Compartmentalization and spatiotemporal organization of macromolecules in bacteria

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Abstract
For many years, the bacterial cells were regarded as tiny vessels lacking internal organization. This view, which stemmed from the scarcity of membrane-bounded organelles, has changed considerably in recent years, mainly due to advancements in imaging capabilities. Consequently, despite the rareness of conventional organelles, bacteria are now known to have an intricate internal organization, which is vital for many cellular processes. The list of bacterial macromolecules reported to have distinct localization patterns is rapidly growing. Moreover, time-lapse imaging revealed the spatiotemporal dynamics of various bacterial macromolecules. Although the regulatory mechanisms that underlie macromolecules localization in bacterial cells are largely unknown, certain strategies elucidated thus far include the establishment of cell polarity, the employment of cytoskeletal proteins, and the use of the membrane properties, that is, curvature, electric potential, and composition, as localization signals. The most surprising mechanism discovered thus far is targeting of certain mRNAs to the subcellular domains where their protein products are required. This mechanism relies on localization features in the mRNA itself and does not depend on translation. Localization of other mRNAs near their genetic loci suggests that the bacterial chromosome is involved in organizing gene expression. Taken together, the deep-rooted separation between cells with nucleus and without is currently changing, highlighting bacteria as suitable models for studying universal mechanisms underlying cell architecture.

Introduction
Clustering of functionally related molecules in a complex system is a thermodynamically favorable process, because such an arrangement is expected to yield optimal functioning with reduced disturbances (Zimmerman & Trach, 1991). Membrane-bounded structures, which maintain the constituents of discrete processes together, represent a strategy for achieving this type of organization within living systems. The main difference between the two major domains of life, eukaryotes and prokaryotes, is that, excluding exceptional cases, prokaryotic cells lack membrane-bounded organelles. Because the differential protein composition of specific eukaryotic organelles reflects their function, the scarcity of such organelles in prokaryotes led to the general assumption that proteins and other macromolecules are randomly distributed throughout the cell. The bacterial cell wall, the membrane, and the nucleoid were regarded as exceptions, as these domains are known to recruit specific proteins. However, the unexpected observation of cytosolic proteins localizing to specific subcellular domains, without an apparent physical structure or a membrane-surrounded organelle (Rudner & Losick, 2010), surprised cell biologists and questioned the traditional view of bacterial cells as bags of freely diffusing macromolecules.

Recent advancements in imaging capabilities enable better visualization of bacterial cells and hence the investigation of their internal organization and dynamics. Among the earlier examples for bacterial proteins that have a distinct subcellular localization were the cell division protein FtsZ and the constituents of the chemotaxis complex in Escherichia coli. In both cases, the proteins were initially visualized by immunoelectron microscopy, FtsZ as a...
discrete ring in the center of dividing cells (Bi & Lutkenhaus, 1991) and the chemoreceptors at the cell poles (Alley et al., 1992). Visualization of the two proteins was improved after fusing the proteins under study to fluorescent proteins, such as GFP and yellow fluorescent protein (YFP), and observing them in live cells (Ma et al., 1996; Sourjik & Berg, 2000), a technique that became the leading method for visualization of proteins in the tiny bacterial cells. Extensive studies that combined fluorescence microscopy with genetic manipulations in three model bacteria, E. coli, Bacillus subtilis and Caulobacter crescentus, led to the identification and characterization of several spatiotemporal regulatory mechanisms (Shapiro et al., 2002; Collier & Shapiro, 2007; Thanbichler & Shapiro, 2008; Kirkpatrick & Viollier, 2010; Rudner & Losick, 2010; Toro & Shapiro, 2010). Recently, new experimental approaches enabled the investigation of mRNA localization in bacterial cells (reviewed in Broude (2011)), thus revolutionizing our view of the spatial relationship between the different stages of gene expression in these allegedly ‘noncompartmentalized’ cells. The observations regarding proteins and RNA localization, combined with the detection of lipid domains within the membrane (Matsumoto et al., 2006), the complex arrangement of the chromosome (Toro & Shapiro, 2010), the active movement of the oriC element to the poles during DNA replication (Toro & Shapiro, 2010), the non-random distribution of the ribosomes (Lewis et al., 2000), and the formation of transcription foci at actively transcribed rRNA genetic locus (Davies & Lewis, 2003) strengthen the view that clustering and compartmentalization of the cytoskeleton are central themes that underlie spatiotemporal control of molecular processes in bacterial cells.

The list of bacterial macromolecules with distinct subcellular localization is increasing rapidly. Using a high-throughput method for quantitative genome-scale analysis of protein localization in C. crescentus, Gitai et al. were able to show that over 10% of the proteins in this organism, around 10-fold more than previously estimated, display a nonuniform distribution (Werner et al., 2009). Notably, the number of localization patterns that have been observed is rather limited, with the majority of the proteins localizing to the poles, to midcell or demonstrating a patchy distribution along the cell axis (Werner et al., 2009). Although relatively little is known about the precise mechanisms that establish the bacterial cell architecture, certain strategies like cell polarity, cytoskeleton dependence, the contribution of various membrane features, and, recently, mRNA targeting were documented as being involved in the spatiotemporal control of protein localization and of protein complex formation. Below, we will touch upon these mechanisms and their involvement in macromolecule localization and compartmentalization in bacteria.

The involvement of the cytoskeleton in protein localization

The discovery of bacterial cytoskeletal proteins, for example, the actin-like proteins MreB and ParM, the tubulin-like protein FtsZ, and the intermediate filament protein crescentin, reinforced the strong similarity between prokaryotes and eukaryotes, thus highlighting the importance of bacteria as a model system for cellular organization (Cabeen & Jacobs-Wagner, 2010). Studies in the last decade demonstrated that many functions of eukaryotic cytoskeletal proteins, such as cell division, chromosome segregation, organelle movement, and cell shape maintenance, are applicable to their prokaryotic homologs (Vats et al., 2009a, b; Cabeen & Jacobs-Wagner, 2010; de Boer, 2010; Erickson et al., 2010; Jockusch & Graumann, 2011).

In addition to homologs of the eukaryotic cytoskeletal structures, which is, actin, tubulin, and intermediate filaments, bacteria contain also distinct cytoskeletal proteins, which are not known to have homologs in eukaryotes (MinD and ParA), and the list of proteins that form cytoskeletal-like structures in bacteria is still growing (Vats et al., 2009a, b; Ingerson-Mahar & Gitai, 2012).

Much of what we know about the involvement of cytoskeletal structures in bacterial protein localization has come from the studies of FtsZ, which assembles in midcell to form the Z-ring, eventually leading to the recruitment of over two dozens of proteins to form the divisome and the mature septal ring (de Boer, 2010). However, the role of the tubulin-like FtsZ in protein localization, extensively reviewed over the years, has been mostly investigated with respect to cell division. The actin-like MreB protein, on the other hand, was suggested to be implicated in localization of proteins that function in diverse cellular processes, for example, cell shape determination, cell growth and differentiation, polarity, motility, chromosome dynamics, and even bacterial pathogenesis (reviewed in Carballido-Lopez et al., 2006; Cabeen & Jacobs-Wagner, 2010; Shaevitz & Gitai, 2010).

