ALTERNATIVES TO BINARY FISSION IN BACTERIA

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Abstract | Whereas most prokaryotes rely on binary fission for propagation, many species use alternative mechanisms, which include multiple offspring formation and budding, to reproduce. In some bacterial species, these eccentric reproductive strategies are essential for propagation, whereas in others the programmes are used conditionally. Although there are tantalizing images and morphological descriptions of these atypical developmental processes, none of these reproductive structures are characterized at the molecular genetic level. Now, with newly available analytical techniques, model systems to study these alternative reproductive programmes are being developed.

NUCLEOID
The highly organized chromosomal DNA of a bacterial cell.

CYTOSKELETON
Internal network of proteins that gives a eukaryotic cell its shape, facilitates its movement and provides a means of internal spatial organization.

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Conceptually, cell propagation by binary fission is a simple process; a cell merely needs to grow to twice its size, and then split in two. But to remain competitive, let alone viable, a prokaryotic cell must divide at the appropriate time and at the correct location in the cell, and must ensure that each progeny daughter cell receives a complete complement of genes with high fidelity. Bacterial models that have been used for the study of cell division include the Gram-negative proteobacterial species *Escherichia coli* and *Caulobacter crescentus*, and the low-GC Gram-positive bacterium *Bacillus subtilis*. These models have provided insight into cell division and continue to reveal surprising findings[1]. The cell biology and genetics of cell division in these and other model organisms have been discussed in recent reviews[2–8]. As important cell-division components are revealed, and their genetic homologues in other systems are discovered and characterized, a framework of core mechanisms that are conserved among prokaryotes is emerging (TABLE 1).

Binary fission: *Bacillus subtilis*

In this review, *B. subtilis* will be used as a model to introduce the general mechanisms and regulation of cell division in bacteria (FIG. 1a). During growth, *B. subtilis*, in common with other rod-shaped bacteria, elongates. When it reaches about twice its starting length, the cell divides in the middle by binary fission. Concurrent with growth, the genetic material of the cell replicates and segregates into incipient daughter cells in a controlled manner[2]. Although the timing of replication initiation with respect to the cell-division cycle, and the maintenance of NUCLEOID position are tightly controlled in bacteria, master cell-cycle regulators have only been identified in *C. crescentus*[9,10]. The mechanisms that are responsible for the observed rapid movement of replication origins to polar positions and the segregation of nucleoids have yet to be discerned[7,11]. However, DNA-binding proteins that maintain nucleoid structure and that seem to have a role in nucleoid segregation (such as SMC in *B. subtilis*) have been identified in various bacteria[2,11–13] (TABLE 1). Other cellular components that are implicated in DNA segregation include the actin-like protein MreB[14].

For cell division to occur, the division apparatus must assemble at the site of future cytoplasmic cleavage. FtsZ, a structural homologue of the eukaryotic CYTOSKELETAL element tubulin[15], assembles into a ring-like structure at the centre of the cell[16]. Other components of the division machinery assemble at the FtsZ ring. These components redirect cell wall growth, and prevent damage to the DNA while the cell envelope invaginates[6,17]. Finally, the cell divides to form two approximately equivalent daughter cells. Although many of the genes that are involved in cell division have been identified, the mechanisms of action of these gene products are still under intense investigation[6,18].

FtsZ is highly conserved among prokaryotes and is one of the first proteins to assemble at the future cell division site. FtsZ is also the target of cellular mechanisms that regulate the timing of cell division as well as the selection of the cell division site[18]. In normal, growing *B. subtilis* cells, nucleoid occlusion — mediated by the unsegregated nucleoid and associated proteins such as Noc[19] — prevents Z-ring assembly at the midcell prior to nucleoid segregation, and the Min system — MinCD and DivIVA — prevents the assembly of Z rings near the poles. There is evidence that other proteins, such as EzrA, affect the stability of FtsZ polymers to enhance Z-ring assembly only at appropriate locations. Additional proteins (such as YneA in *B. subtilis* and SulA in *E. coli*) destabilize Z-ring formation to prevent cell division when DNA damage is detected[20,21].

Table 1 | **Distribution of cell-division proteins among selected bacteria**

E. coli	*B. subtilis*	*C. crescentus*	*S. coelicolor*
Nucleoid structural maintenance			
MukB	SMC	SMC	SMC
Nucleoid partitioning			
	Soj/Spo0J	ParA/B	ParA/B
MreB	MreB	MreB	*mreB*-like*
FtsK	SpoIIIE	*ftsK**	*ftsK/spoIIIE*-like*
Master cell-cycle regulators			
		CtrA	
		GcrA	
Staking the division site‡			
FtsZ	FtsZ	FtsZ	FtsZ
Nucleoid occlusion			
+	Noc	w	w
Prevention of polar Z-ring assembly			
MinCD	MinCD		*minD*-like*
MinE			
	DivIVA		(DivIVA)§
Destabilization of extra Z rings			
	EzrA		
Destabilization of Z rings as part of the SOS response			
SulA			
DpiA			
	YneA		
Other division proteins that assemble with the Z ring			
FtsA	FtsA	FtsA	
FtsI	Pbp2b	FtsI	*ftsI**
FtsQ	DivIB	FtsQ	FtsQ
FtsL	FtsL		*ftsL**
FtsW	FtsW	FtsW	*ftsW**

Homologues — proteins that share a common ancestry and have the same function in a cell — are shown in rows that are shaded the same colour, for example, FtsZ. *Potential homologues that have been identified by sequence comparisons, but that have no assigned function. + indicates a functional mechanism for which no associated genes have been identified. w indicates evidence for a weak phenotype for which no associated gene has been identified. ‡With only a few exceptions, the division protein FtsZ is conserved among prokaryotes and assembles at the incipient site of cell division in many organisms including members of the Archaea, and organelles (such as chloroplasts and mitochondria). §DivIVA of *S. coelicolor* is shown in brackets because it seems to be involved in hyphal tip growth and not in the selection of the cell division site. Genes are italicized. *B. subtilis*, *Bacillus subtilis*; *E. coli*, *Escherichia coli*; *C. crescentus*, *Caulobacter crescentus*; *S. coelicolor*; *Streptomyces coelicolor*.

Several stabilizing and destabilizing factors ensure that the Z ring, and therefore the division apparatus, is properly positioned. Even among the model systems there is astonishing variation and flexibility in the genes and mechanisms that mediate cell division site selection. For example, both *E. coli* and *B. subtilis* use polar localization of the MinCD complex to prevent inappropriate division at the cell poles[18]. Genetic analyses have shown that the topological specificity of the MinCD complex is mediated by DivIVA in *B. subtilis* and by MinE in *E. coli*. Although DivIVA and MinE are functional analogues, they have no significant sequence or structural similarity. In both of these bacteria, MinD interacts with the topological specificity factor (either DivIVA or MinE) to maintain the polar localization of MinC, which in turn prevents assembly of the Z ring. In *E. coli*, MinD travels periodically from one pole of the cell to the other[22]. One complete pole-to-pole oscillation takes place in less than one minute. By contrast, the MinD homologue in *B. subtilis* is pole-associated, but remains static throughout most of the cell cycle[23]. Despite its role in enhancing the fidelity of midcell division in many bacteria, *minD* is not universally conserved — *C. crescentus* lacks a recognizable *minD* homologue[8,24]. Furthermore, other highly conserved genes that are essential for cell division in *E. coli*, *C. crescentus* and *B. subtilis*, most notably *ftsZ*, are not found in all prokaryotes[25,26]. Remarkably, the cell-division machinery is not as evolutionarily conserved as many of the components of central metabolism, as outlined in REF. 27. This propensity to tolerate modifications of an essential process provides opportunities for flexibility to accommodate the different 'lifestyles' of individual organisms.

