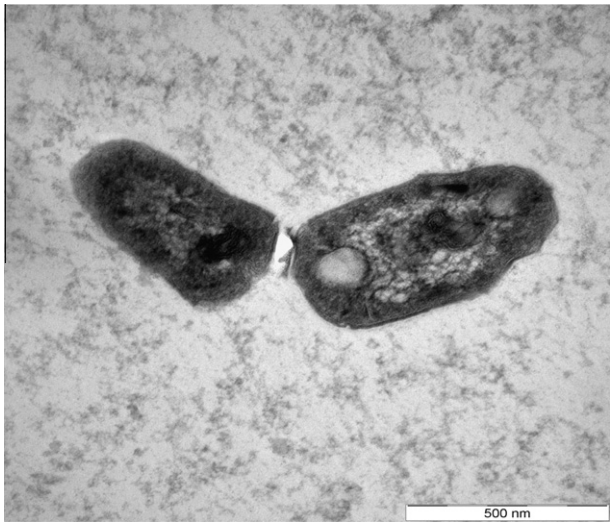

Fig. 9 – Shows V-shape *M. tuberculosis* while they are splitting.

Septal invagination with constriction of the envelope layer

The force necessary for the Z-ring to constrict may be from a passive process, or it may shrink owing to a net loss of FtsZ molecules, facilitated by a rapid turnover of FtsZ. In Mycobacteria, first the generation of a septum is completed and daughter cells get sealed off, then in the later stage, peptidoglycan (PG) hydrolases digest part of the septal PG, releasing the two daughter cells [36] (Fig. 6).

Splitting of the daughter cells

After septum synthesis is complete, the septal cell wall material has to be hydrolyzed so that the two daughter cells can split [47]. The localized hydrolysis of the septal peptidoglycan is catalyzed by a specific group of proteins known as peptidoglycan hydrolases or autolysins (Fig. 7).

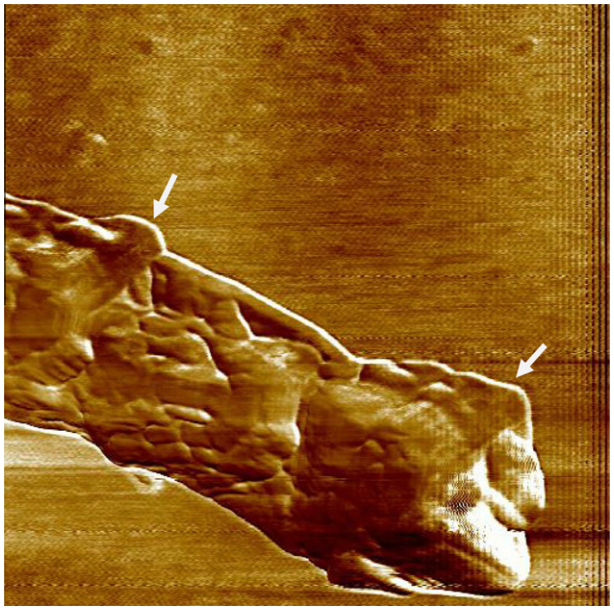

Fig. 10 – AFM shows ridge or division scar in single *M. tuberculosis*.

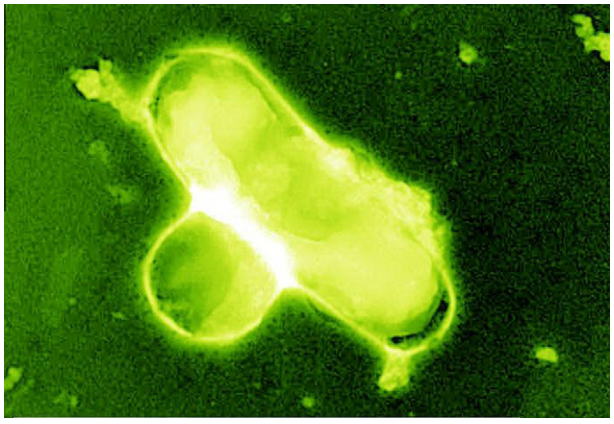

Fig. 11 – Branching or budding produced along the cylinder in XDR-TB strains.

