Dynamic Organization of Molecular Machines in Bacteria

BHUPENDER SINGH
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Abstract

Bacterial cells were once treated as membrane-enclosed bags of cytoplasm: a homogeneous, undifferentiated suspension in which polymers (proteins, nucleic acids, etc.) and small molecules diffused freely to interact with each other. Biochemical studies have determined the molecular mechanisms underlying the biological processes of metabolism, replication and transcription-translation, etc. However, recent advancements in optical techniques armed with fluorescent tags for proteins and nucleic acids have increased our ability to peer into the interior of live bacterial cells. This has revealed an organized layout of multi-protein complexes, or molecular machines, dedicated to specific functions at defined sub-cellular locations; the timing of their assembly and/or rates of their activity being determined by available nutrition and environmental signals from the niche occupied by the organism.

In the present study, we have attempted to identify the intracellular location and organization of the molecular machines assembled for protein synthesis (ribosomes), DNA replication (replisomes) and cell division (divisome) in different bacteria. We have used the model system Escherichia coli as well as Helicobacter pylori and mycobacterial strains (Mycobacterium marium and Mycobacterium smegmatis), which grow at different rates and move to dormancy late into stationary phase.

Bacterial nucleoid plays a major role in organizing the location and movement of active ribosomes, replisomes and placement of divisome. While the active ribosomes appear to follow the dynamic folds of the bacterial nucleoid during cell growth in E. coli, inactive ribosomes appear to accumulate near the periphery. The replisome in H. pylori was visualized as a sharp, single focus upon SSB and DnaB co-localization in growing helical rods but disassembled into diffused fluorescence when the cells attained non-replicative coccolid stage. Our investigation into mycobacterial life-cycle revealed unique features such as an absence of a dedicated mid-cell site for divisome assembly and endosporulation upon entry into stationary phase.

In brief, we present the cell cycle-dependent subcellular organization of molecular machines in bacteria.

Keywords: molecular machines, bacteria, cell cycle, ribosome, replisome, divisome, spore, E. coli, H. pylori, Mycobacteria

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Adrish
This thesis is based on the following papers, which are referred to in the text by their Roman numerals (I-VI).


*Equal contribution

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**Additional reading**

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<tr>
<td>ADP</td>
<td>Adenosine di-phosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine tri-phosphate</td>
</tr>
<tr>
<td>bp</td>
<td>Base pair</td>
</tr>
<tr>
<td>DAPI</td>
<td>4’, 6-diamidino-2-phenylindole</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>ds</td>
<td>Double strand/ed</td>
</tr>
<tr>
<td>FL-Vanco</td>
<td>Fluorescently labeled Vancomycin</td>
</tr>
<tr>
<td>GFP</td>
<td>Green fluorescent protein</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilo Dalton</td>
</tr>
<tr>
<td>Mbp</td>
<td>Mega base pair</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger RNA</td>
</tr>
<tr>
<td>NO</td>
<td>Nucleoid occlusion</td>
</tr>
<tr>
<td>ORF</td>
<td>Open reading frame</td>
</tr>
<tr>
<td>oriC</td>
<td>Origin of chromosome replication region</td>
</tr>
<tr>
<td>PolI</td>
<td>Polymerase I</td>
</tr>
<tr>
<td>PolIII</td>
<td>Polymerase III</td>
</tr>
<tr>
<td>RER</td>
<td>Rough endoplasmic reticulum</td>
</tr>
<tr>
<td>RFP</td>
<td>Red fluorescent Protein</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>spp.</td>
<td>Species</td>
</tr>
<tr>
<td>ss</td>
<td>Single strand/ed</td>
</tr>
<tr>
<td>SSB</td>
<td>Single strand DNA binding protein</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>terC</td>
<td>Termination of chromosome replication region</td>
</tr>
<tr>
<td>tRNA</td>
<td>Transfer RNA</td>
</tr>
</tbody>
</table>
Introduction

Bacteria, next to viruses, are the most abundant form of life on planet earth (Whitman et al., 1998). They are single celled organisms that are fully capable of performing all the vital functions of life. Bacteria were once considered as “bags of enzymes” without a sub-cellular architecture. That description was based on their lack of a nuclear membrane, cell organelles or a cytoskeleton; these features give a characteristic cellular architecture to eukaryotic cells (Guerrero & Berlanga, 2007). With more detailed understanding of cellular ultra-structure and physiology at present, except for the nuclear membrane, there are evidences in bacteria in favor of all above mentioned criteria (Kerfeld et al., 2005, Murat et al., 2010, Smith & Brun, 2005, Shih & Rothfield, 2006).

Bacterial anatomy is simple yet complex. In principle, they consist of a cytoplasm surrounded by a cell membrane, which is covered by the cell wall. Cytoplasm is the site for major intracellular activities and contains various macromolecules such as nucleic acids and proteins. Amongst nucleic acids, double stranded (ds) deoxyribonucleic acid (DNA) forms the bacterial chromosome and plasmids; while ribonucleic acids (RNA) consist of varieties of classes: messenger, transfer, regulatory and ribosomal RNAs (Mayer, 1999). Proteins, on the other hand, furnish structural (cell membrane and cell wall) and functional entities (motors, enzymes) of a cell. Different macromolecules can form complexes with each other, referred to as molecular machines, to carry out complex cellular processes (Mayer et al., 2009).

The intracellular organization of these molecular machines contributes to the major differences in architecture of bacterial and eukaryotic (plant and animal) cells with the latter having fixed sub-cellular locations. For example, protein synthesis machineries, ribosomes, are located on the rough endoplasmic reticulum (RER) near the periphery of the nucleus in eukaryotic cells. The RER provides a platform for protein synthesis and folding. RER and other such membrane bound structures of eukaryotic cells are called cell-organelles. These organelles occupy specific intracellular locations and accommodate unique molecular machines that perform specific cellular functions. One of the major factors leading to differences in the sub-cellular architecture of prokaryotic and eukaryotic cell types is their size. A bacterial cell has typically 1000 fold less cell volume compared to that of a eukaryotic cell, where the majority of intracellular space is occupied by the
chromosome and there is not enough space for a well organized, permanent set-up of cell organelles (Prescott et al., 1996a, Prescott et al., 1996b).

