Minireview

Bacterial cell biology outside the streetlight

Silvia Bulgheresi*
Department of Ecogenetics & Systems Biology,
University of Vienna, Althanstrasse 14, Vienna 1090,
Austria.

Summary

As much as vertical transmission of microbial symbionts requires their deep integration into the host reproductive and developmental biology, symbiotic lifestyle might profoundly affect bacterial growth and proliferation. This review describes the reproductive oddities displayed by bacteria associated – more or less intimately – with multicellular eukaryotes.

“We become what we behold. We shape our tools and then our tools shape us”

Culkin, J.M. (1967). A schoolman’s guide to Marshall McLuhan. Saturday Review, pp. 51-53, 71-72.

Introduction

According to rough estimates, less than 0.1% of the up to 10^9 bacterial species thriving on planet Earth may be cultivated. Yet the reproduction of only a handful of lab-reared bacteria is being studied (Goley, 2013). The current quest to unravel the mechanisms underlying bacterial reproduction is therefore comparable to a drunkard’s search. Its inherent observational bias – the so-called streetlight effect – is treacherous as it may easily generate wrong assumptions or even beliefs. Here, I wish to review cell biological studies on bacterial symbionts, some of which already confuted well-rooted tenets of bacterial cell growth and division (Table 1). I will use the term symbiont sensu lato, that is to say every organism living together with a differently named organism (de Bary, 1879). Traditionally, symbionts are classified in ecto- and endosymbionts depending on whether they are outside or inside the host body respectively. In this review, although formally situated inside the host, I will regard the gut epithelium as superficial and consider its inhabitants – the gut microbiota – together with other ectosymbionts. Owing to space limitations, I will not review systems in which the molecular mechanisms underlying anomalous symbiont growth have not been investigated yet. These are the vast majority and include – for example – the ectosymbionts of unicellular (Desai et al., 2010; Strassert et al., 2010; Brune, 2014; Brune and Dietrich, 2015) and colonial eukaryotes (Bright et al., 2014), the endosymbionts of unicellular eukaryotes (Schulz and Horn, 2015) and of abyssal bathymodiolin mussels Candidate Endonucleobacter bathymodioli (Zielinski et al., 2009). Finally, also due to space limitations, I will be able neither to review our knowledge about mitochondrial and plastid reproduction and its control by the eukaryotic cell, nor to consider bacteria whose cell biology and physiology is dramatically affected by developmental programs [e.g., elementary body–reticulate body transition in Protchlamidiae associated to free-living amoebae (Horn, 2008; Schrallhammer and Schweikert, 2009)]. And now, before I embark on describing abnormal reproductive modes, I must briefly summarize how conventional bacteria grow and divide.

Bacterial growth and division for dummies

A wealth of exhaustive and well-written reviews is available on the fundamental process of bacterial reproduction [among the most recent ones: Bramkamp and Van Baarle (2009), Young (2010); den Blaauwen (2013); Chang and Huang (2014); Rowlett and Margolin (2015)]. The peptidoglycan (PG or murein) wall or sacculus confers physical strength and defines the shape of most bacteria (Egan and Vollmer, 2013; de Pedro and Cava, 2015; Randich and Brun, 2015). It overlies their cell membrane and consists of a mesh-like macromolecule of glycan chains that are cross-linked together via peptide chains. In model rods, two protein assemblies (the elongasome and the divisome) direct the modification and synthesis of the cell wall: during growth, the elongasome inserts freshly synthetized PG along the length of the rod, and during septation a cytokinesis complex called the divisome (Nanninga, 1991) completes the steps of constriction and new PG synthesis at midcell (Typas et al., 2012). Both divisome and elongasome consist of scaffolding cytoskeletal-like proteins (cytoplasmic), inner membrane spanning proteins and a...
| Host | Symbiont | Environment surrounding the symbiont | Reproductive anomaly | Presence of FtsZ | Host factor(s) affecting symbiont division |
|------|----------|--------------------------------------|----------------------|-----------------|-----------------------------------------|
| **Extracellular symbionts** | | | | | |
| *Laxus oneistus* nematode | *Candidatus Thiosymbion oneisti* (Gammaproteobacteria) | Marine sediment | Widening and symmetric longitudinal fission | YES | NN |
| *Eubostrichus fertilis* nematode | *Eubostrichus fertilis* nematode ectosymbiont (Gammaproteobacteria) | Marine sediment | Cell elongation (up to 45 μm) and symmetric transverse fission | YES | NN |
| *Eubostrichus dianeae* nematode | *Eubostrichus dianeae* nematode ectosymbiont (Gammaproteobacteria) | Marine sediment | Cell elongation (up to 120 μm) and symmetric transverse fission | YES | NN |
| *Epulopiscium* spp. | Surgeonfish (Firmicutes) | Gastrointestinal tract | Multiple intracellular offspring | YES | NN |
| *Metabacterium polyspora* | Guinea pig (Firmicutes) | Gastrointestinal tract | Multiple endospore formation or binary fission | YES | NN |
| *Mus musculus* | Segmented Filamentous Bacterium (SFB; Firmicutes) | Gastrointestinal tract | Cell elongation, segmentation and intracellular offspring or sporulation | YES | NN |
| **Intracellular symbionts** | | | | | |
| Legume | Rhizobia (Alphaproteobacteria) | Modified plant cell (nodule) in nodule | Cell elongation (block of cytokinesis) | YES | Nodule-specific cysteine-rich peptides (NCRs) |
| Weevil | *Sodalis pierantonius* | Modified insect cell (bacteriocyte) in bacteriome | Cell elongation (block of cytokinesis) | YES | Coleopterin A (CoIA) |
| Aphid | *Buchnera aphidicola* (Gammaproteobacteria) | Modified insect cell (bacteriocyte) in bacteriome | Gigantism (block of cytokinesis) | YES | Secreted cysteine-rich proteins? |
| *Sulcia muelleri* | Modified insect cell (bacteriocyte) in bacteriome | Gigantism (block of cytokinesis) | YES | NN |
profusion of periplasmic enzymes including PG synthases and hydrolases. In nearly all bacteria, the tubulin-like GTPase FtsZ regulates the localization and activity of divisome components. In canonical rod-shaped bacteria, which divide by transverse binary fission, spatiotemporal regulation of the divisome is fairly understood. During division, the GTP-dependent polymerization of FtsZ creates a ring-shaped structure called the Z-ring at the centre of the
cell (Fig. 1A) and perpendicular to the axis of chromosome segregation (Bi and Lutkenhaus, 1991; Lowe and Amos, 1998; Mukherjee and Lutkenhaus, 1998; Erickson et al., 2010). The Z-ring is attached to the cytoplasmic face of the membrane via membrane-associated proteins and recruits ten essential proteins important for septum assembly and Z-ring constriction (Hale et al., 1997; Pichoff and Lutkenhaus, 2005). In *Escherichia coli*, the mechanism for targeting the Z-ring to midcell involves the Min system, a machinery that inhibits the formation of the contractile ring at the cell poles (Raskin and de Boer, 1999; Hale et al., 2001; Monahan et al., 2014), and nucleoid occlusion (NO), which prevents nucleoid guillotining by the Z-ring (Wödringh et al., 1990; 1991; Bernhardt and de Boer, 2005). Much less is known about the spatiotemporal regulation of the elongasome, for which the actin-like protein MreB appears to be the major scaffold for coordinating PG precursor synthesis and polymerization (Fig. 1; Esue et al., 2005, 2005; Salje et al., 2011; Ozyamak et al., 2013).