Final stage of separation

Plasma membrane and PG form the septa during Z-ring constriction, but the thick layers of arabinogalactan (AG) and mycolic acids (MA) and other lipids remain intact during this stage [36]. When the cell divides by hydrolyzing the PG linking the daughter cells together, the outer layers are still intertwined. The V-shape splitting of cells results from an uneven rupturing of outer cell wall [36,48,50] (Figs. 8 and 9).

Rupturing of the outer material could also be aggravated by polar growth that occurs in the newly formed septum prior to completion of cell division [36]. The visible ridge on the external surface of the mycobacterium was of an underlying septum (Fig. 10). The similar structures were reported in *Actinomyces Israelii* cells [48,49]. A single *M. tuberculosis* bacilli may have numerous ridges on its surface (Fig. 10). The position of external ridges near the ends of cells suggests the cells may have divided and these ridges are division scars.

Generally, these bands are considered as allocation for future septa formation. Cells that are actively dividing were more likely to contain multiple bands while cells of older cultures were relatively smoother in appearance (Fig. 10).

Branching or budding

In *M. tuberculosis*, branches were first seen as a small bud that does not grow to any appreciable size before breaking off as a separate cell [48,50,51]. Thanky et al. suggested that *M. tuberculosis* grows from the ends of bacilli and not along the length of the cylinder as seen in other well-characterized rod-shape bacteria [51]; however, recently branches were seen along the cylinder [22] (Fig. 11).

Asymmetrical type of cell division

Generally, under the environmental stress, *M. tuberculosis* changed its morphology and type of cell-division. Recently, asymmetrical type of cell division was reported in 40% of highly drug-resistant TB bacilli [22,50]. More recently, Aldridge et al. showed within a clonally population of mycobacteria that there is deterministic heterogeneity which arises as a result of unusual growth [52]. These subpopulations of cells may have different elongation rates and sizes [52]. How the cells switch to an asymmetrical-type of cell division is not very clear and needs further investigation.

Conflict of interest

Authors has no conflict of interest.