This review focuses on selected alternative reproduction modes that are found in the Bacteria, which include cell-division programmes that result in the formation of multiple offspring, and budding mechanisms. With the advent of microbial genomics and advances in sensitive microscopic techniques (for example, deconvolution microscopy[28]) and analytical techniques (for example, mass spectrometric imaging[29] and microbeam analyses[30]), we now have the tools to investigate the mechanisms that underlie these diverse processes without the need for elaborate genetic analyses in less tractable bacteria. Understanding the acquisition of these processes in different lineages will not only provide insight into the evolution of these reproductive strategies, but will also shed light on the basic principles of microbial cell biology that dictate cellular asymmetry, nucleoid segregation and cell division. It is useful in this context to consider these mechanisms from an evolutionary perspective. In this review, members of only four major bacterial lineages are featured: the low-GC Gram-positive bacteria, the Cyanobacteria, the Actinobacteria and the Proteobacteria (FIG. 2). A phylogenetic perspective is emphasized because model systems can serve as a foundation on which to build hypotheses to study these alternative systems of reproduction and development.

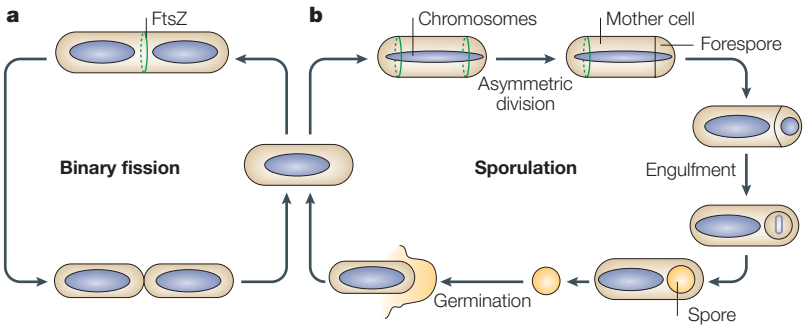


Figure 1 | **Life cycles of *Bacillus subtilis*.** *B. subtilis* has two alternative life cycles that result in different patterns of cell division. **a** | The vegetative life cycle. When conditions are favourable, *B. subtilis* elongates, replicates its chromosome (shown in blue) and divides by binary fission. The division apparatus assembles with FtsZ (green) in a ring-like structure at the midcell, where cell division occurs. **b** | When resources are exhausted, *B. subtilis* can develop a highly resistant and dormant cell to survive the harsh environmental conditions. The two copies of the chromosome adopt a novel configuration that stretches from one pole of the cell to the other. The division machinery assembles at both poles of the cell but cell division occurs at only one pole. A portion of one chromosome is trapped by the division septum. Proteins in the division septum package the chromosome into the smaller cell (known as the forespore). The forespore is then fully engulfed by the larger mother cell. Through the coordinate expression of genes in both cells, the internalized forespore is prepared for dormancy. Specialized proteins bind to and protect the DNA, the cell cytoplasm becomes mineralized and a protective protein barrier is assembled on the outer surface of the cell. When conditions improve, the endospore germinates and *B. subtilis* re-enters a vegetative life cycle.

Multiple intracellular offspring

In the low-GC Gram-positive bacteria (Firmicutes), several lineages have apparently converted ENDOSPORE formation from a provisional programme that produces a single dormant cell to a means of propagation in which multiple dormant offspring (endospores) or multiple active offspring are produced within a parental cell that is known as the mother cell. In some lineages, the formation of intracellular offspring is the primary means of cellular reproduction, and binary fission occurs either occasionally, or not at all.

***Endospore formation:* Bacillus subtilis.** Only low-GC Gram-positive bacteria produce true endospores. *B. subtilis* is used as a model to study this intricate developmental process. This bacterium produces an endospore primarily in response to nutrient depletion, although other environmental and physiological signals influence the initiation of spore formation in an individual cell[31]. Classical genetics and genome studies in *B. subtilis* have revealed a regulatory hierarchy of hundreds of genes that are involved in the production of a markedly resistant endospore[32,33]. The details of endospore formation have been discussed in recent reviews[34–36].

A modified form of binary fission is a pivotal morphological event that marks entry into the endospore formation pathway, but instead of dividing at the midcell, a sporulating cell divides near one pole (FIG. 1b). Asymmetric cell division of *B. subtilis* produces a small forespore (or prespore) and a large mother cell. During this division, approximately one-third of one of the chromosomes is trapped in the forespore. The DNA translocase SpoIIIE, which is located in the division septum, then pumps the rest of the chromosome into the forespore[37]. The other copy of the chromosome is retained in the mother cell. It is worth noting that although a productive cell division occurs at only one pole, wild-type *B. subtilis* prepares for division at both poles. FtsZ rings assemble at both poles[38] and ring-shaped invaginations can be observed near both cell poles early in sporulation[39]. *B. subtilis* has a marked preference for placement of the asymmetric cell division septum near the 'older' cell pole[35]; that is, opposite the pole that was created in the previous vegetative division. The factors that determine this preference are not known. It has been suggested, however, that construction of the cell-division apparatus at both poles allows the cell a 'second chance' if the first asymmetric division fails to capture a chromosome[40]. Some *B. subtilis* mutants have a 'disporic' phenotype[35]. In these cells, sequential bipolar cell division occurs and the two copies of the chromosome are partitioned into the polar forespores, leaving the mother cell devoid of genetic material, which results in the arrest of sporulation. Therefore, it seems that the mechanisms for bipolar division are present in endospore formers.

Once asymmetric division and chromosome translocation are complete, the forespore is engulfed by the mother cell and the internalized forespore matures into a spore. The coordinate regulation of genes in both the mother cell and the forespore is required for spore formation and maturation. When sporulation is complete, the mother cell dies. A mature, quiescent endospore can survive for tens-of-thousands of years, and perhaps even longer[41]. When environmental conditions improve, the spore germinates and the cell that is produced has a normal vegetative growth cycle. The ability to produce a small, dormant, highly resistant spore in response to adverse environmental conditions has provided low-GC Gram-positive bacteria with an exceptional adaptive advantage. Endospores are ubiquitous and dispersible; they are found in all types of environmental samples worldwide, even at long distances from their original environmental niche[41]. However, the *B. subtilis* paradigm should not limit our view of how cell-division programmes are implemented. For some endospore formers, sporulation is part of the normal life cycle and has evolved into an important mode of propagation.