In addition to regulating protein localization, MreB and other bacterial cytoskeletal proteins that form filament-like structures were suggested to play an important role in localizing large protein complexes and even organelles, for example, the magnetosome in Magnetospirillum magneticum (Komeili et al., 2006), the carboxysome in Synechococcus elongates (Savage et al., 2010), the pilus in Pseudomonas aeruginosa and Myxococcus xanthus (Cowles & Gitai, 2010; Mauriello et al., 2010), the stalk in Caulobacter (Wagner et al., 2005; Divakaruni et al., 2007), and inclusion bodies in E. coli (Rokney et al., 2009). However, except for cell shape determination, the molecular mechanisms and the possible MreB-interacting partners that are involved in the different processes are largely unknown.
Recently, the use of novel imaging technologies to document MreB localization and its linkage to the cell wall synthetic machinery opened an interesting and timely discussion on the nature of localization patterns that are inferred from imaging data. Hence, we chose to focus below on MreB and its involvement in setting up the cell morphogenesis.

MreB is widely conserved in rod-shaped bacteria and is dispensable in several cocci (Carballido-Lopez et al., 2006). Certain bacterial species, like B. subtilis, encode more than one MreB paralog (Shaevitz & Gitai, 2010). The mre locus was first identified in E. coli as encoding genes that are involved in cell shape maintenance (Wachi et al., 1987). The idea that MreB functions as a prokaryotic actin-like protein in determining cell morphogenesis was suggested based on sequence similarity (Bork et al., 1992) and supported by the in vivo observation of MreB filamentous structures in B. subtilis (Jones et al., 2001). The ATP/GTP-dependent polymerization of Thermotoga maritima MreB1 into filaments in vitro (van den Ent & Lowe, 2000) reinforced this notion. The MreC, MreD, Pbp2, RodA, and RodZ proteins, as well as several others, were shown to associate with the MreB cytoskeletal structures and to be involved in cell shape determination (Shiomi et al., 2008; Alyahya et al., 2009; Bendezu et al., 2009; Shaevitz & Gitai, 2010; van den Ent et al., 2010).

Many studies pointed at the tight linkage between MreB and cell wall synthesis. The mechanical stiffness and rigidity of MreB filaments were shown to provide additional support to the peptidoglycan layer, the major cell wall component, in maintaining the overall cell shape (Wang et al., 2010). Moreover, MreB and its homologs were shown to be directly involved in cell wall synthesis. By and large, cell wall synthesis in rod-shaped bacteria can be divided into two stages: cell elongation, which is linked to the localization of MreB-associated cytoskeleton, and septal division, which is affected by the FtsZ ring positioning. Efficient functioning of both processes depends on the ability of these two proteins to recruit other proteins involved in the each stage (Mattei et al., 2010). Using fluorescently labeled vancomycin, an antibiotic that binds to peptidoglycan, cell wall elongation in B. subtilis was shown to follow the distribution pattern of Mbl, an MreB homolog, and was suggested to be governed by it (Daniel & Errington, 2003). Both patterns, of MreB and its homologs and of the cell wall synthetic machinery, were interpreted as helical structures, although this interpretation is currently debatable in light of new data (see below).

Recent studies in C. crescentus shed light on how the cytoplasmic MreB can impact the assembly of the cell wall, which occurs outside of the inner membrane. The explanation seems to be provided by the intricate machinery of MreB-interacting partners, which likely function in efficient channeling of metabolic intermediates and site-directed synthesis of peptidoglycan. In an elaborate study, the cytoplasmic proteins MurB, MurC, MurE, MurF, and the inner membrane proteins MurG and MraF, all functioning in initiating peptidoglycan synthesis, were shown to exhibit a similar localization pattern that directly depends on a network of MreB–MreD–RodA interactions (White et al., 2010). This dependence was validated by perturbation of the observed localization pattern, using the MreB-inhibitor A22; the interactions between the proteins were confirmed by two-hybrid assays. Then again, periplasmic proteins involved in the final stages of peptidoglycan synthesis, including PBP2, PBP3, MipA, and MltA, were shown to interact with the transmembrane protein MreC and to exhibit a pattern which is similar to MreB, that is, a helical path (White et al., 2010). This study, together with other studies, suggested that the Caulobacter MreB and MreC affect peptidoglycan precursor synthesis and assembly in the cytoplasm and in the periplasm, respectively (Figgie et al., 2004; Divakaruni et al., 2005; Dye et al., 2005). However, despite the similar localization patterns, an apparent interaction between MreC and MreD or MreB was not demonstrated in two-hybrid experiments (White et al., 2010). Nevertheless, because such interactions were reported to occur in B. subtilis (Kruse et al., 2005), it is still possible that MreB, MreC, and MreD, together with other interacting proteins, create a common interface for the cytoplasmic and periplasmic peptidoglycan synthesizing proteins. Intriguingly, in some species, MreB localization depends on MreC, whereas other species have either MreB or MreC (Kruse et al., 2005), suggesting the existence of species-specific machineries for cell wall synthesis.

Recently, a more detailed picture of the subcellular organization of MreB and the cell wall synthetic machinery has been obtained by three independent studies that imaged the movement of MreB structures and found it to be inconsistent with the previous description of MreB as a continuous helix that extends from pole to pole. Using high-precision particle tracking (Garner et al., 2011) and total internal reflection fluorescence microscopy (Dominguez-Escobar et al., 2011), the MreB in B. subtilis was shown to form discrete patches that move processively and circumferentially along helical tracks, perpendicularly to the cell axis. The directional motions of MreB in the B. subtilis cells were often discontinuous and independent, inconsistent with the existence of a coherent, long-range MreB cytoskeleton. Similarly, the E. coli MreB was also shown to display heterogeneous spots that rotate around the long axis of the cell in a persistent manner (van Teeffelen et al., 2011). The observed localization
pattern was consistent with the lack of long helical filaments also in this case. Significantly, the dynamic rotational movement of the MreB patches was shown in these studies not to be caused by MreB polymerization, but rather by the force exerted by cell wall synthesis. A similar rotational motion was observed also for components of the cell wall synthesis complexes. Together, these studies revealed a mobile, fragmented picture of MreB, rather than a contiguous helical structure. On the other hand, a very recent study demonstrated that the chirality of MreB in rod-shaped bacteria gives rise to a chiral insertion of peptidoglycan into the cell wall and causes cells to twist in the same handedness as their MreB during elongational growth (Wang et al., 2012). Together, these new studies opened a discussion about other bacterial proteins, whose organization has previously been interpreted as continuous helices. Additional analyses are required to unequivocally determine to what extent the interpretation of a continuous vs. a patchy and discontinuous filament is methodology dependent or, in fact, represents different patterns that exist in the bacterial cell. The clusters comprising of cytoskeletal elements and the peptidoglycan synthesizing proteins that move along helical tracks are schematically presented in Fig. 1.

