Bacterial Growth and Division

Investigating Molecular Mechanisms Governing Cell Morphology in Streptomyces venezuelae

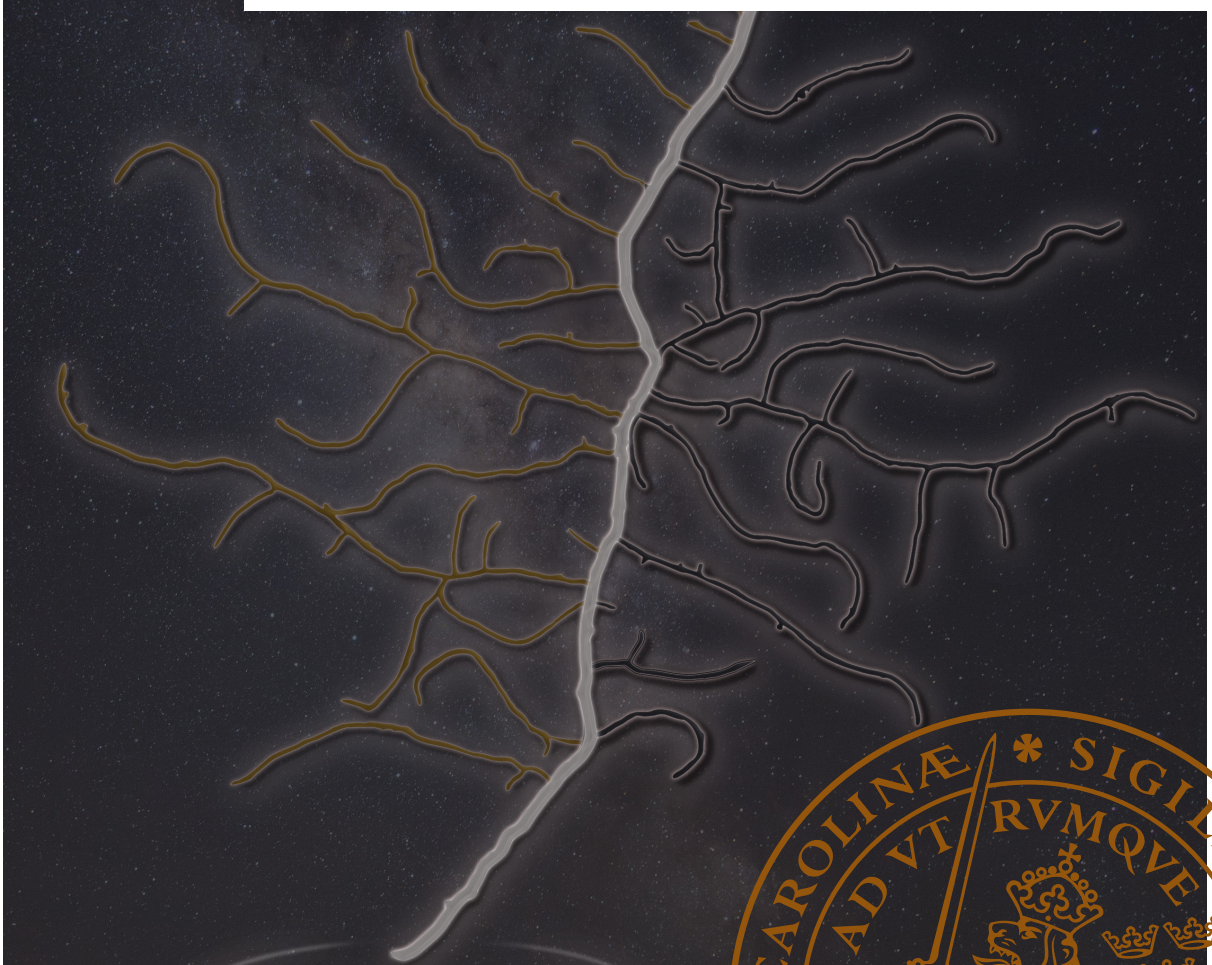
Massri, Dima

2026

Bacterial Growth and Division

Investigating Molecular Mechanisms Governing Cell Morphology in *Streptomyces venezuelae*

DIMA MASSRI
DEPARTMENT OF BIOLOGY | FACULTY OF SCIENCE | LUND UNIVERSITY



“One never notices what has been done,
one can only see what remains to be done”

Marie Curie

Bacterial Growth and Division

Investigating Molecular Mechanisms Governing Cell Morphology in *Streptomyces venezuelae*

Dima Massri

LUND
UNIVERSITY

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Science at Lund University to be publicly defended on 11th of September at 09.00 in Biologihörsalen (A213), Department of Biology, Sölvegatan 35, Lund, Sweden

Faculty opponent
Professor Marie A. Elliot
McMaster University, Department of Biology, Hamilton, ON, Canada

Organization: LUND UNIVERSITY

Document name: Doctoral dissertation **Date of issue:** 2026-08-17

Author(s): Dima Massri

Title and subtitle: Bacterial Growth and Division: Investigating Molecular Mechanisms Governing Cell Morphology in *Streptomyces venezuelae*

Abstract:

Bacteria shape their cells by coordinating the growth and division of their peptidoglycan cell wall. The filamentous *Streptomyces* species, renowned for their complex multicellular development, diverge from the rod-shaped model bacteria in their modes of growth and division, making them powerful models to study mechanisms behind morphogenesis. Instead of elongating at the sidewalls and simply dividing into two daughters, vegetatively growing streptomycetes employ two cellular processes to obtain and sculpt a vegetative mycelium of an inherently multicellular nature: a polarized mode of growth to extend hyphal tips and make branches and a unique type of cell division that does not involve cell separation. During sporulation, synchronized cell division events produce spores. Throughout this life cycle, polar growth and cell division act as an integrated morphogenetic system, enabling development and morphological plasticity. This thesis investigates the two key molecular apparatuses underlying morphogenesis: the DivIVA-based polarisome and FtsZ-based divisome, using *Streptomyces venezuelae* as a model organism. The thesis work that pertains to polar growth describes a dual-operon system implicated in multicellular shape-organizing behaviors and polar growth-related mechanisms. In this work, we perform a systematic investigation of the eight Actinobacterial G protein systems (AGPSs) of *S. venezuelae* that are encoded by the conservons, a large family of operons that are conserved within Actinomycetota and exist in multiple copies in *Streptomyces* genomes. Our analysis primarily shows that AGPSs produced by conservons 1 and 2 are implicated in an apparent hyphal self-avoidance phenotype underlying the mycelial ordered architecture. Further characterization of individual components of conservons 1 and 2 demonstrates functional redundancy across their GTPase regulatory modules, each encoded collectively by *cvnB*, *cvnC*, and *cvnD* genes, and functional divergence of their ATPase sensory components, encoded by the *cvnA* genes, suggesting that this dual-operon system likely integrates operon-specific inputs into a shared GTPase core to regulate cell shape. We further establish a connection to polar growth by showing the enrichment of proteins encoded by these conservons at the hyphal tips, and an effect of the deletion of the two operons on DivIVA's behavior and dynamics. Our work on cell division revisits the role of the ancient Gram-positive divisome member SepF and clarifies the functions of the two extra SepF paralogs, SepF2 and SepF3, that are uniquely encoded by *Streptomyces* genomes. Our work confirms the indispensability of the *bona fide* SepF for cell division and its role as a membrane anchor of the Z-ring. The study additionally uncovers non-essential yet important roles of SepF2 and SepF3 in cell division, with SepF3 being required for septum closure and the coordination between septum formation and chromosome segregation during sporulation, and both SepF2 and SepF3 having synergistic roles in facilitating cell division during vegetative growth.

Key words: *Streptomyces,* polar growth, polarisome, DivIVA, conservons, AGPS, cell division, SepF

Language: English **Number of pages**: 92

ISBN: 978-91-90202-65-4 (*print*); 978-91-90202-66-1 (*electronic*)

Bacterial Growth and Division

Investigating Molecular Mechanisms Governing Cell Morphology in *Streptomyces venezuelae*

Dima Massri

LUND
UNIVERSITY

Copyright

Cover image: *"The mycelial road to a PhD"*. Segmented microscope image of *Streptomyces* mycelium whose expanding network reflects the journey from a research question to new knowledge, through both setbacks (grey) and achievements (gold). © 2026 Dima Massri.

Published by:

Department of Biology

Faculty of Science

Lund University

Lund 2026

ISBN 978-91-90202-65-4 (print)

ISBN 978-91-90202-66-1 (electronic)

Printed in Sweden by Media-Tryck, Lund University,
Lund, 2026

Media-Tryck is a Nordic Swan Ecolabel certified provider of printed material. Read more about our environmental work at www.mediatryck.lu.se

MADE IN SWEDEN

To all children, women, and others who suffer around the world. This work does not address their experiences, but its completion was made possible by opportunities that many are denied. I dedicate my thesis to them as a reminder that knowledge alone is not enough without compassion.

Table of Contents

Popular science summary

Bacteria are the most abundant and adaptable living organisms on earth. They are everywhere, from the environment to our own bodies, where bacterial cells are often said to outnumber our own cells. We tend to think of bacteria as unicellular and free-living cells, although many are social, live as multicellular communities, exchange chemical messages, inspect their environments, and organize behavior with neighboring cells. Bacteria grow into visible colonies, like the *Streptomyces* fuzzy colonies that look like tiny molds. Yet most people have never seen bacterial cells because they are too small to be seen with the naked eye. However, under the microscope, this hidden world is full of shapes, from rods to filaments and spirals.

Bacterial cells are surrounded by a cell wall that protects and gives bacteria their shape. This cell wall is constantly rebuilt as bacteria grow and divide. Where and how new cell-wall material is added during growth and reorganized during division ultimately determines whether a bacterium becomes a sphere, a rod, or a more elaborate shape. For example, bacteria favoring rod shape must build cell wall on sidewalls to elongate their bodies. Some bacteria can grow asymmetrically and insert cell wall material at the cell poles. Cell division is equally important because if a rod continues to grow without separating into daughter cells, it becomes a long filament. This is exactly how streptomycetes make filaments: they grow at the pole to extend hyphae, and their hyphae undergo cell division without cell separation culminating in filamentous shape. They further branch from these filaments forming a continuous multicellular network of hyphae known as the vegetative mycelium. However, this phase, known as vegetative growth, represents only one stage of their life, as streptomycetes later send aerial hyphae into the air and divide them through cell division events into ovoid spores. The spores scatter and remain dormant, then germinate when conditions improve to make new vegetative mycelia, restarting the cycle. Therefore, multicellularity and the different cell shapes streptomycetes adopt across their life are the product of two processes: polar growth and cell division. I aimed through my work at exploring these two processes to understand how streptomycetes achieve and maintain their cell shape.

Cells perform functions through proteins, and manufacture proteins by decoding genes, DNA segments that serve as instruction manuals. Bacteria also organize genes in operons to produce all proteins needed for a certain function at once. We found that when nutrients are limited, streptomycetes use two operons, known as conservons 1 and 2, to achieve hyphal self-avoidance, i.e., keeping hyphae separated

to maintain the shape and organization of their mycelial network. This behavior likely points to a multicellular behavior where hyphae are talking to each other to organize their collective growth pattern. Also, proteins produced by these operons possibly form a signal transduction complex where one protein might sense and transmit an environmental signal to other proteins to initiate an effect on cell shape. This finding represents exciting evidence that streptomycetes actively and genetically coordinate multicellular growth in response to environmental signals, particularly given we still know little about how cell shape in these organisms is controlled in a multicellular context or how it changes in response to their surrounding environment. While we did not experimentally test why they do this, *Streptomyces* hyphae likely spread on the surface to take up more nutrients, so maybe they avoid each other to reduce competition over resources. We further showed that the two operons influence the function of a key polarity protein and that proteins produced by them accumulate at cell poles. This is particularly interesting because polarity is important for cellular organization across the tree of life and connecting polarity to multicellular shape-organizing behavior advances our understanding of how cells translate positional information into functions.

Bacterial cell division starts with the formation of a ring made of the protein FtsZ and the ring is anchored to the cell membrane to act like a construction scaffold, recruiting other proteins needed to split the cell while ensuring that the DNA is evenly separated into daughter cells. In our work, we confirmed the role of SepF, an ancient protein that helps anchor the FtsZ ring to the membrane. Mysteriously, *Streptomyces* has two extra related copies of SepF, SepF2 and SepF3, and their functions are not well-characterized. We found that streptomycetes need SepF2 and SepF3 to make the cross walls that make vegetative hyphae compartmentalized. This process is important for *Streptomyces* fitness, although many questions about its benefit remain unanswered. During sporulation, we observed a cell shape defect in the absence of *sepF3* where *Streptomyces* produced long spore chains instead of single ovoid spores, only to find later that SepF3 is required for the complete closure of septa and for the proper partitioning of DNA between spores.

Overall, our results reveal new players in polar growth and cell division and show that careful control of these processes is required for maintaining shape, particularly in a complex, multicellular bacterium where morphogenesis demands a higher-level coordination. But why is it important for bacteria to have and maintain a certain shape? Bacteria care about their shape and preserve it across generations because it benefits them. While not all benefits are fully understood, bacteria generally adopt shapes that help them thrive in their environments. And why their shape matters to us? It is undoubtedly fun to manipulate bacterial shape in the lab and watch bacteria beautifully transform under the microscope, but the implications for sure go far beyond aesthetics. One example is the importance of cell shape in biotechnological and medical applications. Mechanisms that control shape are promising targets for disarming pathogens. For instance, the cell wall, which defines shape, is a prime

antibiotic target because most bacteria cannot survive without it. Shape also influences pathogenicity as some bacteria rely on their shape to move and colonize tissues. If we think about streptomycetes, these organisms are fascinating in terms of their massive abilities to produce a wide range of useful compounds including antibiotics. Making such compounds is tightly linked to *Streptomyces* development and therefore understanding how they grow, organize themselves, and thrive in their environments can help unlock their capabilities. More generally, cell shape affects how microbes move, obtain resources, and persist in their environments. Therefore, understanding why different shapes have evolved answers questions about how microscopic life functions and contributes to Earth's ecosystems. Because all living things share ancient cellular ancestors, studying cell shape also provides clues about how the first cells became more complex over billions of years, ultimately giving rise to the kind of cells found in plants, animals, and humans today.

Abbreviations

ADP	Adenosine diphosphate
AGPS	Actinobacterial G protein system
ATP	Adenosine triphosphate
BGC	Biosynthetic gene cluster
cAMP	Cyclic adenosine monophosphate
c-di-GMP	Cyclic dimeric guanosine monophosphate
D-Ala	D-alanine
D-Glu	D-glutamic acid
GAP	GTPase activating protein
GDP	Guanosine diphosphate
GEF	Guanine nucleotide exchange factor
GlcNAc	N-acetylglucosamine
G protein	Guanine nucleotide-binding protein
GTP	Guanosine triphosphate
L-Ala	L-alanine
meso-DAP	meso-2,6-diaminopimelic acid
MurNAc	N-acetylmuramic acid
NIT	Nitrate- and nitrite-sensing domain
PG	Peptidoglycan
Rpfs	Resuscitation promoting factors
SSSC	*Streptomyces* spore synthesizing complex
TGase	Transglycosylase
TPase	Transpeptidase
UDP	Uridine diphosphate
UndPP	Undecaprenyl pyrophosphate

List of papers

Papers included in this thesis

I. **Massri, D**., Osgyan, R., Ekdahl, V., and Flärdh, K., Two actinobacterial G protein systems control multicellular mycelial growth and morphology in *Streptomyces venezuelae*. *Submitted.*

II. **Massri, D**., Louro, S., Radojkovic, D.P., and Flärdh, K., Actinobacterial G protein systems encoded by conservons 1 and 2 regulate cell shape through polar growth-dependent mechanisms. *Unpublished manuscript.*

III. **Massri, D**., Sen, B.C., Cantlay, S., McCormick, J.R., and Flärdh, K., Divergent roles of three SepF homologs in *Streptomyces venezuelae* include SepF3 linking cell division to nucleoid partitioning. *Submitted.*

Author contributions

I. **DM** and KF conceptualized, designed and supervised the study. **DM** performed most of the experiments, with contributions from RO and VE to strain construction and preliminary experiments. **DM** and RO analyzed data. **DM** wrote the initial draft of the paper, and KF and **DM** finalized the paper.

II. **DM** and KF conceptualized, designed and supervised the study. **DM** performed most of the experiments with contributions from SL and DPR. **DM** analyzed all data. **DM** wrote the initial draft of the paper, and KF and **DM** finalized the paper.

III. **DM**, BCS, SC, JRM and KF designed experiments and analyzed data. **DM**, BCS and SC performed experiments. **DM**, BCS and SC wrote the initial draft of the paper. **DM**, JRM and KF finalized the paper.

Preface

Bacteria shape their cells by utilizing cytoskeletal systems that instruct the peptidoglycan synthesis machinery when and where to build the cell wall. In multicellular bacteria, including the filamentous bacteria of the genus *Streptomyces*, morphogenesis comprises an additional layer of organization, as it must integrate within the collective cellular architecture and include spatial coordination between individual cells. My PhD work explores molecular mechanisms governing cell shape in streptomycetes. My thesis is structured to first discuss morphogenetic systems in bacteria broadly and then revisit them through the lens of streptomycetes, and concludes with a discussion of my results, and how the work I conducted, in collaboration with colleagues, add to our understanding of these systems.

I initially provide a discussion of bacterial cell shape and how it is generally defined by the peptidoglycan cell wall and cytoskeletal systems. To lay the groundwork for understanding cytoskeletal functions, I dedicate a significant part to describing the elongasome and the divisome systems of the rod-shaped model bacterial species. I further expand this view by discussing more complex systems, highlighting diverse morphologies and mechanisms of their emergence (Chapter I). To extend the discussion of cell shape to higher-order organization, and as this concept is central to understanding streptomycetes, I address bacterial multicellularity, describing its emergence and presenting key examples and defining characteristics (Chapter II).

Building on this foundation, I extrapolate the concepts discussed about model species to apply them to *Streptomyces* species. Streptomycetes display unique features of cell biology, including morphological complexity and a sophisticated multicellular life, and therefore the discussion begins by addressing these features (Chapter III). I proceed to discuss systems governing cell shape regulation in these species, polar growth and cell division (Chapter IV). Finally, as they pertain to paper I and II, the conservon operons in streptomycetes are also addressed (Chapter V).

Throughout the development of this thesis, GAI tools approved by Lund University were used in part to improve the clarity and phrasing of text, support brainstorming, and assist with programming tasks and suggestions for data analysis approaches. Their use was restricted to language enhancement and conceptual and technical guidance. AI tools were not used to generate scientific content, analyze datasets, or write portions of the thesis.

Chapter I. Bacterial cell shape

Structural and molecular basis of morphogenesis

Bacterial cell shape is an emergent feature of molecular machineries that organize cell wall synthesis in space and time, deciding where growth is permitted, where it is inhibited, and where cell cycle progression occurs, thereby resulting in diverse cell shapes. Below is a discussion of how morphogenesis is generally established in bacteria.

Peptidoglycan is the structural determinant of cell shape

Most bacteria are enclosed by the sacculus, a protective sac-like exoskeletal peptidoglycan (PG) layer that functions in preserving cell shape [1, 2]. The cell wall is thought to be the structural blueprint of morphology, as purified sacculi retain the shape of the bacterium [3]. The PG is composed of polysaccharide chains, known as glycan strands, of N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid residues (MurNAc) connected by β-1,4 glycosidic bonds, and further cross-linked by short peptide bridges [2, 4]. The basic structure of PG is conserved, but the chemical composition can vary across organisms, primarily in the stem peptides. Majority of cross-links in *Escherichia coli* is 4-3 linkages, and the peptide sequence is L-Ala-γ-D-Glu-meso-2,6-diaminopimelic acid (Meso DAP)-D-Ala-D-Ala [5, 6]. The sacculus is thick and multi-layered in Gram-positive bacteria and is in contact with extracellular space through covalently bound teichoic acids, whereas in Gram-negative bacteria, is thin, monolayered, and covered by an outer membrane [7].

The structural role of the cell wall arises not only from its polymeric nature, but from the biosynthetic pathways that define its chemistry and mechanical properties. PG synthesis entails a series of reactions that span various cellular compartments [2, 8] (Fig. 1). The process is initiated in the cytoplasm where the cytoplasmic precursor UDP-MurNAc-pentapeptide is synthesized, through the conversion of UDP-N-acetylglucosamine to UDP-N-acetylmuramic acid by MurA and MurB, followed by the stepwise assembly of the peptide stem through the sequential actions of four essential enzymes, MurC, D, E, and F ligases [9]. The membrane steps of PG synthesis culminate in the formation of lipid II. The cytoplasmic precursor must be first linked to undecaprenyl phosphate (UndP or C55-P), a 55-carbon membrane-bound carrier lipid, to form lipid I at the cytoplasmic face of the

membrane, mediated by the action of MraY. Another membrane-bound protein, the glycosyltransferase MurG, helps in transferring a molecule of UDP-linked GlcNAc to lipid I, resulting in its conversion to the final PG precursor lipid II (UndPP-GlcNAc-MurNAc-pentapeptide) [10, 11]. Lipid II is subsequently transported by flippases to the periplasm or the exterior of the cell, in Gram-negative or Gram-positive bacteria, respectively [12]. MurJ fulfills the role of a lipid II flippase, where its central cavity bound to lipid II can alternate between periplasm-facing and cytoplasm-facing conformations [13, 14]. Following its transportation to the other side of the membrane, lipid II serves as a substrate for sacculus synthesis. The undecaprenyl pyrophosphate (UndPP) is dephosphorylated by UndPP phosphatases to UndP, which, with the help of UndP flippases, is translocated back to the inner side of the membrane to produce a new lipid II molecule. The disaccharide-pentapeptide part is utilized by PG synthases for polymerization and association with the PG layer, requiring two common reactions: transglycosylation mediated by transglycosylase (TGase) enzymes, to form the glycan strands, and transpeptidation, mediated by the transpeptidase (TPase) enzymes, required for cross-link formation between the peptide stems [2, 8]. The two reactions are mediated by penicillin-binding proteins (PBPs) and SEDS-family proteins [15, 16]. In addition to PG polymerization, the incorporation of lipid II precursors into the existing PG requires multiple hydrolytic enzymes to break up existing bonds, allowing for the insertion of new material: the D,D-carboxypeptidases, D,D- and L,D-endopeptidases, lytic transglycosylases, and amidases, required for the cleavage of terminal D-Ala residues, amide bonds in stem peptides, bonds between MurNAc and GlcNAc, and stem peptides from the glycan strands, respectively [16].