Although the bacteria treated in the following sections are coccoid, rod-shaped or filamentous and therefore, morphologically, are not significantly different from the conventional ones, they display at least one of the following peculiarities: (i) polymorphism, often within the same population, (ii) reproduction modes other than transverse binary fission and/or (iii) alternation between two different reproduction modes.

**Dressed to cooperate: growth anomalies of ecsymbionts**

Non-conventional reproductive modes seem to be the specialty of the house in the *Stilbomonadinae*, a small family of mesosomamic (interstitial) roundworms: *Laxus oneistus* nematodes are covered by a single layer of rod-shaped bacteria tightly packed with one another, and standing perpendicularly to the worm’s surface as to form a columnar epithelium (Fig. 1B); the filamentous ecsymbionts of two other stilbomonad nematodes, *Eubostrichus fertilis* and *Eubostrichus dianeae*, are attached to the worm cuticle with two or one pole(s) respectively (Fig. 1C and D; Pende et al., 2014). The first one forms a bacterial coat resembling a braided rope, the second one resembling a fur. All the stilbomonad symbionts molecularly characterized fall in the Marine Oligochaete and Nematode Thiotrophic Symbionts (MONTS) cluster (Polz et al., 1994; Bayer et al., 2009; Heindl et al., 2011; Pende et al., 2014), more recently referred to as Candidatus Thiosymbion (Zimmermann et al., 2016). This is a basal group of Gammaproteobacteria related to free-living sulfur-purple bacteria of the *Chromatiaceae*. Candidatus Thiosymbion spp. may synthesize organic carbon compounds by exploiting the energy released by the oxidation of reduced sulfur (Polz et al., 1994; Hentschel et al., 1999; Musat et al., 2007; Bayer et al., 2009). We proved that the L. oneistus-associated rods grow in width, set constricting Z-rings parallel to their long axes and divide longitudinally by default (Fig. 1B; Leisch et al., 2012). Remarkably, the newly described Z-ring appears not only 90° shifted with respect to model rods, but also elliptic and highly discontinuous. As for the symbionts of *E. fertilis* and *E. dianeae*, they reproduce by setting and constricting a single Z-ring transversally, at midcell (Fig. 1C and D, respectively; Pende et al., 2014). Strikingly, though, in the former symmetric FtsZ-based fission occurs in crescents with lengths from 4 to 45 μm leading to an unprecedented cell length variation of one order of magnitude within the same population (Pende et al., 2014). This suggests that cell size may not be the primary trigger of division in these bacteria. As for the *E. dianeae* symbiont, despite lengthening up to 120 μm, it forms and constricts a single FtsZ ring at midcell, which makes it the longest unicellular organism known to divide by symmetric transverse division (Pende et al., 2014). We identified the min operon in *Laxus* and *Eubostrichus* symbionts, but its role in septum positioning is still unknown.
unknown. Moreover, although we identified the mre operon in all the aforementioned stilbonematid symbionts we do not know how MreB coordinates cell wall growth in the nematode ectosymbionts. Finally, we must determine the exact number of genomes that get symmetrically localized and their orientation and segregation mechanisms. Do the different symbiont spatial dispositions, i.e., the different symbiont reductive strategies, represent adaptations to different nematode hosts or to different nutritional regimes (Vadia and Levin, 2015)? Did \textit{L. oneistus} symbiont longitudinal fission or did \textit{E. fertilis} symbiont bipolar attachment evolve to favour symbiont vertical transmission? Are these two symbionts metabolically more dependent on their host than the \textit{E. dianae} symbiont, which can afford to let one daughter cell detach from the host surface?

We hope that omics-based comparisons among stilbonematids occupying different habitats or carrying different types of bacterial coats will clarify whether the latter serve specific, host-symbiont metabolic networks or physiological interdependencies, that – in turn – evolved as adaptations to specific habitats.

**Outside but inside: fish gut residents**

Although extraordinarily long, the \textit{E. dianae} symbiont is not the longest known. The size record holders are indeed the surgeonfish intestinal symbionts \textit{Epulopiscium} spp. Their heterotrophic fish-shaped cells are up to $600 \times 80 \, \mu \text{m}$ and reproduce by forming at least two intracellular daughter cells (Fig. 1E; Angert \textit{et al}., 1993). Cells colonizing the gut of a given host fish synchronize internal offspring initiation and development so that offspring always grows during the day, concomitantly with host feeding. Reproduction begins as two Z-rings form at the mother cell’s poles. As the rings fully constrict, two polar cells form, are engulfed by the mother cell and grow in a membrane-bound cytoplasmic compartment until they completely fill the mother cell. Finally, the mother cell undergoes a form of programmed cell death that likely conserves the biochemical resources accumulated during growth (Ward \textit{et al}., 2009) and offspring emerge through a split in the mother cell envelope. Notably, despite developmental synchrony, inhabitants of a single population vary up to five times in volume (Mendell \textit{et al}., 2008). How can \textit{Epulopiscium} spp. accommodate growth of internal offspring? Although their genome size is unexceptional (ca. 4 Mb), each cell contains up to $10^5$ copies (Mendell \textit{et al}., 2008). However, in contrast to most bacteria, in which chromosomes are distributed throughout the cytoplasm, \textit{Epulopiscium} nucleoids are located at the cell periphery, which may also allow \textit{Epulopiscium} to respond promptly to environmental stimuli.