Bacterial cells were once thought to be devoid of a cytoskeleton. Recent advances in biochemical and microscopy techniques have proved otherwise. Analogues of major eukaryotic cytoskeletal proteins have been identified in bacteria. Bacterial analogues of tubulin (FtsZ, MinCD), actin (MreB, FtsA ParM) and intermediate filament (Crescentin, FilP) proteins play vital roles in cell shape and morphology, sub-cellular architecture, cell division and differentiation (Ausmees et al., 2003, Bagchi et al., 2008, Bi & Lutkenhaus, 1991b, Bork et al., 1992, de Boer et al., 1989, Erickson, 2007).

Cellular differentiation is the key phenomenon in eukaryotic development. Embryonic cells are pluripotent cells that differentiate into specialized cells, such as muscle, liver and brain cells. Single celled bacteria were thought to lack cellular differentiation. However, bacteria possess a remarkable capacity for cellular differentiation (Smith & Brun, 2005). For example, the stalk cell and the swarmer cell of Caulobacter crescentis are morphologically distinct cell types specialized for different cellular functions. Spores are one of the most extensively studied bacterial differentiated cells that are important for tiding over unfavorable conditions.

Focus of the thesis

With the advancement in the understanding of the biochemical nature of bacterial and eukaryotic cells, it is apparent that these two cell types share common molecular constituents but differ in the composition and intracellular organization of molecular complexes (Guerrero & Berlanga, 2007, Gitai, 2005). The present thesis work investigates the subcellular localization of bacterial molecular complexes regulating the cell cycle, and the dynamics of their location in the cell. In this thesis I have studied molecular machines responsible for protein synthesis (ribosomes), chromosome replication (replisome) and cell division (divisome) throughout the cell cycle of diverse bacteria viz., Escherichia coli, Helicobacter pylori, Mycobacterium marinum and Mycobacterium smegmatis. The thesis also presents morphophysiological investigations into the process of cellular differentiation in the life cycles of H. pylori and M. marinum.
“Bugs” used in the thesis

The Bugs used in this thesis are *Escherichia coli*, *Helicobacter pylori* and *Mycobacterium* spp.. The trio represents the most common and historic bacteria associated with humans. All warm blooded animals carry *E. coli* in their gut. Its presence is beneficial as it provides Vitamin K to its host (Bentley & Meganathan, 1982) but it is also associated with most of the enteric and urinary tract disorders, which could vary from mild to lethal (Kaper et al., 2004). *H. pylori* defies the stomach pH as it is present in the upper intestine (pylorus region) of most humans. It is associated with ulceration of the intestine and adenocarcinoma (Zhang et al., 2005, Peek & Blaser, 2002). Different species of *Mycobacterium* produce deadly diseases in warm and cold blooded animals, including tuberculosis and leprosy (Smith, 2003, Hastings et al., 1988, Broussard & Ennis, 2007). The aim of the present thesis work is to understand the cell-cycles of these bacteria.

*Escherichia coli* (*E. coli*)

*E. coli* was first discovered by Theodor Escherich in 1885 and was named after him. It is a Gram-negative bacterium belonging to the enterobacteriaceae family of gamma-proteobacteria. *E. coli* is one of the most extensively studied bacteria and is used as a model system for genetics and biochemical studies (Hobman et al., 2007). It is a rod-shaped bacterium with an approximate size of 1.0 x 5.0 µm. It has a single, circular chromosome with a genome size of 4.6 Mbp (Blattner et al., 1997).

*Helicobacter pylori* (*H. pylori*)

This bacterium was identified by Barry Marshall and Robin Warren in 1982 (Marshall & Warren, 1984). They were awarded Nobel Prize for their discovery in 2005. *H. pylori* is a Gram-negative bacterium belonging to the helicobacteriaceae family of epsilon-proteobacteria. This rod-shaped bacterium has a helical twist in its morphology, hence named *Helicobacter*. Approximate size of a rod is 0.3 x 3.0 µm. *H. pylori* rods can differentiate into nonculturable, metabolically inactive coccoid forms (Benaissa et al., 1996, Costa et al., 1999, Cole et al., 1997, Kusters et al., 1997). Its genome
size is relatively small, which is represented by a single 1.7 Mbp chromosome (Tomb et al., 1997).

**Mycobacterium spp.**

100 years before the discovery of *H. pylori*, Robert Koch in 1882 linked tuberculosis to the tubercle bacillus (*M. tuberculosis*) and received the Nobel prize in 1905 (co-incidentally, 100 years before Marshall and Warren received the prize for their discovery). Mycobacteria are Gram positive rods belonging to the mycobacteriaceae family of actinobacteria (Hett & Rubin, 2008). Like other members of the Phylum Actinobacteria, mycobacteria possess a high G+C content genome. Mycobacterial species differ in their genome size. Chromosome sizes of *M. tuberculosis* H37Ra/v, *M. marinum* M and *M. smegmatis* MC2 155 are 4.4, 6.65 and 7.0 Mbp, respectively (Stinear et al., 2008, Cole et al., 1998, Zheng et al., 2008).
The bacterial cell cycle

The bacterial cell cycle, by definition, is vegetative or asexual reproduction of cell mass via periodic events of cell growth, chromosome replication and division. These events are referred as B, C and D periods, respectively (Nordström, 1999). Different bacteria exhibit similar schemes with individual variations in these events. This section will briefly describe cell cycle events as they occur in E. coli.

Figure 1. The bacterial cell cycle. Most bacteria reproduce by binary fission that constitute periods of cell growth (B-period), chromosome replication (C-period) and cytokinesis (D-period). Chromosomal replication initiates at a fixed position on the chromosome (oriC) shown by cross line in ds circular DNA; two replication forks formed at oriC proceed bi-directionally and meet at a diagonally opposite site from oriC, the terC. Upon duplication, chromosome is segregated such that each daughter cell receives a single copy of genomic material. Two daughter cells of equal size are born as a result of cell division at the middle of the mother cell. Newly born cells can enter into a fresh round of cell-cycle.