Disruption of genes involved in PG synthesis through mutations often results in cell shape defects, demonstrating the importance of biosynthetic integrity for shape maintenance [17-19]. PG hydrolytic enzymes also play a central role in shaping cells by altering the chemical composition of PG through various mechanisms [20].

While these biosynthetic processes define the structural and mechanical framework of the PG layer, the sacculi alone lack the intrinsic information to define their own shape and shape generation requires the precise spatial and temporal control of PG synthesis to translate this framework into morphology, as will be discussed in the following sections.

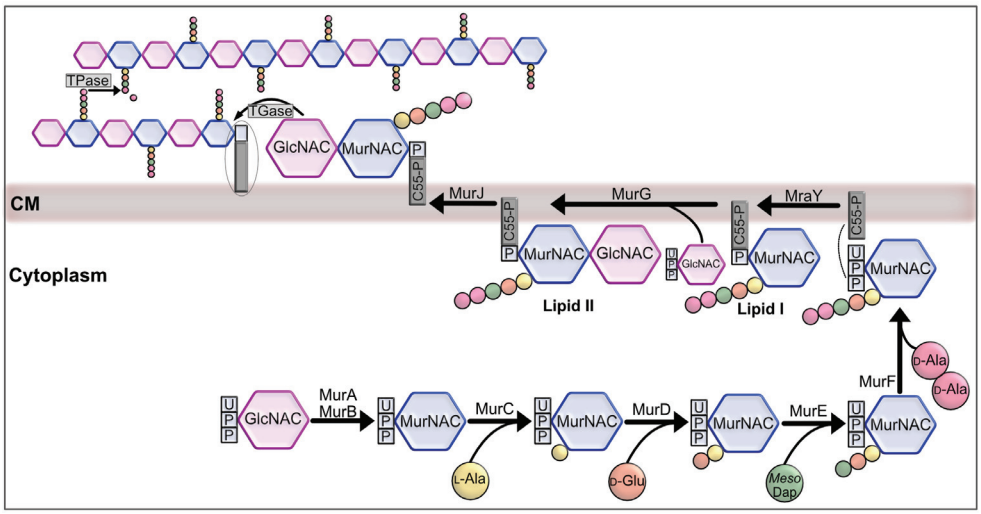


Figure 1. A simplified overview of the minimal steps of PG synthesis. Cytoplasmic PG biosynthetic steps include the conversion of UDP-N-acetylglucosamine to UDP-N-acetylmuramic acid by MurA and MurB, and the assembly of the peptide stem by MurC, MurD, MurE, and MurF, culminating in the cytoplasmic precursor UDP-MurNAc-pentapeptide. In the cytoplasmic membrane (CM), MraY and MurG mediate the formation of lipid I and lipid II, respectively. Lipid II is flipped across the membrane by MurJ and is polymerized into nascent PG by transglycosylation and transpeptidation, mediated by SEDS and PBPs.

Cytoskeletal control of peptidoglycan assembly

Eukaryotic cell organization relies on the three main cytoskeletal elements, the microtubules, the microfilaments, and the intermediate filaments, composed of tubulin, actin, and intermediate filament proteins, respectively [21]. Homologs to all three eukaryotic elements were described in prokaryotes, despite the long-held view that bacteria rely simply on their cell wall for structural maintenance [22]. The best-elucidated cytoskeletal proteins bacteria use to coordinate PG synthesis are the actin homolog MreB, the tubulin homolog FtsZ, and the IF-like protein Crescentin [23, 24]. The protein complexes known as the elongasome and divisome in rod-shaped model bacterial species, centered around MreB and FtsZ, respectively, exert significant influence over bacterial morphogenesis, with the elongasome guiding PG insertion during cell elongation, and the divisome coordinating septal PG synthesis [25, 26] (Fig. 2). The functional roles of the divisome and elongasome in cell shape determination in *E. coli* and *Bacillus subtilis* form the basis of our understanding of bacterial morphogenesis. Accordingly, the two protein complexes are discussed in more detail in the following sections.

The elongation complex

Rods of *E. coli* and *B. subtilis* elongate their bodies by inserting new PG material at the sidewalls, a process mediated by the protein complex known as the elongasome. Core components of the elongasome, referred to here using *E. coli* nomenclature, include MreB, MreC, MreD, RodA, RodZ and the penicillin-binding protein PBP2 [7, 27]. The discussion of the elongasome complex below pertains to *E. coli* and *B. subtilis*, unless otherwise specified (See also Fig. 2).

The mreBCD *operon*

MreB belongs to the actin superfamily, and despite differences in sequence, MreB tertiary structure is similar to that of its eukaryotic counterpart [28]. *mreB*-like genes are prevalent across rod-shaped bacterial species, where all rods, except for those of Actinomycetota and Hyphomicrobiales, encode at least one homolog, and a direct relationship exists between the occurrence of MreB proteins and cell morphology [26, 29]. For instance, perturbation of MreB function in *E. coli* can result in rounding of the cells [30]. MreB forms membrane-bound filamentous structures of an antiparallel organization in the elongated region of the cell [31-33]. These structures develop as short pairs of filaments, associate with the membrane at dispersed sites, and rotate along the cell longitudinal axis, with this circumferential movement being linked to PG synthesis and is independent of MreB polymerization [34-37].

Depletion of the essential membrane proteins MreC and MreD results in phenotypes similar to that of MreB depletion, namely the loss of rod morphology [38]. MreC interacts with MreB, MreD, and PBP2, and is proposed to activate the RodA-PBP2 PG synthase complex by inducing conformational changes in PBP2, whereas MreD counteracts this effect by maintaining PBP2 in an inactive state [38, 39].

RodA-PBP2 complex

The SEDS-PBP complex formed by RodA and PBP2, providing TGase and TPase activities, respectively, constitutes the essential PG synthase component in cell elongation [40, 41]. Like MreB, RodA-PBP2 rotates circumferentially [35, 36]. Cooperation between PBP2 and the bifunctional TGase/TPase PBP1a was also found important for the attachment of newly synthesized PG [42].

RodZ

The membrane protein RodZ self-interacts while associating with MreB and is proposed to function in modulating MreB polymer number and curvature preference and act as a transmembrane linker that couples MreB to the PG synthesis [43-46].

Bacterial cell division

From finding the division site to the complete segregation of DNA and the activation of downstream PG synthesis machinery, bacterial cells navigate a range of complicated tasks to produce viable offspring of correct shape and size [47, 48]. Below is a discussion of the molecular basis of cell division in model bacterial organisms (See also Fig. 2). The discussion pertains to *E. coli* and *B. subtilis*, unless otherwise specified.

The Z-ring is the key architect in bacterial cell division

The first to act in cell division is FtsZ, a broadly conserved cytoskeletal protein that drives cell division in almost all bacterial species [25, 49], some archaeal members [50], as well as chloroplasts in some eukaryotes [51]. FtsZ was first described as implicated in cell division in *E. coli* [52]. A study by Bi and Lutkenhaus in 1991 showed that FtsZ makes a ring-like structure, the Z-ring, at the division site closely beneath the inner membrane [53]. Subsequent studies recognized FtsZ as a GTPase homolog of eukaryotic tubulin and described its self-polymerization into tubulin-like filaments [54-56]. The first crystal structure of FtsZ confirmed homology to tubulin and revealed the presence of N-terminal GTP-binding domain [57]. GTP hydrolysis in FtsZ occurs when the C-terminal T7 synergy loop of one monomer inserts into the GTP-bound nucleotide-binding pocket of the adjacent monomer, forming the active site for hydrolysis [56, 58, 59].

The Z-ring is of a heterogenous discontinuous structure and consists of clusters of short overlapping FtsZ protofilaments [60-62]. Such heterogeneity might result from the inherent subunit exchange dynamics of FtsZ, i.e. the rapid exchange of GDP-bound FtsZ monomers in the ring with the cytoplasmic pool of GTP-bound FtsZ monomers [49]. Further, an apparent directional motion of polymers due to continuous polymerization occurring at the front (plus) end and depolymerization occurring at the opposite (minus) end, aka treadmilling dynamics, were also described for FtsZ filaments [25, 49]. These dynamics were suggested to contribute to PG remodeling around the cell diameter [63, 64].

The Z-ring is positioned to mid-cell by negative regulatory systems, the Min and the NO systems, which inhibit FtsZ polymerization at any site other than mid-cell. The Min proteins help localize the cell division machinery to mid-cell by forming pole-to-pole oscillations, generating a gradient that inhibits Z-ring formation at polar sites [65]. The NO, or nucleoid occlusion, system functions by inhibiting Z-ring formation over regions occupied by nucleoids and therefore is important in the coordination between cell division and chromosome segregation [66].

The Z-ring acts as a precursor for the assembly of the mature divisome, as following its formation, the remainder of the divisome is recruited in a sequential and temporal manner, with early arrivals being required for forming a focused mature Z-ring and interacting directly with FtsZ, followed by successive waves of protein recruitment,

bringing in factors as needed for their specific functions, including those required for septal PG synthesis [25, 47, 67], as will be discussed in the following sections.

Stabilizers of the Z-ring

The association of FtsZ filaments into a coherent mature Z-ring is mediated by the Zap proteins, also known as the stabilizers of the Z-ring [68]. The broadly conserved ZapA promotes bundling of FtsZ polymers [69, 70]. The remaining Zap proteins, ZapB through ZapD, also seem to have the same apparent function in bundling FtsZ filaments and promoting Z-ring maturation and stability [71-74]. The role of another Zap, ZapE, is debated as it had been initially described to destabilize FtsZ filaments [75], but a recent conflicting evidence suggested its role in filament bundling [76].

Anchors of the Z-ring

The Z-ring must be tethered to cytoplasmic membranes for the recruitment of the mature divisome, septum formation, and constriction of the membranes. Since FtsZ lacks inherent membrane binding properties, its anchoring to lipid bilayer relies on membrane-associated FtsZ-interacting partners [77]. FtsA and ZipA fulfill the roles of Z-ring anchors in *E. coli*. The actin-like filament-forming FtsA is recruited to division site in an FtsZ-dependent manner where it binds the membrane by its C-terminal amphipathic helix while interacting with FtsZ to tether it to the membrane [78-81]. ZipA is also recruited to the division site through interaction with FtsZ [82, 83]. However, Gram-positive divisomes lack ZipA [84]. *B. subtilis* has another membrane anchor, SepF [85, 86]. SepF is dispensable in *B. subtilis*, but essential for cell division in species lacking homologs of FtsA and ZipA [87, 88]. Protein interactions were demonstrated between Z-ring anchors and late divisome proteins and PG synthases, including FtsA interactions with FtsK, FtsEX, FtsQ, and FtsN as well as ZipA interaction with PBPs involved in cell division [89-93], supporting a role of membrane anchors in recruiting the late members of the divisome.

Recruitment of late divisome proteins and PG synthesis

Proper septation requires clearing DNA from septum formation site, using DNA translocases, like FtsK in *E. coli* and SpoIIE and SftA in *B. subtilis* [94, 95]. FtsK is considered bifunctional, coordinating cell division and chromosome segregation via functionally distinct domains [96]. The N terminus has four transmembrane helices, while the C-terminal domain is responsible for translocating DNA through ATP hydrolysis and facilitating disassembly of chromosome dimers in coordination with the XerCD-dif resolvase system [94].

Completion of cell division also requires the recruitment of septal PG synthesis machinery, occurring mainly under FtsEX, FtsQLB, FtsWI, and FtsN [67]. These divisome members form a network of interactions that regulates both septal PG synthesis and hydrolysis. The transmembrane ATP-binding cassette-like complex FtsEX is recruited via interactions with FtsZ and FtsA and helps in PG hydrolysis by recruiting EnvC to division sites [90, 97, 98]. EnvC, in turn, activates hydrolytic

amidases responsible for cleaving PG, promoting daughter cell separation [99]. In parallel, septal PG synthesis is mediated by the core enzymatic complex FtsWI, formed through the interaction of the transglycosylase FtsW with the transpeptidase FtsI (PBP3) [100]. The activity of this complex is tightly controlled. The FtsQLB complex, composed of the membrane proteins FtsQ, FtsL, and FtsB, is required for FtsWI activation, with interactions between FtsL and FtsI playing a central role [101]. FtsQLB also exerts an inhibitory role, suppressing the transpeptidase activity of PBP3 and the glycosyltransferase activity of another PBP, PBP1b, preventing premature PG synthesis [102]. This inhibition is relieved upon the arrival of the integral membrane protein FtsN, the last protein recruited to the division site, and which signals divisome maturation. FtsN triggers PG synthesis by interacting with PBP1b and modulating FtsQLB to activate PBP3 [103-105].

Systems supporting chromosome partitioning and condensation

In addition to the role of FtsK in the separation of daughter chromosomes, the tight coupling of cell division with chromosome segregation and condensation involves additional systems. In *B. subtilis*, but not in *E. coli*, the ParAB-*parS* system, consisting of the ATPase ParA (Soj), the DNA-binding protein ParB (Spo0J), and conserved *parS* sites clustered near *oriC*, coordinate chromosome segregation. Mechanistically, ParB, a CTP-dependent sliding clamp, binds *parS* via its helix-turn-helix motif and spreads along DNA, whereas ATP-bound ParA associates non-specifically with chromosomal DNA to form a gradient that guides the movement of ParB-*parS* complexes, ensuring accurate chromosome segregation prior to cell division [106, 107]. ParB also recruits the *B. subtilis* SMC protein, the condesin, onto the chromosome to help in DNA entanglement [108].

Structural Maintenance of Chromosome, or SMC proteins, represent an important group of proteins that feature globular N- and C-terminal head domains, including the Walker A motif for ATP binding and the Walker B motif for ATP hydrolysis, respectively, and are implicated in partitioning and condensing chromosomes across all domains of life [109, 110]. *E. coli* MukB, encoded by an operon with MukE and MukF, was the first SMC-like bacterial protein to be described [111, 112]. MukBEF organizes hundreds of kilobase-sized chromosomal loops in the nucleoid with the help of MatP, which binds specifically to *matS* sites in the terminus (Ter) region, displacing MukBEF from Ter to establish an SMC gradient along the chromosome, while also anchoring Ter to the divisome by connecting to ZapA and ZapB, thus coordinating chromosome partitioning with cell division [113-116].

Systems involved in DNA segregation also involve the type II topoisomerases DNA gyrase and topoisomerase IV, which transiently introduce double strand breaks to pass one DNA duplex through another before resealing the DNA. Composed of GyrA/GyrB and ParC/ParE, respectively, these enzymes play complementary roles during DNA replication and segregation: gyrase relieves positive supercoiling ahead

of the replication fork, while topoisomerase IV decatenates intertwined daughter chromosomes to ensure their proper segregation following replication [117, 118].

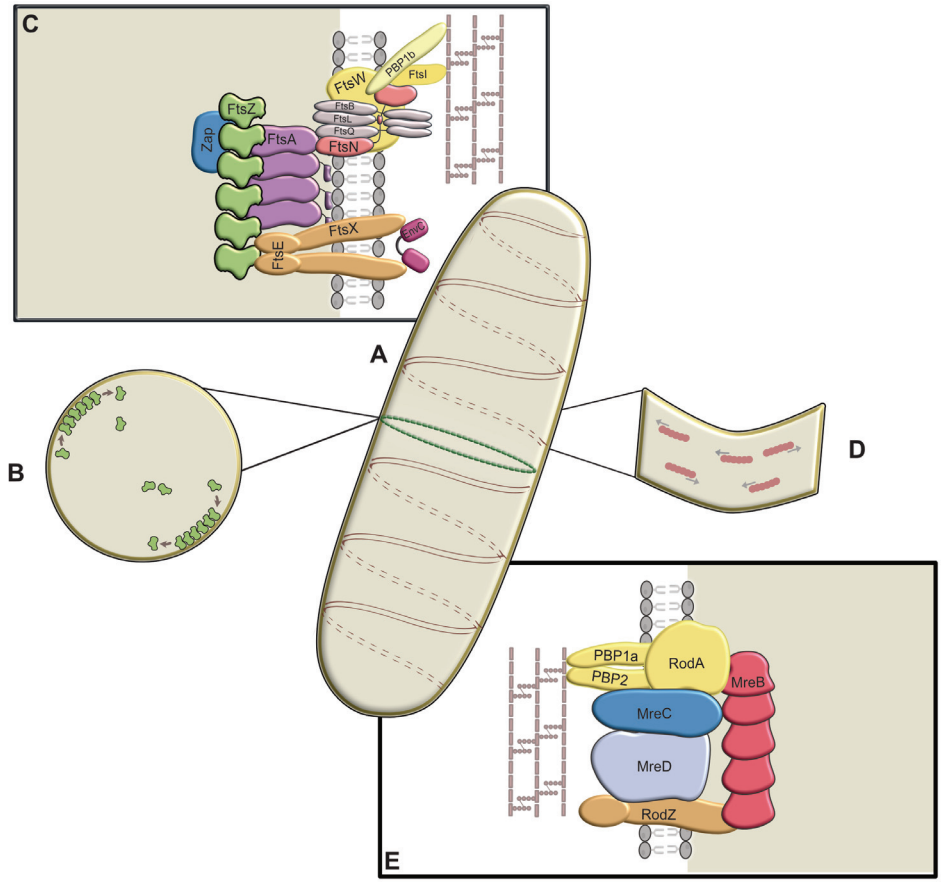


Figure 2. Illustration of cell shape generation in rod-shaped model bacterial species. (A) Rod-shaped *E. coli* and *B. subtilis* achieve their shape through FtsZ-mediated cell division (shown in green) and MreB-based elongation (shown in red). **(B)** A cross-section of the rod cell showing FtsZ oligomers linked to the membrane, with subunit exchange dynamics, where GDP-bound FtsZ monomers in the oligomers are replaced with GTP-bound FtsZ monomers from the cytoplasm, and treadmilling dynamics, where continuous polymerization occurs at the plus end and depolymerization at the minus end. **(C)** An illustration of an active divisome protein complex. **(D)** A section of the inner face of the rod cell showing MreB making short filaments and engaging with the membrane negative curvature. **(E)** An illustration of an active elongasome protein complex.

Manifestations of bacterial morphogenesis: from simple to complex

Bacteria adopt a wide range of shapes beyond the extensively studied rod form, yet the complexity and importance of cell morphology are often underestimated. Far from being simply a descriptive feature, cell shape is evolutionarily selected and may reflect adaptation to specific functional and environmental factors [119-121]. This section outlines general principles of bacterial morphogenesis through selected examples and the mechanisms underlying their generation, highlighting only key determinants associated with each morphology (See also Fig. 3).

Bacterial key cell body geometries

An imperfect sphere

The shape of spherical cocci, such as micrococci and staphylococci, is thought to be the simplest morphology, and the generation of such shapes is proposed to require only septal PG synthesis machinery. Coccoid cells of *Staphylococcus aureus* lack MreB and rely only on cell division for growth and morphogenesis [122, 123]. However, staphylococcal cells are not perfect spheres, as *S. aureus* demonstrates the capacity for some elongation, mediated by the action of the SEDS-PBP pair, RodA-PBP3 and a DivIVA-paralog, GpsB, contributing to the mild elongation of the cell wall near the septum [124, 125].

A slightly ovoid sphere

Ovococcoid, as in the case of lactococci and streptococci, is a morphology that requires peripheral PG synthesis complementing the septal apparatus [122, 123]. Despite lacking MreB, *Streptococcus pneumoniae* retains members of the elongasome, and it is suggested that both septal and peripheral PG synthesis occur at the division site, requiring the coordinated actions of FtsZ, components of the elongasome complex, and distinct types of penicillin-binding proteins [123].

The polarly and laterally growing rods

Rod shape in the extensively studied rods *E. coli* and *B. subtilis* results from the interplay between septal and peripheral PG synthesis guided by FtsZ and MreB, respectively, as discussed in previous sections. However, rod shape can occur without MreB, as in the case of the actinomycetotal genus *Mycobacterium* or the alphaproteobacterium *Agrobacterium tumefaciens* [126]. In these cases, cell elongation is mediated by polar growth, where PG synthesis is directed to poles by other morphogenetic determinants, such as the Growth Pole Ring (GPR) protein and the DivIVA-like protein Wag31 in *A. tumefaciens* and mycobacteria, respectively [127, 128]. Polar growth will be addressed in detail in chapter IV.

A curved rod

Caulobacter crescentus with its arcuate half-moon morphology represents one of the best models to study curved cell shape [129]. MreB is needed for preserving the rod shape in these species [130]. However, the crescent shape is mediated by the IF-like protein Crescentin, whose absence can result in straight rods [131]. The Crescentin induces a bending in the rod by exerting a compressive force on the cell wall, reducing the peptide bond strain and limiting glycan strand insertion, thereby creating a gradient of PG synthesis with higher activity on the side opposite to Crescentin [132]. Curved shapes can also occur independently of the Crescentin, as witnessed in *Vibrio cholerae*, which relies on CrvA, another morphogenetic determinant that assembles in the periplasm at the inner curvature of the cell and induces the asymmetric PG insertion required for curved shape [133].

