Bacterial solutions to multicellularity: a tale of biofilms, filaments and fruiting bodies

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Abstract | Although bacteria frequently live as unicellular organisms, many spend at least part of their lives in complex communities, and some have adopted truly multicellular lifestyles and have abandoned unicellular growth. These transitions to multicellularity have occurred independently several times for various ecological reasons, resulting in a broad range of phenotypes. In this Review, we discuss the strategies that are used by bacteria to form and grow in multicellular structures that have hallmark features of multicellularity, including morphological differentiation, programmed cell death and patterning. In addition, we examine the evolutionary and ecological factors that lead to the wide range of coordinated multicellular behaviours that are observed in bacteria.

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Before James Shapiro's seminal paper on bacterial multicellularity was published 25 years ago[1], bacteria were viewed as archetypal unicellular organisms — dividing by binary fission and feeding independently, they were considered to be the ultimate loners. Although there were several examples of highly complex and coordinated bacterial behaviours occurring in a multicellular context — for example, the developmental transitions of myxobacteria and *Streptomyces* spp. or the swarming of *Proteus* spp. — such observations had long been considered to be exceptions to the rule of the predominantly unicellular lifestyle of bacteria. Following the broader recognition of bacterial multicellularity[1,2], considerable advances in microbiology and evolutionary biology have been made towards understanding the mechanisms and origins of multicellular behaviour in bacteria.

Similarly to eukaryotes, bacteria have evolved from unicellularity to multicellularity several times[3,4]. Bacterial manifestations of multicellularity take different forms (FIG. 1), which range from undifferentiated chains to morphologically differentiated structures; and the behaviour of cells within multicellular structures is coordinated by both shared and unique molecular mechanisms. These functionally diverse forays into multicellularity arose for different reasons — for example, to mitigate predation risk or nutrient stress — and are associated with distinct evolutionary costs and benefits[3,5]. These observations pose a number of questions relating to how widespread

multicellularity is among bacteria, the different forms in which it manifests and the factors that have facilitated its evolution. For ease and clarity of organization, although by no means to indicate a linear progression, we partition multicellularity into several stages, beginning with loosely associated groups of undifferentiated cells (such as filaments or temporarily adhered clusters of cells) to biofilms that display transient multicellularity, through to cellular groups that display essentially permanent multicellularity and that exhibit the hallmark features of complex multicellularity, such as irreversible differentiation, division of labour among different cell types and intercellular cooperation. Our aim in this Review is to revisit the question of bacterial multicellularity in light of recent developments, using *Bacillus subtilis*, myxobacteria, cyanobacteria and the streptomycetes (FIG. 1) as specific examples from the bacterial world to illustrate the advantages of coordinated multicellular behaviours and to highlight how multicellularity might have evolved.

Incipient multicellularity
Before multicellular groups of cells can benefit from the advantages of multicellularity (such as division of labour), they first need to overcome the problem of forming a coherent group of cells. This transition can arise via three distinct processes: aggregation of individual cells (which is observed during the initial stages of biofilm formation)[6], incomplete cell fission after cell division to
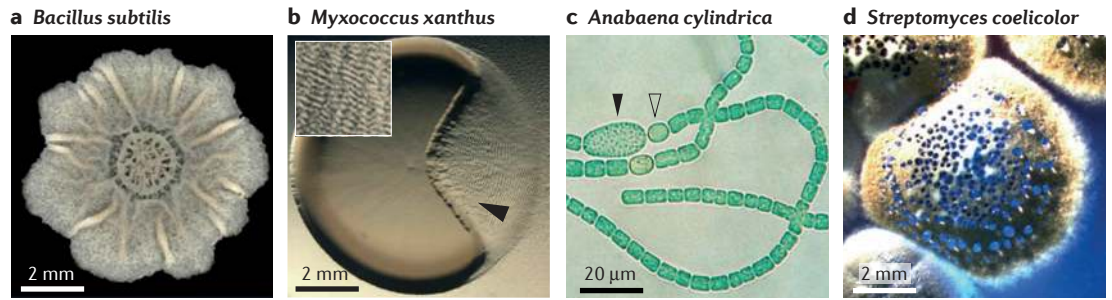
a *Bacillus subtilis*　**b** *Myxococcus xanthus*　**c** *Anabaena cylindrica*　**d** *Streptomyces coelicolor*

Figure 1 | **Bacterial manifestations of multicellularity. a** | A mature *Bacillus subtilis* biofilm. **b** | Predation of an *Escherichia coli* colony (left) by swarming *Myxococcus xanthus* cells (right), which is characterized by a rippling pattern (arrowhead and inset). **c** | Formation of heterocysts (open arrowhead) and akinetes (closed arrowhead) in chains of the filamentous cyanobacterium *Anabaena cylindrica*. **d** | A mature colony of *Streptomyces coelicolor*, which is indicated by the fluffy, grey layer of sporulating aerial mycelium on the colony surface. The colony produces the blue-pigmented polyketide antibiotic actinorhodin. Image in part **a** is reproduced, with permission, from REF. 28 © (2013) Macmillan Publishers Ltd. All rights reserved. Image in part **b** courtesy of S. Müller and J. Kirby, University of Iowa, USA. Image in part **c** courtesy of J. E. Frías and E. Flores, Centro de Investigaciones Científicas, Universidad de Sevilla, Spain.

produce chains of cells (referred to as clustered growth, which is a feature of filamentous cyanobacteria) or the formation of syncytial filaments (which occurs in streptomycetes via the formation of crosswalls that divide the hyphae but do not lead to cell fission)[5] (FIG. 2).

Biofilm formation typically requires an extracellular matrix (ECM), which usually contains extracellular polysaccharides, amyloid fibrils, lipids and nucleic acids[7–9] (FIG. 2a). This matrix holds the constituent cells together and creates a bidirectional barrier to the environment. This barrier excludes toxic substances (such as antibiotics) and retains water and enzymes close to the cell surface, thus enabling members of the community to access remnants of lysed cells and colloidal biopolymers[8]. Similarly to biofilms, swarming cells, such as *Myxococcus xanthus*, are also embedded in an ECM, which not only ensures that the cells remain physically connected to each other but also contributes to directional movement[10].

Physical connections between cells can also emerge by mechanisms that prevent the complete separation of daughter cells after cell division, thus generating so-called chains (FIG. 2b). In fact, in the early stages of biofilm formation by *B. subtilis* and *Lactococcus lactis*, chains are temporarily formed. These chains only later resolve into an aggregate of cells, presumably when matrix production has been activated[11,12]. Whereas chains are temporary in the context of biofilms, permanent chaining occurs in filamentous cyanobacteria, in which dividing cells remain physically attached to one another (note that these chains are often referred to as filaments in the literature, although they are distinct from the syncytial filaments that are formed by streptomycetes; see below). Recent data suggest that there is a proteinaceous complex that is involved in linking the cells in a chain[13,14], which might also influence molecular exchange between the cells (BOX 1). Unlike the cells in chains, individual cells in syncytial filaments of *Streptomyces* spp. are not recognizable; instead, the branched mycelia contain relatively large compartments that are separated by crosswalls, and each compartment contains multiple chromosomes (FIG. 2c).

Filamentation, and even branching, is also observed in cells that have defects in cell division, such as the branched filaments that are formed by *Escherichia coli* cells with mutations in penicillin-binding proteins[15,16]. Although indefinite filamentous or clustered growth is not sustainable, the occasional separation of these multicellular structures into smaller units could lead to the formation of heritable and phenotypically heterogeneous units that contain a variable number of cells. Indeed, such multicellular clusters were recently shown to rapidly evolve in laboratory cultures of the unicellular eukaryote *Saccharomyces cerevisiae*[17]. The multicellular clusters even evolved a division of labour that was akin to germ–soma division in higher animals and plants. Some cells divided normally, whereas others became apoptotic, and the structurally weak junctions between cells that were caused by apoptosis led to the splitting of large 'parental' clusters to create new propagules. Thus, cell death was crucial for cluster propagation. Although this work does not recapitulate the evolution of multicellularity under natural conditions, it highlights the surprising ease with which multicellularity can evolve when the conditions are right. It also emphasizes the value of using experimental systems to understand both the phenotypic dynamics of multicellular transitions and their molecular underpinnings.

Ecological benefits of cluster and filament formation. What evolutionary factors favour the maintenance of multicellular groups when they are in competition with their unicellular predecessors? The effects of filamentous or clustered growth are generally negative, owing to impaired motility, reduced buoyancy in an aquatic environment and increased competition for resources as a result of increased cell density[3,5]. Two main benefits can offset these costs: predation- or stress-resistance and improved resource acquisition among cooperative foragers.

The larger size of filaments and clusters compared to individual cells reduces susceptibility to size-selective phagotrophic predators[18]. Experiments in which individual bacterial cells and flagellate predators were mixed

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Swarming
The coordinated movement of a group of bacterial cells across a surface.

Irreversible differentiation
A process by which cells become irreversibly specialized in form and function.

Syncytial filaments
Filaments that have a multinucleated cytoplasm that is not separated into individual cells.