Quite unexpectedly, partition of MreB in the two daughter cells during cytokinesis of E. coli, C. crescentus, and Rhodobacter sphaeroides was shown to involve the formation of an MreB medial ring in predivisional cells, which then relocates to the original structures just before completion of the division process (Figge et al., 2004; Gitai et al., 2004; Slovak et al., 2005; Vats & Rothfield, 2007). The E. coli MreB cytoskeletal ring was shown to contain also the MreB-interacting proteins MreC, MreD, Pbp2, and RodA proteins. Whereas the MreB, MreC, MreD, and RodA were each able to independently assemble into the cytoskeletal ring, incorporation of Pbp2 into the ring required MreC (Vats et al., 2009b). This dynamic process, which is triggered by the membrane association of the FtsZ cell division protein, apparently leads to equal partition of MreB and the other cytoskeletal proteins between the daughter cells.

Summarily, studies thus far established the central role of bacterial cytoskeleton proteins in determining cell morphology and in maintaining cell shape. Additional studies are required to elucidate their involvement in the spatiotemporal regulation of other processes.

Membrane properties affecting protein localization

Accumulating evidence for compartmentalization and nonrandom localization of proteins in bacterial cells suggests the existence of prelocalized cues, which affect the localization of proteins to certain subcellular domains. Proteins that are not known to require other proteins for their localization may be useful for the identification of these cues. Below, we discuss studies of such proteins, which have identified certain properties of the cytoplasmic membrane, that is, membrane curvature, membrane potential, and lipid composition, as localization signals.

Membrane curvature

Recent studies from Losick’s laboratory identified membrane curvature, which is reflected in cell shape and architecture, as a physical cue for protein localization. Significantly, both concave (negative curvature) and convex (positive curvature) shapes can act as beacons for localization of different proteins. The nonuniformity in membrane curvature is schematically shown in Fig. 2. The first protein, whose localization was shown to be determined by membrane curvature, was the peripheral membrane protein SpoVM, a small amphipathic alpha helix peptide, expressed exclusively in the mother cell during B. subtilis sporulation (Ramamurthi et al., 2006). SpoVM is tethered to the spore surface and, along with SpoIVA, forms the spore coat complex. Because genetic and biochemical techniques did not identify any other protein as necessary for SpoVM localization, it was assumed that
SpoVM recognizes a nonchemical factor that is found exclusively in the forespore wall. A strong candidate for such a cue was the positive curvature of the forespore membrane, because this type of topology is not present anywhere else in the sporulating cell (Fig. 2c). Imaging of GFP-tagged SpoVM in cell division-defective mutants reinforced the notion that positive curvature acts to recruit SpoVM to the spore coats. In a mutant producing incomplete polar septum and hence devoid of convex-shaped positive membrane, SpoVM-GFP localizes indiscriminately to the inner membrane of the mother cell and the outer membrane of the forespore. SpoVM sensing of positive curvature was also validated in vitro by its specific binding to the outer surface of liposomes and in vivo by observing SpoVM localizing to positively curved membrane structures of the heterologous cells of *E. coli* and *Saccharomyces cerevisiae*. All these results implied that SpoVM is capable of sensing positive membrane curvature for its localization (Ramamurthi *et al.*, 2009).

Can negative membrane curvature also act as a cue for protein localization? This question was addressed by two research groups that investigated the subcellular localization of DivIVA. The DivIVA protein of *B. subtilis* is involved in two important processes, namely, prevention of polar septum formation (Bramkamp *et al.*, 2008) and segregation of the chromosomal DNA during sporulation (Ben-Yehuda *et al.*, 2003). Several types of evidence suggested that DivIVA might recognize the negative curvature of the membrane. First, DivIVA-GFP was observed mainly at the division septa, but also at the poles of *B. subtilis* cells (Fig. 2b), and secondly, DivIVA localized mainly at the poles of heterologous *E. coli* (Fig. 2a) and *Schizosaccharomyces pombe* cells (Lenarcic *et al.*, 2009; Ramamurthi & Losick, 2009; Eswaramoorthy *et al.*, 2011). Notably, as opposed to *E. coli* cells, in which the strongest negative curvature is at the poles, in *B. subtilis* cells, the forming septa, which later becomes the new pole, displays even sharper membrane curvature than the existing poles (Ramamurthi, 2010). To further investigate this issue, the subcellular localization of GFP-tagged DivIVA was monitored under conditions that affect the negative curvature of *B. subtilis* membrane. When *B. subtilis* was grown as single cells rather than chains, DivIVA was observed only at the poles. A similar localization pattern was observed upon prevention of septum formation. Moreover, in cells that were made spherical, having uniform negative curvature, by lysozyme treatment, DivIVA localized indiscriminately (Lenarcic *et al.*, 2009; Ramamurthi & Losick, 2009). Taken together, certain bacterial proteins are capable of sensing a physical cue, such as membrane curvature, and localize accordingly.

### Membrane potential

The inner membrane serves as a preferred subcellular compartment for protein localization in bacteria. Whereas the membrane composition and structural features have been given a lot of attention, one cannot rule out that the proton motive force (pmf) generated in the membrane also affects subcellular organization. Generation of pmf in the membrane is a robust process, which involves exchange of ions, creation of electric potential, and subsequently, synthesis of the main energy molecule of the cell, ATP (Ingledeew & Poole, 1984). Could membrane potential affect the localization of proteins to membrane surfaces?

A recent investigation by Strahl & Hamoen (2010) revealed that pmf might play a significant role in protein localization. The authors discovered it by serendipity, when they observed that the use of polylysine-coated slides resulted in loss of MinD polar localization. Because it has previously been reported that polylysine may affect the pmf of bacterial membranes (Katsu *et al.*, 1984), the possible role of pmf in protein localization was investigated by monitoring localization of several proteins in the presence of ionophores that dissipate pmf. Indeed, dissipation of pmf led to promiscuous localization of *B. subtilis* proteins that are involved in cell division (FtsA, MinCD),
cell shape regulation (MreB, Mbl, MreBH, and MreCD) and chromosome segregation (Soj). The observed mislocalization of certain proteins in *E. coli* and *C. crescentus* because of pmf dissipation indicated that the involvement of pmf in protein localization is widespread among bacteria.