Helices and helically coiled spirals

Helical morphologies appear to occur mostly in Gram-negative bacteria, likely due to the biophysical properties of their thinner cell wall, allowing for deviations from rod form through processes of localized modifications or asymmetric PG insertion [119]. For instance, maintenance of helical shape in *Helicobacter pylori* is the result of MreB promoting default rod growth, another protein CcmA driving extra PG insertion along the convex side of the cell, and hydrolases that further modify PG, helping establish and maintain the helical curvature [134]. Spirochetes, on the other hand, have evolved different strategies to acquire their shape. The periplasmic rotating endoflagella is thought to be implicated in deforming PG and imparting the flat-wave shape in some spirochetes [135]. For instance, in *Borrelia burgdorferi*, mutants lacking endoflagella are non-motile and have a straight cell body [136].

Towards more complexity

Cell-wall enclosed appendages

Moving towards more complex architectures, some bacteria exhibit prosthecae, cell envelope extensions whose generation requires processing of PG and cell envelope [137]. For example, *C. crescentus* has a polar prostheca, aka stalk, with an adhesive holdfast at its tip. The biogenesis of the prosthecae through zonal PG remodeling is localized at the pole by SpmX, which confines the cytoskeletal proteins BacA and BacB to act as a scaffold for biogenesis, and is recruited to the pole by PopZ [138]. Other bacteria display surface appendages, the flagella and pili, projecting filamentous structures on the outside of the cell. However, a discussion of surface appendages is outside the scope of this thesis (For reviews see, [139, 140]).

Developmental and post-divisional asymmetry

Here again, *C. crescentus* stands out as an example, now highlighting the complex dynamics of post-divisional asymmetry. Cell division in *C. crescentus* produces two non-identical daughter cells: a motile flagellated swarmer cell and a sessile stalked cell that attaches to surfaces via the stalk. The swarmer cell swims using its flagellum until it adheres to a surface through pili, after which it forms a holdfast to secure attachment. It then undergoes a morphological transition into a stalked division-competent cell [141]. Another example is the asymmetric cell division seen in mycobacteria. Polarly growing mycobacteria exhibit asymmetric growth and cell division with elongation occurring at unequal amounts at the poles and cell division resulting in daughter cells of unequal sizes, with daughters inheriting the old pole being larger than daughters inheriting the new pole [142].

Morphological plasticity and differentiation

Several bacterial species can alter their shape in response to environmental stressors or during predation [143]. For example, the uropathogenic *E. coli* (UPEC) exhibits different morphotypes at different growth phases during infection. UPEC forms communities of rods upon invasion, which later transition into slowly growing coccoids. A filamentation in a subset of these coccoids follows, with the latter believed to help in coping with host immune responses. At later stages, coccoids become bacillary and motile, leading to host cell lysis and the initiation of a new round of infection [144].

Other bacterial species actively sculpt their shapes as they progress in their life cycles. *B. subtilis* reproduces as a simple rod during vegetative growth. However, during the transition to sporulation, several morphological strategies are employed, including an asymmetric cell division resulting in a forespore engulfed by the mother cell. The forespore becomes protected by a coat and a PG cortex. Following spore maturation, the mother cell is lysed, and the highly resistant spore is released into the environment [145]. Another example is the filamentous streptomycetes, whose life begins with ovoid spores. Spore germination results in germ tubes that grow by tip extension and branching into a vegetative mycelium. Hyphae within the mycelium elongate by polar growth and undergo cell division without separation, resulting in a filamentous morphology. At a later stage, aerial hyphae, a second type of filamentous structure, emerge and subsequently differentiate into spores [146].

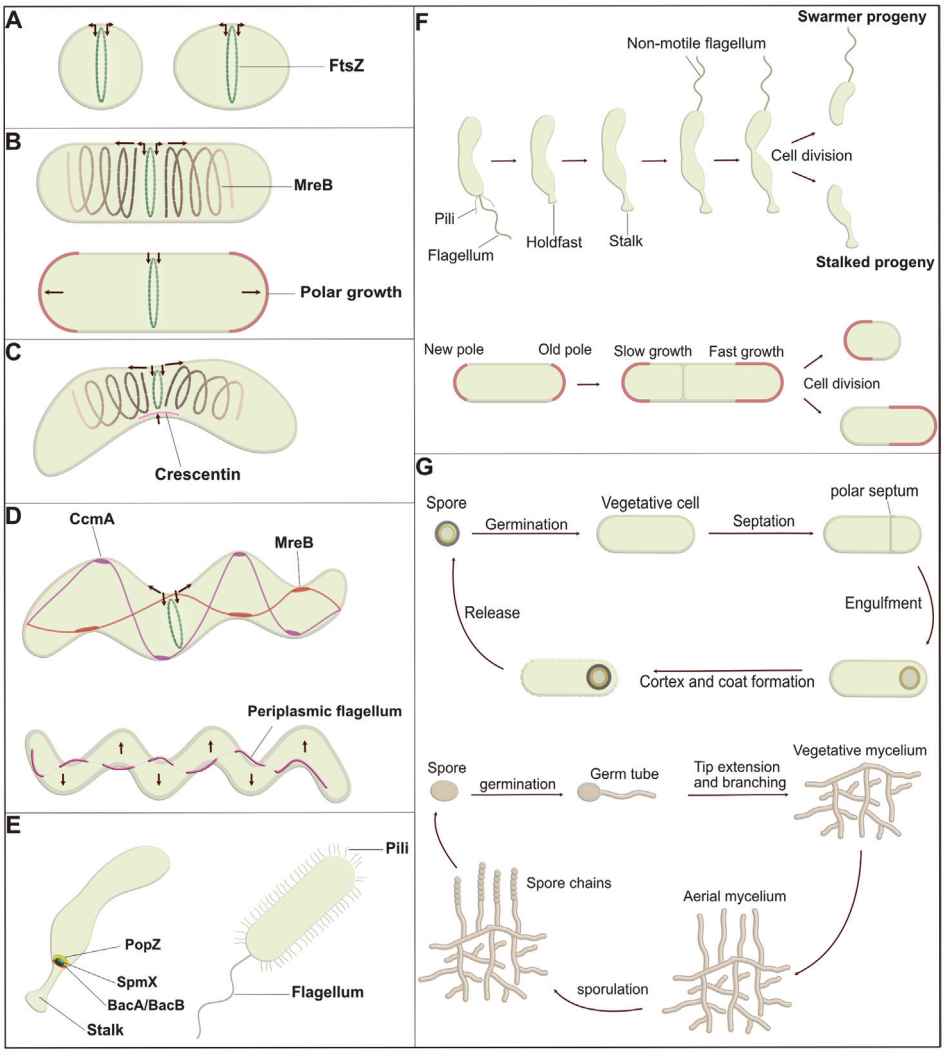


Figure 3. A simplified illustration depicting the generation of various bacterial cell shapes. (A) Coccoids (left) and ovococcoids (Right) rely primarily on FtsZ for morphogenesis. **(B)** Rod-shaped bacteria as in *E. coli* and *B. subtilis* additionally requires the MreB elongasome complex (Top). Rod shape can alternatively emerge by polar growth (Bottom). **(C)** Crescent shape as in *C. crescentus* additionally requires a compressive force on cell wall and uneven PG synthesis mediated by the Crescentin. **(D)** Helical shape exemplified by *H. pylori* is mediated by PG modifying enzymes (not shown) and both MreB and CcmA driving PG synthesis at the concave and convex parts, respectively (Top). The rotating endoflagellum, as in *B. burgdorferi*, can deform the cell wall and generate the flat-wave spiral shape (Bottom). **(E)** Bacteria with pili and flagella, as in *P. aeruginosa* (Right), and prosthecae, like the stalk in *C. crescentus*, positioned at the pole by SpmX, recruited by PopZ and subsequently recruits BacA and BacB for stalk biogenesis (Left). **(F)** *C. crescentus* life cycle illustrated as an example of generating asymmetric progeny (Top). Asymmetric growth and cell division in mycobacteria (Bottom). **(G)** Life cycles of *B. subtilis* (Top) and *Streptomyces* spp. (Bottom) as examples of bacteria that show morphological plasticity throughout their life cycle.

Shaped for success

Bacteria sculpt their shapes carefully for a reason: their morphologies provide selective advantages. While there is no direct evidence of an advantage for each cell shape, the biological relevance of morphology is supported by several arguments, which are well-articulated in the reviews by Young [120, 121]. Bacteria within a genus exhibit a limited range of morphologies despite the variety of possible shapes they can adopt, suggesting that shapes are adaptive and confer advantages. Further, morphological plasticity displayed by some bacterial species during development, pathogenesis, and in response to environmental cues, demonstrates the importance of controlling shape. Finally, and despite coccoids having the simplest bacterial morphology, evidence suggests that first bacteria were rods, and coccoids were derived from rods that had lost their ability to elongate. Such evolutionary progression implies that there are selective forces at work [120, 121].

Numerous studies have investigated or touched upon the biological significance of bacterial morphology. While it would be exhaustive to cover the literature illustrating this, a limited selection of examples can include: the *C. crescentus* curved body facilitating adherence to proximal surfaces under flow [147], the effect of cell shape on nutrient acquisition, as exemplified by enhanced uptake of phosphate by extruding a stalk [148], an effect of cell shape on motility as evidenced by enhanced motility of helically shaped cells through viscous solutions [149], enhanced surface attachment through utilizing morphological features, such as pili [141], and contribution to more successful pathogenesis as demonstrated by UPEC where bacillary cells are selectively targeted over filamentous cells [144].

.

Chapter II. From single cells to multicellular communal structures

A discussion of complex bacterial shapes inevitably brings bacterial multicellularity into focus. In fact, some of the complex morphologies mentioned before are forms of multicellular organization.

Multicellularity in bacteria

Life on earth commenced and remained unicellular for approximately 3 billion years before the appearance of multicellular organisms [150]. Historically, bacteria since their discovery had been considered exclusively unicellular until the 1980s when James A. Shapiro disputed this by arguing that bacteria are capable of cell-cell communication, multicellular organization, and performing specialized functions [151, 152]. Multicellularity in bacteria has evolved independently multiple times, often in response to physiochemical and ecological pressures, and usually confers selective advantages to the organisms [153, 154]. While enhanced survival, more efficient feeding, and resistance to stress and predation are well-known benefits of being multicellular [154, 155], multicellularity can also impose significant costs, such as competition over space and resources [156]. Therefore, success of the two organizational states, multicellularity versus unicellularity, generally depends on the ecological context and selective pressure, and a unicellular state of bacteria should not be seen as inferior or only as an ancestral phase on the way to multicellularity, but rather as a highly successful strategy.

While bacteria can lead multicellular lives, they do so without crossing the threshold into obligate multicellularity, i.e., multicellular bacteria undergo a unicellular phase at some stage in their life cycle. The only known example of obligate multicellular bacteria is a group of unique marine bacteria referred to as the multicellular magnetotactic prokaryotes [154, 157].

Forms of multicellular organization in bacteria

Multicellularity in bacteria can arise through clonal or aggregative processes [153, 154] (Fig. 4). Clonal multicellularity ensues when cells develop by division from a single predecessor, as exemplified by the multicellular filamentous cyanobacterial and actinomycetotal species. In filament-forming cyanobacteria, multicellularity is manifested by the nature of these filaments, consisting of hundreds of connected cells surrounded by a continuous outer membrane, with each cell being enclosed by PG [158]. The filamentous *Streptomyces* species are multicellular during vegetative growth where cross-wall formation creates multi-nucleoid compartments within vegetative hyphae [159]. Unlike filamentous bacteria, where multicellularity is established by physically connected cells, other bacteria can become multicellular through dynamic behaviors like aggregation, as for example during biofilm formation, which is classically initiated by surface-attachment, but requires cellular aggregation for maturation [160, 161]. Fruiting bodies formed by aggregation of *Myxococcus xanthus* swarms also belong to this category [162].

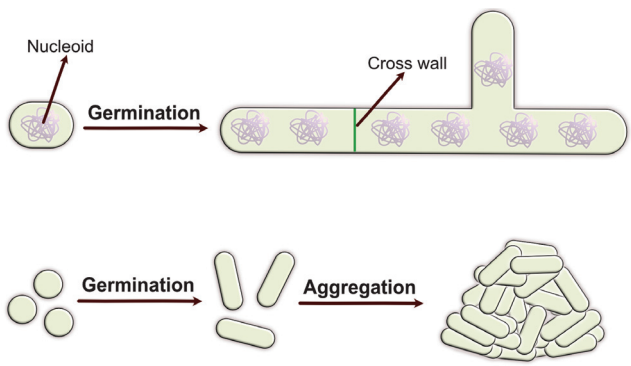


Figure 4. Two roads to bacterial multicellularity. Multicellularity can emerge through clonal or aggregative models as exemplified by *Streptomyces* vegetative hyphae (Top) and the aggregation of *M. xanthus* cells for fruiting body formation (Bottom).

However, limiting the discussion of multicellularity to mechanisms of formation overlooks other fascinating multicellular patterns. Using *M. xanthus* once more as a an example while looking beyond the formation of fruiting bodies, this organism is of social nature, and multicellular social behaviors are manifested in the different phases of its life cycle [163]. Gliding of *M. xanthus* on surfaces is achieved by two independent genetically encoded mechanisms: Adventurous motility and social motility, responsible for movement of individual cells and the swarming movement of cells as a group, respectively [164]. Driven by type IV pili retraction operating on self-produced exopolysaccharides, social motility requires cell-cell proximity and is necessary for cooperative predation and fruiting body formation. Therefore,

M. xanthus exhibit multicellular behaviors during motility, feeding, and survival [163]. Swarming motility in *Proteus mirabilis* also represents a compelling demonstration of multicellular behaviors. The surface-induced differentiation of *P. mirabilis* vegetative swimmer cells results in elongated hyperflagellated swarmers that move in groups across surfaces, and such coordinated movement occurs in cycles of rapid swarming interspaced with periods of cessation of movement, referred to as consolidation [165].

Key features of bacterial multicellularity

Multicellularity in bacteria has key biological features that can be interpreted both as its outcomes and as the underlying drivers of its occurrence. The following section covers some of these features in the light of the previously mentioned examples of multicellular bacteria.

Heterogeneity, differentiation, and specialization, often culminating in division of labor

Multicellular bacteria undergo differentiation and specialization, where monoclonal cells adopt specialized roles. In filaments of *Anabaena*, a well-studied genus of filament-forming cyanobacteria, part of the CO_2-fixing vegetative cells, under conditions of altered nitrogen-carbon balance, differentiate into heterocysts that are capable of fixing atmospheric nitrogen. Heterocysts and photosynthetically active vegetative cells engage in division of labor where heterocysts transfer N_2 fixation products to vegetative cells and the latter transfer reduced carbon compounds to heterocysts [166]. *Streptomyces* multicellular life, on the other hand, involves a reproductive division of labor, where differentiation of vegetative hyphae to spore-bearing aerial hyphae is coupled with lysis of vegetative mycelium, likely to provide nutrients to the developing spores [167, 168] (Fig. 5). The multicellular fruiting bodies of *M. xanthus* comprise three subpopulations of specialized cells that show an altruistic behavior: peripheral rods surrounding and providing structure to the fruiting body, myxospores that are resistant to environmental stressors, and cells that undergo lysis, perhaps to serve as nutritional sources [162] (Fig. 5).

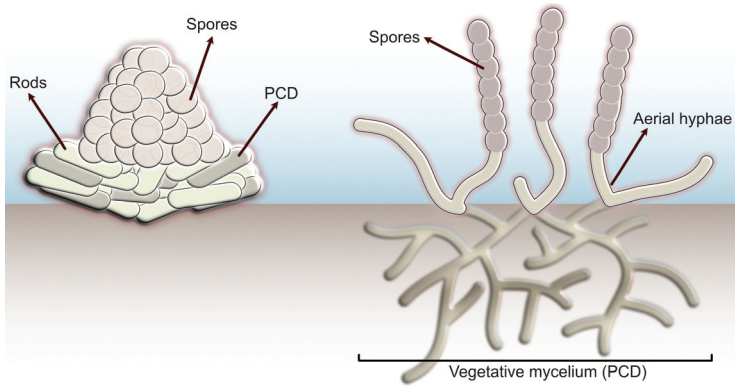


Figure 5. Differentiation and division of labor as features of multicellular life in bacteria. *M. xanthus* fruiting bodies comprise three subpopulations: spores, rods, and cells that undergo programmed cell death (PCD) (Left). *Streptomyces* development involve differentiation into spore-bearing aerial hyphae where vegetative hyphae undergo PCD (Right).

Intercellular communication

Intercellular communication is not limited to multicellular bacteria as single-celled bacteria can also communicate, for example by quorum sensing [169]. However, it remains a feature of multicellular life where such communication is needed for organization and coordination as a community [154]. Intercellular communication in multicellular bacteria can be for example mediated by physical interactions. An example of that is the proteinaceous septal junctions that traverse the PG layer through nanopores connecting adjacent cells in *Anabaena* filaments and modulating molecular diffusion [158]. *M. xanthus* cells also use physical interactions in the form of outer membrane vesicles and tubes and undergo a social process known as outer membrane exchange, where they transiently fuse their outer membranes to facilitate the exchange of proteins and lipids [170].

Intercellular communication can also manifest as kin-biased interactions during which social bacteria recognize and assist genetically similar partners, to maintain viable cooperation within the population and prevent social cheating [171, 172]. In *M. xanthus*, the kin recognition TraAB system has been described where TraA is a polymorphic surface receptor that allows cells to recognize kins based on specific recognition tag. This system is coupled to the outer membrane exchange process described earlier. A proposed profit of this coupling is to repair damaged cells, where cells functionally rescue one another and sustain a homogeneous population of fit individuals [162]. *P. mirabilis* swarmers were also described as being capable of territorial behavior and to possess a self/non-self-recognition system, mediated by the Ids and Idr proteins [173, 174]. Mutations in *idsABCDEF* operon were shown to disrupt the recognition of wild-type parent [174], indicating that such self-recognition systems exhibit high precision.

Chapter III. *Streptomyces* species: overview and cell biology

Streptomyces: the largest genus in the phylum Actinomycetota

Actinomycetota is one of the largest and most diverse bacterial phyla, encompassing Gram-positive species characterized by their high genomic GC content. Members of this phylum are mainly free-living soil bacteria, with some occupying aquatic habitats, and the phylum also includes clinically important pathogens such as *Mycobacterium tuberculosis* [175, 176]. The name of the phylum derives from the type genus *Actinomyces*, from the Greek for "ray fungus", reflecting the filamentous morphology and branching growth pattern that resemble fungal mycelium and are common among many members of the phylum. However, the term captures only a part of their biology, as Actinomycetota have remarkable morphological diversity, encompassing a wide range of shapes including rods (e.g., *Corynebacterium* spp. and *Mycobacterium* spp.), and coccoids (e.g., *Micrococcus* spp.), as well as fragmented hyphal structures (e.g., *Nocardia* spp.), and branched filamentous forms (e.g., *Streptomyces* spp.) [175, 176]. The genus *Streptomyces* is the largest and among the most well-studied genera within Actinomycetota. Streptomycetes are mostly soil-dwelling bacterial species [177]. In addition to free-living, *Streptomyces* symbionts of fungi, plants, and animals have been described [178], and the genus also includes few plant and human pathogens [179, 180].

Streptomycetes have attracted scientific attention for decades, owing to their remarkable biology and ecological importance. Their sophisticated extracellular biology, which underpins their ability to exploit and modify their surrounding environment, is one area of research. For example, as soil-dwelling saprophytes, they thrive on complex organic matter by secreting a diverse collection of hydrolytic enzymes that degrade polymers such as cellulose and chitin, and thereby driving nutrient cycling in soil ecosystems [181]. Streptomycetes are also renowned for their unusual capacity to produce specialized metabolites, many of which have revolutionized modern medicine through their use as antibiotics. In their natural habitat, these compounds, together with a diverse repertoire of enzymes, serve as

chemical arms and signaling molecules that shape interactions within the highly competitive soil environment [182].

Equally remarkable is their multicellular development and complex filamentous shape [146]. Their intricate morphology was so striking that streptomycetes were historically mistaken for fungi but also made these organisms invaluable models for studying the molecular basis of bacterial development and morphogenesis, the primary focus of this thesis. Several *Streptomyces* models have driven major advances in this field. For instance, *Streptomyces avermitilis* and *Streptomyces griseus* have contributed important insights on *Streptomyces* development during early molecular studies. However, our current knowledge of molecular mechanisms governing *Streptomyces* development and morphology has been shaped largely through the study of the two models, *Streptomyces coelicolor* and *Streptomyces venezuelae*. *S. coelicolor* has been widely used due to its production of pigmented antibiotics, providing genetic markers for studying development and metabolism, whereas *S. venezuelae* offered advantages such as rapid growth and sporulation in liquid culture, which, with advances in time-lapse microscopy techniques, enabled direct visualization of the full life cycle and revealed spatiotemporal regulation of developmental processes [183].

Overall, the unique cell biological features of streptomycetes have established them as models for investigating questions in bacterial multicellularity, morphogenesis and development, and in research areas beyond developmental biology including microbial ecology, biotechnology, and medicine. Some of these aspects, along with additional aspects of *Streptomyces* cell biology will be explored further throughout the following sections.