Germ–soma division
The distinction in animals and plants between cells that are reproductively competent (known as germ cells) and those that contribute only to growth and structural maintenance (soma).

Propagules
Materials that enable dispersal and promote continued growth, such as a spore or cluster of cells.
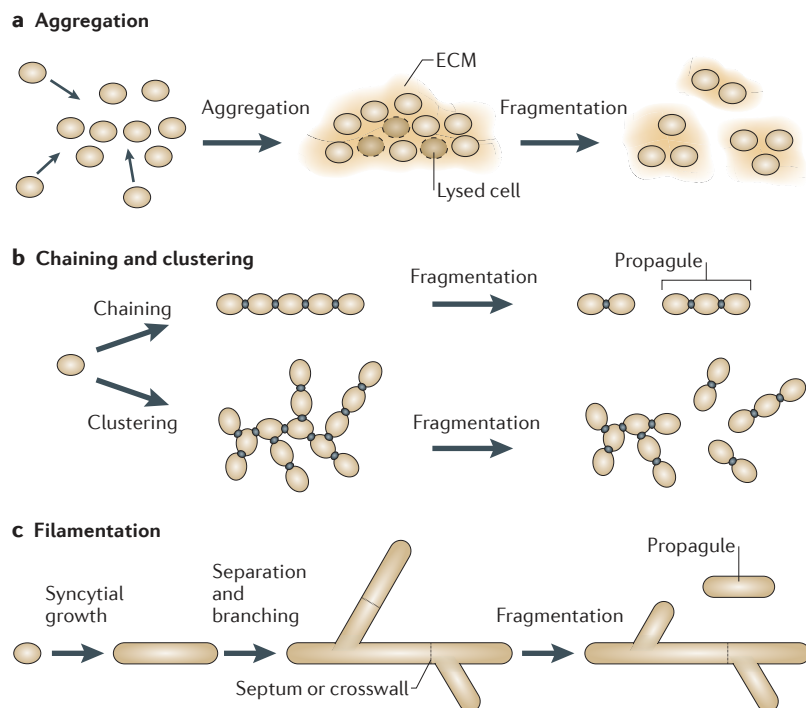
a Aggregation

b Chaining and clustering

c Filamentation

Figure 2 | **Evolution of multicellular clusters.** Multicellular aggregates can form and propagate by distinct mechanisms. **a** | An aggregate of cells that is enclosed in an extracellular matrix (ECM) is known as a biofilm. The matrix is partially formed by stress-induced cell lysis, which releases DNA and proteins into the environment. Fragmentation of the biofilm into smaller aggregates can lead to dispersal and the formation of new biofilms at other sites. **b** | Incomplete cell fission leads to the formation of chains or clusters of cells. Fragmentation of these aggregates can lead to the formation of new propagules. **c** | Cell division arrest leads to the formation of filamentous, syncytial cells. To sustain growth without cell separation, additional mutations that induce septation and branching are required. New propagules are formed by fragmentation.

Sessile
A term used to describe cells that grow while they are attached to a surface substrate.

Planktonic
A term used to describe unattached cells that grow in the bulk liquid of a medium.

Pellicles
Biofilms that form at the water–air interface.

Sporulation
The process of generating spores that are resistant to environmental stresses, such as dessication and starvation.

led to the enrichment of filamentous phenotypes among the bacterial prey[19]; for example, inedible filaments of between three and ten elongated cells rapidly emerged when the freshwater bacterium *Flectobacillus* sp. was cultured with the bacterivorous flagellate *Ochromonas* sp.[20]. Notably, when these filaments were subsequently grown in predator-free medium, they quickly reverted to rapidly growing single cells. This is consistent with the idea that substantial costs are associated with the transition to multicellularity, such that bacteria use inducible, rather than irreversible, strategies to enter a multicellular state, thereby maximizing the benefits of multicellular growth and minimizing its long-term costs[20,21]. Similar responses are observed in the clinical environment; for example, the actinomycete *Mycobacterium tuberculosis* transitions from single cells into filaments during proliferation inside macrophages[22]. This morphological plasticity of pathogenic bacteria, which is often achieved by suppressing cell division, is also seen during antibiotic exposure, leading to the suggestion that filamentation is an adaptive response that is used to increase survival during exposure to exogenous stresses[23].

A second benefit of cluster and filament formation is that it enables growth to occur at resource concentrations

that are inadequate to support unicellular proliferation[24,25]. This has been elegantly illustrated in *S. cerevisiae* cells that were dependent on the enzyme invertase to break down sucrose into glucose and fructose. This dependence led to selection of clustered growth (referred to as incomplete cell separation), as the monosaccharides diffused away from invertase-producing single cells, and clustering enabled direct access to the glucose that was produced by neighbouring cells, thus increasing the per capita efficiency of resource acquisition[24,25]. As clustering of invertase-producing cells also enables only those cells that are within the cluster to access the monosaccharides, this reduces the ability of invertase non-producers outside the cluster to cheat[24]. Similar advantages are anticipated for bacteria — for example, in myxobacteria that cooperatively feed using exo-enzymes[26,27].

Transient multicellularity in biofilms

Biofilms are a widespread mode of bacterial multicellularity and form when individual cells aggregate and proliferate as sessile communities that are embedded in an ECM[28,29]. Many bacterial species have the ability to switch between planktonic and sessile lifestyles, depending on growth conditions and environmental factors[6]. These changes are accompanied by dramatic shifts in gene expression, which result in phenotypic changes. For example, biofilms that are formed by *Staphylococcus aureus, Pseudomonas aeruginosa* and *E. coli* show increased resistance to stresses caused by factors such as exposure to organic acids, solvents (for example, acetic acid, malic acid and ethanol) and antibiotics, or stresses caused by predation[6]. Biofilms are phenotypically diverse, ranging from pellicles at the air–water interface to communities of cells that are associated with a solid surface, such as macrocolonies on agar plates in the laboratory[30] (FIG. 1a). In *B. subtilis*, these macrocolonies typically form elevated structures that are the preferential sites for sporulation[31] (FIG. 3a). Sporulation is preceded by a form of programmed cell death (PCD) known as cannibalism in a subpopulation of the biofilm. In this process, siblings are killed to enable the remaining cells to access the nutrients that are released, and thereby delay sporulation[32]. These differentiation strategies are proposed to have evolved to optimize the persistence of different clonal lineages[33].

As the differentiation of cells is coordinated with a division of labour, biofilm formation superficially resembles more complex multicellular bacterial assemblies (see below), and analogies can also be made with the cellular organization of animals, plants and fungi[28]. For example, in *B. subtilis* biofilms, well-defined channels that resemble circulatory systems transport liquid nutrients and waste over distances for which diffusion would be insufficient[34]. However, the manner in which biofilms develop differs from that of multicellularity in plants and animals. Whereas animal development begins with a single cell that proliferates, biofilms form when cells from distinct lineages or species aggregate, which can result in conflicts of interest between individuals. Accordingly, a recent review[35] questioned whether biofilms are more analogous to multi-organism collectives (such as a

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Some bacteria elaborate on simple forms of multicellular growth by producing channels between adjacent cells[34]. Particularly well studied in this respect are cyanobacteria, in which intercellular channels enable the exchange of molecules between cells. Extensive molecular transfer occurs between heterocysts and vegetative cells; gases and photosynthetically fixed sugars are passed into heterocysts, whereas heterocysts supply vegetative cells with nitrogen[14,62]. Freeze-fraction electron microscopy has demonstrated the presence of what were originally referred to as 'microplasmodesmata' in both heterocyst-forming and non-heterocyst-forming cyanobacteria[95]. These were recently renamed septosomes, to reflect their proteinaceous nature and, thus, their similarity to eukaryotic desmosomes[96]. SepJ and the filament integrity proteins FraC and FraD from *Anabaena* sp. PCC 7120 have been localized to these intercellular septa and are required for intercellular molecular exchange, which has been visualized using fluorescent tracers[14,97]. Interestingly, SepJ can be distinguished from FraC and FraD proteins on the basis of the molecules that they transfer, which suggests that there are functionally different types of cell–cell joining complexes at the septa[14].

It also seems probable that transport occurs through the crosswalls that separate the adjacent compartments in the hyphae of streptomycetes. We define crosswalls as non-fission cell division structures, which are an integral feature of filamentous growth. Crosswalls are placed at irregular intervals in the vegetative hyphae, leading to a syncytial network of connected compartments[74]. Evidence of transport between compartments is provided by conjugation experiments[98,99], in which plasmids spread over long distances through vegetative hyphae, which suggests that the DNA must be transported through the crosswalls. Consistent with this, channels that have a similar appearance to the septosomes that are seen in *Anabaena* sp. PCC 7120, are occasionally seen in transmission electron micrographs of crosswalls[69]. Cell division is not essential for the vegetative growth of *Streptomyces* spp., although a lack of cell division prohibits sporulation[100]. The cell division scaffold protein FtsZ is required for crosswall formation, but crosswalls are formed in mutants that lack canonical cell division genes such as *ftsW* and *ftsI*[101]. Further research of this phenomenon should provide new insights into the mechanisms of cell division.