***Multiple spores:* Metabacterium polyspora.** *M. polyspora* is a spore-forming bacterium that inhabits the gastrointestinal (GI) tract of guinea pigs. Unlike most endospore formers, it produces multiple endospores — up to nine per mother cell[42,43]. Morphologically similar symbionts have been found in various rodent species[44] although no *Metabacterium*-like symbiont has been maintained in culture. The life cycle of *M. polyspora* in its natural host requires the bacterium to cycle through the GI tract and therefore relies on the COPROPHAGOUS nature of the guinea pig for survival[45]. Mature endospores of *M. polyspora* can be isolated from the faeces of a host guinea pig. Only mature endospores survive passage through the mouth and stomach of the host, and germinate in the small intestine (FIG. 3). Some cells undergo binary fission at this stage in the life cycle, but

ENDOSPORE
A specialized dormant cell, that forms within some Gram-positive bacteria, and which is highly resistant to agents (such as heat, solvents and ultraviolet radiation) that would normally harm a vegetative cell.

COPROPHAGOUS
Feeding on faeces.

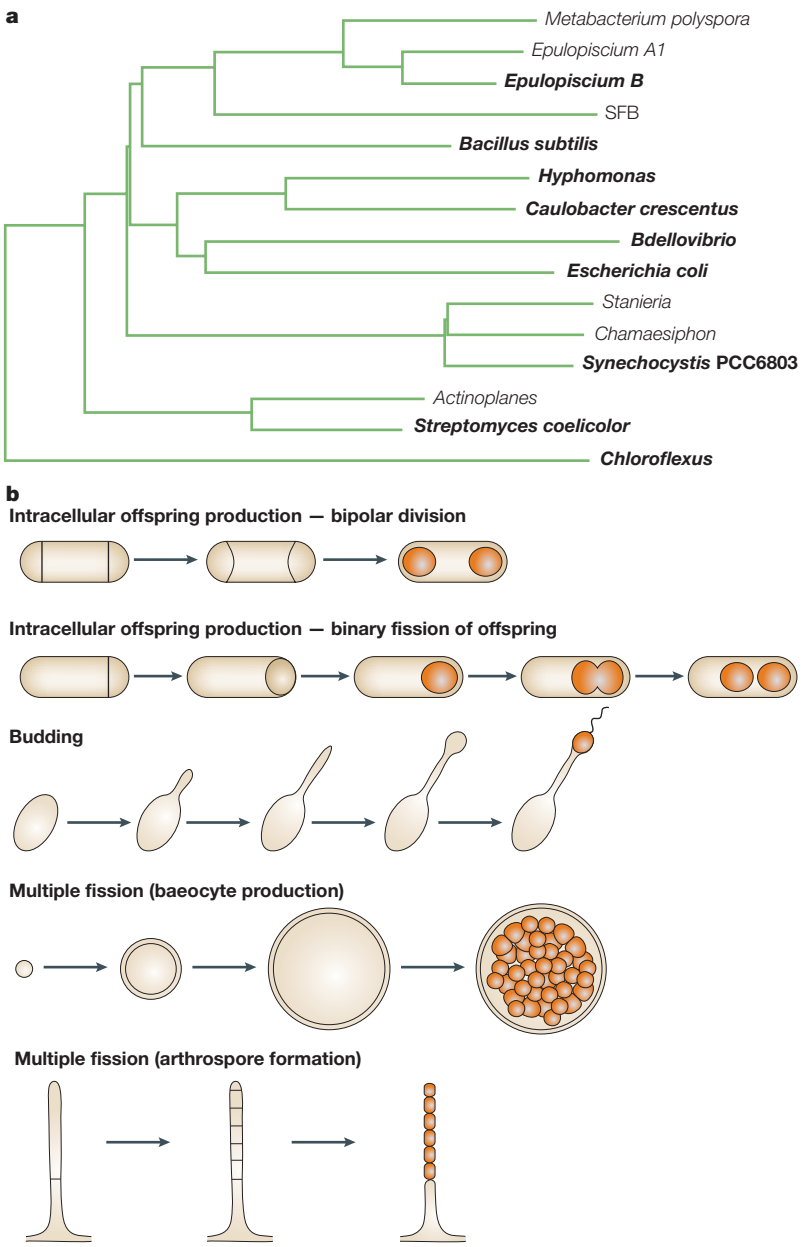


Figure 2 | **Evolutionary relationships of model organisms and bacteria that show unusual reproductive strategies.** This phylogenetic tree (**a**) illustrates the diversity of organisms that use the alternative reproductive strategies shown in (**b**). Bold type indicates complete or ongoing genome projects. Intracellular offspring are produced by several low-GC Gram-positive bacteria such as *Metabacterium polyspora*, *Epulopiscium* spp. and the segmented filamentous bacteria (SFB). Budding and multiple fission are found in the proteobacterial genera *Hyphomonas* and *Bdellovibrio*, respectively. In the case of the Cyanobacteria, *Stanieria* produces baeocytes and *Chamaesiphon* produces offspring by budding. *Actinoplanes* produce dispersible offspring by multiple fission of filaments within the sporangium.

most cells begin to sporulate[45]. From the small intestine, *M. polyspora* cells are deposited in the guinea pig caecum where they complete spore formation. Cells with engulfed forespores or mature endospores do not seem to undergo binary fission. The *M. polyspora* cells pass from the caecum, traverse the lower intestine and are finally eliminated from the host. If ingested by a guinea pig, the life cycle starts again.

HOLDFAST
A tapered protrusion of the segmented filamentous bacterial cell that firmly secures it to an epithelial cell that lines the host intestinal tract.

The process of endospore formation in *M. polyspora* differs from that of *B. subtilis* and many other endospore-forming bacteria[45]. The asymmetric cell division of *M. polyspora* normally takes place at both cell poles. DNA is partitioned into both polar compartments, but some DNA is also retained in the mother cell. After engulfment, the forespores can undergo division to produce multiple forespores that grow and mature into multiple endospores. Unlike sporulating *B. subtilis*, which contains two copies of its genome, *M. polyspora* must contain three or more copies of its genome. For the successful production of multiple intracellular progeny, the bacterium must be able to coordinate genome replication and segregation with offspring formation[45]. The genetic changes in this developmental programme that have led to multiple endospore production have yet to be elucidated.

For most endospore formers, sporulation is a provisional developmental programme that is activated by unfavourable environmental conditions. A single, dormant, highly resistant offspring is produced to aid in dispersal, or to survive the adverse conditions. Few lineages in the low-GC Gram-positive bacteria can form more than two endospores per mother cell. For *M. polyspora*, multiple endospore formation is part of the normal life cycle and is not initiated solely in response to stressful conditions. In this case, sporulation not only provides protection as the spores pass out of the host, but it also enables propagation. The coordination of spore formation with transit through the GI tract, combined with a coprophagous natural host, might favour multiple spore formation over binary fission, thereby alleviating the need for binary fission in the *M. polyspora* life cycle.