The exceptional biology of streptomycetes

Atypical genomic features

Streptomyces genomes, featuring unusual GC-rich composition with over 70% of nucleotides being guanine or cytosine, have sizes that range from approximately 6 to 12 Mb, making them among the largest bacterial genomes [184]. By comparing the smallest *Streptomyces* genomes, such as that of *Streptomyces albus* (6,8 Mb, encoding 5,832 genes) [185] to for instance the genome of the simple free-living eukaryote *Schizosaccharomyces pombe* (13.8 Mb, encoding 4,824 genes) [186], it becomes evident that *Streptomyces* genomes are unusually large with respect to size and number of coding sequences. A closer look at *Streptomyces* genomes further reveals a heavy investment in regulation. For example, in the 8.7 Mb *S. coelicolor* genome, containing 7,825 genes, 965 genes encode regulatory functions, with a pronounced preference for specific regulatory groups, as exemplified by the 65

sigma factors encoded by the genome and the abundance of two-component regulatory systems, including 85 sensor kinases and 79 response regulators, with 53 sensor-regulator pairs [187].

Atypical for prokaryotes, *Streptomyces* genomes are also linear [188]. This linear structure features terminal inverted repeats and telomers at both ends that can form complex secondary structures, as well as terminal proteins covalently linked to their 5' DNA termini, essential for chromosome replication [189, 190]. *Streptomyces* chromosomes consist of a central core flanked by terminal arms, with coding density remaining relatively consistent across the chromosome. The core region houses the centrally placed origin of replication and house-keeping genes, essential for primary cellular functions, such as DNA replication, cell division, and central metabolism. The chromosome arms usually contain less conserved and species-specific genes that mainly encode conditionally adaptive functions and secondary metabolism pathways [191, 192]. Notably, the core region shows significant synteny with the entire circular genomes of other actinomycetes, such as the 4.4-Mb *M. tuberculosis* and the 2.5-Mb *C. diphtheriae* genomes, suggesting a shared evolutionary origin of fundamental cellular processes [187]. The chromosomal arms are highly prone to genomic rearrangements leading to genomic instability, another intriguing feature of *Streptomyces* genomes. Genomic instability in streptomycetes has been attributed to the linear structure with terminal repeats, promoting frequent DNA double-strand breaks, recombination, and large-scale deletions or rearrangements, particularly in chromosomal arms [192].

Prolific secondary-metabolic capabilities

Streptomycetes demonstrate powerful secondary metabolism, owing to the rich repertoire of biosynthetic gene clusters (BGCs) in their genomes [193]. BGCs, comprising co-located genes, encode the enzymes necessary for the synthesis, transport, and regulation of specialized metabolites [194]. Since the discovery of streptomycin from *S. griseus* in 1943 [195], streptomycetes have been broadly acknowledged for their contribution to antibiotic production. Beyond antibiotics, compounds with biological activities (e.g., antifungals, immunosuppressants, immunostimulants, antivirals, and anticancer agents) and agricultural applications (e.g., insecticides and pesticides) have been derived from streptomycetes [193].

Streptomycetes remain a subject of interest due to the large potential for the discovery of new compounds. Advancement in genome mining tools and in-depth examination of sequenced genomes showed that *Streptomyces* species harbor 8 to 83 BGCs per genome [196]. Most of these BGCs remain inactive or cryptic under laboratory growth conditions, which makes further investigation of the required signals and ecological context to trigger their activation necessary for the discovery of new compounds [194].

Morphological and developmental complexity

Streptomyces life cycle

Streptomyces undergo a complex life cycle of different developmental stages (Fig. 3G and Fig 10). Spore germination gives rise to an initial filamentous structure, the germ tube, which grows by tip extension and branching, resulting in a network of vegetative hyphae, known as the vegetative mycelium. In response to certain signals, morphological differentiation to aerial hyphae ensues, and sporulation septa form along the aerial hyphae partitioning them into spore compartments. Following maturation, spores are released into the environment, where they survive a dormant state until germination is triggered. Dormant spores serve to preserve genetic information during adverse conditions and facilitate dispersal to new environments [146]. The life cycle of *Streptomyces* and the molecular mechanisms underlying each stage are explored in the following sections.

Molecular basis of *Streptomyces* development

Germination

Macromolecules required for initiating germination are not produced *de novo* during this process, but rather synthesized prior to dormancy [197]. Within dormant spores, a reservoir of stable mRNAs, a translational machinery, functional ribosomes, and hydrolytic enzymes are stored [198, 199]. Accumulation of trehalose, produced via glycogen conversion during spore maturation, helps in preserving macromolecules during the dehydrated spore state [197, 200]. Exact environmental cues that trigger germination remains to date unidentified. However, at the molecular level, the structural cell wall protein NepA has been proposed to contribute to maintaining dormancy under unfavorable conditions and may influence the likelihood of exiting dormancy [201]. When germination starts, spores undergo uncoating, releasing calcium accumulated in their coats, and rehydration causes spore swelling, restoring enzymatic activity, and reactivating ribosomes to translate proteins from the mRNA stock. PG hydrolases are important during germination, where these lysozyme-like enzymes facilitate changes in spore morphology, breaking down and restructuring PG, and allowing the penetration of nutrients [197]. Rpfs, or resuscitation promoting factors, a subgroup of lysozyme-like transglycosylases are among enzymes that serve this function [202-204]. Efficient germination also requires cAMP (cyclic adenosine monophosphate), and cAMP-receptor protein Crp, which is important for the regulation of a subset of Rpfs [205-207].

Germ tube to vegetative mycelium

The formation of vegetative mycelium requires the DivIVA-based polarisome, an apically localized multiprotein complex that mediates polarized cell wall synthesis to enable hyphal extension at the tip [208, 209]. The polarisome also marks sites of new hyphal branches. The formation of new branches requires polarisome foci along

the lateral walls of hyphae, emerging through a mechanism of polarisome splitting where a daughter polarisome is left behind after being broken off from an existing tip-localized polarisome, grows until achieving a critical size allowing the original tip sufficient time to extend a considerable distance from the branching site [210-212] (Fig. 6). Polar growth will be further discussed in Chapter IV.

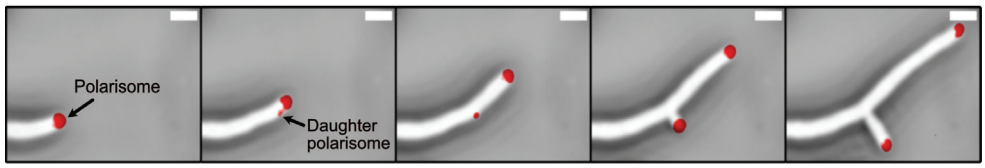


Figure 6. New branches emerge through polarisome splitting. Montage of time-series of merged phase-contrast and fluorescence micrographs taken at 10-minute intervals of *S. venezuelae* expressing DivIVA-mCherry. Scale bars, 2 μm.

Vegetative mycelium to sporulating aerial hyphae

The differentiation into sporulating aerial hyphae is under the regulation of complex developmental regulatory cascades that have been investigated thoroughly for over half a century (For reviews, see ([146, 213, 214]). The isolation of *S. coelicolor* "bald" and "white" mutants that lost the ability to undergo aerial growth and sporulation, respectively, marked the biggening of our understanding of this process, as tracing these mutations to their genomic locations identified the two primary classes of developmental regulators: the Bld (bald) and Whi (white) regulators [215, 216]. Early microscopic studies also showed that aerial hyphae and spores are coated by hydrophobic sheaths [217, 218]. Chaplins [219], rodlins [220], and SapB [221, 222] have been implicated in forming these hydrophobic structures. The surfactant lantibiotic-like peptide SapB is required for aerial differentiation on rich media, whereas the process can proceed in the absence of SapB on minimal media [221-223]. Chaplins and rodlins, encoded by *chp* (*chpA-chpH*) and *rdl* (*rdlA* and *rdlB*) genes, respectively, constitute the major components of the hydrophobic sheath, with chaplins forming the densely packed fibrillar layer responsible for the hydrophobic characteristics of the surface layer, and rodlins organizing chaplin fibrils into a more complex rodlet structure [219, 220, 224-227]. The hydrophobic surface is thought to help aerial mycelia overcome surface tension and emerge as erected structures in the air [146].

Subsequent research revealed the multi-tiered network underlying development and ensuring that transition occurs with precise timing (Fig. 7). The *chp* and *rdl* genes are under the control of sigma factor BldN, and the activity of the latter is controlled by anti-sigma factor RsbN and repressed by the transcription factor BldD [228-230]. *bldM*, encoding an orphan response regulator, is another target of σ^{BldN}-mediated activation, and BldD-mediated repression [229, 231, 232]. The transcription factor

BldC, which itself is repressed by BldD, also activates *bldM* [229, 233]. BldM undergoes self-dimerization and can form a heterodimer with WhiI [234]. The atypical response regulator encoded by *whiI* is activated by the motility-like sigma factor WhiG, and repressed by BldC [233, 235, 236]. The BldM homodimer and the BldM-WhiI heterodimer activate distinct sets of target genes. Initially, the homodimer is formed and activates genes such as *whiB* and *ssgR*, crucial for the differentiation of aerial hyphae into spores. Subsequently, BldM-WhiI heterodimer is made, activating genes like the *smeA-sffA* operon, encoding the small membrane protein SmeA that is responsible for recruiting the putative DNA translocation protein SffA to sporulation septa, and *whiE*, the locus responsible for the spore pigment [234, 237, 238]. The transcription factor WhiG, regulated by anti-sigma factor RsiG and repressed by BldD, has another target, *whiH*, whose activation depends on BldC and functions in late sporulation [230, 233, 239, 240].

WhiB, the founding member of the "Wbl" actinomycetotal family of transcription factors, is repressed by both BldO and BldD, and to some extent by BldC, and pairs with the DNA-binding protein WhiA [229, 233, 241-245]. The WhiA-WhiB complex activates divisome genes required for sporulation septation (e.g. *ftsZ*, *ftsW*, and *ftsK*) and appears to repress the polarisome gene, *filP* [242, 246].

An intriguing aspect of development in streptomycetes is their ability to exploit the absence of TTA codons in essential genes and employ a tRNA as a developmental regulator, namely BldA, the only cognate tRNA for leucine codons [214]. BldA and the transcription factor AdpA, also designated BldH, both repressed by BldD, form a positive feedback loop where BldH activates *bldA* transcription and BldA is required for *bldH* expression [229, 247]. AdpA is a global transcriptional regulator that controls secondary metabolism and a wide range of sporulation genes [248, 249].

BldD, having more than 170 targets including itself, and exerting control over developmental regulation by directly or via cascade mechanism influencing most regulators, as previously stated, as well as key developmental genes including *ftsZ* and genes encoding regulators of FtsZ, such as *ssgA*, *ssgB* and the *smeA-sffA* operon, has been recognized as the master regulator of *Streptomyces* development [214, 229]. BldD is highly conserved in sporulating actinomycetes and all sequenced *bldD* orthologs appear to have c-di-GMP (cyclic di-GMP; 3, 5-cyclic dimeric guanosine monophosphate) binding sites [250, 251]. c-di-GMP acts as a molecular brake on development by associating with BldD as a co-repressor: when its levels are high, differentiation is blocked, but as they drop, BldD repression is lifted, triggering the activation of the downstream cascade [214, 251, 252].

BldC, belonging to MerR family of regulators and a nucleoid-associated protein, is also a significant repressor positioned downstream of BldD in the cascade [229, 253]. While it functions principally as a repressor, the study by Bush *et al*. [233] found that BldC plays a dual role as both a repressor and an activator, turning on

key genes such as *bldM* and *whiH*, while simultaneously shutting down others, including its own gene, *smeA*, *ssgB*, *hupS* encoding a nucleoid-associated protein, cell division genes encoding the dynamin-like proteins DynAB proteins, and *sigF* encoding a sporulation-specific sigma factor [233].

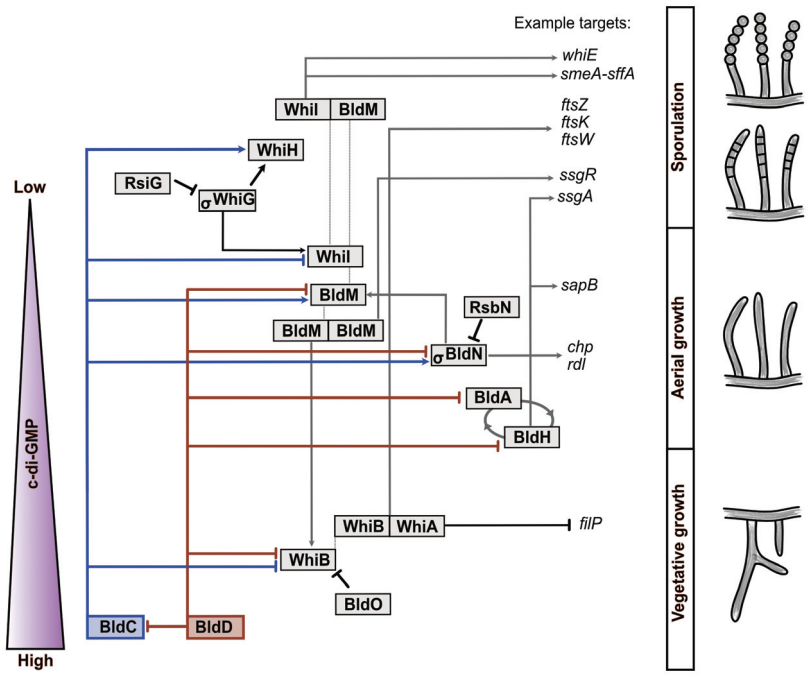


Figure 7. Regulatory network controlling *Streptomyces* development. BldD and BldC are master regulators controlling other regulatory genes that activate or repress target genes, allowing for developmental progression. Although BldD and BldC also directly regulate some of these targets, these interactions are not shown for simplicity. Repression is indicated by blunt arrows (—|) and activation is shown as standard arrows (→).

As a result of these regulatory events and the subsequent activation of genes involved in the cell division machinery, aerial hyphae undergo synchronous cell division events, leading to the formation of a ladder of regularly spaced septa. This synchronized septation, coupled with timely chromosome segregation, function in partitioning the hyphae into chains of pre-spore compartments, each inheriting a copy of the chromosome [254, 255]. Cell division and chromosome segregation will be further discussed in Chapter IV.

From septation to mature spores

Maturation of pre-spore compartments results in dormant spores, characterized by their condensed DNA, buildup of trehalose, deposition of spore-specific pigment, as well as cell wall remodeling and thickening, leading to enhanced stress-resistance [197, 254, 256]. Deposition of the spore pigment of a polyketide nature in mature spores is attributed at the molecular level to the *whiE* gene cluster in *S. coelicolor* [238] and similar clusters in other *Streptomyces* spp. [257, 258].

While polar growth in Actinomycetota is independent of Mre proteins, and members of the phylum generally lack the MreB-based elongasome apparatus involved in the lateral growth in model rod-shaped bacteria, *Streptomyces* genomes harbor rod-shape-determining genes that are dispensable for vegetative growth but function particularly in the formation of the thick spore cell wall [259, 260]. Proteins encoded by these genes form the *Streptomyces* spore wall synthesizing complex (SSSC) [260](Fig. 8). The SSSC primarily comprises MreB, MreC, MreD, RodZ, PBP2, and the putative transglycosylase Sfr, all of which exhibit interaction patterns similar to those of their counterparts in rod-shaped bacteria. The complex also includes the glycopolymer transferase PdtA and the kinases PkaI and PkaH, which phosphorylate MreC, with phosphorylation likely serving as a regulatory signal that coordinates sporulation septation with the synthesis of the spore envelope, ensuring proper spore development [261]. Mutations in SSSC components cause defects ranging from increased sensitivity to stress, irregularly sized spores, and defective spore envelopes to aberrant spore chains containing non-viable or anucleate spores, highlighting the central role of the SSSC complex in spore differentiation and morphogenesis [260, 262-265].

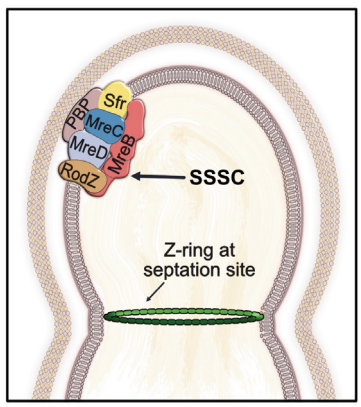


Figure 8. The *Streptomyces* spore wall synthesizing complex (SSSC). The SSSC directs the construction of a thickened spore envelope and primarily comprises MreB, MreC, MreD, RodZ, PBP2, and the putative transglycosylase Sfr. It also includes the glycopolymer transferase PdtA and the kinases PkaI and PkaH (not shown).

Deviation from canonical life cycle: S-cells and exploratory growth

Streptomycetes can adopt different growth patterns, deviating from their classical life cycle, mainly in response to environmental conditions or during interactions with other organisms [213]. For example, streptomycetes can exist as L-form-like S-cells in response to exposure to hyperosmotic conditions [266]. L-forms are wall-deficient cells that proliferate through biophysical membrane dynamics rather than canonical FtsZ-dependent division machinery, and arise by exposing walled cells to cell wall stressors, but become capable of indefinite propagation upon acquiring adaptive mutations [267]. *Streptomyces* S-cells, on the other hand, can be extruded from hyphal tips, and are transient wall-deficient cells that can revert to mycelial growth, albeit with developmental defects [266].

Streptomycetes were further described to undergo exploratory growth, a newly discovered growth pattern that was identified in *S. venezuelae* but expected to be widespread in other streptomycetes. [268, 269]. The characterization of this growth mode stemmed from co-culturing experiments with *Saccharomyces cerevisiae*. *Streptomyces* explorer cells were described to form colonies of wrinkled surface and to rapidly propagate as non-branching, vegetative-like hyphae, with leading edge progressing at rates exceeding classical hyphal extension by approximately ten folds. This growth pattern is glucose-repressible and the requirement for co-culturing with fungi to induce this behavior can be bypassed by depriving the growth medium of glucose [268]. Glycerol was found unique among carbon sources in its ability to noticeably accelerate exploratory growth. This triggered the speculation that *S. venezuelae* may perceive glycerol as an ecological cue of a nearby yeast activity, given that yeasts such as *S. cerevisiae* secrete glycerol when glucose is present [270, 271]. *S. venezuelae* explorers reshape their local environment to gain a competitive advantage. They release trimethylamine, increasing local pH and reducing iron bioavailability. To ensure access to this limited resource, they employ two siderophores with distinct roles: desferrioxamine to primarily capture iron within the colony, and the more diffusible foroxymithine to sequester iron beyond the colony border, resulting in iron-depleted zones, which together with the production of antimicrobial compounds suppress the growth of neighboring competitors [268, 270, 272]. Exploratory growth appears distinct from canonical *Streptomyces* development as supported by transcriptomic evidence of extensive reprogramming of gene expression during exploration, with most prominent changes seen in metabolic pathways, particularly respiration and oxidative stress, rather than in classical developmental regulators [271]. Notably, cell envelope integrity is important for exploratory growth, as mutating the stress-responsive sigma factor *sigE* markedly compromised exploration [269]. However, the notion that exploration is a deviation from canonical development was revised upon the discovery that exploration and sporulation can occur concurrently under specific nutrient conditions such as growth on a medium that supports sporulation but in the presence of glycerol. This dual mode of growth uniquely requires the regulators

BldD and RsiG, retains the requirement for iron sequestration and cell envelope integrity, but can progress without the characteristic alkalinization of the growth medium [269]. More recent work showed that MreB, classically associated with lateral growth in rod-shaped bacteria and linked only to spore wall maturation in streptomycetes as described earlier, contributes to maintaining cell wall integrity during exploration, and that MreB and DivIVA-based polar growth are needed for the rapid exploration phenotype, providing first evidence that MreB- and DivIVA-dependent growth mechanisms can function simultaneously [273].

Aspects of multicellularity in *Streptomyces*

Multicellularity is a central organizational aspect of *Streptomyces* existence and must be considered to understand the biology of these species (for a review, see [274]). The multigenomic vegetative mycelia, that are not transient aggregates but rather stable continuous multicellular bodies in which cells remain physically attached, represent the stage that defines true multicellularity in streptomycetes. While cross walls partition vegetative hyphae into multinucleoid compartments, the exact nature of these walls and the broader role of such compartmentalization in multicellular organization is poorly understood. Interestingly, putative channels traversing these cross walls were described, and although their function remains elusive, they may facilitate molecule exchange between adjacent compartments, potentially representing a mechanism of intercellular communication within the mycelium [159, 275].

However, multicellularity in streptomycetes is not limited to a single developmental stage but rather is a feature of the entire life cycle. Also, manifestations of bacterial multicellularity discussed in Chapter II are also reflected in *Streptomyces* life, which involves differentiation, functional specialization, and division of labor. Together, these characteristics represent interlinked aspects that enable these organisms to behave and thrive as integrated individuals.

Heterogeneity across life cycle

Heterogeneity is an inherent feature of multicellularity, and this feature is seen in streptomycetes across all stages of life cycle. Heterogeneity across the life cycle is described comprehensively in the review by Norte *et al*. [274]. From the earliest stages, spores undergo stochastic germination resulting in variable germ tubes. The developmental program is further characterized by heterogeneity and asynchronous developmental transitions, with processes such as antibiotic synthesis and aerial differentiation occurring in distinct spatial and temporal waves across the colony. Such heterogeneity also culminates in variable sporulation [274].