Other examples of bacterial crosswalls include a crosswall-like barrier in *Caulobacter crescentus*, which is formed by complexes consisting of stalk proteins that prevent the exchange of proteins between the polar stalk and the cell body[102]. Interestingly, almost all Crenarchaea that have been investigated lack *ftsZ*; instead *Pyrobaculum islandicum* produces a crosswall that is composed of S-layer material, and the daughter cells remain associated until the cell ruptures[103].

Kin selection
The evolutionary theory that explains why altruistic behaviours are directed towards individuals that are highly genetically related.

Desmosomes
Eukaryotic cell structures that are specialized for cell–cell adhesion and molecular exchange.

S-layer
A cell envelope layer that is composed of proteins, which encloses the cell surface of many bacteria and archaea, and occasionally divides cells.

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school of fish or a flock of birds) than to multicellular organisms. Individual animals in multi-organism collectives occasionally cooperate — for example, to form a shared defence. However, they more often compete with one another, as cooperative behaviours are costly to the individuals that carry them out. As a consequence, there is a strong selection for 'cheats' that do not cooperate but instead exploit the cooperative behaviours of others.

The typical solution to the evolution of cooperation is kin selection, in which cooperative acts are directed towards individuals with which the organism shares genes (that is, towards their siblings or close relatives)[35,36]. Although some biofilms are largely clonal (for example, those that form on indwelling devices after surgery and consist of a single species), in natural environments they are likely to be composed of divergent species, and thus conflicts are likely to be more common[6,35]. Recent studies have shown that non-cooperative cheats proliferate in biofilms and compromise their thickness and their growth rate, and even increase their antibiotic susceptibility[37]. Although these observations do not contradict the idea of biofilms being true multicellular structures, they do raise important questions relating to the ability of the constituent cells to behave in a coordinated and cooperative manner. Simulations

suggest that biofilm-like structures can arise among non-cooperating cells, owing to resource gradients that are generated during cell growth, such as gradients of secreted extracellular products and gases[35,38]. Such gradients generate phenotypic and structural heterogeneity, which gives rise to structures such as pellicles (in which cells at the surface of the liquid have increased access to oxygen and grow quickly), troughs (in which cells are more nutrient-limited and grow slowly) and water channels for the transport of nutrients and waste[6,29]. Thus, although cooperation between cells can occur on a local level (that is, within defined structures), it is unlikely that all cells in the community cooperate, and moreover, their cooperation is unnecessary for the generation of biofilm architecture. Therefore, caution should be taken when drawing analogies between biofilms and the complex multicellular systems that are found in other types of bacterial communities or, indeed, multicellular eukaryotes.

Patterned multicellularity

A key advantage of multicellularity is that differentiated cell types carry out distinct functional roles that increase the fitness of the whole population, even if the function is costly to the individual cell[3,5]. This permits cellular filaments and aggregates to simultaneously carry out tasks that are functionally incompatible[39,40], such as photosynthesis and nitrogen fixation. Although biofilms have several hallmark features of multicellularity, including intercellular signalling, morphological differentiation (also known as sporulation) and PCD (or cannibalism), they lack a key feature of multicellularity: a well-defined and reproducible shape, which we refer to as patterned multicellularity (FIG. 3). Despite the fact that spores are produced at the tips of projections in a *B. subtilis* biofilm[31], the biofilm lacks the complexity and reproducible organization of myxobacterial fruiting bodies. Thus, they are not considered to be a true form of patterned multicellularity. Patterned multicellular structures are the products of either the combination of division and proliferation of clonal cells (such as those that are formed by cyanobacteria and actinomycetes) or of the aggregation and coordinated movement of groups of independent cells by motility (which occurs in myxobacteria). These fundamental differences in origin have important consequences for the social interactions and stability of these multicellular groups. We discuss several hallmark features of patterned multicellularity, using well-studied model organisms to illustrate common and divergent themes in their evolution.

Aggregative multicellularity in myxobacteria. Myxobacteria are a group of Gram-negative bacteria that are predominantly found in the soil and that engage in social foraging for food; the best studied species of the group is *Myxococcus xanthus*. During starvation, growth is arrested and a developmental programme is initiated, which culminates in the formation of spore-bearing fruiting bodies that have a well-defined shape (FIG. 3b). During growth, myxobacteria move as a coordinated assembly of cells to prey on other microorganisms or

a Bacillus subtilis

Motile cells — Attchment, differentiation and matrix synthesis — Biofilm growth and cannibalism (PCD) — Maturation and sporulation

b Myxococcus xanthus

Cooperative feeding — Fruiting body formation

c Anabaena spp.

d Streptomyces spp.

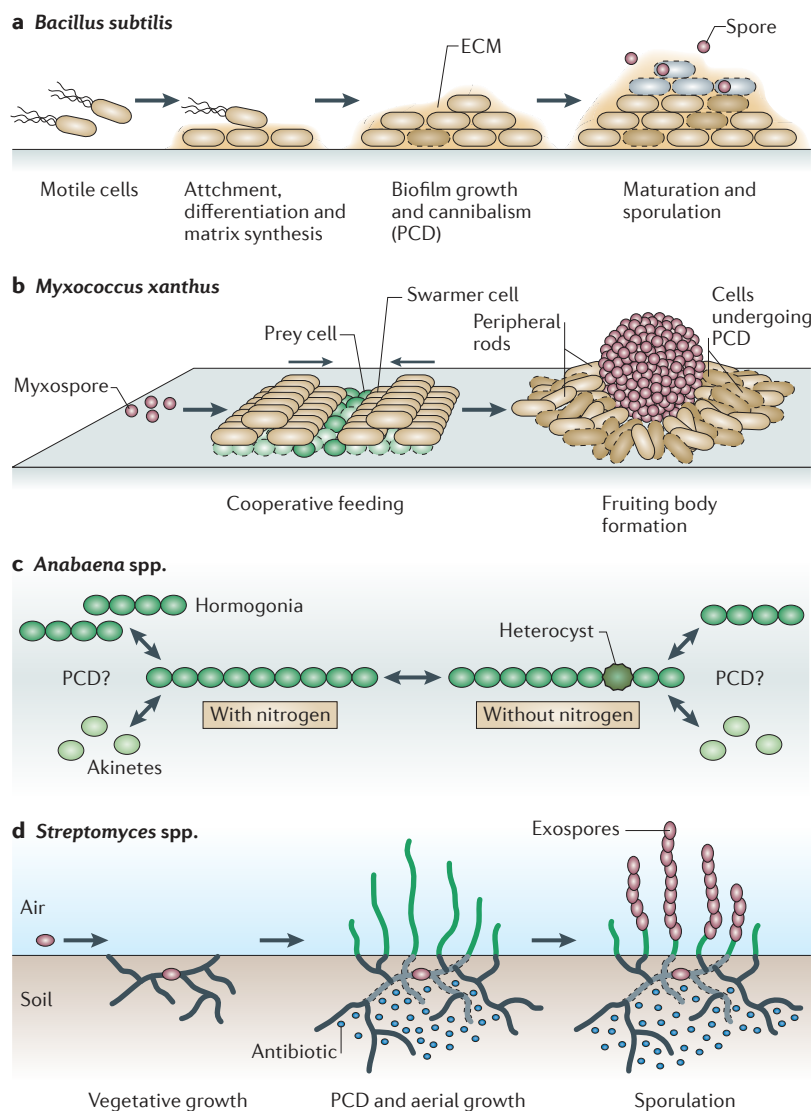Vegetative growth — PCD and aerial growth — Sporulation

Figure 3 | **Different strategies that lead to the development of bacterial multicellularity. a** | Biofilm formation by bacilli, such as *Bacillus subtilis*, is initiated by individual motile cells that adhere to a surface structure and produce an extracellular matrix (ECM)[28]. Cells within this matrix proliferate and differentiate to carry out distinct functions. Some cells are cannibalized (in a process that is akin to programmed cell death (PCD)), which delays the initiation of sporulation[32]. The production of endospores does not require a multicellular structure with a well-defined shape (which is a key feature of multicellularity), although spores tend to form at the tips of projections in a biofilm. **b** | Germinated myxospores produce swarming cells that move in a coordinated manner to forage for food. In the event of starvation, a fruiting body forms and produces spores, some cells lyse and others differentiate into peripheral rods. **c** | Filamentous cyanobacteria, such as *Anabaena* spp., become physically linked owing to the incomplete separation of cells after division. During nitrogen depletion, *Anabaena* spp. differentiate by occasionally producing nitrogen-fixing heterocysts that obtain sugars from neighbouring photosynthetic cells and, in turn, provide these cells with nitrogen. Differentiation can also lead to the formation of hormogonia and akinetes, which are used for dispersal and persistence, respectively, and which might be released in the environment as a result of PCD. **d** | Germination of spores of *Streptomyces* spp., with *Streptomyces coelicolor* as a model organism, leads to the formation of a syncytial vegetative mycelium. Exposure to stress (such as nutrient depletion) induces PCD of the vegetative mycelium and the development of an aerial mycelium to physically separate cells from the stressful environment. Aerial growth coincides with the production of antibiotics that function to protect the pool of nutrients that is released during dismantling of the vegetative mycelium. The aerial hyphae develop into chains of exospores, which, after dispersal, can initiate growth at other sites.

to feed on organic biopolymers by secreting hydrolytic enzymes that degrade target cells and biopolymers[41]. Two processes have evolved to increase the efficiency of this process. First, the simultaneous germination of spores from a fruiting body ensures that foraging cells are at the high densities that are needed to maximize growth rates[26]. As vegetative cells feed by cooperatively secreting proteolytic enzymes, their growth rate correlates with the concentration of these enzymes and thus with cell density[26]. Second, during social foraging, cells organize into a rippling pattern comprised of cells assembled into ridge-like structures that are separated by troughs of low cell density (FIGS. 1b, 3b). The cells in these 'ripples' move as waves across prey bacteria to increase the efficiency by which diffusible growth substrates (which are generated by secreted hydrolytic enzymes) are utilized[42].