**B-Period** (Period Before Chromosome Replication): It is a period of cellular growth characterized by increases in cell mass due to new protein synthesis (Donachie, 1968). Time span of the B-period varies according to growth conditions. Fast growing cells have either shorter or no B-period. During the
slow growth conditions, cells require longer time before committing to chromosome replication. In *E. coli* cells growing at a generation time of 70 minutes, the B-period roughly represent 10 minutes of the cell cycle (Skarstad *et al.*, 1983).

**C-Period** (Period of Chromosome Replication): During this phase, a bacterial cell duplicates its chromosome via new DNA synthesis. Transcription and *de novo* protein synthesis are pre-requisites for the cells to enter into this phase, especially the synthesis of DnaA protein, which initiates the process of chromosome replication (Herrick *et al.*, 1996, Donachie, 1968, Fuller & Kornberg, 1983b). The chromosome replication process can be divided into three stages: initiation, elongation and termination. Replication starts and ends at fixed positions on the chromosome, namely oriC (Origin of Chromosome replication) and terC (termination of Chromosome replication) region, respectively (Masters, 1970, Kuempel *et al.*, 1977). Chromosome replication is bi-directional consisting of left and right replication forks (Cairns, 1963, Prescott & Kuempel, 1972, Masters & Broda, 1971). Compared to the growth phase (B-period) of the cell cycle, duration of this phase is relatively constant. *E. coli* cells complete duplication of its chromosome in about 40 minutes at optimal growth conditions (Helmstetter, 1996).

**D-Period** (Period of Cell Division): Two major events of this period are: separation of daughter nuclei and daughter cells. Segregation of the duplicated chromosomes is followed by the formation of a septum and cell division such that each daughter cell receives a single copy of the genomic material. An *E. coli* cell takes approximately 20 minutes to give birth to two individual cells following chromosome duplication (Cooper & Helmstetter, 1968).

The fate of a newly born cell depends upon the growth conditions. A favorable condition allows cells to undergo a new round of the reproduction cycle; otherwise they enter into the resting phase. Some bacteria carry out morphological changes during the resting phase. For example, in otherwise rod-shaped bacteria, “dormant” *H. pylori* and Mycobacterium cells transform into coccoid or ovoid form (Benaissa *et al.*, 1996, Anuchin *et al.*, 2009).
Molecular machines

Biochemical, genetic and microscopic studies, over a period of time, have led to the development of the concept that biological processes of a cell are carried out by highly organized and efficient molecular complexes, called molecular machines (Piccolino, 2000, Mayer et al., 2009). They are involved in most cellular processes like growth, maintenance of shape, motility, transport, replication, regulation, and metabolism. A few examples of molecular machines and their biological functions are listed in Table 1. However, the actual number of such machines could be much greater (Erhardt et al., 2010, Vendeville et al., 2011, Gavin et al., 2006).

Table 1. Examples of molecular machines in bacteria.

<table>
<thead>
<tr>
<th>Molecular machine</th>
<th>Cellular Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replisome</td>
<td>DNA synthesis</td>
</tr>
<tr>
<td>Divisome</td>
<td>Cell division</td>
</tr>
<tr>
<td>Ribosomes</td>
<td>Protein synthesis</td>
</tr>
<tr>
<td>Degradosome</td>
<td>RNA degradation</td>
</tr>
<tr>
<td>Flagellum</td>
<td>Cell motility</td>
</tr>
<tr>
<td>Pilus</td>
<td>Cell adhesion, DNA exchange, cell motility</td>
</tr>
<tr>
<td>Magnetosome</td>
<td>Cell orientation</td>
</tr>
<tr>
<td>Thylakoid</td>
<td>Photosynthesis</td>
</tr>
<tr>
<td>Chlorosome</td>
<td>Photosynthesis</td>
</tr>
</tbody>
</table>

A temporal and spatial arrangement of such a vast array of molecular complexes could pose an intra-cellular organizational challenge; especially, in a bacterial cell that, unlike their eukaryotic counterpart, lack the plethora of cell organelles. The overall emerging picture of a bacterial cell is revealing a highly structured intra-cellular architecture (Morris & Jensen, 2008, Guerrero & Berlanga, 2007, Costerton, 1979, Gitai, 2005, Losick & Shapiro, 1999, Lewis, 2004), with the bacterial chromosome playing a central role as it occupies the bulk of cytoplasmic space and is itself highly organized to ensure its efficient duplication, timely transcription and sequential movement during segregation (Viollier et al., 2004, Lau et al., 2003, Hiraga et al., 2000, Boccard et al., 2005, Sherratt, 2003). Molecular complexes dedicated to a particular cellular function localize at distinctive locations in a cell and are often dynamic in nature. Not only the intra-cellular
positions of these complexes are specified but also their time of appearance is regulated in a cell cycle dependent manner. For example, the replication machinery is recruited, in growing cells, at the oriC only at the time of initiation of chromosomal replication initiation (Lemon & Grossman, 1998, Reyes-Lamothe et al., 2008); the Z-ring is formed by the FtsZ protein at the cell centre during the D-period of the cell-cycle (Bi & Lutkenhaus, 1991b); molecular machines governing cell chemotaxis, bacteriophage and DNA uptake occupy cell poles (Alley et al., 1992, Edgar et al., 2008, Kaufenstein et al., 2011); partition specific complexes, ParAB, form cylindrical structures to aid chromosome or plasmid segregation (Ebersbach & Gerdes, 2005, Schumacher, 2008); molecular complexes determining bacterial shape, MreB and Mbl, are arranged in a cylindrical fashion in the cell wall (Margolin, 2009).

The current status of bacterial intra-structure and molecular machines is a subject of several reviews (mentioned above). The following discussion will focus on molecular machines for protein synthesis (ribosomes), chromosome replication (replisomes), and cell division (divisome).

The ribosome
The B-period of the bacterial cell cycle is dedicated to growth and protein synthesis, which is required for the cell to undergo DNA replication, cell division, and other physiological and metabolic functions. Unlike eukaryotic cells, bacteria lack a strict check-point regulation for growth, chromosome replication and segregation; rather they have a certain level of overlap (Mitchison, 2003, Haeusser & Levin, 2008, Wang & Levin, 2009). However, a link between cell growth or protein synthesis, which is represented in terms of cell mass, and initiation of chromosome replication has been established (Herrick et al., 1996). Proteins are synthesized by the most universally conserved molecular machines, the ribosomes.