How does pmf affect protein localization? Because the proteins that exhibited delocalization upon pmf dissipation by ionophores require ATP for their activity, and because such a treatment can result in a significant decrease in ATP levels in the cell, a drop in ATP could theoretically account for the observed effect on protein localization. By repeating the experiments in strains impaired in ATP synthesis or with non-ATP-binding mutant proteins, the effect of pmf was shown to be independent of the ATP levels in the cell (Strahl & Hamoen, 2010). The next question to be addressed was which of the two components of pmf, transmembrane electric potential (ΔΨ) or transmembrane chemical potential (ΔpH), is involved in protein localization. Using ionophores that specifically modify each pmf component, it is has been shown that ΔΨ is the key factor that affects localization of proteins. How does ΔΨ influence protein localization? Dissection analysis of MinD revealed that its C-terminal amphipathic helix is sensitive to disturbance of membrane potential. Moreover, binding of this amphipathic helix to lipids was shown to depend on ΔΨ in vitro, raising the possibility that a change in membrane potential results in a subtle conformational change of the amphipathic helix that enhances its binding (Strahl & Hamoen, 2010). On the other hand, membrane fluidity, previously shown to depend on ΔΨ (Schaffer & Thiele, 2004), has also been demonstrated to affect MinD binding (Mazor et al., 2008a), suggesting that membrane potential might change the membrane lipid properties, which facilitates the insertion of amphipathic helices. Further studies are required to elucidate the exact molecular mechanism that underlies the effect of ΔΨ on protein localization.

Finally, protein localization was shown to be affected by depletion of oxygen, which is the major electron acceptor that influences membrane potential (Strahl & Hamoen, 2010). The authors demonstrated that the morphological changes, which depletion of oxygen induces in bacterial cells, correlate with delocalization of cell division and cytoskeletal proteins. These results suggest that the changes in bacterial cell morphology observed in natural habitats, in which oxygen levels fluctuate constantly, can be explained by the effect of membrane potential on protein localization.

**Membrane lipid composition**

Studies on bacterial membrane properties revealed that the phospholipids are not homogenously distributed. Rather, some of them were observed as microdomains within the membrane, which can be compared to the lipid rafts in eukaryotes. This discovery was made possible by the use of efficient lipid-binding fluorescent dyes (Matsumoto et al., 2006). Two well-characterized lipids that form distinct microdomain in bacterial membranes are cardiolipin and phosphatidyglycerol (PG). Whereas cardiolipin was shown to concentrate in the poles and septum of *E. coli* cells (Fig. 3a) (Mileykovskaya & Downham, 2009), PG was observed as spirals in *B. subtilis* cells (Fig. 3b) (Barak et al., 2008).

The lateral heterogeneity of lipids in the bacterial membranes, prompted scientists to examine the possibility that this arrangement acts as a mechanism for protein localization, that is, certain proteins localize by binding to specific lipids. ProP, an osmosensory transporter protein from *E. coli*, was the first protein shown to depend on the polarly concentrated cardiolipin for its localization (Romantsov et al., 2007). It has been shown that increasing osmolarity increases the cardiolipin content in the poles; this cardiolipin microdomain recruits ProP to the poles; ProP then functions to import osmolytes into the cell. Finally, polar localization of ProP was shown to be independent of its expression level but correlated with the proportion of polarly localized cardiolipin (Romantsov et al., 2007). The same research group that studied ProP...
polar localization later identified MscS, an E. coli membrane protein that forms a part of the mechanosensitive channels, also as a cardiolipin-dependent polar-localizing protein (Romantsov et al., 2010). Figure 3a illustrates the mechanism of cardiolipin-dependent polar localization of ProP and MscS.

Lipid composition was suggested to be involved also in the spatial organization of the Min proteins, which are involved in the inhibition of polar septum formation (Mileykovskaya et al., 2003). It has long been known that MinCDE proteins of E. coli oscillate from pole to pole in a dynamic manner (Vats et al., 2009a, b; Lenz & Sogaard-Andersen, 2011). Several studies of the Min system indicated that the lipid composition of the membrane has a direct influence on dynamic localization of the Min proteins and hence on the regulation of cell division. The dimeric ATP-bound form of the MinD protein, which tethers to the membrane through its C-terminal amphipathic alpha helix, designated membrane-targeting sequence (MTS), is stabilized by anionic lipids (Mazor et al., 2008a, b). The pole-to-pole oscillation of MinD follows a helical path, and it has been shown by fluorescence resonance energy transfer analysis that the MinD–GFP helices co-localize with the spirals of the anionic phospholipid PG in B. subtilis cells. Furthermore, MinD spirals were not observed in mutants lacking PG (Barak et al., 2008). MinE, another Min protein, which activates MinD ATPase activity and thereby releases MinD–ADP from the membrane, was also recently shown to bind directly to the membrane by electrostatic interactions (Hsieh et al., 2010).

Other proteins that whose localization was shown to depend on lipid composition include DnaA (Sekimizu & Kornberg, 1988), FtsA (Barak et al., 2008), and the Sec translocon system (Gold et al., 2010). Because DnaA and FtsA also possess an MTS domain, it was assumed that binding of these proteins to the anionic-rich membrane follows a similar pattern as that of MinD (Barak et al., 2008). In the case of the Sec system, cardiolipin was shown to not only promote dimerization of SecYEG, but to also increase the ATPase activity of SecA (Gold et al., 2010). Finally, diverse bacteria were shown to harbor homologs of flotillins, which are ‘raft markers’ found exclusively in lipid rafts within the membrane of eukaryotic cells (Zhang et al., 2005; Donovan & Bramkamp, 2009; Lopez & Kolter, 2010). YuaG, a flotilin homolog from B. subtilis, renamed FloT because of its similarity to the eukaryotic flotillin-1, was shown to localize in discrete foci along the bacterial membrane in a dynamic fashion. Although YuaG co-isolates with anionic phospholipids, its in vivo localization was shown not to depend exclusively on these phospholipids (Donovan & Bramkamp, 2009). The lipids associated with the bacterial rafts were suggested to be polisoprenoids (Lopez & Kolter, 2010). Components of various physiological processes, such as biofilm formation, protein secretion, and the onset of sporulation, were proposed to be organized in lipid rafts (Donovan & Bramkamp, 2009; Lopez & Kolter, 2010).

Taken together, the data that accumulated thus far suggest that lipid microdomains are important higher-order structures that directly affect localization of certain proteins, thus impinging on the regulation of diverse cellular processes in bacteria.

Unidentified polar cues for protein recruitment

Cellular asymmetry is critical for the life cycle of many types of bacteria. Cell polarity, which apparently underlies the establishment of asymmetry, is often visually manifested by the existence of polar organelles, such as pili or flagella (Ebersbach & Jacobs-Wagner, 2007; Dworkin, 2009; Shapiro et al., 2009). Alternatively, spatial and temporal pole-to-pole oscillations of proteins can establish polarity, for example, the Ras-like G proteins that set up the polarity of motility proteins in M. xanthus cells (Kirkpatrick & Viollier, 2010; Lenz & Sogaard-Andersen, 2011). Polarity is also established in cells lacking discernible polar organelles, such as E. coli cells, in which specialized protein complexes localize at or near the poles, despite their apparent symmetrical morphology. Polarization in these cells, which stems from the different division history of two poles (old vs. new), is seemingly exploited for the positioning of protein complexes (Janakiraman & Goldberg, 2004). The list of proteins and complexes that localize to the poles of rod-shaped bacteria is growing rapidly, emphasizing the importance of this domain for cell architecture and organization. Polar positioning of many of these proteins was shown to be crucial to fundamental cellular processes and for orchestrating sophisticated regulatory processes (Kirkpatrick & Viollier, 2010). For example, it has recently been suggested that the polarly localized histidine kinase CckA in C. crescentus is regulated in a protected zone near the pole, without the use of membrane-enclosed compartments (Tsokos et al., 2011). Spatial regulation is also of importance for bacterial pathogenesis, as many virulence factors localize to the poles (Goldberg et al., 1993; Scott et al., 2001; Judd et al., 2005; Jain et al., 2006; Jaumouille et al., 2008; Carlsson et al., 2009; Bowman et al., 2010).