Self-organization at the macro- and microcolony levels

Streptomyces vegetative hyphae extend outward from the colony center to form the vegetative mycelial network by polar tip extension and regulated branching, which in turn defines the hyphal shape [146]. However, the vegetative mycelium is continuous and local growth behaviors occurring at the level of individual hyphae might influence the higher-level organization, i.e., mycelium-level morphology. At the microscopic scale, growth of vegetative hyphae can result in uniform spreading on the surface of the substrate [276]. Also, our recent work, as described later in Paper I, showed that vegetative growth can result in highly ordered microcolonies, with hyphae showing pronounced self-avoidance and remaining spatially separated rather than growing in contact, a behavior that results in an organized mycelial architecture (see Paper I and Fig. 9). Such self-organization requires that individual hyphae grow in a coordinated manner to produce the well-organized microcolony, which raises the possibility of intercellular communication between hyphae to coordinate collective growth. While mechanisms behind this organization remain unresolved, such observations invite speculative interpretations, such as hyphae recognizing self and actively redirecting tip growth in response to meeting other hyphae by employing kin-recognition systems or other signaling mechanisms. Notably, this spatial organization is mostly evident upon nutrient limitation (see [276] and Paper I), suggesting a role of nutrient availability in this spatial patterning.

At the macroscopic level, *streptomyces* colony architecture mirrors the bacterium's developmental life cycle, with vegetative hyphae making the basal layer, followed by the formation of aerial mycelium at the colony surface [277]. This vertical stratification reflects the well-characterized developmental program. Beyond this top-to-bottom organization, *Streptomyces* colonies can display zonation and spatial heterogeneity across the radial axis, i.e., from the colony center to the periphery [278]. Such heterogeneity in *S. coelicolor* colonies was explained by the sequential and spatially separated activation of antibiotic biosynthesis and developmental gene expression [279]. We also show in Paper I that *S. venezuelae* macrocolonies can exhibit zonation patterns, manifested as spatially separated regions comprising a dense central core, a surrounding zone of reduced density, and an outermost colony fringe (See Paper I and Fig. 9).

Our recent work showed that perturbations in regulatory systems encoded by the conservon operons, known as the Actinobacterial G protein systems (AGPSs), can result in defects associated with these multicellular behaviors, where mutations in *S. venezuelae* conservon 1 disrupted the macrocolony-level self-organization by abolishing zonation and leaving only the dense core, and mutations in conservons 1 and 2 affected the microscopic mycelial organization, altering the self-avoidance phenotype and resulting in hyphal aggregation and less-ordered microcolonies (See Paper I). As proteins encoded by the conservons collectively form putative signal transduction complexes, this suggests that self-organization in *Streptomyces* is an

actively regulated multicellular process rather than a simple emergent consequence of growth alone.

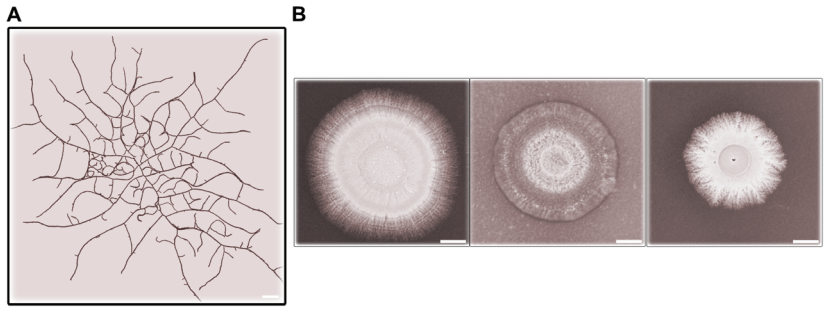


Figure 9. S. Self-organization at the macro- and microcolony levels. (A) Segmented phase-contrast micrographs showing *S. venezuelae* microcolonies displaying ordered mycelial architecture and hyphal self-avoidance. Scale bars, 20 μm. **(B)** Concentric-ring pattern of *S. venezuelae* colonies grown on MYM agar (Left), chitin agar (Middle), and MOPS minimal medium (Right). Images were taken at 7-9 days of incubation. Scale bars, 0.5 cm.

Multicellularity as an adaptive strategy

Features of multicellularity are best understood in terms of selective advantages they provide. The multicellular vegetative mycelium may enhance nutrient foraging and acquisition. The large network arising as a result of periodic branching can help in weaving through soil and penetrating insoluble resources, while the interconnected cytoplasm may enhance nutrient transport compared to extracellular diffusion [274]. Forms of division of labor has also been described in streptomycetes. Reproductive division of labor, as discussed in Chapter II, is demonstrated by the altruistic programmed cell death of vegetative hyphae to provide resources for aerial growth. Additionally, two manifestations of metabolic division of labor were described: the first one is when a fraction of colonies acquire chromosomal deletions that severely reduce their fitness but lead to hyperproduction of antibiotics, and the second is through the aforementioned spatiotemporal patterning of gene expression within colonies [167, 279, 280].

Multicellular features in streptomycetes might also be shaped by ecological context. Soil is characterized by heterogeneity in composition, patchily distributed resources, and intense competition. Patchy resource availability across the mycelium can drive nonuniform precursor availability and interactions, promoting heterogeneity and division of labor [167, 274, 278].

Chapter IV. *Streptomyces* growth and division

Growth and division in streptomycetes are not simply processes that facilitate proliferation and survival. As discussed throughout the previous sections, these processes collectively function as architects of cell shape, underlie *Streptomyces* multicellular nature, and facilitate morphological plasticity, allowing the transition between different cellular forms. Polar growth drives tip extension and branching to generate the filamentous morphology of vegetative hyphae, while cell division partitions the hyphae without cell separation, preserving an interconnected mycelial structure. During the reproductive phase, polar growth extends aerial hyphae, whereas cell division helps in the transition to the next morphological state by subdividing these filaments into compartments that mature into ovoid spores. The molecular basis of *Streptomyces* polar growth and cell division is explored in the following sections, and morphogenesis facilitated by these processes at the different developmental stages of *Streptomyces* life cycle is summarized in Figure 10.

Polar growth

Polar growth is prevalent within Actinomycetota, including non-mycelia-forming rods such as mycobacteria and corynebacteria [126, 281, 282]. In streptomycetes, polar growth is mediated by the action of the polarisome, the core members of which include three coiled-coil proteins with similar domain organization, DivIVA, Scy, FilP, and the recently discovered histidine pseudo kinase PsmA [281, 283](Fig. 10).

DivIVA

DivIVA is essential for *Streptomyces* viability and represents the key element in the polarisome [208]. Widespread among Gram-positive bacteria, DivIVA proteins are membrane-associated, have affinity to negative membrane curvatures, and function primarily as polarity determinants and topological regulators of diverse cellular processes [284, 285]. The structure of DivIVA from *B. subtilis* features two coiled-coil domains. The N-terminal domain adopts a coiled-coil dimer configuration and

is capped by two intertwined loops that expose positively charged and hydrophobic residues for membrane association, whereas the C-terminal domain forms a curved tetramer consisting of two coiled-coils and serves as a platform for protein-protein interactions [286, 287]. *Streptomyces* DivIVA is predicted to contain a highly conserved N-terminal region and another conserved segment near the C-terminus with a high tendency to form a coiled-coil structure [208]. Further, the N-terminal domain of *S. coelicolor* DivIVA is thought to facilitate membrane binding through electrostatic interactions with membrane lipids, with membrane association and orientation depending on lipid composition [288].

B. subtilis DivIVA accumulates at polar regions and division sites [289-291], and engages in many processes, such as limiting the action of MinCD to septal and polar regions during cell division [292, 293], and anchoring chromosomal origins to the pole to help in polar division during sporulation [294]. DivIVA in corynebacteria and mycobacteria, beside its role in mediating polar growth, as will be discussed below, is also recruited to division sites where it fulfills other roles, such as, a polar tether of the origin during chromosome segregation mediated by its interaction with ParB in *Corynebacterium glutamicum* and ParA in *Mycobacterium smegmatis* [295, 296]. Further, DivIVA's activity is often regulated by phosphorylation [297-299].

The role of DivIVA in polar growth is conserved within Actinomycetota. In the rod-shaped mycobacteria and corynebacteria, the effect of modulating DivIVA levels on cell shape, the localization of DivIVA to the poles, its ability to dictate sites of PG growth and interaction with enzymes involved in PG synthesis, as well as other aspects that makes DivIVA a morphogenetic determinant and the moderator of polar growth, have been described [128, 300-303]. In *Streptomyces* spp., DivIVA appears to dictate the PG synthesis machinery to hyphal tips and mark branching sites, as evident by its localization to the aforementioned sites [208, 211, 212]. Manipulation of DivIVA levels in *S. coelicolor* causes polar growth defects, including irregular curly hyphae and branching patterns upon partial depletion, and excessive branching and strongly perturbed cell shape upon overexpression [208]. Regulation of DivIVA through phosphorylation was also described in streptomycetes. In *S. coelicolor*, the process is mediated by AfsK, a tip-localizing Ser/Thr protein kinase. Elevated levels of AfsK can lead to disassembly of the polarisome [304]. SppA, a phosphoprotein phosphatase, was found to counteract AfsK phosphorylation and to significantly influence hyphal morphology and polar growth [305].

Scy

Scy is conserved among filamentous Actinomycetota and represents one of the longest bacterial coiled-coil proteins [306, 307]. As a member of the *Streptomyces* polarisome, Scy binds DivIVA and FilP and co-localizes with DivIVA [306]. Unlike *divIVA*, *scy* is not essential, and its deletion results in a pleiotropic effect on polar growth, including hyperbranching, with branching unusually occurring in aerial

hyphae, irregular hyphal width, tip splitting pattern, and dispersed patchy DivIVA foci. These findings from the study of Holmes *et al.* [306] suggested that Scy has an important role in polarisome assembly, specifically in regulating the number of polarity centers along the hyphae [306].

FilP

FilP was identified based on similarity to intermediate filament-like proteins and exhibits a rod domain of segmented coiled coils. FilP is encoded by a gene that lies immediately downstream of *scy* and was initially *characterized* as necessary for growth and morphology in *S. coelicolor* [246]. FilP assembles into filamentous structures *in vitro* and a deletion mutant of its non-essential gene caused slower growth and made hyphae less stiff, highlighting a role of FilP in maintaining the mechanical strength or rigidity of the hyphal wall [246, 308]. The deletion of *filP* in *S. venezuelae* was associated with morphological defects including more curved and irregularly shaped hyphae [309]. FilP forms polar gradients at hyphal tips with the highest intensity near the tip, and despite evidence of interaction between the two proteins, FilP does not co-localize with DivIVA but rather sits subapically, just below DivIVA, allowing for interactions at the boundaries [308, 309]. Further investigation of FilP and its interplay with the polarisome proteins revealed several key features. FilP's gradient-like assemblies are dynamic and exhibit a rapid subunit exchange between polar and soluble pools. Notably, in the hyperbranching *scy* mutant, FilP loses its characteristic gradient pattern, indicating that its localization depends on Scy. FilP was further suggested to influence the amount and distribution of DivIVA at hyphal tips [309].

PsmA

The histidine pseudo kinase, PsmA, is widely-conserved across streptomycetes and has recently been linked to polar growth [283]. PsmA interacts and co-localizes with DivIVA, and a deletion mutant of its corresponding gene disrupts cell shape and DivIVA behavior, producing widened irregular hyphae with notably rounded tips, as well as hyperbranching and splitting of DivIVA polar foci [283].

Cell division

Streptomycetes undergo two types of cell division, vegetative cross-wall formation and sporulation-specific septation, each corresponds to a developmental stage in their life cycle. Despite structural and functional differences, both division modes rely on the common apparatus centered around FtsZ [159, 275]. Remarkably, *ftsZ* and cell division altogether are dispensable for *Streptomyces* viability, but *ftsz* is essential for cell division and its deletion in *S. coelicolor* or *S. venezuelae* can fully abolish cross walls and sporulation septa [310-312]. The molecular basis of each cell division type is discussed in the following sections (See also Fig. 10).

Vegetative cell division

As noted previously, vegetative hyphae undergo cell division in which cross walls form without constriction or separation, partitioning the hyphae into multigenomic compartments [159, 275]. The exact biological purpose of this type of cell division remains elusive, as hyphae can still grow in the absence of cross walls in the *ftsZ* deletion mutant [311, 312]. Vegetative cell division is also not well-characterized at the molecular level. Apart from FtsZ, only two other proteins were described to be involved, namely SepX and SepF, which are also implicated in sporulation septation. Individual deletion mutants of *sepX* and *sepF* both resulted in vegetative hyphae devoid of cross-walls (see [313] and Paper III). Bush *et al*. [313] in their study highlighted a role of hyphal partitioning in maintaining fitness and hyphal integrity, as the absence of cross walls in the *sepX* mutant increased the incidence of cell lysis. Results from our work also implicated the two additional SepF homologs encoded by streptomycetes, SepF2 and SepF3, in this process, where deletion mutants of *sepF2* and *sepF3* were shown to affect fitness and the frequency of cross-wall formation as described in Paper III.

Sporulation-specific cell division

The formation of the thick double-layered septa during sporulation share common features with cell division in other bacteria, in terms of recruitment of divisome proteins to the Z-ring, segregation and condensation of the chromosomes, as well as cell constriction and separation, ultimately culminating in the detachment of the unigenomic spores and their subsequent dispersal [159]. *Streptomyces* divisomes retain orthologs of the key cell division components of *E. coli* and *B. subtilis*, such as FtsQ, FtsL, FtsB, FtsW, FtsI, FtsEX, and FtsK [159]. Akin to FtsZ, these proteins are not essential for viability but may also be dispensable for cell division as mutants lacking their corresponding genes are mostly impaired during sporulation to varying degrees yet continue forming some hyphal cross walls and sporulation septa [314-318]. On the other hand, homologs for nearly all Z-ring regulators and anchors, such

as MinC, MinD, Noc, FtsA, and ZipA are absent in streptomycetes [159].Therefore, and while our understanding of cell division in *Streptomyces* is still developing, the execution and regulation of cell division in these organisms combine mechanisms that are partly shared with other bacteria and others unique to streptomycetes. Some of the unique regulators in *Streptomyces* cell division are addressed below.

Positive regulation of FtsZ polymerization: SsgA, SsgB, SepG, and SepH

SsgA and SsgB are members of the SALPs (SsgA-Like Proteins) family of proteins that are unique to sporulating actinomycetes [319-321]. The impaired sporulation of *ssgA* mutants and the non-sporulating phenotype of *ssgB* mutants have led to the proposal that SsgA and SsgB are implicated in cell division during sporulation [319-322]. SsgA and SsgB were further suggested to contribute to Z-ring positioning and FtsZ polymerization, with a working model proposing that SsgA recruits SsgB, which in turn associates with FtsZ to promote Z-ring assembly [323].

SepG (also known as YlmG), a small integral membrane protein encoded by one of the last genes in the *d*ivision and *c*ell *w*all (*dcw*) cluster, might contribute to the proper localization of SsgB, as suggested by their interaction and the inability of SsgB to accumulate at division septa in the absence of *sepG* [324].

The recently characterized protein SepH binds FtsZ and was suggested to promote FtsZ polymerization and protofilament stabilization [325].

Negative regulation of FtsZ polymerization: CrgA

The small integral membrane protein CrgA represents a conserved family of Actinomycetota-specific small proteins, with only one single ortholog positioned near the replication origin in all fully sequenced genomes. Although its precise role remains unclear, CrgA might function as a negative regulator of Z-ring formation, based on sporulation defects observed in *crgA* mutants and inhibition of Z-ring assembly upon CrgA overexpression [326, 327].

The three SepFs

SepF (also known as YlmF) is a member of Gram-positive divisomes and a well-studied protein in *B. subtilis* and members of the phylum Actinomycetota. In *B. subtilis*, SepF is encoded by the *ylm* operon, downstream the *dcw* cluster [328]. *B. subtilis* SepF binds FtsZ and promotes its polymerization [329]. SepF was also seen, through electron microscope, to form very large (~50 nm diameter) polymeric rings [330]. SepF can bind the membrane while associating with FtsZ, recruiting the Z-ring to the membrane, and therefore was recognized as a membrane anchor for FtsZ in *B. subtilis* [85]. Because Bacillota encodes other membrane anchors, like FtsA, SepF is dispensable for cell division in this phylum, but becomes essential when these membrane tethers are absent [86, 331]. However, SepF is indispensable in unicellular members of Actinomycetota that lack true homologs of FtsA. For

instance, *M. smegmatis* and *C. glutamicum* SepF proteins are required for viability and their depletion results in abolished septation [87, 88]. Interactions between SepF and FtsZ have been demonstrated within members of this phylum, including streptomycetes [87, 88, 332, 333]. Structural and mutational analyses on SepF revealed that the protein is active in a homodimer form, using an amphipathic helix at its N-terminus to associate with membrane and a C-terminal globular domain to interact with FtsZ. Notably, the C-terminal domain of SepF is critical for FtsZ polymerization [85, 88, 330]. The ability of SepF to bind the membrane and form large rings appears to be conserved across diverse bacterial species and the size of SepF rings was proposed as a determinant of septum thickness [334].

Streptomyces SepF is encoded by an operon within the *dcw* cluster, downstream of *ftsZ* [328]. In addition to the *bona fide* SepF, streptomycetes uniquely encode two extra SepF proteins, SepF2 and SepF3, whose corresponding genes are located elsewhere in the genome [333]. Sequence analysis showed that SepF2 and SepF3 preserve residues required for dimerization and the C-terminal FtsZ-binding domain of SepF, but SepF2 lacks most of the conserved N-terminal membrane-binding residues [333]. All three SepFs in *S. venezuelae* were shown to self-interact and bind each other, but only the canonical SepF can bind FtsZ. SepF2 appeared to interact with other cell division proteins, namely SsgB and DynB. [333]. However, and despite recent progress, the reason streptomycetes encode three SepF proteins and the exact contributions of the additional two are not yet fully resolved. A recent study suggested a role of SepF2 and SepF3 in *S. coelicolor* (designated SflB and SflA, respectively, for SepF-Like) in the correct positioning of both FtsZ and DivIVA [335]. Our recent showed that *S. venezuelae* SepF is essential for cell division and is likely the membrane anchor for FtsZ and further revealed a role of SepF3 in the coordination of chromosome segregation with sporulation septation, as will be discussed in Paper III.

Stabilization of the Z-ring: DynAB and SepX

The bacterial dynamin-like proteins DynA and DynB, belong to the dynamin superfamily of large GTPases, which encompasses classical dynamins, operating predominantly in animal cells, and dynamin-like proteins, found in animal, plant, and prokaryotic cells [333, 336, 337]. DynA and DynB, as components of the *Streptomyces* divisome, are proposed to promote cell division by stabilizing FtsZ. Rather than acting through direct FtsZ interactions, they are linked to FtsZ through a network of division proteins: both DynA and DynB interact with SsgB, DynB additionally interacts with SepF2, and SepF2 interacts with SepF, which binds FtsZ [333]. Furthermore, SsgB is directly associated with FtsZ, as described earlier [323].

The recently discovered SepX was also shown to interact and have a synergistic role with DynB, i.e., both work towards the same cellular function of stabilizing the Z-ring during sporulation, despite an additional role of SepX in vegetative cross-wall formation as previously mentioned [313].

Chromosome segregation and condensation

Systems that drive chromosome segregation and condensation in *E. coli* and *B. subtilis*, including the FtsK/SpoIIIE DNA translocases, the ParAB-*parS* partitioning system, and SMC proteins, have also been identified in *Streptomyces*, where these systems function in ensuring the accurate partitioning of chromosomes into pre-spore compartments and promoting chromosome condensation as spores mature.

Although dispensable for cell division, FtsK localizes to sporulation septa where it plays a role in chromosome segregation [318, 338]. Deletion of *ftsK* results in a relatively mild phenotype, but a truncated FtsK protein lacking the DNA motor caused a more-severe chromosome segregation defect and increased formation of anucleate spores, highlighting the importance of its DNA translocation activity [338]. Consistent with this role, deletion of *ftsK* also caused the loss of large chromosomal regions near the termini, suggesting that chromosome ends fail to arrive at their destination before septum closure [318]. Encoded from the *smeA-sffA* operon, SffA is another putative DNA translocase protein that co-localizes with FtsK at sporulation septa. Deletion of the operon disrupts chromosome segregation, septum placement, and spore maturation, yet SffA appears mechanistically distinct from FtsK and its DNA translocase activity [237].

Chromosome segregation is further facilitated by the ParAB-*parS* system, in which ParB associates with the linear chromosome by binding the origin-proximal *parS* sites and forms regularly spaced complexes along aerial hyphae to help in DNA partitioning, while ParA accumulates upon cessation of aerial growth and helps in the formation and positioning of ParB complexes [339, 340]. Efficient segregation further requires the SMC condensin [338]. The condensin complex is recruited by ParB and was proposed to establish a positive feedback loop on ParB complexes by reducing ParB CTPase activity and turnover, thereby enhancing the complex stability [341, 342]. Consistent with their role in chromosome segregation, ParB and SMC also contribute to large-scale chromosome organization. Analysis of spatial organization of *S. venezuelae* genome revealed extensive rearrangements during sporulation, where the initially separated chromosomal arms become progressively aligned as sporulation proceeds. This inter-arm proximity depends on ParB and SMC, with the nucleoid-associated protein HupS facilitating the organization of the terminal chromosomal regions into distinct domains [341]. HupS is one of several nucleoid-associated proteins involved in chromosome compaction. Together with its HU-family homolog HupA, which predominates during vegetative growth, HupS contributes to chromosome organization and spore development [343, 344].