**Outside but inside: mammalian gut residents**

Another curious gastrointestinal dweller is \textit{Metabacterium polyspora}. Unlike most endospore formers, this guinea pig gut resident produces up to nine endospores per mother cell (Chatton and Pérard, 1913; Robinow, 1957). Although no \textit{Metabacterium}-like symbiont has been maintained in culture, morphologically similar symbionts have been found in various rodent species (Kunstyr \textit{et al}., 1988). The natural life cycle of \textit{M. polyspora} (Fig. 1F) requires the bacterium to cycle through the gastrointestinal tract and therefore relies on the coprophagous nature of the guinea pig for survival (Angert and Losick, 1998). Only mature endospores survive passage through the mouth and stomach of the host, and may germinate in the small intestine. Here, some cells undergo binary fission, but most cells begin to sporulate (Angert and Losick, 1998). From the small intestine, \textit{M. polyspora} cells are deposited in the guinea pig caecum where they complete sporulation. Cells with engulfed forespores or mature endospores do not seem to undergo binary fission and, after traversing the lower intestine, they are finally eliminated from the host with its feces. If a guinea pig ingests the defecated spores, the life cycle starts again. The process of endospore formation in \textit{M. polyspora} differs from that of prototypical endospore-forming bacteria such as \textit{Bacillus subtilis} (Angert and Losick, 1998). The asymmetric cell division of \textit{M. polyspora} normally takes place at both cell poles (rather than at one only) and DNA is partitioned into both polar compartments and it is also retained in the mother cell. Therefore, unlike sporulating \textit{B. subtilis}, which is diploid, \textit{M. polyspora} must contain three or more genomes, which makes coordinating DNA replication and segregation with offspring formation even more challenging (Angert and Losick, 1998). After engulfment, the forespores can undergo division to produce multiple forespores that grow and mature into endospores. Most endospore-forming Firmicutes produce a single, dormant, invulnerable spore only to survive adverse environmental conditions or to increase their dispersal. It is therefore stunning that the reiteration of such a developmental program is part of the normal \textit{M. polyspora} life cycle. The coordination of multiple endospore formation with transit through the gut, combined with a coprophagous natural host, might favour this reproduction mode over binary fission. Additionally, the occasional experience of harsh conditions outside the host would still trigger \textit{M. polyspora} to initiate sporulation for survival and dispersal.

There is another group of low-GC Gram-positive bacteria that forms endospores for reproduction and dispersal in a very unorthodox way: the segmented filamentous bacterium (SFB) or \textit{Candidatus Arthromitus} (Fig. 1G). SFB reside in the intestinal tracts of many vertebrate species such as mice and ourselves (Klaasen \textit{et al}., 1992; Yin
et al., 2013; Schnupf et al., 2015) and have received much interest because of their ability to educate the gut immune system and to induce a healthy level of physiological inflammation (Schnupf et al., 2013; Ivanov et al., 2009). Already four decades ago, ultrastructural studies of murine gut SFB supported the following life cycle: attachment to epithelial cells via the holdfast tip of the so-called “initiating intracellular offspring” leads to SFB embedding among the microvilli followed by filamentous growth. A complex developmental program thereupon starts at the distal tip and ultimately leads to intracellular offspring formation and release (Davis and Savage, 1974; Chase and Erlandsen, 1976; Ferguson and Birch-Andersen, 1979). According to this model, when filaments grow longer than 50 μm in length (most filaments are ≈100 μm and can be up to 1 mm long), the large primary filament segments start to undergo a symmetrical division to form smaller secondary segments. These differentiate by dividing asymmetrically to form a mother and a daughter cell. The latter becomes engulfed and subsequently divides to form two intracellular offspring within the surrounding mother cell segment. Intracellular offspring are then released from the filament by breakdown of the filament septa and reattach to the host. Remarkably, the intracellular offspring have two possible fates, either holdfast-producing differentiation, or maturation to form a spore. In the first case, the active offspring are released into the lumen of the intestine and they attach to the intestinal epithelium to establish new filaments within the host. In the second case, maturation results in two intracellular offspring cells that are encased in a common spore coat, which forms an endospore. As the cells of the intestinal epithelium are constantly shed and renewed, they are not a stable substrate. Therefore, the production of intracellular offspring released from the dying parental filament probably evolved to allow SFB to reposition itself inside the same host. Additionally, the endospore provides an effective alternative for the SFB to disperse. These alternative forms of offspring (either active or dormant) allow the SFB to maintain stable populations within a given host and to colonize new hosts after surviving harsh environments such as aerobic ones or the highly acidic upper gut. The proposed SFB life cycle was recently and completely recapitulated in vitro (Schnupf et al., 2015). This is exciting, as it will permit the investigation of the complex developmental stages of SFB and the detailed dissection of the unique SFB–host interaction at the cellular and molecular levels.

What controls the growth of gut residents?
The host-secreted molecules that determine SFB cell fate, as well as those putatively controlling that of other gut residents are not known. Where should we look for those? It is a challenge for the gut to stably host 100 trillion bacteria belonging to more than 100 different species without this inducing inflammation or without the host falling victim of pathogenic infections (estimates by Qin et al., 2010). Perturbations in intestinal homeostasis are the basis of various diseases such as obesity, diabetes and inflammatory bowel disease. Given that immune homeostasis relies on several, partly overlapping immunological mechanisms we still have not fully grasped how it is achieved (Hooper and Macpherson, 2010). However, several effectors of the innate immune system controlling the number of specific gut residents have already been identified and these are antimicrobial peptides (AMPs). This should not come as a surprise as the growing consensus is that both pathogenic and mutualistic bacteria share similar microbe associated molecular patterns (MAMPs) and trigger an immune response. Nevertheless, its outcome differs between pathogenic and mutualistic associations: in the former, the immune response triggers the elimination of deleterious microbes, in the latter a molecular dialogue is initiated which results in homeostasis and immunotolerance (Nussbaum and Locksley, 2012). In a mutualistic context, antimicrobial peptides produced by the epithelia contribute to maintaining the population structure of the microbiota and prevent these from penetrating the underlying tissues. The epithelia are covered with a thin mucous layer that is kept nearly sterile, while microbes abound in the above-lying lumen (Hooper and Macpherson, 2010). In the small intestine, this is accomplished by the Paneth cells which secrete into the gut AMPs and other antimicrobial proteins thereby limiting contact between the microbiota and epithelial tissues (Kobayashi et al., 2005; Vaishnava et al., 2008). In addition, AMPs such as α-defensins also regulate the composition of the microbiota in the lumen (Salzman et al., 2003; 2010; Wilson et al., 1999). In particular, bacteria known as Firmicutes decreased with higher defensin production by Paneth cells. Similarly, Vaishnava et al. (2011) showed that the antimicrobial lectin RegIII-γ prevents any contact between the mouse commensal microbiota and the epithelial surface of the small intestine. Disruption of this physical separation, by knocking out the RegIII-γ gene, resulted in bacterial proliferation at the intestinal epithelial surface, which subsequently triggered an adaptive immune response against the microbiota. Secretion of the RegIII-γ protein was shown to inhibit bacterial growth within a band extending 50 μm from the epithelium, creating a “no-microbe’s-land” where no commensal can grow or stimulate the host immune system. Given that RegIII-γ is not expressed in the large intestine, this is one of the first cases describing the involvement of the innate immune system in the regulation of mutualism via a local immune response. Also in the fruit flies gut a well-adjusted level of AMP expression under healthy, as well as pathogenic conditions is essential for maintaining homeostasis (Ryu et al., 2008).