Although several of the polarly localized proteins were suggested to be positioned by one of the localization mechanisms discussed above, a large number of proteins are targeted to the poles by a yet unidentified mechanism(s). Careful examination of the list of polarly localized proteins reveals that some of them belong to closely related families that may have evolved to interact simultaneously with the bacterial membranes.
related signaling pathways, which need to communicate to generate an optimal cellular response. Two such linked pathways, which exhibit a similar spatial organization, are the phosphotransferase (PTS) and the chemotaxis systems (see schematic presentation of the spatial organization of both systems in Fig. 4). The chemotaxis system senses nutrients or repellents availability in the environment and controls bacterial taxis along chemical gradients accordingly (Porter et al., 2011). The PTS system determines the hierarchical uptake of carbon sources from the environment and consequently adjusts cell metabolism via the control of global regulatory systems, such as carbon catabolite repression and inducer exclusion (Deutscher, 2008). Together, the PTS and chemotaxis systems sense various nutrients and determine the metabolic status of the cell. Both systems were extensively investigated, but the mechanism that underlies their cross talk remained unknown. A hint about their cooperativity was provided by the suggested interaction between key components of the two systems, that is, the general PTS protein EI and the chemotaxis histidine kinase CheA (Lux et al., 1995).

Knowledge that accumulated in recent years regarding the subcellular localization of the components of both systems suggests a spatial rationale for their cross talk (Lopian et al., 2010; Amster-Choder, 2011).

The chemotaxis system of E. coli is one of the best studied signal transduction systems, and the proteins that compose this pathway were among the first to be observed at the bacterial cell poles. Detailed information regarding the interactions and the mechanism underlying chemotaxis has been reviewed elsewhere (Roberts et al., 2010; Sourjik & Armitage, 2010). Here, we will highlight only the information that accumulated regarding the localization strategies of the chemotaxis proteins. The E. coli chemotaxis system is comprised of five different transmembrane chemoreceptors (Tar, Tsr, Tap, Trg and Aer), a histidine kinase (CheA), an adaptor protein (CheW), a response regulator (CheY), a phosphatase (CheZ), a methyl

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**Fig. 4.** Spatial organization of the PTS and the chemotaxis systems in Escherichia coli. (a) Schematic presentation of the PTS sugar transport system. The general PTS proteins, EI and HPr, localize to the E. coli cell poles (top). Upon the addition of PTS sugars to the growth medium, HPr is phosphorylated by EI; HPr-P is released from the poles and distributes in the cell (bottom); a fraction of HPr-P gets near the membrane to phosphorylate the PTS sugar permeases, allowing them to translocate the PTS sugars into the cell and to phosphorylate them. (b) Schematic presentation of the chemotaxis system. The methyl-accepting chemotaxis proteins (MCPs) form large clusters at the E. coli cell poles. The chemotaxis proteins (for simplicity, only CheW, CheA, and CheY are shown) associate with the polar MCP clusters to form the chemotaxis complexes (top); the small response regulator CheY is phosphorylated by the histidine kinase CheA; CheY-P migrates from the poles to the membrane, where it interacts with proteins in the flagellar motor (bottom). Binding of CheY-P to the flagellar motor changes the rotation from counterclockwise to clockwise and hence the swimming mode from ‘run’ (top) to ‘tumble’ (bottom).
transferase (CheR), and a methyl esterase (CheB). Orchestration of all these proteins’ activity determines the locomotion directionality of the bacterium (Fig. 4b). Extensive studies revealed that this coordination is achieved by clustering the chemotaxis proteins at the poles. Clusters of the chemoreceptors, coupled with CheW and CheA, form the core of the chemotaxis polar complex (Sourjik, 2004). Localization of the other Che proteins was known to be mediated by their association with the core complex, but the mechanism that directs the core complex to the poles remained an enigma (Sourjik & Armitage, 2010). Recently, two models were suggested to explain the formation of the polar complex. One model suggests that stochastic self-assembly of receptors is the mechanistic reason for cluster formation. According to this model, receptor clusters are formed either by the assembly of newly synthesized receptors with already existing clusters or by the formation of new nucleation centers at places of high concentration of the newly synthesized proteins. The interplay between these two assembling patterns was proposed to determine the spatial distribution of receptor clusters in the membrane (Thiem & Sourjik, 2008). It was also suggested that the localization of lateral clusters marks the future division sites and hence the poles to be of the bacterial cell (Thiem et al., 2007). The stochastic self-assembly model was also supported by visualization of cluster assembly using super-resolution light microscopy (Greenfield et al., 2009). The second model suggests that an active process of partitioning, rather than self-assembly is involved in the localization of the chemoreceptor. Time-lapse analysis of newly synthesized Tsr, a major chemoreceptor, fused with GFP revealed that the receptors are first synthesized in a helical fashion in the membrane; these newly synthesized receptors then migrate within the membrane to form large clusters at the poles. The helically distributed lateral receptor clusters co-localizes with the subcellular spirals of the Sec proteins of E. coli, suggesting that the Sec secretion system is involved in spatial partitioning of the chemoreceptors (Shiomi et al., 2006). Further studies using FRAP analysis revealed that the chemotaxis polar clusters are relatively stable over the chemotactic signaling period, with few components, mainly CheY, exhibiting a dynamic behavior and shuttling between the polar complex and the flagellar motors, which are randomly distributed throughout the E. coli membrane (Schulmeister et al., 2008).

In the case of the PTS system, the two general PTS proteins, EI and HPr, were recently shown to localize to the cell poles. Studies of ectopically expressed EI and HPr in a pts deletion mutant revealed that their polar localization is independent of each other. The EI polar foci are relatively stable, whereas HPr was observed to relocate from pole to the cytoplasm upon sugar stimulation. HPr relocation explains its ability to transfer a phosphoryl group from EI to the soluble domain of the different permeases of PTS sugars, which either localize near the cell membrane or are distributed throughout the cytoplasm (Fig. 4a) (Lopian et al., 2010). The PTS system was also shown to cross talk with other regulatory systems by spatial modulation of their components (Lopian et al., 2010). The cues that recruit the PTS components to the poles are not known. Moreover, the factors that are responsible for limiting the diffusion of the soluble EI protein and for maintaining it at the pole, subsequent to its recruitment to this domain, are yet to be identified.