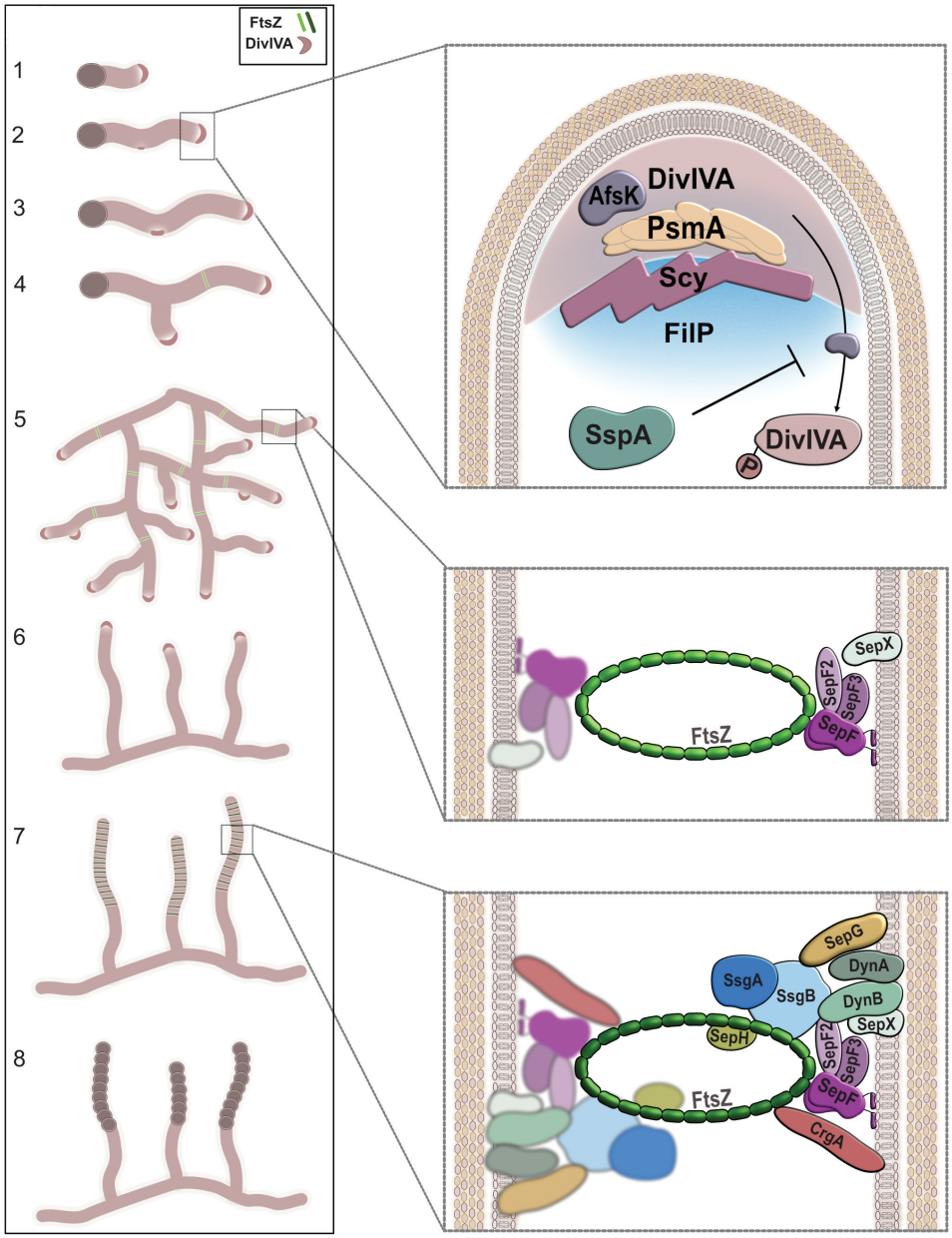


Figure 10. Regulation of *Streptomyces* cell shape through polar growth and cell division. An overview of cell shape determination throughout *Streptomyces* life cycle. Left panel shows the life cycle, including spore germination, polarisome-mediated tip extension, branch formation through polarisome splitting, multicellular vegetative mycelium compartmentalized by cross walls, aerial hyphae formation, and sporulation. DivIVA is shown in pink and FtsZ is shown in green. Right panels show the multiprotein complexes: the polarisome (Top), and the divisome, both during vegetative growth (Middle) and sporulation (Bottom).

Chapter V. The conservon operons

Sequencing of *S. coelicolor* genome revealed the presence of 13 copies of conserved operons, termed the conservons, also recently referred to as Actinobacterial G Protein Systems (AGPSs) [187, 345]. The discovery of these genetic arrangements surfaced as an intriguing feature of *Streptomyces* genomes, pointing to a unique evolutionary aspect. These operons are conserved across Actinomycetota and show an interesting distribution within the phylum: number of the conservon copies positively correlates with genome size, where genomes of genera that undergo complex morphological differentiation such as *Streptomyces* and *Nocardia* often contain multiple copies, whereas rod-shaped mycobacteria generally retains a single locus [345, 346]. A *Streptomyces* conservon locus comprises four core genes, *cvnA* through *D*, where the product of *cvnA* being a membrane protein with a histidine kinase-like ATPase domain, *cvnB* encodes a protein featuring a roadblock/LC7 domain known for GTPase regulation, *cvnC* encodes a protein containing a helix-turn-helix motif and a conserved DUF742 domain of unknown function, and *cvnD* encodes a G protein with GTP-binding consensus sequences similar to eukaryotic Ras-like proteins. The occurrence of the four genes is common across all the *cvn* loci. However, some loci are also accompanied by accessory genes, *cvnE* and/or *cvnF*, which encode a homolog of cytochrome P450 and a GAF domain-containing protein, respectively [345].

CvnA proteins typically possess canonical domains of histidine kinases, including a transmembrane segment and an extracellular sensory (NIT) domain, although not all CvnAs retain the sensory domain. Notably, the absence of the sensory domain was shown to correlate with the presence of the accessory proteins CvnE and CvnF [345]. CvnDs and CvnBs are members of the MglA family of small prokaryotic GTPases and MglB family of GTPase-activating proteins, respectively [347]. In all domains of life, small GTPases function as nucleotide-dependent molecular switches to regulate signal transduction pathways. Due to their low intrinsic hydrolytic activity, they alternate between GDP-bound (inactive) and GTP-bound (active) configurations with the help of guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs), which facilitate GDP dissociation and GTP hydrolysis, respectively [348]. MglA in *M. xanthus* is the first bacterial member of the Ras superfamily of small GTPases to be identified, and MglB functions as its cognate GAP [349, 350]. MglA and MglB have been thoroughly investigated for

their role in polarity reversals, i.e. pole-to-pole relocation of motility proteins, which allows *M. xanthus* to change direction during motility [351].

Beyond *Streptomyces* species, functional insights on the conservons have primarily come from studies in mycobacterial and nocardial species. The pentapeptide repeat protein MfpA, a significant factor in the inherent resistance of mycobacteria to the gyrase-targeting fluoroquinolones antibiotics is preceded and regulated by the sole conservon locus of *M. smegmatis,* which consists of *mfpB* through *E* [352]. A recent study described a role of the conservon locus *nprFGHI* in *Nocardiopsis* spp. The study showed that α-pyrone molecules can trigger secondary metabolites and act as signaling molecules in cell-cell communication in *Nocardiopsis*, through a signaling cascade mediated by NprFGHI [353].

The first experimental evidence assigning a function to a *cvn* locus in streptomycetes was described by Komatsu *et al*. [354]. The study showed that the aerial mycelium-deficient mutant of a key regulator in *S. griseus* development can be suppressed by a truncated version of *cvnA*, located in a *cvn* locus of five genes in *S. griseus*, with its closest homolog being *cvn9* in *S. coelicolor*. Deletion mutants of *cvnA9* and *cvnD9* in both *S. griseus* and *S. coelicolor* had comparable phenotypes: both mutants produced aerial mycelia in the presence of glucose, where such development was repressed in the wild type [354]. Further characterization of the *cvn9*-encoded proteins in *S. coelicolor* confirmed the GTPase and ATPase activity of CvnD9 and CvnA9, respectively, and demonstrated protein interactions among members of Cvn9 [346]. The same study further showed that expression of three *bld* regulators was upregulated in the *cvnA9* mutant [346]. In another study where phenotypic effects of individual deletion mutants of all 13 *cvn* loci in *S. coelicolor* where analyzed, a phenotype was observed only for the *cvn1* mutant, which, in the presence of high glucose levels, formed aerial mycelia and increased production of pigmented antibiotics, two phenotypes that were notably repressed in the wild type under similar conditions. Mutating individual genes within the *cvn1* operon showed that the phenotype is due to deletion of *cvnA1* [355]. A role of consrvon 8 *in S. coelicolor* was also described, where *cvn8*, through transcriptomics and mutational analysis, was found necessary for activating pigmented antibiotic production in *S. coelicolor* during interactions with various *Streptomyces* species. Notably *cvnA8* and the gene immediately downstream of the *cvn8* locus that appeared co-regulated with *cvn8* genes, *cvnF8*, were recognized as the major contributors [356]. CvnF8 was subsequently characterized as a regulator of CvnA8, promoting its ATPase activity and autophosphorylation [345]. *S. venezuelae* has eight uncharacterized conservons [357]. RNA-seq experiment identified these loci as potential BldC targets, where all loci, except for *cvn6* showed significant reduction in expression during vegetative growth in the *bldC* mutant [233].

Furthermore, studies that dissected the conservons to determine gene-specific functions in *Streptomyces* and other organisms suggest that each conservon

constitute a putative signal transduction complex analogous to eukaryotic small G protein-mediated regulatory systems [346, 358, 359](Fig. 11).

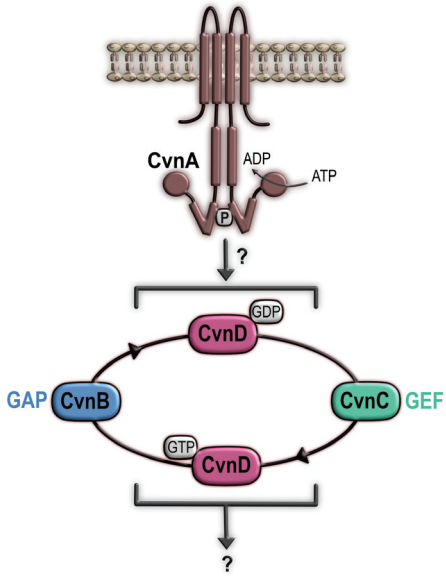


Figure 11. Signal transduction through an AGPS. AGPSs encoded by the conservons are thought to function as signal transduction complexes where the histidine kinase-like ATPase CvnA senses and transmits the signal to the GTPase regulatory module encoded by *cvnB*, *cvnC*, and *cvnD*.

Therefore, few functions have been linked to the conservons across different species within the Actinomycetota phylum. The conservation of these operons within the phylum is noteworthy, but their duplication in species with more complex morphology and development, like streptomycetes, is particularly intriguing, and key questions remain, including the roles they fulfill, the evolutionary significance of their expansion in streptomycetes and the possibility of functional redundancy.

Our recent studies on the conservons, where we systematically investigated the 8 conservons of *S. venezuelae*, addressed some gaps, and specifically revealed intriguing insights about the roles of conservons 1 and 2 in cell shape determination and polar growth in *S. venezuelae*, as will be discussed in papers I and II.

Thesis objectives and discussion of results

Aims of this thesis work

The overall aim of this thesis work is to clarify molecular mechanisms governing cell shape determination in *Streptomyces* and how such mechanisms contribute to multicellular organization, by investigating the two key systems involved in *Streptomyces* morphogenesis: polar growth and cell division, using *S. venezuelae* as a model organism. The part of my PhD work that pertains to polar growth aims at clarifying the roles of the conservon operons in relation to cell polarity and cell shape. In the part concerning cell division, my aim is to elucidate the roles of the three SepF proteins. Specific research questions addressed by each paper include:

Paper I: What are the roles of the AGPSs encoded by the conservons and the purpose behind their multiplication in *Streptomyces* genomes? Do these regulatory modules exhibit any functional overlap?

Paper II: Results in Paper I implicated AGPSs encoded by conservons 1 and 2 in cell shape and aspects of multicellular organization. Therefore, we asked in Paper II how these two AGPSs mechanistically contribute to morphogenesis, dissecting the roles of individual genes within each operon and exploring heir connection to DivIVA-based polar growth

Paper III: Does the *bona fide* SepF fulfill the role of a membrane anchor for FtsZ in *Streptomyces* cell division? Why do streptomycetes possess two additional SepF homologs and what roles have SepF2 and SepF3 evolved to fulfill?

Paper I. Two actinobacterial G protein systems control multicellular mycelial growth and morphology in *Streptomyces venezuelae*

Streptomyces genomes show remarkable focus on signaling and a huge investment in regulation, challenging the traditional view of bacteria as simple streamlined systems. This becomes particularly interesting knowing that streptomycetes commit to a multicellular life, where they must organize cellular processes across space, time, and developmental stage. An intriguing feature of *Streptomyces* genomes is the presence of multiple copies of the Actinobacteria G protein systems (AGPSs), encoded by the conservons. Given the central role of G proteins in regulatory decision-making, such multiplication is unlikely to be simple redundancy but rather points to a fine-tuned regulation that is likely needed for coordinating complex behaviors within these organisms.

In this paper, we aimed at investigating and assigning potential roles to the eight AGPSs of *S. venezuelae*. The foundational stage of this work involved a systematic mutagenesis of all conservon loci to obtain single operon deletions and ultimately a *cvn*-free mutant, lacking all copies. We subsequently conducted comprehensive macroscopic and microscopic analyses of mutant phenotypes under different growth conditions. Previous studies that aimed at clarifying the roles of the conservons relied on single operon deletion mutants, which could not rule out the possibility of functional redundancy. Therefore, obtaining a strain lacking all *cvn* copies was a significant advance, as this strain allowed for testing whether overlapping functions masked phenotypes in single mutants. However, our analysis revealed phenotypes only for conservons 1 and 2, with the *cvn*-free strain, under the conditions tested, showing no additional defects beyond what was observed for these two conservons.

Conservons 1 and 2 contribute to cell shape and the multicellular organization underlying mycelial architecture

The key finding in this work was the implication of *cvn1* and *cvn2* in the regulation of cell shape and mycelial architecture. Mycelial networks of wild type *S. venezuelae* exhibit a fascinating self-organizing behavior when grown on minimal medium: Individual hyphae have curly appearance and engage in dispersed organized growth where they seem to avoid each other in an apparent self-avoidance pattern, resulting in ordered microcolonies. On rich medium, hyphae of the wild type appear straighter and do not show a similar degree of self-avoidance. In contrast, mutants lacking *cvn1* and/or *cvn2* have straighter hyphae that, regardless of nutritional state, appear to bundle and self-associate, resulting in disordered microcolonies. Therefore, the wild type appears to respond to nutrient limitation by altering morphology, whereas the mutants cannot elicit such a response (Fig. 12). Our results also confirmed that *cvn1* and *cvn2* function non-redundantly as both

operons are required to rescue the phenotype of the *cvn1-cvn2* double mutant. Our interest became therefore centered on how *S. venezuelae* generates this fascinating hyphal self-avoidance phenotype and the organized mycelial growth patterns and what mechanisms underlie them.

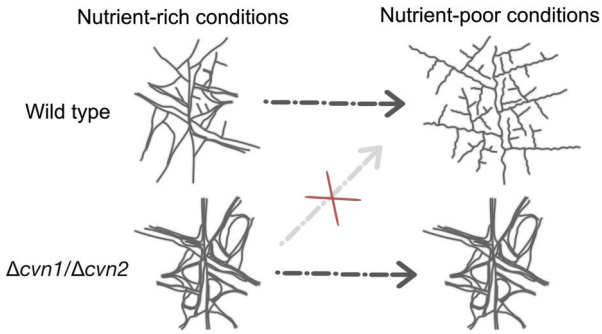


Figure 12. Conservons 1 and 2 are required for ordered mycelial architecture under nutrient-limited conditions. A schematic illustration showing wild-type *S. venezuelae* adaptation to nutrient limitation, characterized by increased hyphal curling and self-avoidance to organize the microcolony, a response disrupted by deletion of conservons 1 and/or 2.

Further microscopic experiments suggested that the hyphal bundling phenotype of the mutant is contact-dependent, as hyphae of co-growing wild type and mutant appeared to associate with each other while those of the wild type remained spatially separated on the same plate. Two observations emerged from this analysis. First, the wild type continued to display its unique self-avoidance phenotype suggesting that bundling between mutant and wild type is primarily driven by the mutant. Second, each of the co-cultured wild-type and mutant strains retained their characteristic phenotype during co-growth, suggesting the phenotype is unlikely to be mediated by diffusible factors. The latter was supported by another microscopic test, which showed that each of the wild type and the mutant retain their own phenotypes when cultivated on a medium conditioned by the opposite strain.

The self-organizing phenotype displayed by the wild type represents a manifestation of a multicellular behavior and can be seen from different biological perspectives. From one perspective, it can result by intercellular communication, or coordination between hyphae within the multicellular vegetative mycelium. Within this context, one compelling concept that emerges is kin recognition. As discussed earlier in Chapter II, multicellular bacteria have evolved kin-recognition systems to promote social behaviors and confer benefits, and as exemplified by the systems described for *M. xanthus* and *P. mirabilis*, these mechanisms involve cell-cell contact, likely to ensure the fidelity of the recognition. Such systems have not been described for streptomycetes, but observations of hyphae avoiding each other may indicate that

they actively sense one another, through a yet-unknown mechanism, and respond by, for instance, changing direction, a behavior that is disrupted in *cvn1* and/or *cvn2* mutants. Observations that hyphal bundling is contact-dependent further strengthens this notion.

From another perspective, the phenotype might arise due to alterations in cell shape affecting individual hyphae. Mutant hyphae are straight, whereas those of wild type adopt a curly shape. While this is a local cell shape defect occurring at the level of single hyphae, local effects can influence the higher-order organization due to the continuous nature of the mycelial network. For instance, straighter hyphae in the mutant might promote increased bundling through indirect physical effects.

Irrespective of whether it is a cell shape defect or lack of response across individual hyphae, both scenarios indicate a connection to polarity events at the hyphal tips. Regulation of *Streptomyces* cell shape is mediated by polar growth, where members of the polarisome complex regulate various parameters of hyphal shape [208, 283, 306, 309]. *Streptomyces* tip growth can also be viewed as a low-cost form of motility that allows exploration of different microenvironments. Accordingly, if hyphae achieve self-avoidance by steering away from one another upon contact, the process likely involves mechanisms acting at the hyphal tip. Such assumption can also be drawn by analogy to hypha-orientating mechanisms mediated by polarity complexes in fungi [360] or flipping the direction of movement in *M. xanthus* by the MglA-based polarity complexes [351], though the bacterial analog is mechanistically distant given that *M. xanthus* is motile. Nevertheless, a link to polar growth remains to be established by, for instance, performing subcellular localization experiments to test whether proteins encoded by two conservons accumulate at hyphal tips or testing for possible protein interactions with members of the polarisome.

We attempted to gain insights on this phenotype by comparing the transcriptome of *cvn1-cvn2* double mutant with wild type under the conditions where the phenotype is pronounced, but none of the *S. venezuelae* morphogenetic determinants was detected in our analysis. The analysis however revealed an intriguing feature of the mutant: a strong bias towards gene upregulation, suggesting that conservon 1 and/or conservon 2 play negative regulatory roles. It also revealed an upregulation of genes involved in exopolysaccharides metabolism and others involved in aerial growth and sporulation, including chaplins and rodlins. In the light of the this, it can be speculated that mutant hyphae have altered cell surface properties due to increased production of certain exopolysaccharides or chaplins and rodlins rendering the surface hydrophobic, which in turn result in self-association.

Conservon 1 contributes to the zonal patterning at the macroscopic colony level, and is involved in *S. venezuelae* development

Beyond the hyphal self-avoidance, an intriguing macroscopic phenotype was seen exclusively in mutants lacking *cvn1*. Mutant colonies had a distinct organization: within-colony heterogeneity and the concentric-ring pattern of wild-type colonies,

manifested as a dense center surrounded by a low-density zone with both being enclosed by a diffuse fringe-like outer ring, was abolished in colonies of *cvn1* mutants, which lacked the fringe and appeared uniformly dense. This phenotype is also a manifestation of multicellular behaviors, as described for heterogeneity in *S. coelicolor* colonies arising from the spatial separation of specialized cells involved in development and antibiotic production across the colony [279]. The spatial patterning in *S. coelicolor* colonies was interrupted by disrupting the developmental regulator AdpA [279], proposing that the *cvn1* mutant phenotype may also be linked to impaired development. Mutants lacking the *cvn1* operon also appeared defective during exploratory growth, where they showed less spreading and formed aerial mycelia on a medium that is non-permissive for development. These observations marked the first indication of a developmental role of conservon 1, but several lines of evidence followed. First, suppressor strains, isolated from *cvn1* mutant colonies, and are capable of restoring the wild-type colony phenotype, harbored mutations in genes involved in c-di-GMP-mediated developmental program. Second, mutants lacking *cvn1* produced more spores as compared to wild type at an early stage of growth. Finally, the transcriptomic analysis on *cvn1-cvn2* mutant versus the wild type, conducted under conditions where such development is not typically expected, revealed upregulation of development-associated genes. Our results collectively, and given that *cvn1* is a target of BldC and BldD [233] and *cvn1* promoter is bound by WhiAB [242], made us hypothesize that conservon 1 participates in a negative feedback loop that acts to limit or buffer the progression of development (Fig. 13).