The first signs of fruiting body formation are evident 4–6 hours after the exhaustion of resources. This is accompanied by changes in cell motility[43] and the formation of aggregation centres. Within 24 hours, the aggregation process is complete, and the nascent fruiting bodies each contain approximately 10^5 densely packed cells that differentiate into spores. Fruiting body formation is induced by the stringent response, which is initiated by accumulation of the alarmone (p)ppGpp and is regulated by complex signal transduction pathways, including several two-component systems, Ser/Thr protein kinases and intercellular signals[44]. This complex regulation results in temporally coordinated changes in motility and gene expression, with genes being turned on or off at specific time points during development[44]. Similarly, intercellular signalling is essential for fruiting body formation, the regulation of gene expression and cell motility. Three subpopulations that show a division of labour arise: 10% of cells differentiate into spores; 30% of cells differentiate into peripheral rods that remain on the exterior of the fruiting body[45]; and the remaining cells undergo PCD[46,47].

The formation of a fruiting body is thought to provide several benefits to cells in comparison to unicellular growth. Spores promote survival during periods of starvation and abiotic stress, and are capable of dispersing to unexploited resource patches. Some *M. xanthus* cells (specifically, the peripheral rods) are capable of growth in nutrient-limited conditions, which are insufficient to induce the germination of spores[48]. Lysed cells are thought to provide nutrients for the other cell types[42]. Despite these benefits, the presence of non-cooperating cheats can disrupt or disable multicellular coordination. In cells that aggregate during each stage of the myxococcal life cycle, and within clonal groups, altruistic behaviour can be explained by kin selection[27,36]; however, in genotypically diverse aggregates, which can arise from mutations, the indirect benefits of self-sacrifice are reduced[36]. This favours the selection of individual cells that act in their own interest rather than in the interests of the group. For example, during foraging, social cheats consume resources without enduring the costs that are associated with the production of hydrolytic enzymes[27]. During fruiting body formation, cheating is even more pronounced, as a large fraction of cells undergo PCD and consequently sacrifice their own reproduction for

that of the differentiated spores and peripheral rods. Both in the laboratory and among natural isolates, there is extensive evidence that such cheats exist; for example, studies have shown that socially defective mutants (some of which are completely incapable of sporulation) gain disproportionate advantages when they are grown in the presence of wild-type cells[27,49,50]. This can reduce group productivity as the total number of potential spore producers declines, or — more dramatically — such behaviour can cause cooperative cells to become extinct[49,51].

How do myxobacteria mitigate this social burden and maintain social cohesion? First, social cheats may often trade-off the advantage of cheating with concomitant deficits in other phenotypes, such as motility or the ability to forage[50,52]. Second, as there is a highly polymorphic system of self–non-self discrimination known as allorecognition, non-identical genotypes recognize and exclude one another during aggregation and fruiting body formation. This increases the clonality of fruiting bodies and reduces opportunities for cheating[27,49,53]. Self–non-self recognition in *M. xanthus* was recently found to be determined by a highly polymorphic cell surface-associated protein, TraA[54]. Cells that have high sequence similarity at the *traA* locus form recognition groups comprised of genotypes that socially interact, whereby TraA–TraA recognition results in cell fusion and the exchange of outer membrane components between cells, whereas cells that have a divergent *traA* allele are excluded. Moreover, cells that belong to the same *traA* recognition group are mutually immune to bacteriocin-mediated killing, whereas cells from different recognition groups are able to kill one another[54]. Similar mechanisms of discrimination have been described in other bacteria that also exhibit complex multicellular phenotypes; for example, neighbouring colonies of the swarming Gram-positive bacterium *Paenibacillus dendritiformis* secrete lethal factors at the boundary where the two populations meet, killing all of the cells at the interface[55]. A more complex scenario is seen at the boundary between socially swarming colonies of the Gram-negative bacterium *Proteus mirabilis*. Bacteriocin-mediated killing occurs at the boundary of neighbouring populations, but this is preceded by kin recognition of polymorphic alleles at the identification of self (*ids*) locus[56]. Irrespective of the precise mechanism that is involved, such processes function to simultaneously increase the relatedness of co-aggregating cells and reduce the risks of exploitation by cheaters. The identification of these mechanisms is important for understanding myxobacterial sociobiology and is an area in which further work is needed.

Filamentous growth and differentiation of cyanobacteria. Complex multicellularity in cyanobacteria evolved approximately 2.5 billion years ago, coinciding with the increased oxygenation of the atmosphere that occurred in the so-called Great Oxidation Event[57,58]. Since then, multicellularity has been lost and regained several times in these bacteria, which has led to the current distribution of both unicellular and multicellular cyanobacterial forms[57,58]. In contrast to the aggregative and motility-dependent multicellularity of myxobacteria, filamentous cyanobacteria become multicellular as a result of the incomplete separation of daughter cells after cell division. They also show a division of labour owing to the phenotypic differentiation of filamentous cells into distinct cell types. As the cells in cyanobacterial filaments are always clonal, there are fewer opportunities for social conflicts to arise among phenotypically differentiated cells[40]. Cyanobacteria depend on sunlight for photosynthesis and to fix atmospheric nitrogen. Whereas unicellular forms carry out these functions sequentially, filamentous genera such as *Anabaena* and *Nostoc* undergo cellular differentiation, which leads to at least four specialized cell types: photosynthetic cells, nitrogen-fixing heterocysts, akinetes (also known as resting cells) and small filaments of motile cells known as hormogonia[59] (FIG. 3C). Photosynthetic and nitrogen-fixing cells have roles in energy capture and conversion during growth, whereas akinetes and hormogonia ensure persistence and dispersal, respectively, in time and space.

Vegetative and heterocyst cells are mutually interdependent owing to complementary metabolism; heterocysts fix nitrogen but lack components of the photosynthetic machinery[60]. The patterned formation of heterocysts in cyanobacterial filaments depends on intercellular communication, which is thought to involve diffusion of the PatS peptide along the length of the filaments. In turn, the products of nitrogen fixation control the expression of PatS and, in this way, also define the pattern of heterocyst formation[61]. Using intercellular channels, heterocysts import photosynthetically fixed sugars that are produced by vegetative cells[62] and provide nitrogen to vegetative cells[63] (BOX 1). In addition, although vegetative cells are able to reproduce and generate the other cell types, heterocysts cannot revert back to a vegetative state. Thus, this differentiation approximates the germ–soma division in eukaryotic multicellular species. Theoretical models have shown that the spatial division of labour is more efficient at energy capture and conversion than a temporal separation of functions[64]. Moreover, these models show that, although cheater cells that forego heterocyst production for further vegetative growth can arise, their success is short-lived because they only destroy the aggregate in which they emerge but spread no further[65].

Several lines of evidence suggest that PCD might occur in filamentous cyanobacteria. Cell death of different *Anabaena* species on exposure to stress coincides with morphological deformation, fragmentation and the subsequent autolysis of cells. Similarly to cell division, cell death is controlled by circadian rhythms in *Anabaena* spp., which implies that cell death is carefully programmed in these organisms[66,67]. Similarly, cell death is observed in *Trichodesmium* spp. in response to phosphorus starvation, iron starvation, high levels of irradiation and oxidative stresses[68]. Cyanobacterial hormogonia can resume growth when conditions become more favourable[68] and are thus analogous to bacterial spores, in the sense that they preserve a given genotype.

Stringent response
A bacterial stress response that is induced during unfavourable growth conditions (such as lack of amino acids), which creates a negative-feedback loop that shuts down macromolecule biosynthesis and other metabolic activity.

(p)ppGpp
(Guanosine pentaphosphate or tetraphosphate). An alarmone molecule that signals the stringent response.

Allorecognition
The process by which organisms are able to distinguish self from non-self.

Bacteriocin
A narrow- or broad-spectrum antimicrobial peptide, which is ribosomally synthesized by bacteria and is able to kill other bacteria by different mechanisms.

Therefore, it is possible that PCD in cyanobacteria (that is, death of vegetative cells that have not become hormogonia) contributes to the release of hormogonia, and thereby genotype preservation, although this remains to be elucidated.