Ribosomes are one of the largest molecular complexes made up of both proteins and RNAs. Bacterial ribosomes are classified as 70S type (figure 2A), which consists of two subunits: the larger subunit (50S) and the smaller subunit (30S). In E. coli, the 50S subunit contains 31 ribosomal proteins (Large subunit ribosomal proteins), and 23S and 5S ribosomal RNA molecules. The 30S subunit is formed from 21 ribosomal proteins (Small subunit ribosomal proteins) and the 16S ribosomal RNA (Ramakrishnan, 2002, Nierhaus, 1991, Dunkle & Cate, 2010, Dunkle et al., 2010). Ribosomes carry out the process of protein synthesis or translation by inter-bridging the amino-acids on a messenger RNA template. Two subunits work in concert, 30S subunits first binds to messenger RNA (mRNA), followed by its binding with the 50S subunit to make a complete 70S ribosome. The amino-acids are presented by transfer RNA (tRNA), in an orderly pattern
dictated by the mRNA codon sequence, to the ribosomes where individual amino acids are linked together by the peptide bond to form a polypeptide chain (Simonetti et al., 2009).

Ribosomes represent one of the most abundant molecular machines in a bacterial cell. Their actual number is several thousand per cell that varies with the growth conditions (Fegatella et al., 1998, Vendeville et al., 2011). Their vast number is a reflection of extensive protein synthesis in cells, especially during the exponential phase. Unlike eukaryotic cells where ribosomes are primarily localized at the membrane of RER, bacteria lack an organelle that can provide a fixed intra-cellular localization for ribosomes. Miller and Thomas provided the first evidence of organization of the huge repertoire of ribosomes in growing cells; their electron microscopic observation revealed: (i) presence of either a single ribosome or multiple ribosomes on a single mRNA, called polysomes, and (ii) mRNAs were translated while they were being produced on DNA templates leading to the concept of coupled transcription and translation in close proximity of the nucleoid (Miller et al., 1970). This coupling between transcription and translation plays an important role in subcellular architecture by influencing the nucleoid organization (Kleppe et al., 1979, Norris, 1995, Woldringh, 2002, Woldringh & Nanninga, 2006), as it was altered in absence of either transcription or translation or both (Ryter & Chang, 1975, Binenbaum et al., 1999, Cabrera et al., 2009).

Paper I (Localization of ribosomes in E. coli)
The subcellular architecture of a bacterial cell is organized to achieve and maintain a specific orientation of the domains of chromosomal DNA. Proteins with fluorescent tags binding at DNA segments inserted at different locations on the E. coli chromosome shows that left and right replichores occupy spaces left and right of the mid-plane in the bacterial cell and retain their specific orientations through the cell cycle and through subsequent generations (Lau et al., 2003, Sherratt, 2003, Bates & Kleckner, 2005). Since there is no clear evidence for the presence of a direct mechanism regulating such a high level of organization, coupled transcription and translation along with insertion of the polypeptide chain (transertion) seems to be the key regulator of chromosomal organization and subcellular architecture (Kleppe et al., 1979, Norris, 1995, Binenbaum et al., 1999). Active sites for transcription in bacteria are localized within the margins of the nucleoid (Ryter & Chang, 1975, Cabrera et al., 2009); while localization of translating ribosomes seems to differ among bacteria. During the bacterial growth, B. subtilis ribosomes have been shown to be marginalized towards the cell periphery (Lewis et al., 2000, Mascarenhas et al., 2001), while they were spread into the interior of the cell in Caulobacter crescentus (Montero Llopis
et al., 2010). In paper I, we present the localization of ribosomes in yet another bacterium, *E. coli* strain MG1655.

**Figure 2. Ribosomes at rest and action in *E. coli*. (A) *E. coli* ribosome structure as redrawn from the co-ordinates of crystal structure available on PDB database using PyMol (www.pymol.org); PDB id 3OAT and 3OAQ (Dunkle et al., 2010). Fluorescence tagged ribosomes (L9 fused with the fluorescent protein mCherry) were located in *E. coli* in during stationary phase (B) and in growing cells (C). (Size bar = 5 µm; Red: ribosomes; Green: chromosome).

We used fluorescence tagged ribosomes, aided with a fluorescence microscope, to locate their intracellular position/s in stationary phase cells and in actively growing cells. The 50S ribosomal protein L9 and the 30S ribosomal protein S6 were linked to mCherry and green fluorescent protein (GFP), respectively. Our data shows action-specific localization of ribosomes in *E. coli*; at rest they were located at the periphery of stationary phase cells (figure 2B), while they were spread to the cell interior during the active translation phase. Unlike a uniform spread in other bacteria (Lewis et al., 2000, Mascarenhas et al., 2001, Montero Llopis et al., 2010), *E. coli* active ribosomes were present in a helical form interspersed between nucleoid domains made visible by the DNA stain DAPI (figure 2C). Furthermore, we found that the helical arrangement of ribosomes was closely associated with helices of the bacterial cytoskeletal element MreB (Shih et al., 2003). Disruption of the MreB cytoskeleton also disrupts the helical distribution of ribosomes and the chromosome. A role of MreB in chromosome organization has been shown (Kruse et al., 2003); however, it is not clear whether redistribution of ribosomes or chromosomal reorientation upon MreB disruption is a result of a direct interaction between MreB-chromosome or MreB-ribosome or both. We looked at the localization and distribution of ribosomes throughout the entire cell cycle following a single cell in real time. Ribosome localization was dynamic as they were relocating in helical pattern with the progression of the cell cycle.
At the time of cell division, the bulk of ribosomes occupied mid-cell regions overlapping with the septal FtsZ-ring.

Next, we demonstrated the role of dynamic organization of ribosomes in determining subcellular architecture in *E. coli*. Interruption of either ribosomes-in-action or RNA synthesis abolished the helical arrangement of ribosomes and nucleoid domain architecture. Altogether, we have shown the localization of the ribosomes near the periphery at rest, and their mobility to the cell interior during active translation, signifying a dynamic action-specific localization of the ribosomes in *E. coli* cells.