In conclusion, a respectable body of data indicates that vertebrate-secreted AMPs target and regulate the number
of gut micro-residents. Although their capacity to affect the morphology or reproduction mode of commensals has not been shown yet, this is likely, as research reviewed in the following section indicates.

“Staying put”: reproductive anomalies of endosymbionts with non-reduced genomes

If cell gigantism and associated polyploidy have been observed in numerous microbial symbionts, only in those of legumes and weevils the molecular triggers, i.e., the host-secreted molecules that block bacterial cytokinesis have been nailed (Van der Velde et al., 2010; Login et al., 2011). In most cases, the benefit of plant and insect symbionts is privileged acquisition of nutrients and a growth niche. Whether the symbionts reside extracellularly, in luminal spaces between cells and tissues, or live an intracellular existence they are closely associated with the host cells. When legumes interact with nitrogen-fixing rhizobia, the symbiosis leads to formation of new organs, the root nodules (Fig. 2A). These organs house millions of endosymbiotic rhizobia. Within symbiotic nodule cells, these Alphaproteobacteria become capable of reducing atmospheric nitrogen to ammonium, which is transferred to the plant and used for its growth. These irreversibly differentiated rhizobia (also referred to as bacteroids; Fig. 2B) have altered physiology and metabolism. In some legumes, as in the model plant Medicago truncatula, bacteroids have increased membrane permeability, highly amplified genome content and – whether elongated or branched – are much larger than soil-dwelling rhizobia. These bacteroids are incapable of cell division and reproduction (Mergaert et al., 2006; Maroti et al., 2011). Although this terminal differentiation of bacteroids is not observed in all legumes and is therefore not essential per se for symbiotic nitrogen fixation, it improves the symbiotic efficiency of the bacteroids (Oono and Denison, 2010). In M. truncatula, symbiotic nodule cells produce nodule-specific AMPs of a particular family called NCR for nodule-specific cysteine-rich peptides (NCRs; Mergaert et al., 2003; Alunni et al., 2007). Remarkably, the M. truncatula NCR gene family consists of several hundred genes, which are all specifically expressed in Rhizobium-infected nodule cells. The NCRs are responsible for the terminal differentiated state of the endosymbiont Sinorhizobium meliloti in M. truncatula nodules (Fig. 2C; Van der Velde et al., 2010). Similarly to other plant and animal AMPs, they effectively kill both Gram-positive and Gram-negative bacteria in vitro at prototypical concentrations. NCRs are transported via exocytosis to the bacteroids and some of the NCRs enter the bacterial cytosol and likely have intracellular bacterial targets. The in vivo and in vitro effects of NCRs on S. meliloti are dramatically different. The peptides quickly kill the rhizobia in vitro, whereas bacteroids do not grow but maintain active metabolism. The difference between the in vivo and in vitro effect of NCRs could be explained if we assume that, in vivo, several tens or hundreds of native peptides, each likely present at very low concentrations, have a different effect that a single, highly concentrated peptide applied in vitro. Moreover, particular conditions prevalent in nodules, such as the low free oxygen concentration, needed for activity of the oxygen-sensitive nitrogenase, could modulate the bacterial responses to the NCRs in such a way that the bacteroids survive without growing. Some NCRs inhibit bacterial division in vivo and in vitro, leading to cell elongation. Such NCRs were localized at the division site of S. meliloti cells, and may therefore interfere with the bacterial cytokinetic machinery. However, the high sequence variability of NCRs suggests diversity in their functions, mode of actions and targets involved in different aspects of bacteroid metabolism, but could also be an adaptation to the high diversity of soil rhizobia. Analysis of the sequence of NCRs showed that they are subject to diversifying evolution, which is compatible with such a hypothesis (Alunni et al., 2007). Moreover, it is remarkable and yet unexplainable that most plants contain hundreds of genes encoding for cysteine-rich proteins similar to innate immunity-dedicated AMP genes (Silverstein et al., 2007), even if they do not employ them to form symbioses or to defend themselves. The NCRs are indeed expressed only in nodules and are not induced during treatment of M. truncatula with pathogens.

In conclusion, legumes may adopt effectors of the innate immune system to dominate their endosymbionts in order to maximize their own profits. In a striking case of convergent evolution, this is also the case for Sitophilus weevils (Fig. 2D–G; Login et al., 2011, Login and Heddi, 2013). Cereal weevils house Sodalis pierantonius (Fig. 2F; Oakeson et al., 2014; formerly known as Sitophilus Primary Endosymbiont, or SPE) permanently, in the female germ cells from which they are transmitted to the progeny. Early during embryogenesis, S. pierantonius induces the differentiation of bacteriocyte cells that form the bacteriome (Heddi et al., 1999), a specific organ that secludes endosymbionts (Fig. 2E; Anselme et al., 2008). These host cells, in response, express an adapted response in order to maintain the symbionts within the bacteriome (Anselme et al., 2008; Login et al., 2011). Sequestering symbiotic bacteria in the bacteriome – where only a few immune effectors are expressed – protects them from exposure to and elimination by a standard, non-tailored systemic immune response. This humoral response does indeed attack S. pierantonius, when this bacterium is injected in the insect hemolymph (Nakabachi et al., 2005; Ratzk et al., 2011). In the weevil bacteriome, instead, Coleoptericin A (ColA) is the only antimicrobial expressed constitutively and ColA transcript levels correlate with S. pierantonius density (Login and Heddi, 2012; Anselme
et al., 2008; Login et al., 2011; Masson et al., 2015). While ColA exhibits bactericide activity against Gram-negative bacteria and kills them at high concentrations, it exhibits a bacteriostatic activity against *S. pierantonius* at the low concentrations likely existing in the bacteriocytes. Microscopic observations showed that inhibition of cytokinesis by ColA led to filamentation, namely to up to 50 μm-long *E. coli* cells (Fig. 2G) and up to 200 μm-long *Nardonella* cells, the primary endosymbiont of the weevil *Rhynchophorus ferrugineus*. Additionally, it was also showed that *S.
pierantonius reduced significantly in size in vivo following functional knockout of the coIA gene (Login et al., 2011). However, whether this bacterial size reduction is due to a resumption of bacterial cytokinesis or a multiplication of small bacteria remains unclear. In vivo silencing of the coIA gene also resulted in an extensive dispersion of the symbiont outside of the bacteriome, which strongly supports the idea that, besides its morphology, CoIA controls symbiotic bacteria through a specific and local inhibition of bacterial cytokinesis. How does CoIA block bacterial fission? Without affecting the eukaryotic cell, CoIA might interact with bacterial outer membrane protein C (OmpC) and OmpA and, in the bacterial cytoplasm, with the chaperonin GroEL. groEl gene deletion led to filamentation due to the misfolding of FtsE (Susin et al., 2006; Fujiiwara and Taguchi, 2007). Considering that both CoIA peptide activity and groEL gene deletion induce filamentation, it was proposed that CoIA hampers cell division through inhibition of the GroEL chaperonin activity. The presence of accessible hydrophobic patches is the major feature guiding the interaction of GroEL with its substrate. The hydropathy plots showed that CoIA N-terminus is positively charged and it might therefore form stable complexes with GroEL. Interestingly, the interaction of CoIA with GroEL is extremely specific since no interaction was detected with other eukaryotic chaperones, which may explain why CoIA does not harm weevil bacteriocytes (Login et al., 2011).