***Multiple spores:* Anaerobacter polyendosporus.** Other endospore-forming bacteria can produce more than one endospore. *A. polyendosporus*, for example, is a highly pleomorphic anaerobic bacterium that was isolated from rice paddy soil[46]. Under certain laboratory growth conditions — synthetic media supplemented with galactose — an *A. polyendosporus* cell can produce up to 7 endospores, but more often these cells produce only 1–2 endospores, and sometimes might not sporulate[47]. The conditions that regulate the formation of multiple endospores and the ecological significance of this process are not understood.

Multiple spores: segmented filamentous bacteria. Another group of low-GC Gram-positive bacteria that uses a modified pathway to form endospores for reproduction and dispersal is the segmented filamentous bacteria (SFB). The SFB, also known as '*Arthromitus*', have been found in the intestinal tracts of various animals[48], but the SFB of rodents are by far the best characterized. SFB develop as a multicellular filament that is anchored to the epithelial lining of the distal ileum[49,50]. Each SFB originates as a single, HOLDFAST-BEARING cell that embeds itself among the microvilli on the epithelial cell surface (FIG. 4). Once it is firmly attached, the SFB grows and divides, eventually forming a filament that can be up to

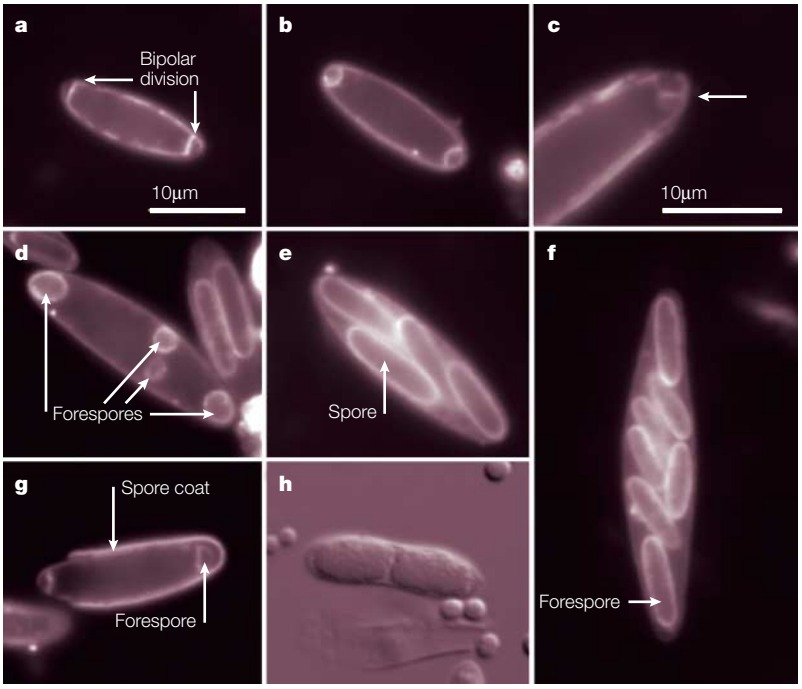


Figure 3 | **Formation of multiple dormant offspring in *Metabacterium polyspora*.** Parts **a**–**g** show fluorescence micrographs of *M. polyspora* cells that have been stained with FM 1-43, which is specific for the cell membranes and spore coats. **a** | Cells divide at both poles. **b** | Forespores are engulfed by the mother cell. **c** | Forespores are capable of binary fission (the arrow indicates a newly formed division septum that is bisecting a forespore). **d** | Forespores continue to divide and grow. **e** | Forespores mature into endospores. **f** | A large *M. polyspora* with seven forespores. **g** | A cell emerging from its spore coat has divided at both poles and begun to sporulate. **h** | Nomarski differential interference contrast micrograph of *M. polyspora* undergoing binary fission, which is a rare event. Scale bar in **a** applies to all panels except **c**. Reproduced with permission from REF. 45 © (1998) National Academy of Sciences, USA.

1 mm long, although most filaments are roughly 100 μm in length[51]. As the cells of the intestinal epithelium slough off and are constantly renewed, the intestinal lining is not a stable substrate for attachment, and the SFB must reposition itself[52]. It does so by producing intracellular offspring, which are released from the dying parental filament. The cell-division programme is initiated at the unattached end of the filament and is sequentially triggered in cells that are closer to the holdfast. Studying an individual filament can therefore document the progression through the developmental process, which has allowed researchers to decipher the complete SFB life cycle by using transmission electron micrographs of longitudinal thin sections of reproductive-stage filaments[52,53].

Overall, but with some exceptions, the process resembles endospore formation in *B. subtilis*. The cell divides asymmetrically, although published electron micrographs[52,53] indicate that the division septum is preferentially placed close to the 'newer' cell pole (FIG. 4). The smaller cell is then engulfed by the larger mother cell and, after engulfment, the internalized cell divides. At this stage, the intracellular offspring have one of two fates, either differentiation, or maturation to form a spore. Differentiation produces holdfast protrusions. These active offspring are released into the lumen of the intestine after the mother cell lyses and they attach to the intestinal epithelium to establish new filaments within the host. Maturation results in two intracellular offspring cells that are encased in a common spore coat, which forms an endospore. The endospore provides an effective dispersal mechanism for the SFB. In fact, exposure to airborne endospores alone can establish an SFB population in a naive host[51]. These alternative forms of offspring (either active or dormant) allow the SFB to maintain local populations and to survive inhospitable environments before colonizing amenable hosts. The factors that determine cell fate — either viviparity or sporulation — are not known. Although the SFB cannot be cultured outside of the host GI tract, populations can be maintained as monoassociations with mice and have been useful models for studying immune development[54,55]. The SFB could also be used as a model system for studies of intracellular communication and replication control. Cell density signals and nutritional status affect the 'decision' to sporulate in *B. subtilis*[31]. Do these factors affect the developmental fate of the intracellular offspring of SFB? Do cell–cell signalling mechanisms coordinate the sequential development of cells in the SFB filament (similar to the intercompartmental communication systems that function in sporulating *B. subtilis*[35])?

The production of a dormant cell to survive harsh environmental conditions is a well-known strategy and has allowed free-living endospore formers to exploit a wide variety of niches. In the SFB, endospore formation in an anaerobic bacterium is coordinated with the production of active intracellular offspring for reproduction or for dispersal through harsh environments such as aerobic conditions or the upper GI tract. One Firmicute lineage, *Epulopiscium*, seems to have taken this process one step further.

Multiple live offspring:* Epulopiscium *species. *Epulopiscium* spp. colonize the intestinal tracts of certain species of herbivorous and detritivorous surgeonfish[56–58]. Their limited host range indicates a commensal or mutualistic symbiosis, in which *Epulopiscium* spp. aid in the breakdown of food ingested by the fish. Depending on the host species and gut location, *Epulopiscium* spp. can be an abundant constituent of the intestinal microbiota. Although *Epulopiscium* spp. comprise a phylogenetically and morphologically diverse group of low-GC Gram-positive bacteria, some cigar-shaped individuals can reach more than 0.6 mm in length, making members of the *Epulopiscium* some of the largest bacteria identified so far[59]. These bacteria produce multiple intracellular offspring that resemble the large endospores that are formed by *M. polyspora*[60], however, *Epulopiscium* spp. produce active (rather than dormant) offspring[56,58]. Usually two offspring are produced, although, in a newly discovered *Epulopiscium* morphotype, up to 12 offspring per mother cell have been observed (E.R.A., K. D. Clements and J. H. Choat, unpublished observations).