The replisome

The Replisome is a multi-protein molecular machine dedicated to chromosome duplication (Johnson & O'Donnell, 2005). During the C-period of the cell cycle, replisomes assemble and initiate bacterial chromosome replication at a unique site, oriC (Masters, 1970). In *E. coli*, the minimum oriC is 245 bp long (figure 3), which contains three repeats (L, M and R) of 13-mer AT-rich regions on the left, and the initiator protein DnaA binding sites, DnaA boxes, on the right (Mott & Berger, 2007). There are five 9-mer DnaA boxes (R1, M, R2, R3, and R4), which bind indifferently to DnaA-ATP and DnaA-ADP; while three 6-mer DnaA boxes (I1, I2 and I3) bind preferentially to DnaA-ATP (Roth & Messer, 1995, Roth & Messer, 1998, Speck et al., 1999, McGarry et al., 2004).

![Figure 3. The minimal oriC of *E. coli* (highlighted) contains AT-rich LMR sites that are opened up by the binding of initiator protein DnaA at DnaA-boxes (R1, M, I1, I2, R2, R3, I3 and R4).](image)

Binding of the DnaA to its boxes marks the initiation of chromosomal replication by opening ds-DNA at the LMR region of the oriC (Fuller et al., 1981, Fuller & Kornberg, 1983a). Opening of the oriC region allows loading of the DnaB helicase, which is the main replicative helicase, with the help of the helicase loader protein, DnaC. Following DnaB loading on DNA, the DnaC protein has to leave the complex in order for DnaB to perform its helicase function (Wahle et al., 1989, Funnell et al., 1987). The DnaG primase binds to DnaB and the pair forms a primosome complex. DnaG
primase generates RNA primers that are utilized by DNA polymerase for the synthesis of a new DNA molecule (van der Ende et al., 1985). The presence of the single-strand-binding protein (SSB) is required for the stabilization of the primosome complex at oriC (Baker et al., 1986).

**Figure 4.** Replication forks and the minimum replisome at the replication fork. (A) Binding of DnaA, followed by subsequent loading of primosome and replisome at oriC creates left and right replication forks that move bidirectionally (large arrowheads); (B) each replication fork contains two units of PolIII holoenzyme linked to each other in parallel, due to which the lagging strand needs to form a trombone loop (Dixon, 2009).

A replication bubble thus formed due to DnaA-mediated unwinding of the oriC region, followed by DnaB loading, results in the formation of two replication forks (figure 4A). Thereafter, the assembly of the other components of the replisome follows at replication forks, including the DNA Polymerase III (PolIII) holoenzyme, which is comprised of PolIII* and the β sliding clamp, and is responsible for the new DNA synthesis. PolIII* consists of the core subunits (αθε), the γ complex (δψξψ) and the τ-subunit that connect two PolIII*, engaged in leading- and lagging-strand synthesis, together at the replication fork. A minimum replisome assembled at one replication fork is shown in figure 4B. Two DNA PolIII* are physically linked to each other in parallel (Pomerantz & O'Donnell, 2007, Kelman & O'Donnell, 1995); thus a minimum of two PolIII holoenzymes are present at one replisome (Dervyn et al., 2001). PolIII holoenzymes can synthesize new DNA only in the 5’- to 3’- direction. Therefore, DNA synthesis on one of the
template strands, the leading strand, continues uninterrupted; while it is discontinuous on the other template strand, the lagging strand, which forms a trombone loop to present the template DNA in a correct orientation (Park et al., 1998, Sinha et al., 1980). Another component of the replisome, Polymerase I (PolI) along with the RNaseH, removes RNA primers from the daughter chromosome during lagging strand synthesis (Okazaki et al., 1971, Kelly et al., 1970, Ogawa & Okazaki, 1984). PolI also fills the gaps that are subsequently being sealed by DNA ligase (Lehman, 1974). Single-stranded (ss) DNA regions at the replication fork are coated with SSB proteins, which protect the ssDNA regions as well as interact with different components of replisome; it modulates the activity of DnaB, Gyrase, PolI, and PolIII* (Baker et al., 1986, Michaels et al., 1986, Glover & McHenry, 1998, Arai & Kornberg, 1979, Kelman et al., 1998). Topoisomerases resolve the DNA supercoils, required for the progression of replication forks (Khodursky et al., 2000).

Paper II (H. pylori ORF HP1245 is an SSB protein)

The single-strand binding protein (SSB) is an essential protein involved in many vital cellular functions such as replication, recombination, repair and transformation. A common feature of these cellular functions is the presence of an ssDNA region, which is covered by SSB protein. SSB interact with the other factors involved in these cellular processes and modulate their activities (Meyer & Laine, 1990). During DNA replication, SSB remains an essential component of the replisome, it localizes at the ssDNA region of the replication forks (figure 4B), and has been shown to interact with DNA polymerase and helicase to facilitate their cellular functions (Kelman et al., 1998, Biswas et al., 2002, Glover & McHenry, 1998). Thus, fluorescence-tagged SSB could function as a reliable reporter for the active replication forks in live cells (Reyes-Lamothe et al., 2008).

H. pylori is a human pathogen, which causes gastric ulcers. We have begun to understand the mechanism of its replication and composition of its replisome (Nitharwal et al., 2011). In paper II, we reported that open reading frame (ORF) HP1245 in the annotated genome of H. pylori strain 26695 is a true SSB protein and it is an integral part of the replisome. We demonstrated that:

a) the molecular weight of the purified SSB protein is approximately 20 kDa and that it specifically binds only to ssDNA using gel retardation and electrophoretic mobility shift assay.

b) like E. coli SSB (Weiner et al., 1975), HP1245 too is present as a tetramer in solution.

c) in vivo, HP1245 can complement the loss of SSB function in E. coli. When fused with a fluorescent marker, it forms definitive foci only in exponentially growing cultures of H. pylori, and in immunofluorescence
assays, it co-localizes with the main replication helicase DnaB. This specific pattern of localization was absent in round, dormant forms. These data suggest that HP1245 is a true SSB protein and a component of the *H. pylori* replisome.

d) HP1245 directly interacts with DnaB and modulates its enzymatic activity in a concentration dependent manner. In a Glutathione S-transferase (GST) pull-down assay, HP1245 interaction was detected with GST-DnaB, while GST alone showed no interaction.