The reproductive minimalists: reproduction of intracellular endosymbionts with highly reduced cytokinetic and/or cytoskeletal machineries

Could AMPs-induced cytokinesis also keep at bay symbionts with extremely reduced genomes? This question is not easy to answer given that many of these not only lack divisome genes but also a canonical PG. Like weevils, aphids carry an obligatory mutualistic endosymbiont, Buchnera aphidicola, which harbours from 20 to several hundreds genome copies, varying from cell to cell (Komaki and Ishikawa, 1999; 2000). Notably, the genomic copy number of this gammaproteobacterium is low in aphid embryos, increases during postembryonic development to adulthood, and decreases during insect ageing. Moreover, aphids have two different morphs. While the aphid colony usually consists mostly of apterae, the wingless morphs, some environmental cues increase the population of alatae, the winged morphs. Alatae-associated Buchnera have twice as many genomic copies per cell as apterae-associated ones. The amplification of the genomic DNA by Buchnera might be a genetic counterbalance against accumulating mutations drift, which leads to long-term massive genome reduction (Moran, 1996; Baumann et al., 1996; Andersson and Kurland, 1998; Fares et al., 2002). This low genome copy number of Buchnera in aphid embryos might result from the elimination of mutated copies during symbiont transmission from mother to progeny. During host postembryonic development, DNA replication not followed by cytokinesis restores the high number of genomic copies of Buchnera characteristic of adult host insect. Apart from the copy number, also the physical conformation of the Buchnera genome varies in response to the physiological state of the host insect (Komaki and Ishikawa, 2000).

We do not know the molecular mechanisms that link host physiology with the number of Buchnera genome copies per cell. Curiously, a novel class of genes that encodes small, secreted, often cysteine-rich, proteins appears to be transcribed in bacteriocytes (Shigenobu and Stern, 2013). These genes are first expressed in developing aphids, exactly when the prospective bacteriocytes engulf the symbionts, and bacteriocyte-specific expression is maintained throughout the aphid’s life. The expression pattern suggests that recently evolved secretion proteins act within bacteriocytes, perhaps to mediate the symbiosis with beneficial bacterial partners, which is reminiscent of the aforementioned leguminous plant NCRs (Shigenobu and Stern, 2013).

Polyploidy has also been suspected (Wu et al., 2006) and subsequently proven (Woyke et al., 2010) for the Bacteroidetes Sulcia muelleri, another sap-feeding insect endosymbiont (Fig. 2H–J). This was estimated to contain 180–880 genome copies per cell. As in the case of Buchnera, it is not known what controls the number of Sulcia genomes or cells.

How do obligate endosymbionts reproduce without a cell wall? Since the original report of Klieneberger in 1935...
Molecular genetic analysis of the L-form variant of B. subtilis showed that conversion into a form that can replicate reasonably efficiently in the absence of a cell wall requires only two genetic changes (Leaver et al., 2009). Remarkably, despite the limited mutational changes required, L-form cells completely abandon the normally essential cell division machinery used by virtually all extant bacterial cells, and proliferate instead by a mechanism of membrane tubulation or blebbing referred to as extrusion-resolution. This process is, at least for B. subtilis, completely independent of the cell wall precursor synthetic pathway and the major cytoskeletal proteins, MreB and FtsZ. A recent report on Listeria L-forms described vesiculation, a process involving similarly complex membrane dynamics (Dell’Era et al., 2009). A wide range of bacteria is thought to be able to enter the L-form state, including both Gram-positive and -negative lineages (Domingue and Woody, 1997). Modern extant cells may have retained L-form production as a back-up process in case of cell wall defective synthesis or damage. These eventualities are likely ancient, given the widespread production of PG active antibiotics, such as β-lactams, glycopeptides and lipopeptides, by various primitive groups of bacteria (Goodfellow and Fiedler, 2010; Gupta, 2011). Consistently, a considerable body of experimental and clinical evidence supports the pathogenicity of CWDB (Domingue and Woody, 1997). Probably all known bacterial species can be converted to L-forms by a variety of inducing agents. Among the best known of such agents are cell wall-inhibiting antibiotics, high concentrations of amino acids, and peptidases and PG lytic enzymes. It is therefore not surprising that (i) obligate PG-deficient endosymbionts can reproduce and that (ii) L-form bacteria have also been found to form non-pathogenic symbioses with a wide range of plants, where they confer resistance against subsequent challenge by bacterial pathogens (Paton, 1987). Examples include the systemic protection by L-forms of the pathogen Pseudomonas syringae of bean plants against halo-blight (Amijee et al., 1992) and the protection of cabbage against Xanthomonas campestris (Waterhouse et al., 1996). More recently, L-forms of the endophyte Bacillus amyloliquesciens have been observed in vanilla crops where they likely protect them from diseases (White et al., 2014).

But between bacterial symbionts bearing complete cytokinetic machinery and L-forms other exotic cases have been reported. Chlamydias are important pathogens and symbionts lacking the cell-division protein FtsZ but it was recently shown that some environmental ones have cell wall sacculi, albeit consisting of a novel PG type (Pilhofer et al., 2014, Jacquier et al., 2015). The discovery of chlamydial PG challenges the current hypothesis that it is the absence of a cell wall, to make FtsZ non-essential.

Concluding remarks and open questions

The recent extension of cell biological studies to the microbial symbioses field already confuted a number of tenets well-rooted in this discipline, underscroing the dangers of the so-called streetlight effect. Even considering the few systems discussed in this non-systematic – and therefore not exhaustive – review only, it is obvious that many more long-standing cell biological dogmas will be broken. More generally, the study of cell wall growth, divisome assembly and positioning, and chromosome segregation in organisms displaying atypical growth modes will help us to elucidate what lies at the core of the bacterial cytokinetic machinery and how it evolved. This information is most precious as it can be easily exploited to design new antibiotics, thereby filling the present “discovery void” (Silver, 2011; 2014; Li and Ma, 2015). Of note, Gammaproteobacteria are not only the most common microorganisms associated with animals (Sachs et al., 2011), but they also include several common, still challenging pathogens.