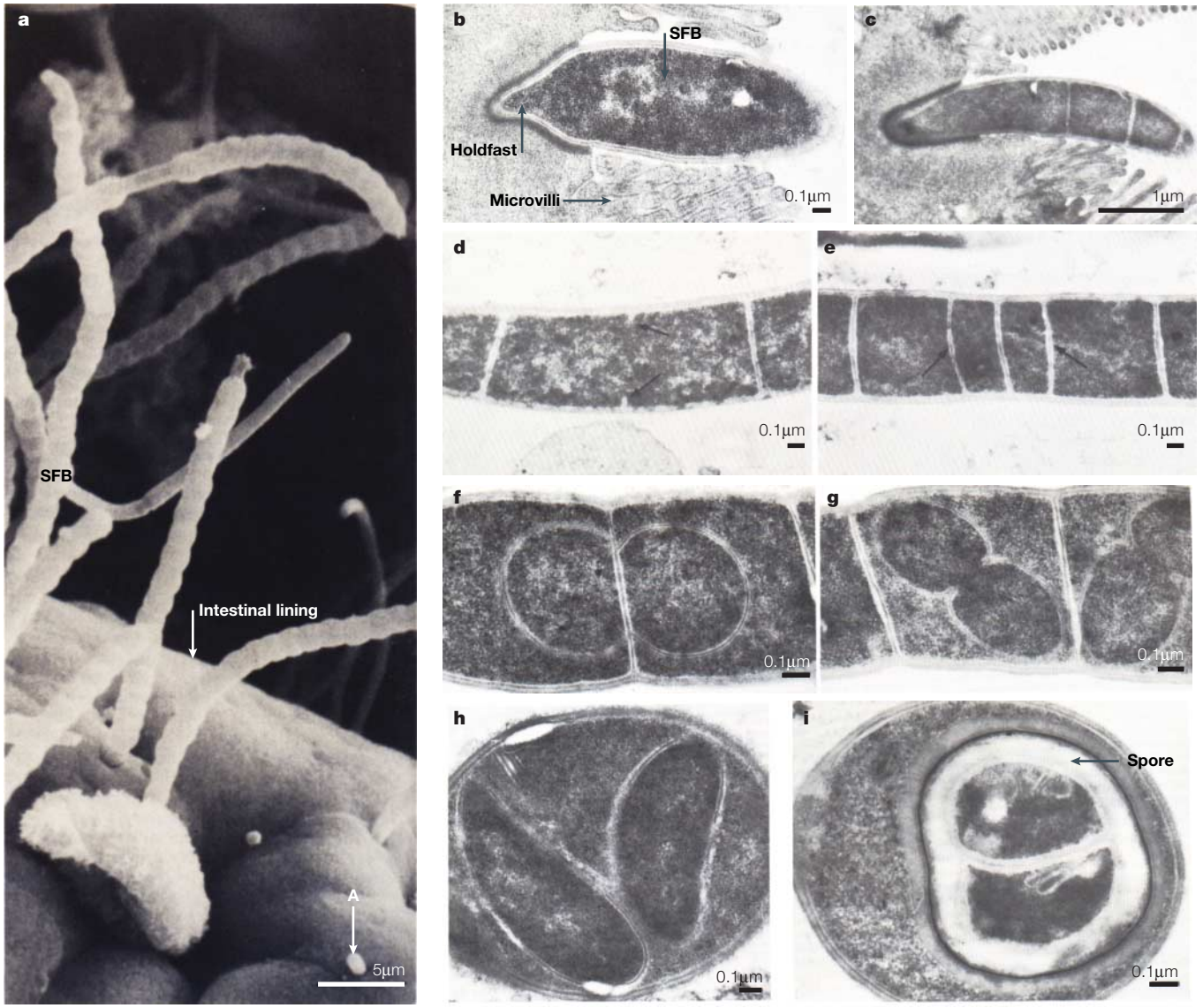


Figure 4 | **Intracellular offspring of the segmented filamentous bacteria. a** | Scanning electron micrograph of the internal wall of the small intestine reveals filamentous bacteria embedded in the epithelial lining. Note the small nub in the lower right corner marked with 'A', which is the start of a new filament. **b** | A single holdfast-bearing cell that is imbedded among the microvilli of the intestinal lining. **c** | Once firmly established, the segmented filamentous bacteria begins to grow and divide. **d** | When reproduction is initiated, primary cells of the filament undergo one division. **e** | Each cell then divides asymmetrically. **f** | The larger cell engulfs the smaller cell. **g** | The intracellular offspring divides once and follows one of two fates: either intracellular offspring form holdfasts (**h**) and are released by the mother cell, or (**i**) the two cells are encased in a common spore coat. Parts **b–i** are transmission electron micrographs. Part **a** is reproduced with permission from REF. 52 © (1976) American Society for Microbiology. Parts **b–i** are reproduced with permission from REF. 53 © (1979) Munksgaard International Publishers.

The earliest stages of intracellular offspring formation in *Epulopiscium* spp. resemble the initiation of endospore formation in *B. subtilis* (FIG. 5)[61]. The close phylogenetic relationship between *M. polyspora* and *Epulopiscium* spp. indicates an evolutionary scheme in which a 'stopgap' survival programme to produce a dormant cell has evolved, in a stepwise fashion, to viviparity[60]. The conditions that drive this evolutionary development are unclear, but might be involved in maintaining the host–microorganism symbiosis.

Endospore formation is a complex developmental process. Genes that are essential for spore formation in *B. subtilis* are dispersed throughout the chromosome[62]. The distribution of this process in the low-GC Gram-positive bacteria might indicate that it is an ancient genetic programme, but few clues remain to readily decipher the steps that led to the acquisition of this complex trait[63]. Contemporary live offspring-bearing Firmicutes, which include *Epulopiscium* and the SFB, most likely arose from an endospore-forming ancestor. But it is also possible that live intracellular offspring production was an early step in the progression toward the development of the first endospore[61].