Differentiated multicellular growth in streptomycetes. Similarly to cyanobacteria, multicellularity in streptomycetes is initiated from a single cell (in this case, a spore), rather than from the aggregation of cells. However, in contrast to the linear arrangement of cyanobacterial cells, bacteria that belong to the genus *Streptomyces* are mycelial organisms that grow as a complex hyphal network by a combination of tip growth and branching[69] (FIG. 3d). The complex multicellular nature of streptomycetes is highlighted by the formation of connected and potentially communicating compartments, which are separated by crosswalls, in vegetative hyphae. Indeed, nutrients and plasmids are transported over long distances through the hyphae, and channels are occasionally observed in the crosswalls[70] (BOX 1). After a stress signal, such as nutrient depletion, a fraction of the hyphae escape the moist soil environment to grow into the air and differentiate into aerial spore-bearing structures. This coincides with the onset of antibiotic production[71,72] (FIG. 3d). Spores are coated with hydrophobic proteins that assemble into a matrix-like, water-repellent structure that surrounds the hyphal surface and facilitates spore dispersal[73].

Several benefits of hyphal growth can be envisioned, particularly benefits relating to the acquisition and discovery of resources. Single motile bacterial cells can only migrate along a linear transect, whereas hyphal growth permits radial expansion and therefore facilitates migration to an extended range of microenvironments. This is expected to be particularly relevant for soil bacteria that grow as saprophytes on abundant, but discontinuously distributed, resources. The high surface area-to-volume ratio of a hyphal mass might also facilitate nutrient transfer, assuming that nutrients are efficiently allocated and distributed across the breadth of the mycelium; however, these potential advantages remain to be tested. Notably, it is unclear whether such advantages were driving forces in the evolution of mycelial growth or whether this mode of growth evolved for other reasons but provides secondary benefits in terms of resource acquisition.

As in cyanobacteria and myxobacteria, cell death has a crucial role in the morphological development of *Streptomyces coelicolor*[74]. When hyphae commit to aerial growth, the vegetative mycelium is dismantled and the nutrients that are released are used to feed the aerial mycelium[75]. An intriguing relationship between PCD and antibiotic production has evolved in streptomycetes. Antibiotics, which are produced by the vegetative mycelium, are hypothesized to protect the pool of nutrients that is released after PCD by preventing other saprophytic bacteria from accessing them; however, antibiotic-mediated lysis of competitors might also constitute a form of preying and thereby supply an additional source of nutrients[71]. Cell wall-derived

N-acetylglucosamine (GlcNAc), which is released during PCD, is an important trigger for antibiotic production in streptomycetes[76–78]. During PCD, GlcNAc accumulates around colonies, resulting in the intracellular accumulation of GlcN-6P (glucosamine-6-phosphate); this then functions as a ligand for the antibiotic repressor DasR (which is a HTH-type transcriptional repressor), resulting in the activation of antibiotic production[79].

One of the most obvious differences between the streptomycetes and unicellular bacteria is manifested in the control of cell division. Most bacteria divide by binary fission, which involves the formation of a dynamic ring structure (known as the Z ring) by the tubulin homologue FtsZ at division sites[80,81]. However, in streptomycetes, the long aerial hyphae differentiate into chains of spores after a uniquely coordinated cell division event. Instead of a single divisome localized at midcell, distinctive ladders of FtsZ are observed that almost simultaneously form up to 100 septa, which eventually leads to the formation of long chains of haploid spores[82]. Thus, the process of sporulation-specific cell division in *Streptomyces* spp. is a key example of patterned multicellularity, and the correct timing of division seems to be a key switch for the development of multicellularity. For example, a number of *whi* regulatory genes function together to control one or more checkpoints that need to be passed before FtsZ accumulates in sufficient amounts for sporulation to occur[83,84]. Sporulation-specific cell division in *S. coelicolor* is controlled by the membrane-associated SsgB protein, which directly recruits FtsZ[85]. SsgB is a member of the SsgA-like proteins (SALPs), which are exclusively found in morphologically complex actinomycetes[86]. Interestingly, the number of SALPs that are encoded by a specific actinomycete species positively correlates with the complexity of its development[86]: species that encode just one paralogue typically produce a single spore per hypha, whereas those that encode multiple (up to 14) paralogues produce long chains of spores or complex sporangia[87]. Thus, this is a rare example of a protein family that controls the number of new cells that are produced during the life cycle of a bacterium. This mode of cell division and its complex regulation might have been a major adaptation that was required to facilitate the transition from unicellular to filamentous growth in sporulating actinomycetes (BOX 2).

The evolution of bacterial multicellularity
Bacteria are thought to have independently developed complex multicellular behaviour several times during evolution, which has resulted in several different routes that lead to multicellularity, with or without ensuing cellular differentiation. Furthermore, although bacterial multicellular structures (for example, mycelia and fruiting bodies) might superficially resemble certain morphologically similar structures in eukaryotes (such as fungal mycelia and *Dictyostelium discoideum* fruiting bodies), these manifestations of multicellularity are evolutionarily unrelated as they emerged independently in these distant groups. The association of cells in a primitive ECM or the formation of adhered clusters
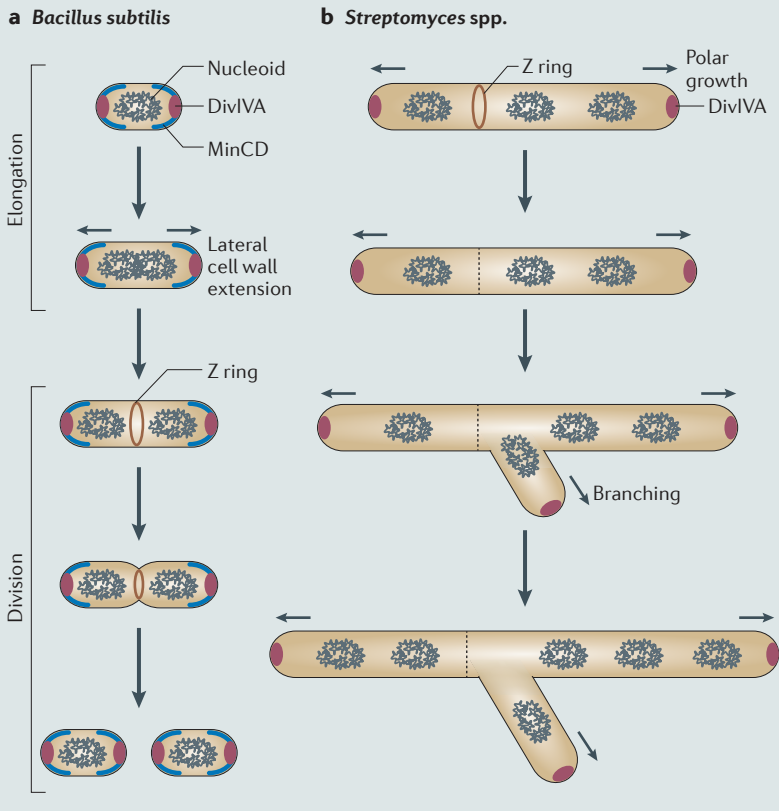

Paralogue
A term used to describe evolutionarily related genes that have duplicated and reside in different locations within the same genome.

Sporangia
Specialized structures in which spores are formed and contained.

Box 2 | Mechanisms of hyphal growth

Vegetative cell division in the low GC Gram-positive bacterium *Bacillus subtilis* is orchestrated by the cell division scaffold protein FtsZ, which assembles into a Z ring at midcell, eventually leading to the formation of two identical daughter cells (see the figure, part **a**). Before division, the cell elongates by extension of the lateral wall. By contrast, *Streptomyces* spp. grow from the cell pole to produce hyphae (see the figure, part **b**). Polar growth is ancestral to the actinomycetes species, even in species that show no tendency towards multicellularity, such as *Corynebacterium glutamicum*[104] and *Mycobacterium smegmatis*[105,106]. The transition from lateral to polar growth requires the repurposing of DivIVA[107]. In *Bacillus* spp., DivIVA functions in concert with the Min system to restrict division to midcell[108,109] (see the figure, part **a**), whereas the septum site-determining MinCD proteins are absent in actinomycetes and instead DivIVA is required to drive tip growth. This repurposing of DivIVA might have been an important factor in the transition from unicellular to filamentous, multicellular growth in the actinomycetes. Indeed, overexpression of DivIVA is sufficient to enable mycobacteria to switch from bipolar to multipolar (also known as mycelial) growth[104]. Mycobacteria and mycelial actinomycetes might be more similar to each other than their morphologies suggest[110]. It has been suggested that *Mycobacterium marinum* might produce endospores[111], although the validity of these findings is under debate, and the experiments could not be reproduced by other laboratories[112]. Several actinomycetes produce single exospores from vegetative hyphae, and multiple modes of cell division might have facilitated the transition from single exospores to chains of spores or sporangia.

a *Bacillus subtilis*

Elongation

- Nucleoid
- DivIVA
- MinCD

Lateral cell wall extension

Z ring

Division

b *Streptomyces* spp.