Besides informing cell biology and biomedicine, by study how symbionts divide we can learn whether (and possibly how) – in evolutionary time – the associative lifecycle shaped bacterial reproduction. As symbiont morphologies and spatial dispositions defy easy explanations, the host role or that of abiotic factors in shaping them is an open question. For example, the L. oneistus symbiont (Leisch et al., 2012) and, possibly, that of Kentrophoros (Fenchel and Finlay, 1989) divide longitudinally allowing both daughter cells to keep contact with the host surface. Nevertheless, longitudinal division was also suggested for endosymbionts of the gutless oligochaete Olavius (Giere and Krieger, 2001; reviewed in Bright and Giere, 2005) and of the deep-sea mussel B. puteoserpentis (Zielinski et al., 2009) although these, as endosymbionts, unlikely evolved this fission mode to better transmit the associative lifestyle to both their offspring.

Unfortunately, many symbionts, including the vast majority of the ones mentioned here are not cultivable yet. Moreover, we ignore how their respective free-living counterparts, if existing, divide. It is possible that they either retained or lost their capacity to switch between canonical and non-canonical reproduction depending on their free-living or symbiotic condition. More efforts are necessary to cultivate these symbionts or – at least – to characterize the morphology and reproduction mode of their environmental counterparts, if existing.

Finally, not to incur into yet another observational bias by merely moving from one streetlight to the next, it is necessary to extend cell biological investigations to other environmental organisms from both the Archaea and Eukarya domains (Bernander et al., 2011), as well as other ecological niches such as extreme environments. Only by bringing cell biology outside the streetlight we can identify
conserved mechanisms of cell growth and reproduction and find new ways to block it for biomedical purposes.

Acknowledgments

I do not have any conflict of interest to declare. I was supported by the Austrian Science Fund (FWF) project P22470. Many thanks to Abdelaziz Heddi, Andreas Brune, Esther Angert, Joerg A. Ott, Nikolaus Leisch, and Nika Pende for their helpful comments on the manuscript’s first draft. I am deeply grateful to two anonymous reviewers for their precious and constructive comments.

References

Alunni, B., Kevei, Z., Redondo-Nieto, M., Kondorosi, A., Mergaert, P., and Kondorosi, E. (2007) Genomic organization and evolutionary insights on GRP and NCR genes, two large nodule-specific gene families in medicago truncatula. Mol Plant Microbe Interact 20: 1138–1148.

Amijee, F., Allan, E.J., Waterhouse, R.N., Glover, L.A., and Alunni, B., Kevei, Z., Redondo-Nieto, M., Kondorosi, A., Angert, E.R., Clements, K.D., and Pace, N.R. (1993) The large bacterium. Nature 362: 239–241.

Angert, E.R., Clements, K.D., and Pace, N.R. (1993) The largest bacterium. Nature 362: 239–241.

Angert, E.R. (2005) Alternatives to binary fission in bacteria. Nat Rev Microbiol 3: 214–224.

Angert, E.R., and Losick, R.M. (1998) Propagation by sporulation in the guinea pig symbiont metabacterium polyspora. Proc Natl Acad Sci U S A 95: 10218–10223.

Angert, E.R., Clements, K.D., and Pace, N.R. (1993) The largest bacterium. Nature 362: 239–241.

Anselme, C., Perez-Brocal, V., Vallier, A., Vincent-Monegat, B., Bernhard, T.G., and de Boer, P.A. (2005) SlmA, a nucleoid-associated, FtsZ binding protein required for blocking septal iome tissue. BMC Biol 6: 43.

Baumann, P., Baumann, L., and Clark, M.A. (1996) Levels of Buchnera aphidicola Chaperonin GroEL during growth of the aphid Schizaphis graminum. Curr Microbiol 32: 279–285.

Bayer, C., Heindl, N.R., Rinke, C., Lucke, S., Ott, J.A., and Bulgheresi, S. (2009) Molecular characterization of the symbionts associated with marine nematodes of the genus Robbea. Environ Microbiol Rep 1: 136–144.

Bernander, R., Lind, A.E., and Ettema, T.J. (2011) An archaean origin for the actin cytoskeleton: implications for eukaryogenesis. Commun Integr Biol 4: 664–667.

Bernhardt, T.G., and de Boer, P.A. (2005) SimA, a nucleoid-associated, FtsZ binding protein required for blocking septal ring assembly over chromosomes in E. coli. Mol Cell 18: 555–564.

Bi, E.F., and Lutkenhaus, J. (1991) FtsZ ring structure associated with division in Escherichia coli. Nature 354: 161–164.

Bramkamp, M., and van Baarle, S. (2009) Division site selection in rod-shaped bacteria. Curr Opin Microbiol 12: 683–688.

Bright, M., and Giere, O. (2005) Microbial symbiosis in Annelida. Symbiosis 38: 1–45.

© 2016 The Authors. Environmental Microbiology Reports published by Society for Applied Microbiology and JohnWiley & Sons Ltd, Environmental Microbiology, 18, 2305–2318
animal colony in Denmark. Acta Pathol Microbiol Scand B 87: 247–252.

Fujiwara, K., and Taguchi, H. (2007) Filamentous morphology in GroE-depleted Escherichia coli induced by impaired folding of FtsZ. J Bacteriol 189: 5860–5866.

Giere, O., and Krieger, J. (2001) A triple bacterial endosymbiosis in a gutless oligochaete (annelida): ultrastructural and immunocytochemical evidence. Invertebr Biol 120: 41–49.

Goley, E.D. (2013) Tiny cells meet big questions: a closer look at bacterial cell biology. Mol Biol Cell 24: 1099–1102.

Goodfellow, M., and Fiedler, H.P. (2010) A guide to successful actinobacterial systematics. Antonie Van Leeuwenhoek 98: 119–142.

Gupta, R.S. (2011) Origin of diderm (Gram-negative) bacteria: antibiotic selection pressure rather than endosymbiosis likely led to the evolution of bacterial cells with two membranes. Antonie Van Leeuwenhoek 100: 171–182.

Hale, C.A., and de Boer, P.A. (1997) Direct binding of FtsZ to ZipA, an essential component of the septal ring structure that mediates cell division in E. coli. Cell 88: 175–185.

Hale, C.A., Meinhardt, H., and de Boer, P.A. (2001) Dynamic localization cycle of the cell division regulator MinE in Escherichia coli. Embo J 20: 1563–1572.

Heddi, A., Grenier, A.M., Khatchadourian, C., Charles, H., and Nardon, P. (1999) Four intracellular genomes direct weevil biota. Invertebr Biol 118: 2316–2321.

Horn, M. (2008) Chlamydiae as symbionts in eukaryotes. Annu Rev Microbiol 62: 113–131.

Ivanov, I.I., Atarashi, K., Manel, N., Brodie, E.L., Shima, T., Karaoz, U., et al. (2009) Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell 139: 485–498.

Jacquier, N., Viollier, P.H., and Greub, G. (2015) The role of segmented filamentous bacteria in the intestinal tract. Curr Opin Microbiol 28: 485–498.