The picture that emerges from the studies described above is that the PTS and the chemotaxis systems utilize a similar strategy for information processing and signal transduction, that is, they localize their respective ‘command centers’ to the poles and rely on small proteins, HPr and CheY, respectively, that shuttle between the poles and other cellular domains, where they execute their function (Fig. 4). It is reasonable to assume that the similar spatial organization of the two systems provides a basis for their cross talk, that is, their spatial proximity may facilitate communication between their components to coordinate their actions and generate an optimal metabolic response (Amster-Choder, 2011). It remains to be studied whether recruitment of the chemotaxis and PTS systems to the poles is interdependent.

**Localized translation**

The question of where translation of mRNA transcripts takes place in the cell has been investigated over the years, with novel techniques changing our understanding of this issue time and again. The first observation which suggested that translation might occur at specific cellular compartments was the detection of ribosomes bound to the membrane of the endoplasmic reticulum (ER) half a century ago (Palade, 1958). In the 1970s, Sabatini et al. detected mRNA molecules that remained attached to the ER membranes even after the removal of the ribosomes (Lande et al., 1975). On the basis of these results, it has been suggested that mRNA is involved in assembling membrane-bound polysomes, which actively synthesize proteins that are secreted via the ER membrane (Lande et al., 1975). In the early 1980s, evidence for the signal recognition particle (SRP) model accumulated. These studies suggested that many proteins localize via a signal peptide to the ER (Walter & Blobel, 1980; Gilmore et al., 1982; Walter & Blobel, 1982) or to the inner membrane (Poritz et al., 1988; Bernstein et al., 1989; Romisch et al., 1989), usually while the nascent chain is still associated with the ribosome. The SRP model established a link...
between mRNA localization and protein targeting and encouraged scientists to pursue imaging of intracellular distribution of mRNAs. An asymmetric distribution of specific mRNAs in the cytoplasm was first visualized in the early 1980s by in situ hybridization to detect β-actin mRNA in ascidians embryos (Jeffery et al., 1983). In the last 10 years, monitoring mRNA trafficking in live cells became possible because of the development of RNA labeling methods by fluorescent RNA-binding proteins (Bertrand et al., 1998; Beach et al., 1999). Hence, the current view is that intracellular localization of mRNA is a common mechanism to regulate spatial and temporal protein expression in most eukaryotic model organisms, thus controlling the development and physiology of these cells (Donnelly et al., 2010).

Is the concept of localized translation relevant for prokaryotes, or did the mechanisms that enable these pathways coevolve with the nucleus? In fact, protein localization via mRNA targeting has not been considered to occur in bacteria because of the lack of nucleus and to the coupling between transcription and translation (Ramamurthi, 2010). Therefore, although components of the bacterial mRNA degradosome machinery, as well as the ribosomes, were shown to have specific intracellular distributions (Herskovits & Bibi, 2000; Taghbalout & Rothfield, 2007; Khemici et al., 2008), the localization of mRNA in bacterial cells remained poorly characterized. Very recent studies of mRNA localization in bacterial cells shed new light on this long-neglected field. These studies made use of the main tools that were used for visualizing mRNA in eukaryotic cells, which were adapted to bacterial cells. Below is a short description of these tools, their adaptation to bacteria, and the novel findings regarding mRNA localization in bacterial cells.

Methods for studying mRNA localization

The two main tools for mRNA visualization are fluorescent nucleic acid probes, which enable the direct detection of specific nucleic acid sequences (fluorescent in situ hybridization, FISH), and fluorescent RNA-binding proteins, which mark the location of transcripts that are bound by these proteins. Owing to the improvement in imaging technologies and the development of better fluorescent reagents, both methods have been recently applied to detect RNA in bacterial cells, despite the inherent limitations, that is, the small cell size and the low copy number of mRNAs per cell (five copies or fewer) (Taniguchi et al., 2010).

The in situ hybridization technique has been extensively used over the years to visualize DNA and later RNA with ever-increasing sensitivity. Methods for improved labeling allowed direct detection of RNA with fluorescent single-strand probes (Kislauskis et al., 1993). The synthesis of locked nucleic acid significantly increased the hybridization capabilities of the probes and allowed for a higher sensitivity and specificity (Obika et al., 1997; Koshkin et al., 1998). In recent years, a large variety of indirect and direct labeling tools have been developed, allowing one to choose from a broad spectrum of detection methods.

Compared with FISH, the methodology that enables monitoring of mRNA trafficking in live cells is fairly new. It is based on the use of RNA-binding proteins, which are fused fluorescent proteins, and requires tagging of the target RNA with an array of aptamers or binding motifs. Binding of the fluorescent RNA-binding protein to its binding sites on the target RNA enables the visualization of the RNA-protein complex. The most widely used RNA-binding protein is the MS2 phage coat protein, which in many cases is simply referred to as MS2 protein. This method was first applied to visualize the Ash1 mRNA in yeast (Bertrand et al., 1998; Beach et al., 1999). Visualization of mRNA by the MS2 protein was adjusted to E. coli by Golding and Cox, who fused a tandem dimer of the MS2 protein to the N-terminus of an enhanced version of GFP and placed it under the control of an inducible promoter (Golding & Cox, 2004).

The background signal, which is generated by the use of intact fluorescence proteins to label RNA, triggered the development of several bimolecular fluorescence complementation (BiFC) systems. The BiFC methodology is based on formation of a fluorescent complex through the association of two fragments of a fluorescent protein, which are brought together because of interaction between proteins that are fused into these fragments. This methodology, developed to monitor protein–protein interaction (Hu et al., 2002; Michnick, 2003), has been adjusted for observing RNA–protein interaction by Rackham and Brown (Rackham & Brown, 2004). In the latter case, the two fragments of a spilt fluorescence protein are fused into two different RNA-binding proteins. Two adjacent sets of aptamers, which are recognized by the RNA-binding proteins, are then introduced into the target RNA. Interaction of the RNA-binding proteins with their target RNA sites brings the two fragments of the spilt fluorescence protein together, close enough to reassemble and to generate a fluorescent signal. This variation in the BiFC methodology, which was described by Rackham and Brown as a trimolecular fluorescence complementation (TriFC) system, has been further developed over the last few years and was adapted to several organisms including bacteria (Valencia-Burton et al., 2007; Yiu et al., 2011). A more detailed description of this methodology has been reviewed elsewhere (Broude, 2011).
The major advantage of in situ hybridization is that the targets are nonmodified RNA or DNA; its drawback is that it can only be used to image dead and fixed cells and therefore does not allow monitoring of real-time dynamics of the nucleic acids. The main advantage of labeling mRNA in live cells and therefore enables real-time measurements of the target RNA dynamics; the drawbacks are that the target RNA is modified, and an array of protein molecules is being attached to it. Hence, mRNA localization should ideally be studied by comparing data obtained with the two approaches.