Z ring

Polar growth

DivIVA

Branching

Min system
A system that ensures the correct localization of the septum during cell division by directing polymerization of the cell division scaffold protein FtsZ away from the cell poles and towards midcell.

seem to be very different, single mutations can lead to the filamentous growth and branching of *E. coli*[16,88–90]. Conversely, stimulation of cell division induces the fragmentation of vegetative hyphae in *S. coelicolor*[91,92], which effectively induces a reversal from multicellular to unicellular growth.

As outlined in the examples that are described in this Review, the switch to multicellularity can confer several advantages, but it also has disadvantages. For example, when single cells aggregate and replicate as a single unit, some individuals may die by PCD or become reproductively sterile. It has recently been shown that these dead altruistic cells evolve more frequently among multicellular groups that form from a single cell[93]. Moreover, such clonal groups, which lack the internal conflicts of non-clonal aggregates, tend to phenotypically differentiate into more cell types. This predicts fewer social conflicts in streptomycetes and cyanobacterial aggregates, which are highly clonal, than in biofilms or myxobacterial fruiting bodies, which develop via aggregation.

Future work will need to address several outstanding issues, including the conditions that are required to maintain multicellularity among disparate bacterial groups. Nutrient or other stresses, including predation, seem to be common driving forces towards multicellularity, but the benefits in many groups remain unclear or, at least, untested. For example, although it is often argued that myxobacteria form fruiting bodies to facilitate dispersal into novel or unoccupied habitats that have a wider spectrum of resources, this conjecture has not been directly tested. Equally, although there are several potential advantages of mycelial growth in streptomycetes, these are mere speculations at this point. Since the recognition of bacterial multicellularity as a more widespread phenomenon[1], novel modes of multicellularity have been discovered, and the mechanisms of bacterial development, signalling and differentiation have been elucidated to some extent. Future advances require these exciting developments to be integrated within a clear ecological and evolutionary context.

In addition, we advocate looking beyond simple phylogenetic comparisons in order to understand phenotypic transitions and recommend focusing greater attention on experimental studies that seek to understand the role of key ecological parameters in the evolution of multicellularity. The *S. cerevisiae* studies that are outlined above[17] as well as related work that shows the experimental evolution of multicellularity in the unicellular algae *Chlamydomonas reinhardtii*[94] provide an excellent starting point for similar studies in bacteria, and we should aim to understand the population dynamics and underlying mechanisms of bacterial multicellular evolution. Such studies should ideally use selective environments that have a natural analogue[24,25]. Although this set-up cannot completely recapitulate the evolutionary changes that have occurred in the past, such studies are a powerful approach to understanding what is evolutionarily possible as well as understanding the manner in which ecological factors interact to drive phenotypic transitions in bacteria.

or filaments are probably starting points from which more complex multicellular structures evolved (FIG. 2). Bacterial responses that are induced by nutrient stress or predation stress and that give rise to heterogeneity, PCD and division of labour might have necessitated cooperative activity between cells in the initial stages of multicellularity. However, an important question that has not yet been answered relates to the ease with which transitions between single cells and multicellular structures occur. Although single cells and mycelia might