Kereszt, A., Mergaert, P., Uchiumi, T., Alunni, B., Evanno, G., Cheron, A., and Heddi, A. (2015) Systemic infection generates a Local-like immune response of the bacterium in rodent intestines. Insect Biochem Mol Biol 36: 253–258.

Kunstyr, I., Schiel, R., Kaup, F.J., Uhr, G., and Kirchhoff, H. (1988) Giant gram-negative noncultivable endosymbiotic bacteria in rodent intestines. Naturwissenschaften 75: 525–527.

Leaver, M., Dominguez-Cuevas, P., Coxeard, J.M., Daniel, R.A., and Errington, J. (2009) Life without a wall or division machine in bacillus subtilis. Nature 457: 849–853.

Leisch, N., Verheul, J., Heindl, N.R., Gruber-Vodicka, H.R., Pende, N., den Blauwen, T., and Bulgheresi, S. (2012) Growth in width and FtsZ ring longitudinal positioning in a gammaproteobacterial symbiont. Curr Biol 22: R831–R832.

Li, X., and Ma, S. (2015) Advances in the discovery of novel antimicrobials targeting the assembly of bacterial cell division protein FtsZ. Eur J Med Chem 95: 1–15.

Login, F.H., and Heddi, A. (2013) Insect immune system maintains long-term resident bacteria through a local response. J Insect Physiol 59: 232–239.

Login, F.H., Balmant, S., Vallier, A., Vincent-Monegat, C., Vigneron, A., Weiss-Gayet, M., et al. (2011) Antimicrobial peptides keep insect endosymbionts under control. Science 334: 362–365.

Lowe, J., and Amos, L.A. (1998) Crystal structure of the bacterial cell-division protein FtsZ. Nature 391: 203–206.

Maroti, G., Kereszt, A., Kondorosi, E., and Mergaert, P. (2011) Natural roles of antimicrobial peptides in microbes, plants and animals. Res Microbiol 162: 363–374.

Masson, F., Vallier, A., Vigneron, A., Balmant, S., Vincent-Monegat, C., Zaidman-Remy, A., and Heddi, A. (2015) Systemic infection generates a Local-like immune response of the bacteriome organ in insect symbiosis. J Inn Immun 7: 290–301.

Mendell, J.E., Clements, K.D., Chast, J.H., and Angert, E.R. (2008) Extreme polyplody in a large bacterium. Proc Natl Acad Sci U S A 105: 6730–6734.

Mergaert, P., Nikovics, K., Kelemen, Z., Maunoury, N., Vaubert, D., Kondorosi, A., and Kondorosi, E. (2003) A novel family in Medicago truncatula consisting of more than 300 nodule-specific genes coding for small, secreted polypeptides with conserved cysteine motifs. Plant Physiol 132: 161–173.

Mergaert, P., Uchiyuni, T., Alunni, B., Evano, G., Cheron, A., Catrice, O., et al. (2006) Eukaryotic control on bacterial cell cycle and differentiation in the Rhizobium-legume symbiosis. Proc Natl Acad Sci U S A 103: 5230–5235.

Monahan, L.G., Liew, A.T., Bottomley, A.L., and Harry, E.J. (2014) Division site positioning in bacteria: one size does not fit all. Front Microbiol 5: 19.

Moran, N.A. (1996) Accelerated evolution and muller’s rachet in endosymbiotic bacteria. Proc Natl Acad Sci U S A 93: 2873–2878.

Moran, N.A., Tran, P., and Gerardo, N.M. (2005) Symbiosis and insect diversification: an ancient symbiont of sap-feeding insects from the bacterial phylum Bacteroidetes. Appl Environ Microbiol 71: 8802–8810.
Mukherjee, A., and Lutkenhaus, J. (1998) Purification, assembly, and localization of FtsZ. *Methods Enzymol* **298**: 296–305.

Musat, N., Giere, O., Gieseke, A., Thiermann, F., Amann, R., and Dubilier, N. (2007) Molecular and morphological characterization of the association between bacterial endosymbionts and the marine nematode *Astomoma* sp. from the Bahamas. *Environ Microbiol* **9**: 1345–1353.

Nanninga, N. (1991) Cell division and peptidoglycan assembly in Escherichia coli. *Mol Microbiol* **5**: 791–796.

Nakabachi, A., Shigenobu, S., Sakazume, N., Shiraki, T., Hayashizaki, Y., Caminci, P., et al. (2005) Transcriptome analysis of the aphid bacteriocyte, the symbiotic host cell that harbors an endocellular mutualistic bacterium, Buchnera. *Proc Natl Acad Sci U S A* **102**: 5477–5482.

Nussbaum, J.C., and Locksley, R.M. (2012) Infectious (non)-tolerance-frustrated commensalism gone awry? *Cold Spring Harb Perspect Biol* **4**: 2016 The Authors. *Environmental Microbiology* Reports published by Society for Applied Microbiology and JohnWiley & Sons Ltd, *Environ Microbiol* **1345–1353.

Oakeson, K.F., Gil, R., Clayton, A.L., Dunn, D.M., von Oono, R., and Denison, R.F. (2010) Comparing symbiotic efficiency between swollen versus nonswollen rhizobial bacteria: an unusual trade-off that drives the post-natal maturation of the gut immune system. *Semin Immunol* **25**: 99–103.

Oono, R., and Denison, R.F. (2010) Comparing symbiotic efficiency between swollen versus nonswollen rhizobial Bacteria. *Plant Physiol* **154**: 1541–1548.

Ozyamak, E., Kollman, J.M., and Komeili, A. (2013) Bacterial actin and their diversity. *Biochemistry* **52**: 6928–6939.

Paton, A.M. (1987) L-forms: evolution or revolution? *J Appl Bacteriol* **63**: 365–371.

Pende, N., Leisch, N., Gruber-Vodicka, H.R., Heindl, N.R., Ott, J., den Blaauwen, T., and Bulgheresi, S. (2014) Size-independent symmetric division in extraordinarily long cells. *Nat Commun* **5**: 4803.

Pichoff, S., and Lutkenhaus, J. (2005) Tethering the Z ring to the membrane through a conserved membrane targeting sequence in FtsA. *Mol Microbiol* **55**: 1722–1734.

Pilhofer, M., Aisteltner, K., Ladinsky, M.S., Konig, L., Horn, M., and Jensen, G.J. (2010) Architecture and host interface of environmental chlamydiae revealed by electron cryotomography. *Environ Microbiol* **12**: 417–429.

Pichoff, S., and Lutkenhaus, J. (2005) Tethering the Z ring to the membrane through a conserved membrane targeting sequence in FtsA. *Mol Microbiol* **55**: 1722–1734.