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REFERENCES

[1] E.M. Brieger, H.M. Fell, Warm-stage observation on the initial development of the avian tubercle bacillus cultivated in embryo extract, J. Hyg. Camb. 44 (1946) 158–165.
[2] E.M. Brieger, C.F. Robinow, Demonstration of chromatinic structures in avian tubercle bacilli in the early stages of development, J. Hyg. Camb. 45 (1947) 413–420.
[3] C. McCarthy, Electronic counting in growth studies of *Mycobacterium avium*, Appl. Microbiol. 22 (1971) 546–551.
[4] E.M. Brieger, A.M. Glauert, A phase-contrast study of reproduction in mycelia strains of avian tubercle bacilli, J. Gen. Microbiol. 7 (1952) 287–302.
[5] C. McCarthy, Effect of palmitic acid utilization on cell division in *Mycobacterium avium*, Infect. Immun. 9 (1974) 363–372.
[6] C. McCarthy, Synthesis and release of sulfolipids by *Mycobacterium avium* during growth and cell division, Infect. Immun. 14 (1976) 1241–1252.
[7] C. McCarthy, Ammonium ion requirement for the cell cycle of *Mycobacterium avium*, Infect. Immun. 19 (1978) 304–311.
[8] T. Imaeda, J. Convit, Electron microscope study of *Mycobacterium leprae* and its environment in a vesicular leprous lesion, J. Bacteriol. 83 (1962) 43–52.
[9] T. Yamamoto, M. Nishiura, N. Harada, T. Imaeda, Electron microscopy of ultra-thin sections of lepra cells and *Mycobacterium leprae*, J. Leprosy 26 (1958) 1–8.
[10] T. Imaeda, M. Ogura, Formation of intracytoplasmic membrane system of mycobacteria related to cell division, J. Bacteriol. 85 (1963) 150–163.
[11] R.P. Edwards, Electron-microscope illustrations of division in *Mycobacterium leprae*, J. Med. Microbiol. 3 (1970) 493–499.
[12] KÖlbel H. Das, substrat der variabilitat von Mykobakterien electronenmikroskipische bild, Pneumonology 142 (1970) 137–164.
[13] C. Ratledge, J. Stanford, The Biology of the Mycobacteria, vol. 1, Academic Press, 1982. pp. 2–52.
[14] A. Csillage, Development of form 2 mycobacterium on autoclaved LJ medium, Tubercle 43 (1962) 433–439.
[15] A. Csillage, The mycococcus form of mycobacteria, J. Gen. Microbiol. 34 (1964) 341–352.
[16] P. Lafont, J. Lafont, Modification transmissible induite in vitro chez *M. tuberculosis* et mycobacterium bovis, Can. J. Microbiol. 25 (1979) 462–474.
[17] G.R.F. Hilson, Taxonomic characteristics of so-called “form 2 mycobacteria”, J. Gen. Microbiol. 39 (1965) 407–421.
[18] A. Csillage, A simple method to obtain the mycococcus form of *Mycobacterium Phlei*, J. Gen. Microbiol. 62 (1970) 251–259.
[19] K.A. Bisset, J. Tallack, R. Bartlett, Electron microscopy of the L-cycle in *Bacillus licheniformis* Var endoparasiticus, J. Gen. Microbiol. 12 (1979) 469–472.
[20] I.D. Burdett, M.L. Higgins, Study of pole assembly in *Bacillus subtilis* by computer reconstruction of septal growth zones seen in central, longitudinal thin sections of cells, J. Bacteriol. 133 (1978) 959–971.
[21] N. Rastogi, H.L. David, Growth and cell division of *Mycobacterium avium*, J. Gen. Microbiol. 126 (1981) 77–84.
[22] P. Farnia, M.R. Masjedi, M.A. Merza, P. Tabarsi, G.K. Zhavnerko, T.A. Ibrahim, et al, Growth and cell division in extensive (XDR) and extremely drug resistant (XXDR) tuberculosis strains: transmission and atomic force observation, Int. J. Clin. Exp. Med. 3 (2010) 320–326.
[23] S.T. Cole, R. Brosch, J. Parkhill, T. Garnier, C. Churcher, D. Harris, et al, Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence, Nature 393 (1998) 537–544.
[24] S. Sreevatsan, X. Pan, K.E. Stockbauer, N.D. Connell, B.N. Kreiswirth, T.S. Whittam, et al, Restricted structural gene