1. Shapiro, J. A. Bacteria as multicellular organisms. *Sci. Am.* **256**, 82–89 (1988).
   This work challenges the concept of bacteria as strictly unicellular organisms.
2. Shapiro, J. A. Thinking about bacterial populations as multicellular organisms. *Annu. Rev. Microbiol.* **52**, 81–104 (1998).
3. Bonner, J. T. The origins of multicellularity. *Int. Biol.* **1**, 27–36 (1998).
4. Rokas, A. The origins of multicellularity and the early history of the genetic toolkit for animal development. *Annu. Rev. Genet.* **42**, 235–251 (2008).
5. Grosberg, R. K. & Strathmann, R. R. The evolution of multicellularity: a minor major transition? *Annu. Rev. Ecol. Evol. Systemat.* **38**, 621–654 (2007).
6. Monds, R. D. & O'Toole, G. A. The developmental model of microbial biofilms: ten years of a paradigm up for review. *Trends Microbiol.* **17**, 73–87 (2009).
7. Flemming, H. C., Neu, T. R. & Wozniak, D. J. The EPS matrix: the "house of biofilm cells". *J. Bacteriol.* **189**, 7945–7947 (2007).
8. Flemming, H. C. & Wingender, J. The biofilm matrix. *Nature Rev. Microbiol.* **8**, 623–633 (2010).
9. Gebbink, M. F., Claessen, D., Bouma, B., Dijkhuizen, L. & Wosten, H. A. Amyloids — a functional coat for microorganisms. *Nature Rev. Microbiol.* **3**, 333–341 (2005).
10. Li, Y. *et al.* Extracellular polysaccharides mediate pilus retraction during social motility of *Myxococcus xanthus. Proc. Natl Acad. Sci. USA* **100**, 5443–5448 (2003).
11. Kobayashi, K. *Bacillus subtilis* pellicle formation proceeds through genetically defined morphological changes. *J. Bacteriol.* **189**, 4920–4931 (2007).
12. Perez-Nunez, D. *et al.* A new morphogenesis pathway in bacteria: unbalanced activity of cell wall synthesis machineries leads to coccus-to-rod transition and filamentation in ovococci. *Mol. Microbiol.* **79**, 759–771 (2011).
13. Flores, E. *et al.* Septum-localized protein required for filament integrity and diazotrophy in the heterocyst-forming cyanobacterium *Anabaena* sp. strain PCC 7120. *J. Bacteriol.* **189**, 3884–3890 (2007).
14. Merino-Puerto, V. *et al.* FraC/FraD-dependent intercellular molecular exchange in the filaments of a heterocyst-forming cyanobacterium, *Anabaena* sp. *Mol. Microbiol.* **82**, 87–98 (2011).
    This study clarifies the mechanistic basis of intercellular communication across cyanobacterial septa.
15. Nelson, D. E. & Young, K. D. Penicillin binding protein 5 affects cell diameter, contour, and morphology of *Escherichia coli. J. Bacteriol.* **182**, 1714–1721 (2000).
16. Potluri, L. P., de Pedro, M. A. & Young, K. D. *Escherichia coli* low-molecular-weight penicillin-binding proteins help orient septal FtsZ, and their absence leads to asymmetric cell division and branching. *Mol. Microbiol.* **84**, 203–224 (2012).
17. Ratcliff, W. C., Denison, R. F., Borrello, M. & Travisano, M. Experimental evolution of multicellularity. *Proc. Natl Acad. Sci. USA* **109**, 1595–1600 (2012).
    This study shows the ease with which multicellular clusters can evolve *de novo* in *S. cerevisiae* under laboratory conditions.
18. Jousset, A. Ecological and evolutive implications of bacterial defences against predators. *Environ. Microbiol.* **14**, 1830–1843 (2012).
19. Boraas, M. E., Seale, D. B. & Boxhorn, J. E. Phagotrophy by a flagellate selects for colonial prey: a possible origin of multicellularity. *Evol. Ecol.* **12**, 153–164 (1998).
20. Corno, G. & Jurgens, K. Direct and indirect effects of protist predation on population size structure of a bacterial strain with high phenotypic plasticity. *Appl. Environ. Microbiol.* **72**, 78–86 (2006).
21. Blom, J. F., Zimmermann, Y. S., Ammann, T. & Pernthaler, J. Scent of danger: floc formation by a freshwater bacterium is induced by supernatants from a predator-prey coculture. *Appl. Environ. Microbiol.* **76**, 6156–6163 (2010).
22. Chauhan, A. *et al. Mycobacterium tuberculosis* cells growing in macrophages are filamentous and deficient in FtsZ rings. *J. Bacteriol.* **188**, 1856–1865 (2006).
23. Justice, S. S., Hunstad, D. A., Cegelski, L. & Hultgren, S. J. Morphological plasticity as a bacterial survival strategy. *Nature Rev. Microbiol.* **6**, 162–168 (2008).
24. Koschwanez, J. H., Foster, K. R. & Murray, A. W. Sucrose utilization in budding yeast as a model for the origin of undifferentiated multicellularity. *PLoS Biol.* **8**, e1001122 (2011).
25. Koschwanez, J. H., Foster, K. R. & Murray, A. W. Improved use of a public good selects for the evolution of undifferentiated multicellularity. *eLife* **2**, e00367 (2013).
26. Rosenberg, E., Keller, K. H. & Dworkin, M. Cell density-dependent growth of *Myxococcus xanthus* on casein. *J. Bacteriol.* **129**, 770–777 (1977).
27. Velicer, G. J. & Vos, M. Sociobiology of the myxobacteria. *Annu. Rev. Microbiol.* **63**, 599–623 (2009).
28. Vlamakis, H., Chai, Y., Beauregard, P., Losick, R. & Kolter, R. Sticking together: building a biofilm the *Bacillus subtilis* way. *Nature Rev. Microbiol.* **11**, 157–168 (2013).
29. Webb, J. S., Givskov, M. & Kjelleberg, S. Bacterial biofilms: prokaryotic adventures in multicellularity. *Curr. Opin. Microbiol.* **6**, 578–585 (2003).
30. Lemon, K. P., Earl, A. M., Vlamakis, H. C., Aguilar, C. & Kolter, R. Biofilm development with an emphasis on *Bacillus subtilis. Curr. Top. Microbiol. Immunol.* **322**, 1–16 (2008).
31. Branda, S. S., Gonzalez-Pastor, J. E., Ben-Yehuda, S., Losick, R. & Kolter, R. Fruiting body formation by *Bacillus subtilis. Proc. Natl Acad. Sci. USA* **98**, 11621–11626 (2001).
    This is the first to recognize the complex multicellular behaviour of *B. subtilis* biofilms.
32. Gonzalez-Pastor, J. E., Hobbs, E. C. & Losick, R. Cannibalism by sporulating bacteria. *Science* **301**, 510–513 (2003).
    This study shows that a subpopulation of *B. subtilis* communities can delay sporulation by PCD-mediated cannibalism of their siblings.
33. Veening, J. W., Smits, W. K. & Kuipers, O. P. Bistability, epigenetics, and bet-hedging in bacteria. *Annu. Rev. Microbiol.* **62**, 193–210 (2008).
34. Wilking, J. N. *et al.* Liquid transport facilitated by channels in *Bacillus subtilis* biofilms. *Proc. Natl Acad. Sci.* **110**, 848–852 (2013).
    This paper reports on the importance of microchannels for the distribution of nutrients and the removal of waste products from biofilms.
35. Nadell, C. D., Xavier, J. B. & Foster, K. R. The sociobiology of biofilms. *FEMS Microbiol. Rev.* **33**, 206–224 (2009).
36. West, S. A., Diggle, S. P., Buckling, A., Gardner, A. & Griffins, A. S. The social lives of microbes. *Annu. Rev. Ecol. Evol. Systemat.* **38**, 53–77 (2007).
37. Popat, R. *et al.* Quorum-sensing and cheating in bacterial biofilms. *Proc. Biol. Sci.* **279**, 4765–4771 (2012).
38. Nadell, C. D., Foster, K. R. & Xavier, J. B. Emergence of spatial structure in cell groups and the evolution of cooperation. *PLoS Comput. Biol.* **6**, e1000716 (2010).
39. Pfeiffer, T. & Bonhoeffer, S. An evolutionary scenario for the transition to undifferentiated multicellularity. *Proc. Natl Acad. Sci. USA* **100**, 1095–1098 (2003).
40. Rossetti, V. & Bagheri, H. C. Advantages of the division of labour for the long-term population dynamics of cyanobacteria at different latitudes. *Proc. Biol. Sci.* **279**, 3457–3466 (2012).
41. Reichenbach, H. The ecology of the myxobacteria. *Environ. Microbiol.* **1**, 15–21 (1999).
42. Berleman, J. E., Chumley, T., Cheung, P. & Kirby, J. R. Rippling is a predatory behavior in *Myxococcus xanthus. J. Bacteriol.* **188**, 5888–5895 (2006).
    This study shows that rippling is a feeding behaviour that occurs when *M. xanthus* cells make direct contact with prey or large macromolecules.
43. Jelsbak, L. & Søgaard-Andersen, L. Pattern formation by a cell surface-associated morphogen in *Myxococcus xanthus. Proc. Natl Acad. Sci. USA* **99**, 2032–2037 (2002).
44. Whitworth, D. E. (ed.) *Myxobacteria: multicellularity and differentiation* (ASM, 2008).
45. O'Connor, K. A. & Zusman, D. R. Development in *Myxococcus xanthus* involves differentiation into two cell types, peripheral rods and spores. *J. Bacteriol.* **173**, 3318–3333 (1991).
    This study provides the first evidence that *M. xanthus* peripheral rods are a cell type that is distinct from vegetative cells and spores.
46. Nariya, H. & Inouye, M. MazF, an mRNA interferase, mediates programmed cell death during multicellular *Myxococcus* development. *Cell* **132**, 55–66 (2008).
47. Wireman, J. W. & Dworkin, M. Developmentally induced autolysis during fruiting body formation by *Myxococcus xanthus. J. Bacteriol.* **129**, 798–802 (1977).
48. O'Connor, K. A. & Zusman, D. R. Behaviour of peripheral rods and their role in the life cycle of *Myxococcus xanthus. J. Bacteriol.* **173**, 3342–3355 (1991).
49. Fiegna, F. & Velicer, G. J. Exploitative and hierarchical antagonism in a cooperative bacterium. *PLoS Biol.* **3**, e370 (2005).
50. Velicer, G. J., Kroos, L. & Lenski, R. E. Developmental cheating in the social bacterium *Myxococcus xanthus. Nature* **404**, 598–601 (2000).
    This study shows that anti-social behaviours are common in natural populations of *M. xanthus*.
51. Kraemer, S. A. & Velicer, G. J. Endemic social diversity within natural kin groups of a cooperative bacterium. *Proc. Natl Acad. Sci. USA* **108**, 10823–10830 (2011).
52. Velicer, G. J., Kroos, L. & Lenski, R. E. Loss of social behaviors by *Myxococcus xanthus* during evolution in an unstructured habitat. *Proc. Natl Acad. Sci. USA* **95**, 12376–12380 (1998).
53. Vos, M. & Velicer, G. J. Social conflict in centimeter and global-scale populations of the bacterium *Myxococcus xanthus. Curr. Biol.* **19**, 1763–1767 (2009).
54. Pathak, D. T., Wei, X., Dei, A. & Wall, D. Molecular recognition by a polymorphic cell surface receptor governs cooperative behaviors in bacteria. *PLoS Genet.* **9**, e1003891 (2013).
55. Be'er, A. *et al.* Lethal protein produced in response to competition between sibling bacterial colonies. *Proc. Natl Acad. Sci. USA* **107**, 6258–6263 (2010).
56. Gibbs, K. A. & Greenberg, E. P. Territoriality in *Proteus*: advertisement and aggression. *Chem. Rev.* **111**, 188–194 (2011).
57. Schirrmeister, B. E., Antonelli, A. & Bagheri, H. C. The origin of multicellularity in cyanobacteria. *BMC Evol. Biol.* **11**, 45 (2011).
58. Tomitani, A., Knoll, A. H., Cavanaugh, C. M. & Ohno, T. The evolutionary diversification of cyanobacteria: molecular-phylogenetic and paleontological perspectives. *Proc. Natl Acad. Sci. USA* **103**, 5442–5447 (2006).
59. Flores, E. & Herrero, A. Compartmentalized function through cell differentiation in filamentous cyanobacteria. *Nature Rev. Microbiol.* **8**, 39–50 (2010).
60. Kumar, K., Mella-Herrera, R. A. & Golden, J. W. Cyanobacterial heterocysts. *Cold Spring Harb. Perspect. Biol.* **2**, a000315 (2010).
61. Yoon, H. S. & Golden, J. W. PatS and products of nitrogen fixation control heterocyst pattern. *J. Bacteriol.* **183**, 2605–2613 (2001).
62. Golden, J. W. & Yoon, H. S. Heterocyst development in *Anabaena. Curr. Opin. Microbiol.* **6**, 557–563 (2003).
63. Mullineaux, C. W. *et al.* Mechanism of intercellular molecular exchange in heterocyst-forming cyanobacteria. *EMBO J.* **27**, 1299–1308 (2008).
64. Rossetti, V., Schirrmeister, B. E., Bernasconi, M. V. & Bagheri, H. C. The evolutionary path to terminal differentiation and division of labor in cyanobacteria. *J. Theor. Biol.* **262**, 23–34 (2010).
65. Rodrigues, J. F. M., Rankin, D. J., Rossetti, V., Wagner, A. & Bagheri, H. C. Differences in cell division rates drive the evolution of terminal differentiation in microbes. *PLoS Comput. Biol.* **8**, e1002468 (2012).
66. Lee, D. Y. & Rhee, G. Y. Circadian rhythm in growth and death of *Anabaena flos-aquae* (cyanobacteria). *J. Phycol.* **35**, 694–699 (2002).
67. Ning, S. B., Guo, H. L., Wang, L. & Song, Y. C. Salt stress induces programmed cell death in prokaryotic organism *Anabaena. J. Appl. Microbiol.* **93**, 15–28 (2002).
68. Berman-Frank, I. The demise of the marine cyanobacterium, *Trichodesmium spp.*, via an autocatalyzed cell death pathway. *Limnol. Oceanogr.* **49**, 997–1005 (2004).
69. Flärdh, K., Richards, D. M., Hempel, A. M., Howard, M. & Buttner, M. J. Regulation of apical growth and hyphal branching in *Streptomyces. Curr. Opin. Microbiol.* **15**, 737–743 (2012).
70. Jakimowicz, D. & van Wezel, G. P. Cell division and DNA segregation in *Streptomyces*: how to build a septum in the middle of nowhere? *Mol. Microbiol.* **85**, 393–404 (2012).
71. Hopwood, D. A. *Streptomyces in nature and medicine: the antibiotic makers* (Oxford Univ. Press, 2007).
72. van Wezel, G. P. & McDowall, K. J. The regulation of the secondary metabolism of *Streptomyces*: new links and experimental advances. *Nature Prod. Rep.* **28**, 1311–1333 (2011).