Pilhofer, M., Aisteltner, K., Ladinsky, M.S., Konig, L., Horn, M., and Jensen, G.J. (2014) Architecture and host interface of environmental chlamydiae revealed by electron cryotomography. *Environ Microbiol* **16**: 417–429.

Pölz, M.F., Distel, D.L., Zarda, B., Amann, R., Felbeck, H., Ott, J.A., and Cavanaugh, C.M. (1994) Phylogenetic analysis of a highly specific association between ecotsymbiotic sulfur-oxidizing bacteria and a marine nematode. *Appl Environ Microbiol* **60**: 4461–4467.

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S., Manichanh, C., et al. (2010) A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**: 59–66.

Randich, A.M., and Brun, Y.V. (2015) Molecular mechanisms for the evolution of bacterial morphologies and growth modes. *Front Microbiol* **6**: 580.

Raskin, D.M., and de Boer, P.A. (1999) Rapid pole-to-pole oscillation of a protein required for directing division to the middle of *Escherichia coli*. *Proc Natl Acad Sci U S A* **96**: 4971–4976.

Ratzka, C., Liang, C., Dandekar, T., Gross, R., and Feldhaar, H. (2011) Immune response of the ant *Camponotus floridanus* against pathogens and its obligate mutualistic endosymbiont. *Insect Biochem Mol Biol* **41**: 529–536.

Razin, E., Hecht, D., and Hoch, Y. (2006) GroES/GroEL and DnaK/DnaJ have distinct roles in bacterial cell biology. *Trends Cell Biol* **16**: 339–346.

Shigenobu, S., and Stern, D.L. (2013) Aphids evolved novel secreted proteins for symbiosis with bacterial endosymbiont. *Proc Biol Sci* **280**: 20121952.

Silver, L.L. (2011) Challenges of antibacterial discovery. *Clin Microbiol Rev* **24**: 71-109.

Silver, L.L. (2014) Antibacterials for any target. *Nat Biotechnol* **32**: 1102–1104.

Silverstein, K.A.T., Moskal, W.A., Wu, H.C., Underwood, B.A., Graham, M.A., Town, C.D., and VandenBosch, K.A. (2007) Small cysteine-rich peptides resembling antimicrobial peptides have been under-predicted in plants. *Plant J* **51**: 262–280.

Strassert, J.F., Desai, M.S., Radek, R., and Brune, A. (2010) Identification and localization of the multiple bacterial symbionts of the termite gut flagellate *Joenia annectens*. *Microbiology* **156**: 2068–2079.

Sultan, M.F., Baldini, R.L., Gueiros-Filho, F., and Gomes, S.L. (2006) GROEs/GROEL and DnaK/DnaJ have distinct roles in stress responses and during cell cycle progression in *Caulobacter crescentus*. *J Bacteriol* **188**: 8044–8053.

Typas, A., Banzhaf, M., Gross, C.A., and Vollmer, W. (2012) From the regulation of peptidoglycan synthesis to
bacterial growth and morphology. Nat Rev Microbiol 10: 123–136.
Vadia, S., and Levin, P.A. (2015) Growth rate and cell size: a re-examination of the growth law. Curr Opin Microbiol 24: 96–103.
Vaishnava, S., Behrendt, C.L., Ismail, A.S., Eckmann, L., and Hooper, L.V. (2008) Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface. Proc Natl Acad Sci U S A 105: 20858–20863.
Vaishnava, S., Yamamoto, M., Severson, K.M., Ruhn, K.A., Yu, X., Koren, O., et al. (2011) The antibacterial lectin RegIIIgamma promotes the spatial segregation of microbiota and host in the intestine. Science 334: 255–258.
Van der Velde, W., Zehirov, G., Szatmari, A., Debreceny, M., Ishihara, H., Kevei, Z., et al. (2010) Plant peptides govern terminal differentiation of bacteria in symbiosis. Science 327: 1122–1126.
Ward, R.J., Clements, K.D., Choat, J.H., and Angert, E.R. (2009) Cytology of terminally differentiated epulopiscium mother cells. DNA Cell Biol 28: 57–64.
Ward, R.J., and Angert, E.R. (2008) DNA replication during endospore development in metabacterium polyspora. Mol Microbial 67: 1360–1370.
Waterhouse, R.N., Buhariwalla, H., Bourn, D., Rattray, E.J., and Glover, L.A. (1996) CCD detection of lux-marked pseudomonas syringae pv phaseolicola L-forms associated with Chinese cabbage and the resulting disease protection against Xanthomonas campestris. Lett Appl Microbial 22: 262–266.
White, J.F., Jr., Torres, M.S., Sullivan, R.F., Jabbour, R.E., Chen, Q., Tadych, M., et al. (2014) Occurrence of bacillus amyloliquefaciens as a systemic endophyte of vanilla orchids. Microsc Res Technol 77: 874–885.
Wilson, C.L., Ouellette, A.J., Satchell, D.P., Ayabe, T., Lopez-Boado, Y.S., Stratman, J.L., et al. (1999) Regulation of intestinal alpha-defensin activation by the metalloproteinase matrilysin in innate host defense. Science 286: 113–117.
Woldringh, C.L., Mulder, E., Huls, R.G., and Vischer, N. (1991) Toporegulation of bacterial division according to the nucleoid occlusion model. Res Microbial 142: 309–320.
Woldringh, C.L., Mulder, E., Valkenburg, J.A., Wientjes, F.B., Zaritsky, A., and Nanninga, N. (1990) Role of the nucleoid in the toporegulation of division. Res Microbial 141: 39–49.
Woyte, T., Tighe, D., Mavromatis, K., Clum, A., Copeland, A., Schackwitz, W., et al. (2010) One bacterial cell, one complete genome. PLoS One 5: e10314.
Wu, D., Daugherty, S.C., Van Aken, S.E., Pai, G.H., Watkins, K.L., Khouri, H., et al. (2006) Metabolic complementarity and genomics of the dual bacterial symbiosis of sharpshooters. PLoS Biol 4: e188.
Yin, Y., Wang, Y., Zhu, L., Liu, W., Liao, N., Jiang, M., et al. (2013) Comparative analysis of the distribution of segmented filamentous bacteria in humans, mice and chickens. ISME J 7: 615–621.
Young, K.D. (2010) Bacterial shape: two-dimensional questions and possibilities. Annu Rev Microbial 64: 223–240.
Zielinski, F.U., Pertenthaler, A., Duperron, S., Ragg, L., Giere, O., Borowski, C., and Dubillier, N. (2009) Widespread occurrence of an intranuclear bacterial parasite in vent and seep bathymodiolin mussels. Environ Microbial 11: 1150–1167.
Zimmermann, J., Wentrup, C., Sadowski, M., Blajezak, A., Gruber-Vodicka, H., Kleiner, M., et al. (2016) Closely coupled evolutionary history of ecto- and endosymbionts from two distantly-related animal phyla. Mol Ecol doi: 10.1111/mec.13554