Our report is the first that demonstrates physical and physiological properties of HP1245 as an SSB protein, viz.: ssDNA binding, formation of tetramer, and its *in vivo* and *in vitro* interaction with replication protein DnaB. Furthermore, the intracellular location of the active replication centers were demonstrated as sharp fluorescent foci in exponentially growing cells of *H. pylori*. Absence of such foci in the morphological variant, coccoid cells, indicated these to be devoid of DNA replication activity.

The divisome

The D-period in the bacterial cell cycle comprises the events that lead to cell division and birth of daughter cells. Cell division is initiated by the polymerization of the tubulin-like septal GTPase protein, FtsZ, in a ring shape, called the Z-ring (figure 5C - i, ii; Adams & Errington, 2009, Bi & Lutkenhaus, 1991b, Bi & Lutkenhaus, 1991a). Assembly of the other components of the divisome follows the formation of the Z-ring (figure 5C - iii); these components stabilizes the Z-ring (ZipA), resolves the chromosome dimer and translocates DNA across the closing septum (FtsK), initiates downstream events that lead to septum maturation and constriction (figure 5AC - iv), and eventual cell division (de Boer, 2010, Errington *et al.*, 2003, Goehring & Beckwith, 2005, Rothfield *et al.*, 1999).

Spatio-temporal organization of the Z-ring is tightly regulated and well characterized in rod-shaped bacteria like *E. coli* and *B. subtilis* where Z-ring formation and divisome assembly occur at the precise mid-cell position. Mechanisms that (actively) prevent the formation of the Z-ring at positions other than mid-cell are fairly well understood in *E. coli* and *B. subtilis* (Lutkenhaus, 2007). At all stages of the cell cycle, Z-ring formation at the poles is inhibited by the MinCD system (figure 5A, B - ii). MinC is inhibitory to FtsZ polymerization and is localized at the poles with the help of MinD in *E. coli* (de Boer *et al.*, 1989, Dajkovic *et al.*, 2008). In *E. coli*, the MinCD complex oscillates from pole to pole such that at any point its time-integrated concentration is higher at polar regions than at the mid-cell position (Juarez & Margolin, 2010). Oscillations are driven by the MinE protein that destabilizes the polar MinCD complex (Raskin & de Boer, 1999). *B. subtilis*, however, has a fixed polar localization of MinCD with the
help of the DivIVA protein (Marston et al., 1998, Edwards & Errington, 1997, Cha & Stewart, 1997). Absence of the MinCD activity in E. coli has been shown to cause frequent polar septum formation (figure 5B - i) leading to minicell formation (de Boer et al., 1989).

Figure 5. The divisome and its organization in rod-shaped bacteria. Rod shaped bacteria like E. coli and B. subtilis divide by binary-fission following septation at the precise mid-cell position. (A) Formation of the Z-ring near the mid-cell in wild type bacteria as it grows and replicates. (B) Formation of the Z-ring in absence of MinCD or NO system (i, ii) and in non-replicating cells (iii). GFP fused FtsZ forming a ring at mid-cell position in wild type E. coli (iv). (C) Assembly of the Z-ring and divisome.

The MinCD system alone is not sufficient to guide the septum to its mid-cell location as cells lacking this regulation still have a high frequency of mid-cell division (Levin et al., 1998). This suggests the presence of additional mechanisms for precise FtsZ localization. Non-replicating and replicating cells lacking visible chromosome segregation prevent formation of the Z-ring over the nucleoid by a nucleoid occlusion (NO) mechanism (figure 5A, B - i, iii) mediated via the SImA and Noc proteins in E. coli and B. subtilis, respectively (Bernhardt & de Boer, 2005, Wu & Errington, 2004, Wu et al., 2009). In the absence of NO, the Z-ring could be seen over the nucleoid (figure 5B - ii).

Although formation of the Z-ring has been shown to be independent of chromosome replication initiation (figure 5B - iii) (Gullbrand & Nordström,
2000), a Z-ring could only be formed at the mid-cell position when cells have almost finished its replication cycle and the majority of the chromosome has segregated (Wu et al., 1995). Moreover, Noc localization has been shown to be highly concentrated near the oriC region in comparison to its association with the terminus region (Wu et al., 2009). Thus, a potent negative regulation near poles and over the nucleoid leaves only the mid-cell region of the cell for polymerization of the FtsZ.

Paper III (cell division in Mycobacteria)

Mycobacterial cell cycle stages and their regulation are relatively unknown. Mycobacteria display variant life styles from inhabiting intra- and extra-cellular niches to the formation of biofilms, and pleomorphy in their cell morphology even though they are classified as rod shaped bacteria (Carter et al., 2003, Monack et al., 2004, Vaerewijck et al., 2005, Wayne, 1994). The enormous amount of work on cell division in the model bacteria, *E. coli* and *B. subtilis*, provides detailed insight into the mechanism of cell-division in rod-shaped bacteria. As discussed above, these bacteria divide at a fixed mid-cell position (Bramkamp & van Baarle, 2009). Placement of the septum at positions other than the mid-cell is prevented by negative regulations imposed by MinCD and NO mechanisms (de Boer et al., 1989, Bernhardt & de Boer, 2005, Wu & Errington, 2004). Interestingly, neither determinant of septum position (MinCD or NO) has yet been identified in mycobacteria (Hett & Rubin, 2008). Both *E. coli* and *B. subtilis* form septa at aberrant positions in absence of these regulators (Lutkenhaus, 2007). In paper III, we looked at the site of cell division in mycobacteria that appear to lack any known regulatory mechanism for division site selection.