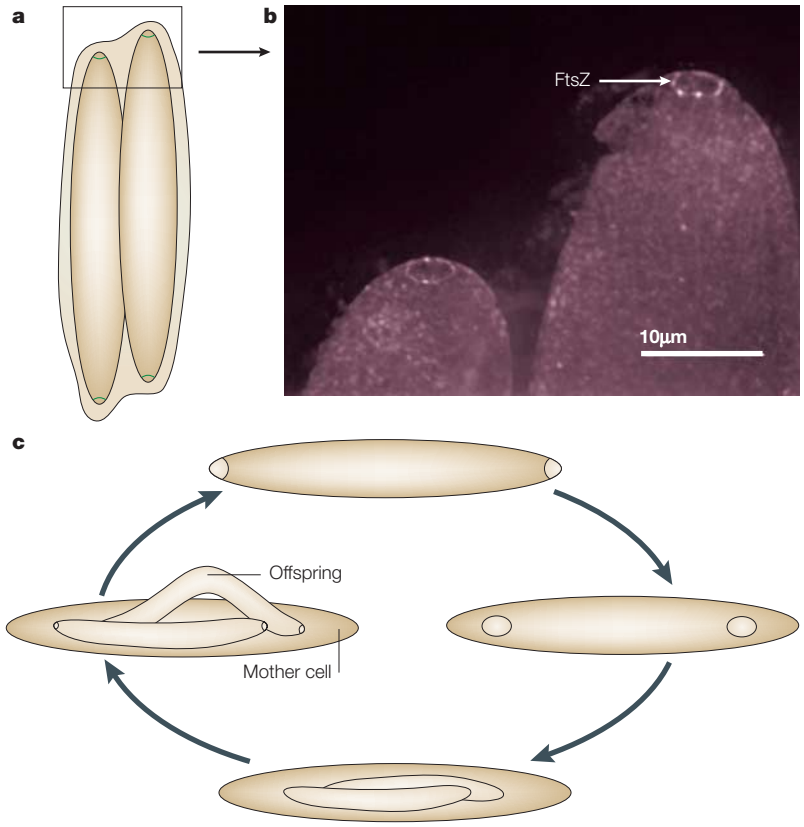


Figure 5 | **Viviparity in *Epulopiscium*.** In a modified form of endospore formation, *Epulopiscium* spp. produce several intracellular offspring. The boxed region in part **a** represents the portion of the cell that is shown in **b**. In this morphotype, offspring are initiated at the poles of a cell prior to release from the mother cell. **b** | A three-dimensional reconstruction from immunofluorescence microscopy experiments that shows immunolocalization of the key cell-division protein FtsZ, which forms ring-like structures at extreme polar locations. **c** | The life cycle of *Epulopiscium* spp. Cell division that takes place close to the cell poles produces small daughter cells that are engulfed by the larger mother cell. Both the mother cell and the internal offspring grow, but eventually growth of the offspring overtakes that of the mother cell. As development progresses, the mother cell inevitably deteriorates (ribosomes disassemble and DNA degrades) and the offspring emerge from an opening in the weakened mother cell envelope. Parts **a** and **b** are reproduced with permission from REF. 61 © (2004) Blackwell Scientific Publications.

Multiple offspring by multiple fission

In at least three lineages, the Cyanobacteria, the Proteobacteria and the Actinobacteria, a rapid succession of multiple rounds of fission of an enlarged multinucleoid spherical cell, or synchronous division at many sites along the length of a multinucleoid filament, leads to the formation of multiple vegetative offspring or spores. A multiple-fission reproductive phase is often induced by depletion of a nutrient, and might be used to aid offspring dispersal.

The pleurocapsalean cyanobacteria. The Pleurocapsales have specialized reproductive cells that undergo multiple fission to produce offspring known as baeocytes (Greek, "small cells")[64]. Some genera undergo limited binary fission, forming coherent clusters of cells or small filaments prior to baeocyte formation. But, even more interestingly, some unicellular pleurocapsalean lineages never reproduce by binary fission.

The *Stanieria* (*Dermocarpa*) life cycle begins with a baeocyte, a spherical cell that is 1–2 μm in diameter (BOX 1). The baeocyte produces an extracellular matrix that is known as the F layer. Accretion of this layer continues throughout the life cycle. The composition of this matrix is not known, but it is probably a polysaccharide and it aids in the attachment of the baeocyte to a solid surface. During vegetative growth, the attached cell enlarges to measure up to 30 μm in diameter. As the cell grows, its genomic DNA replicates and the nucleoids segregate in the cytoplasm. In the subsequent reproductive stage, a rapid succession of cytoplasmic fissions leads to multiple baeocyte formation. The number of baeocytes produced (4 to more than 1,000) depends on the volume of the reproductive-phase mother cell. Multiple fission is not accompanied by a notable increase in total cytoplasmic volume, and each round of division produces sequentially smaller offspring cells, which distinguishes this process from binary fission[64]. Eventually the extracellular matrix tears open, releasing the baeocytes. It is not known which factors trigger the reproductive cycle in *Stanieria*, but environmental cues seem to have a role[64]. This process is not characterized at the genetic or molecular level. *Stanieria* provide an exceptional opportunity to study nucleoid segregation in three-dimensional space, and Z-ring assembly in concert with a complex arrangement of nucleoids.

Other pleurocapsalean cyanobacteria. These species have retained a limited capacity for binary fission, or use a combination of asymmetric cell division and multiple fission for reproduction[64] (see BOX 1). Methods for studying the cell biology of division in the model cyanobacterium *Synechocystis* sp. PCC 6803 are under development[65] and should be applicable to the Pleurocapsales. In these lineages, what are the signals that govern the transition from binary fission to multiple fission? Is cell–cell signalling used to coordinate the alternative division programmes within cell clusters? Or, are the alternative cell fates determined by asymmetry that is established prior to the first cell division? Two even more intriguing questions are: why, and how, have some of these lineages abandoned binary fission? The regulation of alternative cell fates and syncytial-to-cellular transitions are essential in the embryonic development of many metazoan species[66]. The pleurocapsalean cyanobacteria might provide another opportunity for studying development, cell–cell signalling and cell fate in a simple model system. Perhaps when microbial genome projects become sufficiently diverse, some of these questions can be addressed at the molecular level.

The Actinobacteria. The developmental biology of the streptomycetes (FIG. 6), which have a distinct filamentous growth phase, has been extensively studied[67–69]. Vegetative tip growth produces branched filaments that only septate occasionally, forming long cells that contain multiple nucleoids. In response to nutrient depletion, some streptomycetes alter their pattern of growth to produce aerial mycelia and dispersible spores. A complex extracellular signalling cascade, which might provide

Box 1 | **Diversity of division patterns**

Multiple fission of enlarged cells in the pleurocapsalean cyanobacteria

The life cycle of all of the Pleurocapsales starts with a baeocyte. During vegetative growth, the cell enlarges and produces a thick extracellular matrix, known as the F layer. *Stanieria* (see figure, part a), never undergo binary fission. Within the growing cell, DNA replicates and the nucleoids segregate. Eventually, the cell begins its reproductive phase. A rapid succession of cytoplasmic fissions leads to the formation of several baeocytes. The extracellular matrix tears open, releasing the baeocytes. As *Myxosarcina* grow, they divide by binary fission. The pattern of sequential division, which takes place in three planes at right angles to one another, forms a more-or-less cube-shaped, coherent cluster of cells. When the cluster undergoes multiple fission, almost every cell produces baeocytes. *Pleurocapsa* (see figure, part b) grow and divide by binary fission — but in an irregular pattern — which forms filamentous aggregates of vegetative cells prior to the transition of some or all of the cells to produce baeocytes. In the reproductive cycle of the unicellular *Dermocarpella,* (see figure, part c) the pattern of cell division and baeocyte formation is more complex. The cell enlarges asymmetrically and, just before the reproductive cycle begins, the cell divides asymmetrically. The larger reproductive cell undergoes multiple fission, whereas the smaller cell might divide once, but is withheld from further reproduction. Once the baeocytes are released, the remaining parental cell resumes vegetative growth and will eventually enter a reproductive cycle.