polymorphism in the *Mycobacterium tuberculosis* complex indicates evolutionarily recent global dissemination, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 9869–9874.
[25] J.C. Palomino, Tuberculosis 2007: from basic science to patient care, Antwerp, Belgium, 2007.
[26] L. Li, J.P. Bannantine, Q. Zhang, A. Amonsin, B.J. May, D. Alt, et al, The complete genome sequence of *Mycobacterium avium* subspecies paratuberculosis, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 12344–12349.
[27] P.R. Marri, J.P. Bannantine, G.B. Golding, Comparative genomics of metabolic pathways in Mycobacterium species: gene duplication, gene decay and lateral gene transfer, FEMS Microbiol. Rev. 30 (2006) 906–925.
[28] M.H. Qin, M.V. Madiraju, S. Zachariah, M. Rajagopalan, Characterization of the *oriC* region of *Mycobacterium smegmatis*, J. Bacteriol. 179 (1997) 6311–6317.
[29] M.H. Qin, M.V. Madiraju, M. Rajagopalan, Characterization of the functional replication origin of *Mycobacterium tuberculosis*, Gene 233 (1999) 121–130.
[30] J. Dziadek, M. Rajagopalan, T. Parish, N. Kurepina, R. Greendyke, B.N. Kreiswirth, et al, Mutations in the CCGTTCACA DnaA box of *Mycobacterium tuberculosis oriC* that abolish replication of *oric* plasmids are tolerated on the chromosome, J. Bacteriol. 184 (2002) 3848–3855.
[31] J.M. Kaguni, DnaA: controlling the initiation of bacterial DNA replication and more, Annu. Rev. Microbiol. 60 (2006) 351–371.
[32] A. Zawilak, A. Kois, G. Konopa, A.S. Krawczyszyn, J.Z. Czerwińska, *Mycobacterium tuberculosis* DnaA initiator protein: purification and DNA-binding requirements, Biochem. J. 382 (2004) 247–252.
[33] D. Bramhill, A. Kornberg, Duplex opening by DnaA protein at novel sequences in initiation of replication at the origin of the *E. coli* chromosome, Cell 52 (1988) 743–755.
[34] K. Sekimizu, D. Bramhill, A. Kornberg, ATP activates DnaA protein in initiating replication of plasmids bearing the origin of the *E.coli* chromosome, Cell 50 (1987) 259–265.
[35] K.M. Carr, J.M. Kaguni, Stoichiometry of DnaA and DnaB protein in initiation at the *Escherichia coli* chromosomal origin, J. Biol. Chem. 276 (2001) 44919–44925.
[36] E.C. Hett, E.J. Rubin, Bacterial growth and cell division: a mycobacterial Perspective, Microbiol. Mol. Biol. Rev. 72 (2008) 126–156.
[37] G. Di Lallo, M. Fagioli, D. Barionovi, P. Ghelardini, L. Paolozzi, Use of a two-hybrid assay to study the assembly of a complex multi-component protein machinery: bacteria septosome differentiation, Microbiology 149 (2003) 3353–3359.
[38] G. Karimova, N. Dautin, D. Ladant, Interaction network among *Escherichia coli* membrane proteins involved in cell division as revealed by bacterial two-hybrid analysis, J. Bacteriol. 187 (2005) 2233–2243.
[39] M. Vicente, A.I. Rico, The order of the ring: assembly of *Escherichia coli* cell division components, Mol. Microbiol. 61 (2006) 5–20.
[40] E. Bi, J. Lutkenhaus, Isolation and characterization of FtsZ alleles that affect septal morphology, J. Bacteriol. 174 (1992) 5414–5423.
[41] J. Errington, R.A. Daniel, D.J. Scheffers, Cytokinesis in bacteria, Microbiol. Mol. Biol. Rev. 67 (2003) 52–65.
[42] N. Maki, J.E. Gestwicki, M. Lake, L.L. Kiessling, J. Adler, Motility and chemotaxix of filamentous cells of *Escherichia coli*, J. Bacteriol. 182 (2000) 4337–4342.
[43] A. Chauhan, M.V. Madiraju, M. Fol, M.H. Lofton, E. Maloney, R. Reynolds, et al, *Mycobacterium tuberculosis* cells growing in macrophages are filamentous and deficient in FtsZ rings, J. Bacteriol. 188 (2006) 1856–1865.
[44] T.G. Bernhardt, P.A. Boer, SlmA, a nucleoid-associated FtsZ binding protein required for blocking septal ring assembly over chromosomes in *E. coli*, Mol. Cell 18 (2005) 555–564.
[45] E. Harry, L. Monahan, L. Thompson, Bacterial cell division: the mechanism and its precison, Int. Rev. Cytol. 253 (2006) 27–91.
[46] P.A. de Boer, R.E. Crossley, L.I. Rothfield, A division inhibitor and a topological specificity factor coded for by the minicell locus determine proper placement of the division septum in *E. coli*, Cell 56 (1989) 641–649.
[47] J. Errington, R.A. Daniel, D.J. Scheffers, Cytokinesis in bacteria, Microbiol. Mol. Biol. Rev. 2 (2003) 52–65.
[48] J.L. Dahl, Electron microscopy analysis of *Mycobacterium tuberculosis* cell division, FEMS Microbiol. Lett. 240 (2004) 15–20.
[49] H. Akiyama, Studies on cell division and branching in *Actinomyces israelii*, Shigaku 65 (1977) 707–723.
[50] P.J. Cardona, Understanding Tuberculosis – Deciphering the Secret Life of the Bacilli, In tech-open publication, 2010. pp. 149–162.
[51] N.R. Thanky, D.B. Young, B.D. Robertson, Unusual features of the cell cycle in Mycobacteria: polar-restricted growth and the snapping-model of cell division, Tubercle 87 (2007) 231–236.
[52] B.B. Aldridge, M. Fernandez-Suarez, D. Heller, V. Ambravaneswaran, D. Irimia, M. Toner, S.M. Fortune, Asymmetry and aging of mycobacterial cells lead to variable growth and antibiotic susceptibility, Science 335 (2011) 100–104.