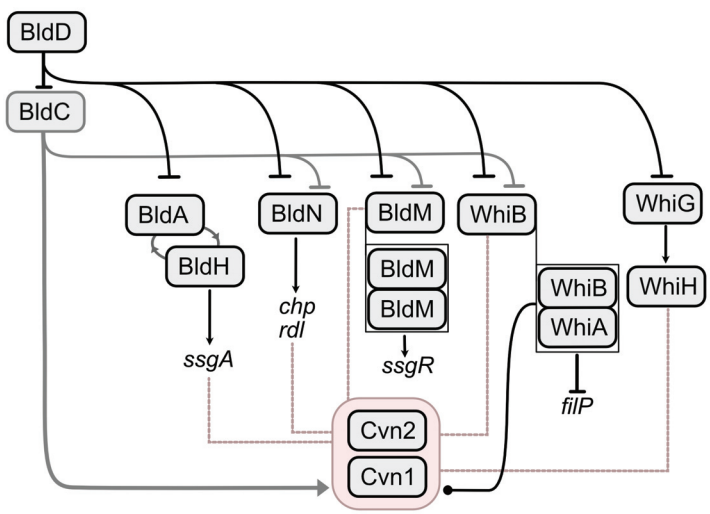


Figure 13. Conservon 1 contributes to *S. venezuelae* development. Conservon 1 promoter is bound by WhiAB and both conservons 1 and 2 are regulated by BldC. Transcriptome of *cvn1-cvn2* double deletion mutant showed upregulation of genes involved in *Streptomyces* development. The dotted pink lines indicate genes upregulated in the mutant. Repression is indicated by blunt arrows (—|) and activation is shown as standard arrows (➔).

Are the microscopic and macroscopic layers of organization coupled?

We like to think that the microscopic phenotype, manifested as loss of hyphal self-avoidance and the disordered mycelial shape, and the two macroscopic phenotypes of forming aerial mycelia during exploration and loss of the concentric ring pattern, represent two layers of multicellular organization arising from distinct processes. However, before considering how they diverge, we should first address whether and how the two macroscopic phenotypes might be linked. We assume the macroscopic phenotypes are related, as both can be explained in light of the developmental role of conservon 1. The premature formation of aerial mycelia can be intuitively viewed as arising due to altered development. Likewise, the lack of colony fringe can be explained through the same framework as heterogeneity within colonies generally reflects the emergence of spatially distributed specialized populations, a process that depends on coordinated differentiation and a functional developmental program. In this sense, both phenotypes point toward perturbations in developmental regulation. On the other hand, the microscopic and macroscopic phenotypes appear to be driven by different mechanisms. First, their genetic requirements differ: the self-avoidance behavior depends on both *cvn1* and *cvn2*, whereas the colony fringe and exploratory growth require *cvn1* alone. Second, the conducted suppressor analysis further separates these processes. Suppressors isolated in *cvn1*-mutant background were able to restore the macroscopic phenotype yet had no effect on the microscopic phenotype. These results collectively suggest that the phenotypes reflect distinct levels of multicellular organization and are not necessarily linked.

Concluding remarks

Our work in this paper highlights two manifestations of multicellular organization in *S. venezuelae*. The first describes an apparent hyphal-self avoidance pattern that underlies the establishment of mycelial morphology and ordered microcolonies, and the second is about heterogeneity and zonation patterning at the macrocolony level. Notably, at the molecular level, the two processes require AGPSs encoded by the conservons, but differ in their requirements where the microscopic phenotype relies on *cvn1* and *cvn2*, and the macroscopic phenotype is primarily dependent on *cvn1* (Fig. 14).

The work also sheds light on the evolutionary implications of gene duplication and conservation. The presence of multiple copies of the conservon operons within *Streptomyces* genomes suggests functional redundancy, where individual copies can compensate for one another. Our results challenge this expectation. Among the eight conservon copies, detectable phenotypes were seen only for *cvn1* and *cvn2*, while deletion of the entire set did not result in additional defects beyond those observed for two operons. The absence of redundancy in such a large and conserved family points toward functional diversification, where individual conservon operons may have acquired specialized roles, but not all roles were readily revealed under the experimental conditions used. Since each conservon encodes a signal transduction

complex, providing a mechanistic understanding of how AGPSs encoded by *cvn1* and *cvn2* are executing their roles would consequently uncover molecular insights into the regulation of cell shape and multicellular organization in *Streptomyces*.

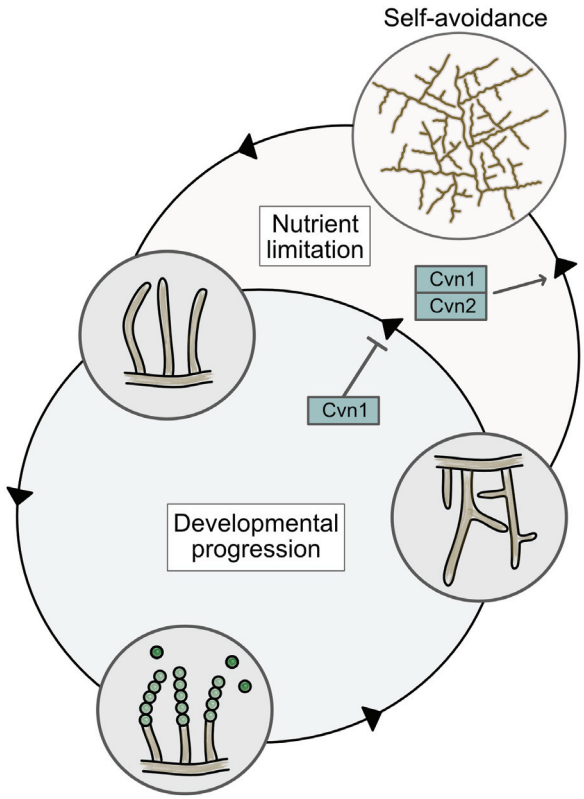


Figure 14. Working model of Paper I. Conservon 1 limits the premature progression of development, whereas both conservons 1 and 2 are involved in hyphal self-avoidance underlying the ordered mycelial architecture in *S. venezuelae*.

Paper II. Actinobacterial G protein systems encoded by conservons 1 and 2 regulate cell shape through polar growth-dependent mechanisms

Polarity creates asymmetry in the cell to establish regions with specialized functions and organize cellular processes. This feature is found across the tree of life, from neurons that differentiate into dendrites and axons and plant cells that direct growth guided by environmental signals, to bacteria where polarity underlies a wide range of functions, including growth, division, and morphogenesis, such as the polarized PG synthesis that results in the filamentous vegetative hyphae of streptomycetes. Once polarity is established, it draws a map for proteins to follow and perform their functions. How proteins decode this map and localize with precision is an attractive question in cell biology. DivIVA, for instance, finds the pole by identifying negative membrane curvatures and acts as a landmark polarity protein to capture proteins for downstream processes. Cell polarity can also be dynamic and switchable, rather than fixing cells in a permanent shape as DivIVA does, as exemplified by the *M. xanthus* small GTPase MglA and its regulators, which establish front-rear polarity (for reviews on polarity in bacteria, see [361-363]).

The involvement of AGPSs encoded by conservons 1 and 2 in mycelial organization and shape determination suggested that, among other things, hyphal tip-associated polar processes are affected in these mutants. Also, each AGPS comprises two core components, the GTPase module consisting of CvnB, CvnC, and CvnD and the sensor histidine kinase-like CvnA, both being co-encoded from a single operon. These features emphasized the need to resolve the roles of individual members within each conservon to understand how they regulate cell shape. Here we analyzed the contribution of individual genes within *cvn1* and *cvn2* by generating deletion mutants and assessing their phenotypic consequences. We further focused on the key members, the predicted small GTPase and the histidine kinase-like ATPase, to explore their functional roles through molecular characterization. Finally, we established a connection to polar growth, providing a potential explanation for the cell shape effects exerted by *cvn1* and *cvn2*.

Conservons 1 and 2 constitute a dual-operon system, likely integrating distinct inputs through a shared GTPase regulatory core to control cell shape

Single gene deletions within *cvn1* and *cvn2* showed interesting patterns. The cell shape defect associated with the deletion of *cvnA* from conservon 1 was particularly pronounced and appeared to account for the phenotype of the whole-operon deletion. Within conservon 2, *cvnA* and *cvnB* contributed to morphological defects to varying extents, with *cvnB2* deletion exhibiting the strongest effect, and none of the individual *cvn2* genes was sufficient to rescue the phenotype of the whole operon deletion. Although our results in Paper I found no evidence of redundancy at the

operon level, here we confirmed redundancy between gene pairs encoding components of the GTPase module across conservon 1 and 2, namely the *cvnD1*/*cvnD2* and *cvnC1*/*cvnC2* pairs. This indicated that the two conservons share a common G protein regulatory core. In contrast, mutants in genes encoding the sensor kinases had distinct phenotypes, suggesting functional divergence.

AGPSs are often compared to *M. xanthus* polarity complex that integrates sensory inputs into G protein signaling, through the Frz pathway coupled with the MglA-based GTPase regulatory module [351]. However, the conservons remain unique in encoding all components from a single compact operon, an arrangement that might facilitate coordinated expression and rapid activation, and for a soil bacterium, may confer advantages such as quick and efficient adaptation of growth. Maintaining the module as a single unit may also preserve functional integrity and facilitate transfer of the entire system to organisms adapted to similar ecological niches.

Also, both conservons 1 and 2 affect cell shape and share a GTPase regulatory core, which led us to view them as a dual-operon system rather than two independent signaling modules (Fig. 15). It is tempting to speculate that such arrangement provides robustness and flexibility, where redundancy within the GTPase module may buffer against the loss of individual components and ensure a robust output, and divergence of the sensory elements may help respond to distinct cues. A question for future bioinformatic analysis is whether the two operons consistently co-occur across streptomycetes, which would provide further evidence that they function as an evolutionarily conserved dual-operon system.

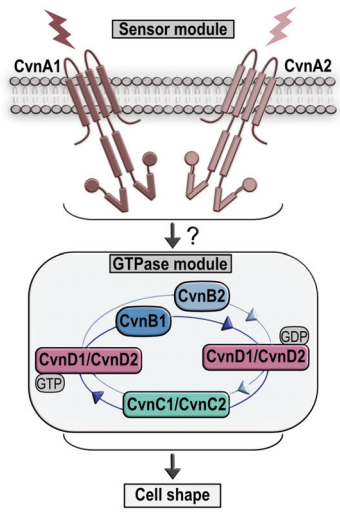


Figure 15. A Conservons 1 and 2 constitute a dual-operon system that controls cell shape in *S. venezuelae*. The sensor modules of AGPSs encoded by *cvn1* and *cvn2* exhibit functional divergence, whereas components of the GTPase regulatory modules are shared between the two systems.

The GTPase regulatory module is functional and polarly localized at hyphal tips

Having established that genes encoding the GTPase regulatory module contribute to cell shape through both direct and redundant functions, we showed that CvnD2 functions as a *bona fide* GTPase *in vivo*, by showing that plasmids carrying *cvnD2* variants with substitutions in residues predicted to impair nucleotide binding and hydrolysis, conformational switching, or regulation by its cognate GAP, failed to rescue the mutant phenotype. Notably, the variant predicted to lock CvnD2 in a constitutively active state produced a phenotype resembling that of the deletion mutant when expressed in the wild-type background, despite the presence of the endogenous protein. This dominant negative behavior suggests that GTP-locked CvnD2 is functionally inhibitory, and its activity depends not only on its catalytic ability but also on the precise control of its activation by the GAP. Consistent with this, intrinsic and GAP-mediated GTP hydrolysis is completely abolished by similar constitutively active MglA variants, and the mutation behaved similarly to *mglB* deletion mutant [364, 365].

The G cycle components CvnD2 and CvnB2 localized to hyphal tips. Localization of CvnD2 was heterogeneous as CvnD2 was not detected at all tips, and appeared dynamic, with fluorescence intensity at individual tips increasing and decreasing over time. Signal from CvnD2-mNG was reduced in mutants lacking *cvn2* or both *cvn1* and *cvn2*. Interestingly, CvnD2 localizes not only to the pole but also as band-like structures across the hyphae, which may be consistent with division cross walls, although this was not experimentally tested. CvnB2 also accumulated at hyphal tips, albeit with substantially weaker signal. Localization of CvnC2 was less conclusive, either because of technical limitations or because its role does not require robust tip localization.

The MglA-based polarity system of *M. xanthus* again provides a useful framework for understanding our system. In *M. xanthus*, MglA is maintained at either the front or the rear pole through the opposing activities of GAP and GEF, which help stabilize a polar pool of MglA to ensure it is restricted to a single pole [351]. Our results about CvnB2 and CvnD2 might imply a similar principle, despite functioning in a permanently polarized hypha with single growth pole. The reduced localization of CvnD2 in the whole-operon deletion mutant indicates that GAP and GEF are not only controlling the nucleotide state but also promoting CvnD2 retention at the tip. The heterogeneous accumulation of CvnD2 suggests that polar CvnD2 pools are dynamic, with cycles of release and recruitment rather than stable recruitment. The weak accumulation of CvnB2 at the pole may also be consistent with local regulation of CvnD2 activity directly at the tip. While localization of CvnC2 remains unclear, it may accumulate at levels below detection limit or transiently exerts its effect without establishing prominent polar localization. Most importantly, localization of components of the GTPase regulatory module at hyphal tips, combined with the requirement of a tightly regulated CvnD2 activity, support a

model in which this system functions at the poles where it is regulating growth and morphology.

The sensor kinase component exhibits atypical catalytic features and is polarly localized at hyphal tips

Structural predictions of CvnA1 showed that it forms a stable dimer and retains the overall domain architecture of histidine kinases but lacks the conserved histidine. CvnA1 also has a NIT sensory domain and contains a disordered C-terminal region. Mutational analysis showed that the NIT domain is important for activity, consistent with ligand-mediated activation. Complementation of *cvnA1* deletion mutant with a *cvnA1* variant lacking the unstructured C-terminal region resulted in partial complementation, indicating that this region is not a key determinant of activity. Also, phenotypic evidence from variants with substitutions in canonical and non-canonical residues that appeared to engage with the bound nucleotide suggested that ATP binding and hydrolysis are important for CvnA1 activity. Therefore, CvnA1 preserves structural features of sensory ATPases, but appears to function through a non-canonical mechanism. Given the apparent lack of redundancy between CvnA1 and CvnA2, it would be important to determine whether the mutational effects identified for CvnA1 are similar in CvnA2.

CvnA1 and CvnA2 localized to hyphal tips, and a strong signal persisted at the tip even in the absence of *cvn1* and/or *cvn2* genes, indicating that polar recruitment of CvnA1 and CvnA2 occurs independently of the remaining conservon components. Rather than remaining static, however, their localization patterns appeared dynamic and changing over time, transitioning between foci, crescent-shaped assemblies, and diffuse signals with varying intensities. The two proteins also accumulated as cross-wall-like structures, as described for CvnD2. The dynamic tip localization may be linked to their biological function. If CvnA1 and CvnA2 act as sensor kinase-like ATPases, such dynamics may reflect shifting between different states, such as activation, signal processing, signal transmission, or association with downstream partners.

Collectively, results here show that the sensor kinase component exhibits atypical catalytic features, conserved ATP-dependent function, and consistent association with the hyphal tips where it might be executing processes related to polar growth.

Conservons 1 and 2 influence DivIVA assemblies at branch points

In the absence of either or both of *cvn1* and *cvn2*, DivIVA displayed altered behavior, occasionally forming band-like structures or two opposing foci at branching sites rather than the typical single sharp focus from which one branch emerges. These structures were associated with hyperbranching, where multiple branches emerge from each branch point.

Branches normally form by a mechanism of polarisome splitting, where the polarisome at the active tip sheds daughter polarisomes that act as seeds for new

branch emergence [210-212]. While DivIVA exhibits distorted behavior at branch points in the absence of conservons1 and/or 2, it still localizes normally at the hyphal tip, implying that these conservons do not influence global DivVIA behavior, but rather become important when new polarity axes are being established away from the main tip. Although supporting evidence is lacking, we can consider different possibilities to explain this behavior. One is that the two conservons function in focusing polarity as if branch initiation normally starts with multiple weak foci and the conservons focus these into a single dominant DivIVA cluster. Another possibility is that the conservons function upstream of DivIVA to define where branch formation is permitted, or as negative regulators of competing polarity DivIVA foci, suppressing polarity establishment at a nearby site rather than promoting DivIVA focusing directly or defining the branch site.

Concluding remarks

While the implication of AGPSs encoded by conservons 1 and 2 in multicellular organizational and morphological processes is intriguing in itself, linking these functions to polarity-related mechanisms adds another compelling dimension to their biological importance (Fig. 16).

Despite insights gained in this work, the exact mechanisms underlying the function of this dual-operon system remain unanswered. Future work is needed to describe the nature of the signal sensed by the sensor modules and to clarify how information is transmitted to the downstream GTPase machinery and subsequently converted into a regulatory output. Equally important is understanding how components within the GTPase module cooperate. Protein interactions between individual components of other conservons have been established [346], but it remains important to test interactions within and across proteins encoded by conservons 1 and 2. Also, our genetic data support the functionality of the GTPase module, but direct biochemical validation is still needed. Purification of individual proteins and reconstitution of the module *in vitro* would enable analysis of GTP hydrolysis, as well as the regulatory relationships among the GTPase and its regulators. Together, these tests will provide a mechanistic model for understanding how the dual-operon system integrates sensory information to regulate cell shape and polarity.

The implication of conservons 1 and 2 in polarity-related mechanisms also deserves further investigation. For instance, to understand the effect on DivIVA behavior, it is important to understand the temporal and spatial relationship between proteins encoded by conservon 1 and 2 and DivIVA by microscopically investigating their co-localization. Also, a relevant experiment would be to test co-localization and interactions with other polarisome members, which could clarify whether these proteins are recruited to the cell pole by DivIVA or other polarisome proteins.

CvnA proteins and CvnD2 were further seen to accumulate as transverse bands in vegetative hyphae, and it is important to test whether these bands are located at division sites, for instance, by examining co-localization with FtsZ. If they

correspond to cross-wall-related structures, these conservons could instead be involved in compartmentalization, and it is possible in this case that polarity defects arise indirectly through altered cellular organization.

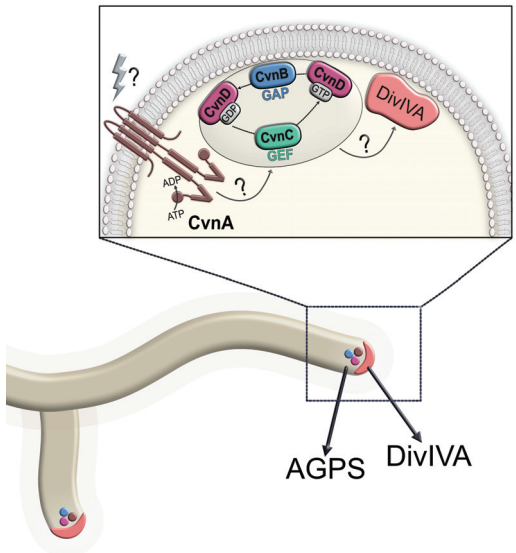


Figure 16. Working model for Paper II. AGPSs encoded by conservons 1 and 2 localize to hyphal tips where they mediate their function in polar growth and regulation of cell shape, likely by directly or indirectly modulating DivIVA behavior.

Paper III. Divergent roles of three SepF homologs in *Streptomyces venezuelae* include SepF3 linking cell division to nucleoid partitioning

Cell division is typically a mechanism that ensures bacterial proliferation and maintains cellular morphology. In *Streptomyces*, however, it has acquired broader morphological and developmental roles. Vegetative cross walls likely contribute to the multicellular organization of the mycelium, while sporulation septation supports the formation of spores. The final shape of *Streptomyces* spores is the result of highly synchronized division events that divide the long aerial hypha into pre-spore compartments. Accordingly, the precise positioning of septa and the successful completion of septation directly defines spore size and shape.

SepF proteins function as a membrane anchor for FtsZ and in stabilizing the Z-ring during cell division in Gram-positive bacteria. In *B. subtilis*, the role of SepF in FtsZ tethering overlaps with the functions of other membrane tethers encoded by these species [86, 331], whereas in actinomycetotal members, which lack membrane anchors other than SepF, it becomes essential for cell division [87, 88]. Unlike most Gram-positive bacteria, *Streptomyces* genomes contain two extra homologs, *sepF2* and *sepF3* in addition to the canonical *sepF* gene. Given the significance of SepF in cell division, we aimed at clarifying the roles of the three SepF proteins of *Streptomyces*. Our results show that while their exact roles have diverged, all three SepF proteins are involved in cell division, both during vegetative growth and sporulation (Fig. 17).

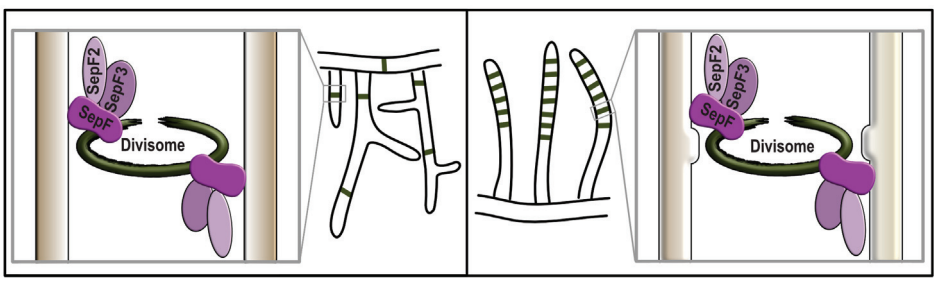


Figure 17. All three SepFs are involved in cell division, both during vegetative growth and sporulation.

SepF is indispensable for cell division in *S. venezuelae* and likely acts as a membrane anchor of the Z-ring

We confirmed the indispensability of SepF in cell division, a conclusions that was drawn based on SepF localization to division sites both during vegetative growth and sporulation, its co-localization with FtsZ, the abolished sporulation upon *sepF*

deletion, and the inability of the mutant to form normal-looking Z-rings during sporulation. We further showed that the putative amphipathic helix of SepF likely targets other proteins to the membrane, supporting a role of SepF as a membrane anchor. Our results on SepF, combined with evidence from the literature including SepF interaction with FtsZ [333], collectively show that SepF is crucial for Z-ring assembly and reinforce the notion that it functions as a membrane anchor for FtsZ in *S. venezuelae*. The pronounced phenotype of the *sepF* mutant also suggested at this stage that SepF function does not overlap with that of SepF2 and SepF3. By redemonstrating the role of SepF in our model organism, our work strengthens the evidence that SepF represents a conserved component of the prokaryotic cell division machinery. Notably, SepF is an ancient protein and homologs of SepF function as FtsZ anchors in some archaea [366], raising the possibility that SepF function reflects an ancient solution to a cellular challenge that has persisted since early prokaryotic evolution.