Multiple fission of multinucleoid filamentous cells

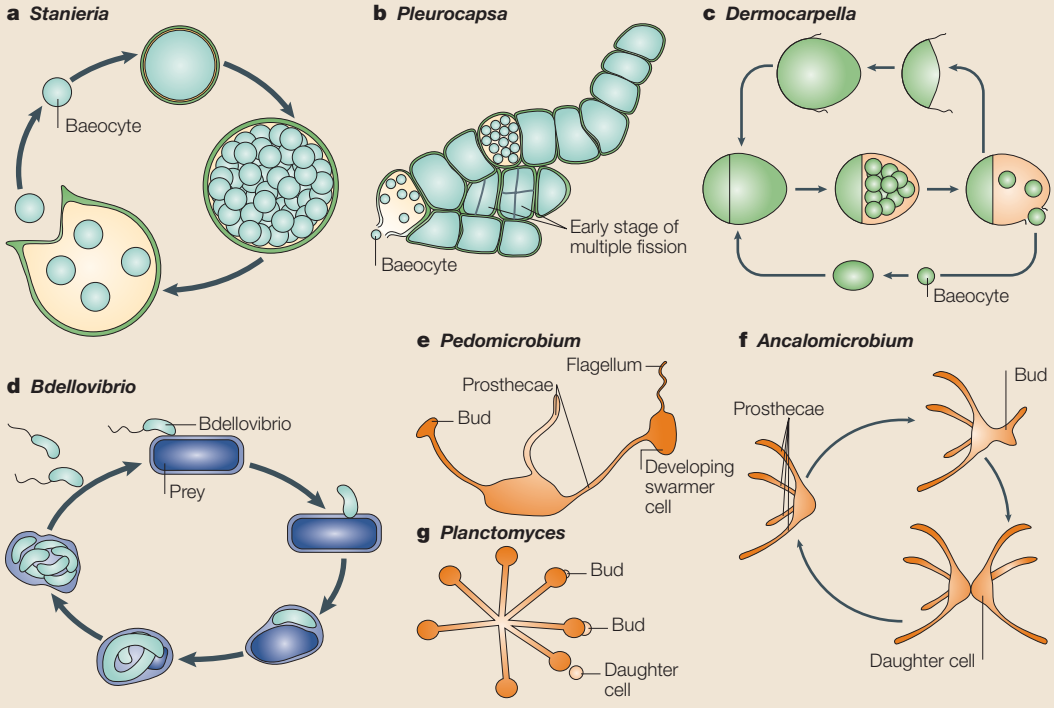
Bdellovibrio are δ-proteobacteria and obligate predators. Wild-type cells only grow within the periplasm of a prey bacterium, forming a multinucleoid filamentous cell. When the host's resources are exhausted, the filament undergoes multiple fission. The offspring produce flagella and emerge from the 'spent' host to search for other prey (see figure, part d).

Budding in the prosthecate α-proteobacteria

Hyphomonas (and *Hyphomicrobium*) start life as a swarmer cell. The swarmer cell cannot replicate its DNA or reproduce; its role is dispersal. Eventually, this motile cell differentiates. It ejects the polar flagellum and in its place grows a single prostheca. The prosthecate cell can replicate its DNA and produce offspring. During reproduction, the body of the stalked cell does not grow markedly, but instead, an offspring cell buds from the distal end of the stalk. The mature offspring assembles a flagellum and swims away (see FIG. 2b). Sessile *Pedomicrobium* produce several prosthecae that bud to form offspring cells (see figure, part e). The offspring produce a single flagellum and swim away, or occasionally attach directly to a surface and grow prosthecae. *Ancalomicrobium* are conical cells with two or more tapered prosthecae. They never produce flagella and have no swarmer cell stage in their life cycle (see figure, part f). To reproduce, a bud develops at the apex of the conical body of the cell. The bud starts as a small protuberance, but as it grows it differentiates and produces prosthecae. The mature offspring is almost a mirror image of the parent. Buds are always produced from the same location on the parent cell and never from the ends of the prosthecae.

Budding in *Planctomyces*

Reproduction by budding is prevalent in another major bacterial phylum, the Planctomycetes (see figure, part g). Although nothing is currently known about the conservation of division mechanisms in this lineage, several genome projects are underway which should reveal potential targets for study.



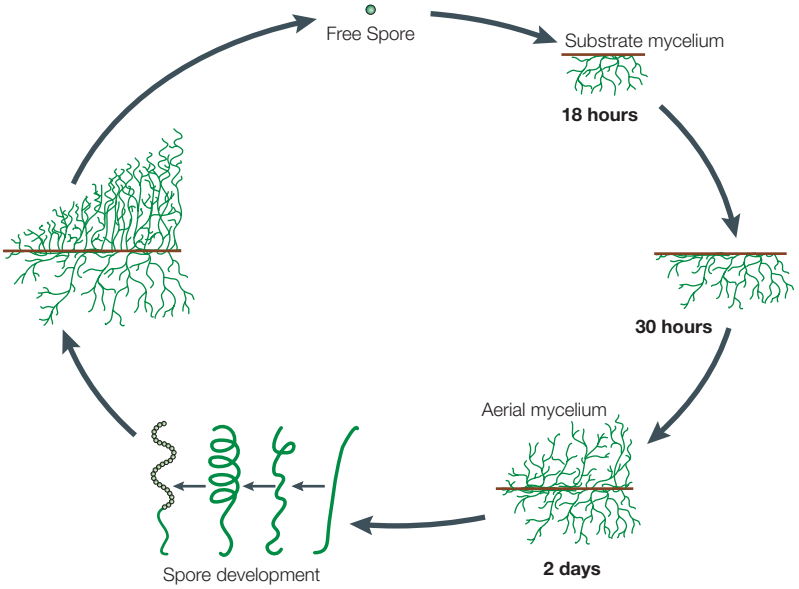


Figure 6 | ***Streptomyces coelicolor* life cycle.** A vegetative hypha emerges from a germinating spore and the hyphal filament grows by tip extension. Occasionally, cell division occurs and the filament branches, which produces a thick network of hyphae, known as the substrate mycelium. As local nutrients are depleted, a complex signalling cascade triggers the production of a surfactant that coats some emerging filaments, which allows them to grow away from the substrate. These aerial filaments are developmentally different from those of the substrate mycelium. The unbranched cell at the ends of some of these aerial filaments differentiates. Each cell divides synchronously at many sites along its length forming uninucleoid cells that further develop into spores.

checkpoints to ensure the coordination of secondary metabolism in a developing colony, controls the transition from vegetative growth to reproduction[69]. In some of the aerial filaments, synchronous cell division occurs at regular intervals to produce uninucleoid cells, which develop into spores[70]. Mechanisms which act on Z-ring assembly in other bacteria (such as nucleoid occlusion and the Min system) do not seem to be as conserved in the streptomycetes[70]. Although *Streptomyces coelicolor* is a technically challenging model system, recent advances in genetic techniques and the completion of the *S. coelicolor* A3(2) and *Streptomyces avermitilis* MA-4680 genome projects have led to rapid advances[70–73]. Perhaps, owing in part to hyphal growth, cell division in laboratory culture is dispensable in this organism[74]. This allows for the generation of non-conditional mutants that are defective in this usually essential process, which provides a distinct and unique advantage to studying cell division in streptomycetes.