There is no clear evidence regarding the choice of a specific site of cell division in mycobacteria. Apart from a single report suggesting asymmetry in mycobacterial cell division (Thanky et al., 2007), most cell division studies have taken mid-cell as the division site in mycobacteria for granted without any detailed measurement (Maloney et al., 2009, Nguyen et al., 2007, Kang et al., 2008). The issue of site-selection for cell division in mycobacteria therefore remains unclear and needs to be investigated further.
In paper III, we studied the positions of septa in the marine pathogenic mycobacterium *M. marinum* NCTC 2275 (ATCC 927) and the non-pathogenic *M. smegmatis* mc2 155 (ATCC 700084). Positions of septa were located by examining cells stained with fluorescently labeled Vancomycin (FL-Vanco) and the membrane dye FM4-64, using fluorescence microscope. FL-Vanco fluorescence indicated the site of ongoing peptidoglycan synthesis; these sites in the case of mycobacteria are the poles and the septa. FM4-64 labeled the entire cell contour including the septa. Both methods satisfactorily indicated the position of mature septum in growing cells. Our data showed that:

a) the positions of septa in rod-shaped mycobacteria is not fixed at the mid-cell. Septal positions in growing populations of *M. marinum* and *M. smegmatis* cells exhibit a wide range of deviation, ranging from the pole to the mid-cell. About 23% of the *M. marinum* and 40% of the *M. smegmatis* cells form septa at positions other than at the mid-cell.

b) we demonstrated probable translocation of the nucleoid through polar and medial septa. The *M. smegmatis* protein MSMEG2690 is annotated as a putative FtsK/SpoIII like DNA translocase. We found that it is associated with septa in dividing cells and thus might represent a true DNA translocase in *M. smegmatis*.

c) non-medial cell division would give rise to two unequal sized daughter cells. With time lapse observation of cell growth and division in real time, we demonstrated that differences in progeny size due to non-medial septa were often compensated by cell growth.

Our observations clearly show that the formation of non-medial septa, in mycobacteria, is not due to the result of differences in the bi-polar growth. However, differences in bi-polar growth might add some level of asymmetry to the size of the daughter cell population. We have also shown that the non-
medial septa are present in a “considerably” larger frequency in mycobacterial cells than in model systems.

**Cell differentiation: Sporulation**

Bacteria inhabit a wide range of ecological niches, which is reflected in their diversity in metabolic processes (Whitman *et al.*, 1998). In their respective natural niches, bacteria face varied masses of environmental challenges, such as: nutritional deprivation to starvation, host immune system, physical stresses of heat, desiccation, radiation etc. Their survival through these challenges and their proliferation under unfavorable circumstances depends upon their adaptability in response to the stress imposed upon them (Aertsen & Michiels, 2005). Bacteria differ in their response and adaptation to various stresses; these responses are regulated via complex networks of gene expression and are aimed to repair the damages imposed by the stresses, adjustment to the altered nutritional regimen (Hecker & Völker, 2001, Foster, 2005), or to cease cellular functions to enter a stage of dormancy until the return of favorable conditions for growth (Mukamolova *et al.*, 2003).

During dormancy, some bacteria undergo a process of cellular differentiation and develop into a more resistant, morphologically distinct, form like a cyst or a spore (Smith & Brun, 2005). Of all forms of cellular differentiation found in bacteria, sporulation is one of the most extensively studied. In *B. subtilis*, which form endospores, the entire process of sporulation requires a huge plethora of over one hundred proteins (Piggot & Coote, 1976, Stragier & Losick, 1996), and is an elegant example of sequential and interdependent regulation of gene expression in time and space (Errington & Scanzocchio, 2003). Developmental changes that lead to the formation of endospores are well characterized, such as: formation of a forespore (prespore) following asymmetrical septation, engulfment, formation of a cortex and an outer coat followed by release of mature spores (figure 7). Released spores, thus, have different morphological features distinct upon light and electron microscopic examination (Errington, 1993, Levin & Grossman, 1998).
Paper IV (Sporulation in Mycobacteria)

The class Actinobacteria contains G+C rich bacteria that are extremely different in their morphological features as they can be found as rods or long filamentous forms. These bacteria inhabit very challenging environments such as soil, water, plants and animals (Locci, 2001), and have evolved mechanisms to cope with different stresses imposed upon them (Ventura et al., 2006, Deb et al., 2009). Formation of a quiescent dormant form and sporulation, in response to different stresses, is common among bacteria belonging to this class (Deb et al., 2009, Locci, 2001). A few examples among the members of subclass Actinobacteriadae are: morphologically and physiologically distinct “dormant” forms are found in species of Micrococcus, Arthrobacter and Mycobacterium (Mulyukin et al., 2010, Shleeva et al., 2011, Mulyukin et al., 2009, Demkina et al., 2000, Mukamolova et al., 2003); “Aleuriospores” has been shown in species of Micromonospora (Hardisson & Suarez, 1979), species of Streptomyces form “exospores” (arthrospores) (Chater, 1993), and “endosporulation” has been reported in S. avermitilis (Filippova et al., 2005).
Figure 8. Sporulation in Mycobacterium marinum. Red Fluorescent Protein (RFP) or Green Fluorescent Protein (GFP) containing integration-vectors pDEAM2 and pDEAM1 (Gift from Dr. Don Ennis) were introduced in M. marinum NCTC 2275 (ATCC 927) strain (Gift from Dr. B.M. Fredrik Pettersson), resulting in strains BSm124 and BSm118 respectively. Phase-Fluorescent microscopic examination shows presence of spores with fluorescence in cultures. (Size bar = 5 µm)

Mycobacteria are defined as rod shaped non-sporulating Actinobacteria. However, they are pleomorphic under different conditions, including differentiation into a round circular dormant form (Csillag, 1961, Csillag, 1963, Chang & Andersen, 1969, Lack & Tanner, 1953, Häggblom et al., 1994, Anuchin et al., 2009, Mulyukin et al., 2010, Shleeva et al., 2011). During infection, dormant mycobacteria remain quiescent leading to chronic infection (Monack et al., 2004, Russell, 2007). There have been reports indicating yet another form of differentiation, sporulation, in mycobacteria (Brieger & Glauert, 1956, Csillag, 1961, Csillag, 1970, Csillag, 1963). But
due to inconsistency in the phenomenon and the possibility of a contamination, it was never clear whether mycobacteria produced spores or not (Hilson, 1965).