RNA localization in bacteria

The first to look at mRNA in bacteria were Golding & Cox (2004), who used the MS2 system to study the motion of model RNAs. These authors found that RNA motion is limited, in most cases, in space to near the center or the quarter points of the cell. This localized motion is consistent with Brownian motion of RNA polymers tethered to their template DNA, which in this case was an F-plasmid, previously shown to be distributed at the center and quarter points of the E. coli cell (Gordon et al., 1997; Pogliano, 2002). Occasionally, some RNAs exhibited a polymer chain dynamics that can be ascribed to transcription elongation, whereas in other cases, the transcripts were observed as freely diffusing in the cytoplasm (Golding & Cox, 2004). However, the histogram of the RNA location implied that in the latter case the mRNAs lingered near the poles (Golding & Cox, 2004). This behavior can be explained by hydrodynamic coupling between the RNA and the cell wall, which would be expected to give an effective pair potential and to decrease the diffusion rate, causing the transcripts to spend more time near the cell poles (Lin et al., 2002; Golding & Cox, 2004). In a later study, Broude et al. used the elF4A split protein, a variation in the BiFC methodology, to explore the localization of three different RNAs in E. coli, encoding lacZ, 5S rRNA, or a short artificial untranslated RNA (Valencia-Burton et al., 2007). The three mRNAs were encoded from the same plasmid, but showed different localization patterns: the lacZ mRNA was distributed evenly in the cytoplasm, the 5S RNA transcripts localized to foci, similarly to ribosomes localization to sites outside the nucleoid, and the short untranslated RNA was observed primarily at the cell poles. Moreover, using time-lapse imaging, the localization pattern of the untranslated RNA transcripts was shown to change dramatically over time. The above studies were pioneering in the field of mRNA localization in bacterial cells and demonstrated that mRNAs can diffuse and specifically localize in these cells.

Further demonstration of mRNA localization in bacteria was provided by three thought-provoking studies, published in 2009. In the first, Broude et al. extended their study of RNA localization in live E. coli cells by the use of the elF4A split protein. In addition to localization of RNA at the mid and quarter points of the cell, they observed RNA that localized laterally along the cell axis, in a pattern suggesting the existence of ordered helical RNA structures (Valencia-Burton et al., 2009). In the second study, Russell and Keiler used FISH to investigate for the first time the localization pattern of an endogenous bacterial RNA, the tmRNA in C. crescentus. The tmRNA, a ubiquitous small RNA, which participates in trans-translation by entering the ribosome and helping with the addition of a peptide tag on a nascent polypeptide, was shown to localize in a helix-like pattern within the cell. The same localization pattern was revealed for the tmRNA-binding protein, SmpB, fused to GFP. SmpB demonstrated a helical pattern also in the absence of tmRNA, but not vice versa, suggesting that SmpB is responsible for the localization of tmRNA (Russell & Keiler, 2009). In the third study, Schleifer et al. documented the localization of nifH, which codes for the enzyme dinitrogen reductase that acts in the process of dinitrogen fixation. The nifH RNA, detected by FISH in Klebsiella oxytoca and Azotobacter vinelandii cells, exhibited an uneven distribution, especially in K. oxytoca cells, in which the mRNA localized to one or both poles (Pilhofer et al., 2009). This localization pattern might correlate with the subcellular regions in which dinitrogen fixation takes place, suggesting localized translation of NifH via targeting of the nifH RNA transcripts. Together, these three studies reinforced the notion that RNA is not randomly localized in bacterial cells.

Recently, two different models for mRNA localization in bacteria were suggested, based on the use of FISH and live cell imaging (Fig. 5a and b). Jacobs-Wagner et al. imaged several mRNAs expressed from the chromosome of C. crescentus, as well as lacZ transcripts in E. coli (Montero Llopis et al., 2010). They observed very limited dispersion of the transcripts from their site of transcription during their lifetime (Fig. 5b). It is worth mentioning that the E. coli lacZ distribution observed in this study differed from the pattern observed by Broude et al. (Valencia-Burton et al., 2007), maybe due to differences in the mRNA expression systems, from the chromosome or from a plasmid, respectively, or due to the different visualization methodologies that have been applied in the two studies. On the basis of their results, Jacobs-Wagner et al. suggested that bacteria use the chromosome layout to limit post-transcriptional processes, that is, translation and potentially mRNA decay, to a defined spatial space, thus increasing the subcellular concentration of the
encoded proteins and their probability to interact and to form complexes. The restriction in mRNA diffusion and in ribosome motility that the authors documented in *C. crescentus* can be explained by the coupling between transcription and translation in bacteria, that is, translation of an mRNA that is still tethered to the DNA can limit its diffusion. Additionally, the presence of multiple ribosomes that translate one mRNA, and the nascent polypeptides emerging from each ribosome may limit both mRNA and ribosome mobility. Significantly, Jacobs-Wagner *et al.* show that the *C. crescentus* RNase E appears associated with chromosome, unlike the *E. coli* RNase E that has been observed in a helix-like pattern and in association with the membrane (Taghbalout & Rothfield, 2007, 2008). One interesting implication of this model is that the chromosome organization can provide a mean to compartmentalize complex formation, thereby explaining clustering of genes that encode interacting proteins in bacterial chromosomes. The idea that chromosome organization is important and essential for complex formation is supported by several studies, which demonstrated that chromosome organization can provide a mean to compartmentalize complex formation, thereby explaining clustering of genes that encode interacting proteins in bacterial chromosomes. The idea that chromosome organization is important and essential for complex formation is supported by several studies, which demonstrated that the chromosome organization can provide a mean to compartmentalize complex formation, thereby explaining clustering of genes that encode interacting proteins in bacterial chromosomes.

A different picture emerges from the study of Amster-Choder *et al.*, who detected mRNAs at particular positions within *E. coli* cells. In addition to FISH and live cell imaging, the authors also monitored the presence of specific mRNAs in the cytosolic or in the membrane fractions of cells, after their disruption, by reverse transcription and PCR amplification. Three types of localization patterns were observed: (1) a helix-like pattern in the cytoplasm; (2) around the cell circumference, that is, at or near the cytoplasmic membrane, and (3) at the cell poles (Fig. 5a) (Nevo-Dinur *et al.*, 2011). Significantly, localization of the mRNAs that have been looked at correlated with the position of their future protein products. Moreover, targeting of transcripts was shown to be in tight correlation with the requirements for complex formation, that is, an mRNA of a transcription factor was detected either at the membrane, when co-expressed with its cognate membrane sensor with which it forms a complex, or at the cell poles, where it associates with another protein, when expressed by itself. On the basis of these results, the authors suggested that, like in eukaryotic cells, localization of bacterial proteins can be achieved via targeting of their mRNA transcripts. Up until now, the prevailing view has been that protein localization in bacteria depends solely on targeting signals within the protein, because of the lack of nucleus and the coupling between transcription and translation. However, Amster-Choder *et al.* have shown that bacterial mRNAs can localize in a translation-independent manner because of *cis*-acting sequences within the mRNAs. A hierarchy in zip codes localization capacity was observed: a membrane-encoding sequence was found to be dominant to a hydrophilic-encoding sequence in determining transcript localization. Intriguingly, the targeting signals seem to be conserved across the eukaryotic–prokaryotic divide, because transcripts encoding a transmembrane protein from *Drosophila melanogaster* localized to the inner membrane when expressed in *E. coli* (Nevo-Dinur *et al.*, 2011). This observation might be explained by the results of a recent bioinformatic study, which showed that the sequences of mRNAs that encode membrane-spanning domains of integral membrane proteins in prokaryotes as well as in eukaryotes are enriched in uracils (Prilusky & Bibi, 2009), a feature that may serve as a membrane-targeting signal.