The diversity of actinobacterial species provides opportunities for comparative studies with streptomycetes to determine modifications and commonalities in developmental programmes. The *Actinoplanes*, *Pilimelia* and morphologically similar genera are readily isolated from soil and decaying organic matter[75–77]. Although economically less important than the streptomycetes, strains in these lineages also produce bioactive secondary compounds[78,79]. But, unlike the streptomycetes, *Actinoplanes* and *Pilimelia* construct complex sporangia in the absence of aerial mycelia. These fruiting bodies seem to contain bundles of filaments that divide to form spores[80,81]. The genetic mechanisms that control spore formation and the development of sporangia in these genera have not been characterized. Do complex signalling cascades coordinate the development of sporangia in these actinobacteria?

The Proteobacteria. *Bdellovibrio* spp. are tiny predatory δ-proteobacteria that invade the periplasm of the prey bacterium and systematically consume it[82,83]. Attack-phase *Bdellovibrio* cells are highly motile as they search for susceptible prey (BOX 1). Once host-contact is made, *Bdellovibrio* cells penetrate the outer membrane of a prey cell. Concealed within the host, *Bdellovibrio* lyses the cell wall, which reduces the prey to a spheroplast, and then the *Bdellovibrio* assimilates organic compounds from the prey cytoplasm. *Bdellovibrio* grows in the prey periplasm, but this growth is not accompanied by cell division — instead, the cell elongates. Once the host cytoplasm is consumed, the *Bdellovibrio* reproduces: the filament divides, and cells differentiate into motile attack-phase cells. Although *Bdellovibrio* are obligate parasitic predators, facultatively parasitic strains of *Bdellovibrio bacteriovorus* have been isolated and can be grown saprophytically in laboratory culture[84,85]. Under these growth conditions, *B. bacteriovorus* has a filamentous growth phase that is followed by a reproductive, multiple-fission phase. A complete genome sequence is now available for *B. bacteriovorus* HD100 (REF. 86). With this information and available transformation protocols[87], the genetic dissection of the attack phase, growth phase and division phase transitions should be feasible. It is not clear why *Bdellovibrio* have evolved complex life cycles that separate growth from cell division. It will be interesting to see if simple genetic disruptions can result in obligate binary fission, and if this change will impact on fitness. *Bdellovibrio* provide another genetically tractable model for studying multiple nucleoid segregation as well as checkpoints that regulate the start of cytoplasmic cleavage.

Budding

Budding has been observed in many lineages in the Bacteria, including the low-GC Gram-positive bacteria[88], the Cyanobacteria[89], the prosthecate proteobacteria and the Planctomycetes[90]. Although hypothetical mechanisms that control bud formation in these and other bacteria have been proposed, the molecular mechanisms that regulate bud formation are not characterized. The Proteobacteria hold great promise for the development of model systems for the analysis of bud formation in bacteria.

The prosthecate α-proteobacteria are morphologically diverse[90]. They have been studied because of their importance in geomicrobiology (such as metal oxide deposition, degradation of methyl halides and other one-carbon compounds[91]) and their role in the ecology of OLIGOTROPHIC environments, but they have not yet fully realized their potential as models for cellular differentiation and reproduction[92]. The variety of unusual forms provides interesting comparative models for

OLIGOTROPHIC
A low nutrient environment.

PROSTHECA
A cellular extension, also known as a stalk or hypha, which contains cytoplasm and is bound by the cell envelope of the organism.

determining common mechanisms that drive bud formation as well as the modifications that accommodate different cell architectures. *Hyphomonas*, *Pedomicrobium* and *Ancalomicrobium* are all bud producing α-proteobacteria, although each has a distinct morphology and pattern of bud formation (BOX 1).

C. crescentus, another prosthecate α-proteobacterium, has been developed as a model for examining the role of cell asymmetry in establishing daughter cell fate[9,93]. Studies of *C. crescentus* have informed the current view of the mechanisms of bacterial cell division and cell architecture[94,95]. Developmental progression in *C. crescentus*, DNA replication, cell division and polar organelle development, is temporally well defined. Synchronized cultures can be reliably obtained, which has allowed for the precise molecular characterization of these interdependent events. Furthermore, key global response regulators, such as CtrA and GcrA, which govern progression, have been identified[9,10]. It is possible that the regulatory mechanisms that have been discovered in *C. crescentus* are conserved in other prosthecate α-proteobacteria and could be involved in bud-site determination and development. Are homologues of CtrA and GcrA found in budding bacteria like *Hyphomonas*? If so, what do they regulate? Is DNA replication tightly controlled in cells of *Pedomicrobium* that produce multiple buds simultaneously? If swarmer cells can be harvested to establish synchronized cultures, expression patterns with respect to bud formation might be followed.

Cellular asymmetry is important for the development of polar organelles in *C. crescentus*[94]. Similarly, cellular asymmetry establishes the site of bud formation and directs the deposition of materials to support bud development in eukaryotic microorganisms such as *Saccharomyces cerevisiae*[96]. Mechanisms to establish cellular asymmetry must also be important for bud formation in bacteria. *Hyphomonas* is of particular interest owing to its potential as a model for studies of bud formation. *Hyphomonas* resembles *C. crescentus*, but in hyphomonads, the swarmer daughter cell buds from the distal end of the PROSTHECA[97–99]. A *Hyphomonas neptumium* genome project is underway[100] and comparative studies with *C. crescentus* should provide a productive framework for characterizing the molecular biology of bud formation.

Conclusion

This review is not exhaustive, but these examples illustrate the most well-characterized unusual modes of offspring production in the Bacteria, and those with the greatest potential for further investigation. Many of these processes seem to have evolved to aid dispersal or for the maintenance of bacterial symbionts in host species, but the evolutionary advantages of some of these processes remain a puzzle. Characterization of these unusual reproductive strategies has the potential to identify fundamental cellular mechanisms that are used to establish cellular asymmetry and to redistribute genetic material. Some of the lineages that have been described in this review cannot be cultured and others lack robust genetic systems. With the maturation of microbial genomics, renewed interest in microbial cellular biology and the development of sensitive cell biology tools, this is the right time to revisit and explore these novel lineages. We are following this approach of genomics coupled with cell biological tools to understand the evolution and cellular biology of intracellular offspring formation in low-GC Gram-positive bacteria, including *M. polyspora* and *Epulopiscium*[45,60,61]. Nearly a century's worth of research into *B. subtilis* provides a substantial foundation for genomic and mechanistic comparisons that can be used to build hypotheses for the mechanisms that underlie cell division. An *Epulopiscium* genome project is currently underway, which will provide the basic information to support comparative studies of these as-yet-uncultured symbionts. Similarly, comparative analyses in the organisms discussed above with related genetic models can provide a basis for exploration.

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Competing interests statement

The author declares no competing financial interests.

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