73. Claessen, D., de Jong, W., Dijkhuizen, L. & Wösten, H. A. Regulation of *Streptomyces* development: reach for the sky! *Trends Microbiol.* **14**, 313–319 (2006).
74. Manteca, A., Fernandez, M. & Sanchez, J. A death round affecting a young compartmentalized mycelium precedes aerial mycelium dismantling in confluent surface cultures of *Streptomyces antibioticus. Microbiology* **151**, 3689–3697 (2005).
75. Chater, K. F. & Losick, R. in *Bacteria as multicellular organisms* (eds Shapiro, J. A. & Dworkin, M.) 149–182 (Oxford Univ. Press, 1997).
76. Colson, S. *et al.* Conserved *cis*-acting elements upstream of genes composing the chitinolytic system of streptomycetes are DasR-responsive elements. *J. Mol. Microbiol. Biotechnol.* **12**, 60–66 (2007).
77. Nazari, B. *et al.* Chitin-induced gene expression involved in secondary metabolic pathways in *Streptomyces coelicolor* A3(2) grown in soil. *Appl. Environ. Microbiol.* **79**, 707–713 (2012).
78. Rigali, S. *et al.* The sugar phosphotransferase system of *Streptomyces coelicolor* is regulated by the GntR-family regulator DasR and links *N*-acetylglucosamine metabolism to the control of development. *Mol. Microbiol.* **61**, 1237–1251 (2006).
79. Rigali, S. *et al.* Feast or famine: the global regulator DasR links nutrient stress to antibiotic production by *Streptomyces. EMBO Rep.* **9**, 670–675 (2008).
   This study provides the first evidence of a direct correlation between PCD and the onset of aerial growth and antibiotic production in *S. coelicolor.*
80. Adams, D. W. & Errington, J. Bacterial cell division: assembly, maintenance and disassembly of the Z ring. *Nature Rev. Microbiol.* **7**, 642–653 (2009).
81. Lutkenhaus, J. Assembly dynamics of the bacterial MinCDE system and spatial regulation of the Z ring. *Annu. Rev. Biochem.* **76**, 539–562 (2007).
82. Schwedock, J., Mccormick, J. R., Angert, E. R., Nodwell, J. R. & Losick, R. Assembly of the cell division protein FtsZ into ladder like structures in the aerial hyphae of *Streptomyces coelicolor. Mol. Microbiol.* **25**, 847–858 (1997).
83. Flärdh, K., Leibovitz, E., Buttner, M. J. & Chater, K. F. Generation of a non-sporulating strain of *Streptomyces coelicolor* A3(2) by the manipulation of a developmentally controlled *ftsZ* promoter. *Mol. Microbiol.* **38**, 737–749 (2000).
84. Willemse, J., Mommaas, A. M. & van Wezel, G. P. Constitutive expression of *ftsZ* overrides the *whi* developmental genes to initiate sporulation of *Streptomyces coelicolor. Antonie Van Leeuwenhoek* **101**, 619–632 (2012).
85. Willemse, J., Borst, J. W., de Waal, E., Bisseling, T. & van Wezel, G. P. Positive control of cell division: FtsZ is recruited by SsgB during sporulation of *Streptomyces. Genes Dev.* **25**, 89–99 (2011).
86. Xu, Q. *et al.* Structural and functional characterizations of SsgB, a conserved activator of developmental cell division in morphologically

complex actinomycetes. *J. Biol. Chem.* **284**, 25268–25279 (2009).
87. Girard, G. *et al.* A novel taxonomic marker that discriminates between morphologically complex actinomycetes. *Open Biol.* http://dx.doi.org/10.1098/rsob.130073 (2013).
88. Akerlund, T., Nordstrom, K. & Bernander, R. Branched *Escherichia coli* cells. *Mol. Microbiol.* **10**, 849–858 (1993).
   This work shows that single mutations can introduce branching in populations of *E. coli* cells.
89. Gullbrand, B., Akerlund, T. & Nordstrom, K. On the origin of branches in *Escherichia coli. J. Bacteriol.* **181**, 6607–6614 (1999).
90. Nilsen, T., Ghosh, A. S., Goldberg, M. B. & Young, K. D. Branching sites and morphological abnormalities behave as ectopic poles in shape-defective *Escherichia coli. Mol. Microbiol.* **52**, 1045–1054 (2004).
91. Kawamoto, S., Watanabe, H., Hesketh, A., Ensign, J. C. & Ochi, K. Expression analysis of the *ssgA* gene product, associated with sporulation and cell division in *Streptomyces griseus. Microbiology* **143**, 1077–1086 (1997).
92. van Wezel, G. P. *et al.* Unlocking *Streptomyces* spp. for use as sustainable industrial production platforms by morphological engineering. *Appl. Environ. Microbiol.* **72**, 5283–5288 (2006).
93. Fisher, R. M., Cornwallis, C. K. & West, S. A. Group formation, relatedness, and the evolution of multicellularity. *Curr. Biol.* **23**, 1120–1125 (2013).
94. Ratcliff, W. C. *et al.* Experimental evolution of an alternating uni- and multicellular life cycle in *Chlamydomonas reinhardtii. Nature Commun.* **4**, 2742 (2013).
95. Giddings, T. H. & Staehelin, L. A. Observation of microplasmodesmata in both heterocyst-forming and non-heterocyst forming filamentous cyanobacteria by freeze-fracture electron microscopy. *Arch. Microbiol.* **129**, 295–298 (1981).
96. Wilk, L. *et al.* Outer membrane continuity and septosome formation between vegetative cells in the filaments of *Anabaena* sp. PCC 7120. *Cell. Microbiol.* **13**, 1744–1754 (2011).
97. Merino-Puerto, V., Mariscal, V., Mullineaux, C. W., Herrero, A. & Flores, E. Fra proteins influencing filament integrity, diazotrophy and localization of septal protein SepJ in the heterocyst-forming cyanobacterium *Anabaena* sp. *Mol. Microbiol.* **75**, 1159–1170 (2010).
98. Kataoka, M., Seki, T. & Yoshida, T. Regulation and function of the *Streptomyces* plasmid pSN22 genes involved in pock formation and inviability. *J. Bacteriol.* **173**, 7975–7981 (1991).
99. Hopwood, D. A. & Kieser, T. in *Bacterial Conjugation* (ed. Clewell, D. B.) 293–311 (Plenum, 1993).
100. McCormick, J. R., Su, E. P., Driks, A. & Losick, R. Growth and viability of *Streptomyces coelicolor*

mutant for the cell division gene *ftsZ. Mol. Microbiol.* **14**, 243–254 (1994).
   This paper shows that *ftsZ*-null mutants of *S. coelicolor* are viable; this is the first example of a free-living bacterium that can grow without cell division.
101. Mistry, B. V., Del Sol, R., Wright, C., Findlay, K. & Dyson, P. FtsW is a dispensable cell division protein required for Z-ring stabilization during sporulation septation in *Streptomyces coelicolor. J. Bacteriol.* **190**, 5555–5566 (2008).
102. Schlimpert, S. *et al.* General protein diffusion barriers create compartments within bacterial cells. *Cell* **151**, 1270–1282 (2012).
103. Sonobe, S. *et al.* Proliferation of the hyperthermophilic archaeon *Pyrobaculum islandicum* by cell fission. *Extremophiles* **14**, 403–407 (2010).
104. Letek, M. *et al.* DivIVA is required for polar growth in the MreB-lacking rod-shaped actinomycete *Corynebacterium glutamicum. J. Bacteriol.* **190**, 3283–3292 (2008).
105. Kang, C. M., Nyayapathy, S., Lee, J. Y., Suh, J. W. & Husson, R. N. Wag31, a homologue of the cell division protein DivIVA, regulates growth, morphology and polar cell wall synthesis in mycobacteria. *Microbiology* **154**, 725–735 (2008).
106. Nguyen, L. *et al.* Antigen 84, an effector of pleiomorphism in *Mycobacterium smegmatis. J. Bacteriol.* **189**, 7896–7910 (2007).
107. Flärdh, K. Essential role of DivIVA in polar growth and morphogenesis in *Streptomyces coelicolor* A3(2). *Mol. Microbiol.* **49**, 1523–1536 (2003).
108. Edwards, D. H. & Errington, J. The *Bacillus subtilis* DivIVA protein targets to the division septum and controls the site specificity of cell division. *Mol. Microbiol.* **24**, 905–915 (1997).
109. Marston, A. L., Thomaides, H. B., Edwards, D. H., Sharpe, M. E. & Errington, J. Polar localization of the MinD protein of *Bacillus subtilis* and its role in selection of the mid-cell division site. *Genes Dev.* **12**, 3419–3430 (1998).
110. Scherr, N. & Nguyen, L. *Mycobacterium* versus *Streptomyces* — we are different, we are the same. *Curr. Opin. Microbiol.* **12**, 699–707 (2009).
111. Ghosh, J. *et al.* Sporulation in mycobacteria. *Proc. Natl Acad. Sci. USA* **106**, 10781–10786 (2009).
112. Traag, B. A. *et al.* Do mycobacteria produce endospores? *Proc. Natl Acad. Sci. USA* **107**, 878–881 (2010).