A thorough investigation into the life cycle of mycobacteria was thus needed, especially in the scenario that different mycobacterial species cause acute, chronic and latent infections in various hosts including humans; that information regarding their life cycle and survival strategies in varieties of environments is not well understood, which could provide more insight into the treatment or control regimen (Singh et al., 2010). In paper IV, we have followed changes in (i) cell morphology using phase contrast microscopy, and (ii) DNA contents indicating the state of chromosome replication using flow-cytometry to analyze *M. marinum* T CCUG 20998 (ATCC 927) inocula from a 2 months old stock through exponential growth and into late stationary phase. Our observations show the presence of spore-like particles in a 2 months old stock of *M. marinum*, which disappeared during the growth phase with ongoing chromosome replication as indicated by flowcytometric-analysis. We verified these particles as true spores by scanning and transmission electron microscopy (SEM & TEM). TEM data showed a multilayered morphology typical of spores. The old cultures were resistant to wet-heat at 65°C for 15 minutes, had increased levels of dipicolinic acid and showed increased expressions of putative sporulation genes, analogues from *B. subtilis*, identified in a bioinformatics search. We also reported the presence of spores in old cultures of *M. bovis* Bacillus Calmette-Guérin, suggesting a possible wide-spread occurrence of sporulation in mycobacteria.

This finding, however, has been challenged. Traag B.A. and co-workers have reported the absence of spore in the same strain of *M. marinum* under the growth conditions reported in paper IV, raising a suspicion of possible contamination (Traag et al., 2010). In the article mentioned in the section “additional reading” (Singh et al., 2010), we have reported the presence of spores in the same strain of *M. marinum* that contained the gene encoding GFP with constitutive expression. The GFP encoding gene was integrated into the mycobacterial genome with the help of an integration vector, refuting the possibility of contamination. In an independent experiment, I examined the RFP and GFP expressing strains of *M. marinum* strain NCTC 2275 (ATCC 927). Presence of the fluorescence marker is a strong indicator of the desired bacteria. We could reproducibly demonstrate the presence of spores in *M. marinum* cultures; the presence of fluorescence in these spores confirms *M. marinum* to be their origin (figure 8). The observation strongly supports “sporulation” as a valid differentiated state for mycobacteria.
Overall conclusions

This thesis work was aimed to describe some of the major life-cycle events during growth and reproduction of bacteria, in terms of the molecular complexes/machines assembled to perform protein and DNA synthesis, division and morphological variation. I explored different bacteria for the spatially and timely localization of ribosomes, replisomes and divisome during distinctive cell cycle stages, and for their morphological differentiation in response to stresses imposed by conditions in old cultures. Overall, this study has demonstrated the following:

Paper I
1. The dynamic distribution of ribosomes from the cell-periphery at rest to the interior of the cell during growth in *E. coli* cells.
2. The critical role of active transcription and translation machineries in maintaining nucleoid shape and the subcellular architecture.

Paper II
1. *H. pylori* ORF HP1245 is an SSB protein.
2. An HP1245 interaction with the replicative helicase protein.
3. The presence of the replication complex only in growing helical rods, and its absence in dormant coccoid cells.

Paper III
The absence of a fixed site for cell division in rod-shaped mycobacteria.

Paper IV
*Mycobacterium* spp. form spores.
Svensk sammanfattning


En bakteriecell utför alla sina funktioner med ett nätverk av molekylära maskiner. Dessa maskiner består av olika komponenter som proteiner och ribonukleinsyror (RNA) som passar ihop med varandra, på många sätt likt hur delarna i en vanlig mekanisk maskin passar ihop. Eftersom dessa maskiner utför sina uppgifter på molekylär nivå i cellen kallas de molekylära maskiner. Liksom en mekanisk maskin utför den molekylära maskinen en specifik uppgift i cellen och alla delar av maskinen arbetar tillsammans för att utföra dess uppgift. Som exempel på molekylära maskiner kan nämnas:

1. ribosomer; molekylära maskiner som gör nya proteiner i cellen,
2. replisomer; molekylära maskiner som gör nytt DNA i cellen,
3. divisomer; molekylära maskiner som gör nya celler genom att dela en modercell i två dotterceller.

tillväxtförhållanden. Under den här fasen som benämns stationär fas vilar bakterierna. Vissa bakterier omvandlar sig under denna fas till en annan celltyp kallad spor. En spor är mer motståndskraftig mot tuffa miljöförhållanden.

I min avhandling har jag undersökt var de molekylära maskinerna tillverkar proteiner, var de kopierar kromosomen, och var de gör så att cellen delar sig. För att kunna lokalisera maskinerna i cellen märkte jag dem med ett fluorescerande protein som ger ifrån sig en fluorescerande signal när man lyser på det med ljus av en specifik våglängd. Signalen man ser visar positionen för de molekylära maskinerna inuti cellen. För att kunna se bakterierna och den fluorescerande signalen inuti dem använde jag mig av ett fluorescensmikroskop.

Ribosomer: I vilande *E. coli*-celler i stationär fas där ingen proteintillverkning pågår befinner sig ribosomerna i cellens perifera delar. I växande celler däremot, där proteintillverkning är nödvändig, lämnar ribosomerna periferin och förflyttar sig till cellens centrala delar (figur 2). Denna omflyttning av ribosomer ändrar också formen på kromosomen och cellens inre arkitektur.

Replisomer: Vi undersökte replisomerna i *H. pylori*, bakterien som orsakar magsår. Dessa bakterier bildar helixformade stavar när de växer och omformas till en rund cirkulär form i vila. Vi visade att replisomer bara existerar i de helixformade stavarna och inte i de runda cellerna. Detta betyder att dessa bakterier bara kopierar kromosomen när de är i helix-stavform.

Divisomen: Stavformade bakterier som *E. coli* delar sig genom binär fission och resulterar i två lika stora dotterceller. Detta är endast möjligt om divisomen delar modercellen exakt i mitten. Jag har undersökt var divisomen är lokalisera i mycobakterier, också de stavformade bakterier. Jag kom fram till att mycobakterieceller inte har divisomen exakt i mitten av cellen. En del celler har den i mitten, medan andra celler har den vid sidan av (figur 7). Det verkar vara så att mycobakterier kan dela sig var som helst, något som är unikt bland bakterier eftersom nästan alla kända bakterier delas från en förutbestämd position.

När vi tittade på mycobakterier i stationär fas eller vilofas upptäckte vi att de bildar sporer (figur 8). Detta är en helt ny upptäckt eftersom mycobakterier alltid ansetts inkapabla att bilda sporer.

I korthet: i den här avhandlingen har jag tittat på var bakterier bildar proteiner (ribosomer), hur de kopierar sin kromosom (replisomer), och hur de delar sig (divisomer).
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References


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