In a very recent study, Guieiros-Filho *et al.* detected another pattern of mRNA localization, that is, to the forming septum in midcell. The authors have found that ComN, which plays a role in the post-transcriptional control of the late competence *comE* operon in *B. subtilis*, promotes accumulation of its target *comE* mRNA to septal and polar sites (Dos Santos *et al.*, 2012).
basis of their results, the authors speculated that localized translation of ComE proteins, facilitated by mRNA-mediated targeting of their encoding mRNA, may be required for efficient competence development.

The above results suggest that mRNA targeting might be important for localized translation in bacteria. This is in line with the reported localization of ribosomes in *B. subtilis* and in *E. coli* to regions outside of the nucleoid, as opposed to RNA polymerase, which resides principally within the nucleoid (Lewis et al., 2000; Bakshi et al., 2012). In fact, based on the evident separation of the nucleoid and the transcription machinery from the translation machinery, Weisshaar et al. suggested that, at least in *E. coli* and probably also in *B. subtilis*, most translation is not coupled to transcription; rather, complete mRNA molecules find their way to the ribosome-rich regions (Bakshi et al., 2012). As mentioned before, the limited dispersion of mRNA in *C. crescentus* is in correlation with the dispersion of both the ribosomes and the DNA throughout the cytoplasm in this organism (Montero Llopis et al., 2010).

RNA targeting may also correlate with other post-transcriptional processes, such as mRNA decay. This suggestion is in agreement with the detection of components of the *E. coli* degradosome, RNase E and RNA helicase B, near the membrane and in a helix-like pattern (Taghabout & Rothfield, 2007; Khemici et al., 2008). The imaging of Hfq, an RNA-binding protein that plays a role in post-transcriptional regulation, and of RodZ, an RNA-binding cytoskeleton anchoring protein, close to the bacterial membrane (Diestra et al., 2009; Mitobe et al., 2011) suggests that RNA localization might be linked to different regulatory mechanisms in the cell. Again, the limited distribution of the transcripts in *C. crescentus* correlates with the distribution of RNaseE in this organism (Montero Llopis et al., 2010). The parallels between the subcellular distribution of mRNA transcripts and of the various post-translational machineries strongly suggest a link between cell architecture and function.

Regardless of the different models that emerge for mRNA distribution, both models agree that, despite the scarcity of membrane-bounded organelles, bacterial cells have the capacity to spatially organize mRNA molecules. It is possible that the two models operate in different organisms or even coexist in the same organism. The field of mRNA localization in bacteria is still in its infancy, and additional studies are required to unravel the relevant mechanisms.

**Concluding remarks**

Studies published in the last decade completely changed our view of bacterial cells. We have learnt that the division to cells with and without membrane-enclosed organelle, first and foremost a nucleus, does not imply that the two main kingdoms of cells differ in having intricate internal organization. The mechanisms that are responsible for cell architecture in bacteria are currently unraveling. In this review, we described several strategies that help establish the bacterial cell architecture and regulate vital processes spatially and temporally. Nevertheless, there are obviously other strategies that we did not cover in this review or that have not been discovered yet. This hypothesis is supported by the finding that in addition to the obvious subcellular domains, that is, the membrane, the chromosome milieu, the poles, and the septum, there are other separate physical compartments in bacterial cells. One example is the metabolosomes, which are metabolically active structures that are bound by a proteinaceous shell and are located within the cytoplasm (Yeates et al., 2008). New studies shed light on the composition, production, and structure of these protein-based microcompartments, which act as simple organelles by compartmentalizing functionally related enzymes that compose specific metabolic pathways (Parsons et al., 2010; Tanaka et al., 2010). The existence of such organelles, evidently developed to cope with metabolic stress, draws parallels with eukaryotic organelles and expands our view of cell compartmentalization. Undoubtedly, the development of super-resolution microscopes and novel fluorescent reagents will lead to the discovery of additional nonconventional subcellular compartments.

The thermodynamic rationale behind cell compartmentalization is valid for bacteria as it is for eukaryotes. In fact, clustering of functionally related molecules and colocalization of signaling pathways seems a requirement considering the high macromolecules concentration in exponentially growing bacterial cells that, apparently, limits diffusion rates in this environment (Zimmerman & Trach, 1991). The large number of factors and complexes, including pathogenesis factors, observed at the poles of bacterial cells suggests that the poles have evolved, because of their special characteristics, for example, composition, geometry, or structure, to serve as hubs that allow for the association of components of complex systems, for the coordination of bacterial signaling systems and, in general, for enabling a tighter regulation (Amster-Choder, 2011).

The demonstration that bacteria can localize RNAs to subcellular regions in the cell revolutionized our view of gene expression in prokaryotes. Similarly, unexpected was the finding that localization of bacterial transcripts may occur in a translation-independent manner. Once again, the orthodox distinction between prokaryotes and eukaryotes is not valid anymore, as it is clear that the mechanistic basis for separating transcription and translation did not coevolve with the nucleus, but, rather, has developed in the
primitive bacterial cell. All in all, these results raise numerous questions regarding the mechanisms underlying RNA localization: How do the mRNA transcripts localize to the different subcellular regions? In eukaryotes, mRNA localization was suggested to occur by facilitated diffusion or by active transport along cytoskeletal filaments (Czaplinski & Singer, 2006; Palacios, 2007). The average short half-life of bacterial mRNAs (Bernstein et al., 2002), together with the short generation time of bacterial cells, suggests that active mechanisms are involved in mRNA targeting, at least in some cases. Careful monitoring of single molecules in live cells should be applied to distinguish between the different possibilities. New tools are constantly being developed for observing RNA within cells, for example, the RNA aptamers that bind fluorophores mimic GFP (Paige et al., 2011). In the case that active mechanisms are involved, the factors that recognize the mRNA zip codes and help transport the transcripts to their destination will need to be identified. The role of mRNA targeting in post-transcriptional processes is another relevant question. In this respect, it is worth mentioning that the recent documentation of binding of the membrane-anchored cytoskeletal protein RodZ to certain mRNAs in Shigella (Mitobe et al., 2011) provoked the authors to suggest that RodZ provides a scaffold for post-transcriptional regulation. Finally, how do sRNA and other regulatory noncoding RNA localize? All these questions await further investigation using advanced methodologies.

Summarily, the evidence that accumulated thus far about the intricate organization of bacterial cells makes them suitable models for studying spatial regulation of cellular processes and for elucidating the mechanisms that underlie cellular architecture in higher organisms. Efforts to unravel these mechanisms will pay off, as they will reveal universal strategies that are relevant to all types of cells, as well as bacterial-specific mechanisms, that can provide targets for the development of novel antibacterial drugs.

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