Similar to the *bona fide* SepF, SepF2 and SepF3 accumulated at division septa and co-localized with FtsZ. However, and unlike SepF, they appeared dispensable for sporulation as evidenced by the ability of mutants of their corresponding genes to sporulate on agar, and later by showing that Z-rings and sporulation septa can still form in these mutants. Despite their dispensability, mutants of *sepF2* and *sepF3* had effects on vegetative cell division, and only the *sepF3* mutant showed defects during sporulation.

SepF2 and SepF3 emerge as previously unrecognized players in vegetative cell division

Deletion of either of *sepF2* or *sepF3* resulted in slightly smaller colonies and slower growth and development. A synergistic effect of *sepF2* and *sepF3* deletion on colony development was shown by finding that a double mutant of the two genes exacerbated both effects. Such phenotypes implied an effect on cell division during vegetative growth, which was later confirmed by showing that the frequency of cross-wall formation was largely affected in the double deletion mutant (Fig. 18). The severe reduction in cross-wall frequency in the double mutant, together with defects in growth and development, suggest a functional link between vegetative compartmentalization and fitness. Consistent with this interpretation, deletion of *sepX*, which abolishes cross-wall formation, was also shown to compromise fitness [313]. While both SepF2 and SepF3 accumulated at division sites during vegetative growth, SepF2 had a stronger signal, suggesting a more prominent contribution to vegetative cell division. Nevertheless, the substantially more severe phenotype observed in the *sepF2-sepF3* double mutant compared with either single mutant indicates that SepF2 and SepF3 perform partially overlapping functions in this process. It remains unclear how SepF2 and SepF3 exert their roles on cross-wall formation. One possibility could be that hyphal extension rate or other aspects of polar growth are affected in these mutants as previously suggested [335]. Importantly, the identification of SepF2 and SepF3 as contributors to cross-wall

formation expands the current understanding of its molecular basis, as vegetative cell division remains poorly resolved at the molecular level, with only FtsZ and SepX being previously implicated.

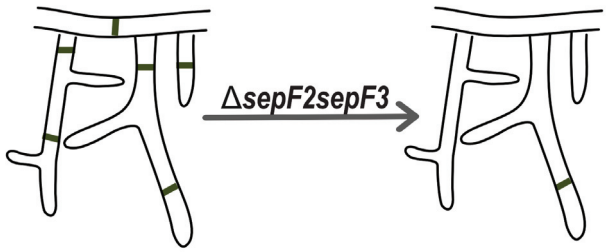


Figure 18. SepF2 and SepF3 perform partially overlapping functions during vegetative cell division. Deletion of both *sepF2* and *sepF3* reduce the frequency of cross-wall formation.

SepF3 couples chromosome segregation to septum closure during sporulation

The central finding of this work is the involvement of SepF3 in several aspects related to sporulation-specific cell division. The *sepF3* mutant initially displayed a morphological defect, forming exceptionally long spore chains instead of the short chains and single spores produced by the wild type, indicating a defect in spore detachment. Cell wall and DNA staining in these long chains revealed striking effects on chromosome segregation, including septa forming over unsegregated nucleoids and a large fraction of spore compartments being void of nucleoids (Fig. 19). The presence of anucleate spore compartments resembles phenotypes observed upon deletion of genes involved in chromosome segregation, including those encoding SMC, DNA translocases, and the ParAB partitioning system [237, 338].

Subsequent experiments provided two lines of evidence to elucidate this phenotype. Firstly, we showed by doing FRAP that *sepF3* deletion causes incomplete closure of sporulation septa, as evidenced by the cytoplasmic continuity and diffusion of proteins between mutant spore compartments. Transmission electron microscopy further showed that septa in spore chains remain with different extents of openings. Secondly, we established a link between SepF3 and FtsK, the DNA translocase that mediates chromosome clearance from closing septa [95]. FtsK did not show regular septal localization in the *sepF3* mutant, suggesting that SepF3 is required for the proper recruitment of FtsK to septa.

The improper septum closure together with inefficient chromosome segregation proposed a model of how defects in the *sepF3*-deficient spore chains emerge: at late stages of sporulation, unsegregated nucleoids become trapped under sporulation septa and are later pulled back during condensation through the improperly closed septa leaving some spore compartments empty of nucleoids (Fig. 19). Improper

septum closure may also result in long spore chains by preventing spore separation. Although factors governing spore detachment remain poorly understood, the incompletely closed septum may be perceived as an unfinished cell division event, preventing the action of downstream factors, like PG modifying enzymes. Further, the inadequate recruitment of FtsK, and while it provided a possible explanation of the chromosome segregation defect, is unlikely to be the sole contributor as the defect the in the *sepF3* mutant is considerably more severe than that reported for *ftsK* deletion mutants in *S. coelicolor* [318, 338], suggesting that additional factors may be involved. However, the consequences of *ftsK* deletion have not yet been described for *S. venezuelae*, and therefore this comparison should be interpreted with caution. This, however, suggests that SepF3 may act together with additional factors to ensure faithful chromosome segregation. Identifying these factors and defining their functional relationship with SepF3 will require further investigation.

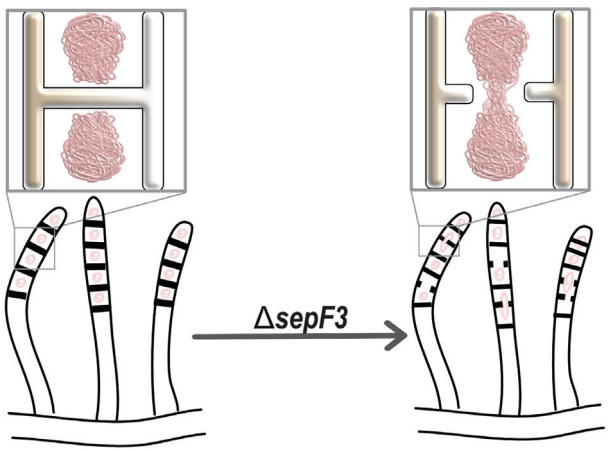


Figure 19. *sepF3* mutant is defective in chromosome segregation and septum closure. Spore chains of *sepF3* mutant show defects including spore compartments void of nucleoids and septa forming over the nucleoids. Unsegregated nucleoids remain trapped under closing septa and are later pulled back during condensation through the open septa leaving pre-spore compartments empty of nucleoids.

Concluding remarks

Overall, our data here confirmed the indispensability of SepF in Z-ring assembly, implicated both SepF2 and SepF3 in vegetative cell division, and identified SepF3 as being involved in three key events during sporulation-specific cell division, the exact mechanisms of which are poorly understood: septa closure, spore detachment, and the coordination between sporulation septation and chromosome segregation. One aspect to clarify is if there is an association between FtsK and SepF3, whether it is a direct association or mediated by other divisome proteins. Other chromosome partitioning system, like ParAB [339, 340], or the decatenation via Topoisomerase

IV [367] might also be responsible for the nucleoid partitioning defect, a possibility that can be investigated by looking at subcellular localization of these proteins. It is equally important to generally determine interaction partners of SepF2 and SepF3, as this will give critical insights on their function.

Further work is also needed to fully elucidate the mechanistic roles of SepF2 and SepF3 on vegetative cell division. This is especially interesting given our limited understanding of this process. Identifying contributors to vegetative cell division and dissecting their mechanisms may help address a range of broader questions: What advantage does a continuous compartmentalized mycelium provide over either a fully syncytial or fully separated structure? Have cross walls evolved as protective, developmental, or metabolic structures? Do cross walls allow for molecular exchange between neighboring compartments or possibly facilitate division of labor between different regions of the mycelium?

Acknowledgments

I would like to thank my supervisor Klas for his guidance and support throughout my PhD. Thank you for welcoming me into your lab and giving me the opportunity to learn from your expertise. I truly appreciate everything you have taught me.

I also thank the rest of my supervision committee, Claes and Allan, for their continuous support and encouragement.

Fredric: A huge thank you for everything. I have learned so much from you, both professionally and personally. I am truly grateful for all the ways you have helped me along the way.

Thank you, Olivier, for always being kind and for taking a part in my examination. Courtney, I really appreciate your consistent kindness and the times you helped me with bioinformatics.

María and Sara, the two girls who came into my PhD life late but made a huge difference: Thank you for your friendship and for bringing so much joy!

Rikard: Thank you for your continuous support at all levels. I appreciate our conversations and the opportunity to get to work with you. I would also like to thank Veronica for her guidance during my master's and for her support during my PhD.

Elisabeth: Thank you for being such a nice and thoughtful office mate and for consistently helping everyone.

Judith, Esther, Teresa, Héloïse, Vivian, Abraham, Cuong, Alberto, Irene, Humberto, Karla, Katie, and Mridula: Each of you has contributed to my journey in your own way and I am truly thankful for your friendship, support, and all the fun memories we've shared. And Katie, thank you for being the final pair of eyes on my thesis!

Ewa, Nik, Juanita, Rawana, Jorge, Valeria, Viktor, Jaume, Marco, and Shakhira: Thank you for the memorable moments we shared, both inside and outside work, and for making my time here enjoyable. Shakhira, Thank you for all the good food!

To all the students who crossed paths with our lab through projects, Heran, Sofija, Sara, Ara, Samuel, Enikö, Lina, Jonathan, Dimitrios, Ada, Nerea, Maria, Nasiya, Emil, Citlalli, Lucia, Linnéa, Mafalda, Naveenkumar, Jose Pablo, Sofia, Patrick, Cornelia, Axel, Maja, Joel, and Vytautas, it was a pleasure working with you, and I learned a lot from you.

Thank you, Mel and Zack, for giving Chiquita a second home while I was busy PhDing and for being consistently thoughtful and kind.

The Bounni Ladies: Thank you for the support across the distance and for caring for me and my family with such kindness and warmth.

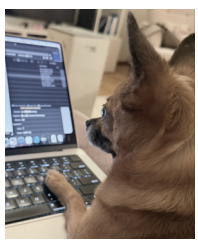
Mama and Baba: Thank you for believing in me, for pushing me beyond my limits, and for, very untraditionally for Middle Eastern parents, telling me to slow down and take care of myself. Thank you for all the love and warmth I carried with me throughout my PhD, even if every goodbye after a visit home came with its fair share of drama. I expect slightly less emotional goodbyes after graduation!

My little sister who somehow became wiser than me and has been teaching me life ever since: Thank you for always supporting me “sistaaa”. I still learn from the woman you are becoming every day. And Becher, thank you for being there and for all the conversations that I’ve truly valued and learned from. Most importantly, thank you both, Rama and Becher, for bringing Sami into the world. Honestly, that feels like your greatest achievement during my PhD!

Baby Sami: Thanks for the perfectly timed video calls that rescued me from becoming one with my thesis, I owe you my sanity! Thank you for all your details and the joy you bring just by being you.

To my second family, Tante Hind, Ammo Fadi, Hiba, Rama, and Amjad, thank you for being there for me throughout and for always showing such kindness. And Ammo Fadi, thank you for inspiring me more times than you probably know.

Firas: Thank you for being my calm in the PhD chaos, for endless hugs, endless patience, and somehow still loving me through all my breakdowns. I love you!



Chiquita, my silent co-author and emotional anchor who could not read a single word of this book but understood every single emotion behind it.

One day you may no longer be here, but I needed you to live on in this thesis, where so much of our life together is quietly held. Thank you, Chichi!

References

1. van Teeseling MCF, de Pedro MA, Cava F. Determinants of bacterial morphology: From fundamentals to possibilities for antimicrobial targeting. Front Microbiol. 2017;8:1264. https://doi.org/10.3389/fmicb.2017.01264
2. Garde S, Chodisetti Pavan K, Reddy M. Peptidoglycan: Structure, synthesis, and regulation. EcoSal Plus. 2021;9(2). https://doi.org/10.1128/ecosalplus.ESP-0010-2020
3. de Pedro MA, Quintela JC, Höltje JV, Schwarz H. Murein segregation in *Escherichia coli*. J Bacteriol. 1997;179(9):2823–34. https://doi.org/10.1128/jb.179.9.2823-2834.1997
4. Egan AJF, Errington J, Vollmer W. Regulation of peptidoglycan synthesis and remodelling. Nat Rev Microbiol. 2020;18(8):446–60. https://doi.org/10.1038/s41579-020-0366-3
5. Schleifer KH, Kandler O. Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriol Rev. 1972;36(4):407–77. https://doi.org/10.1128/br.36.4.407-477.1972
6. Glauner B, Höltje JV, Schwarz U. The composition of the murein of *Escherichia coli*. J Biol Chem. 1988;263(21):10088–95. https://doi.org/10.1016/S0021-9258(19)81481-3
7. Rohs PDA, Bernhardt TG. Growth and division of the peptidoglycan matrix. Annu Rev Microbiol. 2021;75:315–36. https://doi.org/10.1146/annurev-micro-020518-120056
8. Galinier A, Delan-Forino C, Foulquier E, Lakhal H, Pompeo F. Recent advances in peptidoglycan synthesis and regulation in bacteria. Biomolecules. 2023;13(5):720. https://doi.org/10.3390/biom13050720
9. Barreteau H, Kovac A, Boniface A, Sova M, Gobec S, Blanot D. Cytoplasmic steps of peptidoglycan biosynthesis. FEMS Microbiol Rev. 2008;32(2):168–207. https://doi.org/10.1111/j.1574-6976.2008.00104.x
10. Bouhss A, Trunkfield AE, Bugg TD, Mengin-Lecreulx D. The biosynthesis of peptidoglycan lipid-linked intermediates. FEMS Microbiol Rev. 2008;32(2):208–33. https://doi.org/10.1111/j.1574-6976.2007.00089.x
11. Teo AC, Roper DI. Core steps of membrane-bound peptidoglycan biosynthesis: Recent advances, insight and opportunities. Antibiotics. 2015;4(4):495–520. https://doi.org/10.3390/antibiotics4040495
12. Kumar S, Mollo A, Kahne D, Ruiz N. The bacterial cell wall: From lipid II flipping to polymerization. Chem Rev. 2022;122(9):8884–910. https://doi.org/10.1021/acs.chemrev.1c00773
13. Kumar S, Rubino FA, Mendoza AG, Ruiz N. The bacterial lipid II flippase MurJ functions by an alternating-access mechanism. J Biol Chem. 2019;294(3):981–90. https://doi.org/10.1074/jbc.RA118.006099
14. Ruiz N. Bioinformatics identification of MurJ (MviN) as the peptidoglycan lipid II flippase in *Escherichia coli*. Proc Natl Acad Sci U S A. 2008;105(40):15553–7. https://doi.org/10.1073/pnas.0808352105
15. Sauvage E, Terrak M. Glycosyltransferases and transpeptidases/penicillin-binding proteins: Valuable targets for new antibacterials. Antibiotics. 2016;5(1):12. https://doi.org/10.3390/antibiotics5010012

16. Shaku M, Ealand C, Matlhabe O, Lala R, Kana BD. Chapter Two - Peptidoglycan biosynthesis and remodeling revisited. In: Gadd GM, Sariaslani S, editors. Adv Appl Microbiol. 112: Academic Press; 2020. p. 67–103.
17. Elamin AA, Steinicke S, Oehlmann W, Braun Y, Wanas H, Shuralev EA, et al. Novel drug targets in cell wall biosynthesis exploited by gene disruption in *Pseudomonas aeruginosa*. PLoS One. 2017;12(10):e0186801. https://doi.org/10.1371/journal.pone.0186801
18. Nelson DE, Young KD. Penicillin binding protein 5 affects cell diameter, contour, and morphology of *Escherichia coli*. J Bacteriol. 2000;182(6):1714–21. https://doi.org/10.1128/jb.182.6.1714-1721.2000
19. Popham DL, Young KD. Role of penicillin-binding proteins in bacterial cell morphogenesis. Curr Opin Microbiol. 2003;6(6):594–9. https://doi.org/10.1016/j.mib.2003.10.002
20. Lee TK, Huang KC. The role of hydrolases in bacterial cell-wall growth. Curr Opin Microbiol. 2013;16(6):760–6. https://doi.org/10.1016/j.mib.2013.08.005
21. Pollard TD, Goldman RD. Overview of the cytoskeleton from an evolutionary perspective. Cold Spring Harb Perspect Biol. 2018;10(7):a030288. https://doi.org/10.1101/cshperspect.a030288
22. Erickson HP. Evolution of the cytoskeleton. Bioessays. 2007;29(7):668–77. https://doi.org/10.1002/bies.20601
23. Ingerson-Mahar M, Gitai Z. A growing family: The expanding universe of the bacterial cytoskeleton. FEMS Microbiol Rev. 2012;36(1):256–66. https://doi.org/10.1111/j.1574-6976.2011.00316.x
24. Wagstaff J, Löwe J. Prokaryotic cytoskeletons: Protein filaments organizing small cells. Nat Rev Microbiol. 2018;16(4):187–201. https://doi.org/10.1038/nrmicro.2017.153
25. Cameron TA, Margolin W. Insights into the assembly and regulation of the bacterial divisome. Nat Rev Microbiol. 2024;22(1):33–45. https://doi.org/10.1038/s41579-023-00942-x
26. Shi H, Bratton BP, Gitai Z, Huang KC. How to build a bacterial cell: MreB as the foreman of *E. coli* construction. Cell. 2018;172(6):1294–305. https://doi.org/10.1016/j.cell.2018.02.050
27. Wang Y, jiang Y, Song Z, Zhu C, Tang Y, Peng J, et al. MreB: Unraveling the molecular mechanisms of bacterial shape, division, and environmental adaptation. Cell Commun Signal. 2025;23(1):377. https://doi.org/10.1186/s12964-025-02373-y
28. Amos LA, van den Ent F, Löwe J. Structural/functional homology between the bacterial and eukaryotic cytoskeletons. Curr Opin Cell Biol. 2004;16(1):24–31. https://doi.org/10.1016/j.ceb.2003.11.005
29. Daniel RA, Errington J. Control of cell morphogenesis in bacteria: Two distinct ways to make a rod-shaped cell. Cell. 2003;113(6):767–76. https://doi.org/10.1016/s0092-8674(03)00421-5
30. Ouzounov N, Nguyen JP, Bratton BP, Jacobowitz D, Gitai Z, Shaevitz JW. MreB orientation correlates with cell diameter in *Escherichia coli*. Biophys J. 2016;111(5):1035–43. https://doi.org/10.1016/j.bpj.2016.07.017
31. Dempwolff F, Reimold C, Reth M, Graumann PL. *Bacillus subtilis* MreB orthologs self-organize into filamentous structures underneath the cell membrane in a heterologous cell system. PLoS One. 2011;6(11):e27035. https://doi.org/10.1371/journal.pone.0027035
32. Jones LJ, Carballido-López R, Errington J. Control of cell shape in bacteria: Helical, actin-like filaments in *Bacillus subtilis*. Cell. 2001;104(6):913–22. https://doi.org/10.1016/s0092-8674(01)00287-2
33. van den Ent F, Izoré T, Bharat TA, Johnson CM, Löwe J. Bacterial actin MreB forms antiparallel double filaments. Elife. 2014;3:e02634. https://doi.org/10.7554/eLife.02634

34. Billaudeau C, Yao Z, Cornilleau C, Carballido-López R, Chastanet A. MreB forms subdiffraction nanofilaments during active growth in *Bacillus subtilis*. mBio. 2019;10(1):e01879–18. https://doi.org/10.1128/mBio.01879-18

35. Domínguez-Escobar J, Chastanet A, Crevenna AH, Fromion V, Wedlich-Söldner R, Carballido-López R. Processive movement of MreB-associated cell wall biosynthetic complexes in bacteria. Science. 2011;333(6039):225–8. https://doi.org/10.1126/science.1203466

36. Garner EC, Bernard R, Wang W, Zhuang X, Rudner DZ, Mitchison T. Coupled, circumferential motions of the cell wall synthesis machinery and MreB filaments in *B. subtilis*. Science. 2011;333(6039):222–5. https://doi.org/10.1126/science.1203285

37. van Teeffelen S, Wang S, Furchtgott L, Huang KC, Wingreen NS, Shaevitz JW, et al. The bacterial actin MreB rotates, and rotation depends on cell-wall assembly. Proc Natl Acad Sci U S A. 2011;108(38):15822–7. https://doi.org/10.1073/pnas.1108999108

38. Kruse T, Bork-Jensen J, Gerdes K. The morphogenetic MreBCD proteins of *Escherichia coli* form an essential membrane-bound complex. Mol Microbiol. 2005;55(1):78–89. https://doi.org/10.1111/j.1365-2958.2004.04367.x

39. Liu X, Biboy J, Consoli E, Vollmer W, den Blaauwen T. MreC and MreD balance the interaction between the elongasome proteins PBP2 and RodA. PLoS Genet. 2021;16(12):e1009276. https://doi.org/10.1371/journal.pgen.1009276

40. Cho H, Wivagg CN, Kapoor M, Barry Z, Rohs PDA, Suh H, et al. Bacterial cell wall biogenesis is mediated by SEDS and PBP polymerase families functioning semi-autonomously. Nat Microbiol. 2016;1(10):16172. https://doi.org/10.1038/nmicrobiol.2016.172

41. Nygaard R, Graham CLB, Belcher Dufrisne M, Colburn JD, Pepe J, Hydorn MA, et al. Structural basis of peptidoglycan synthesis by *E. coli* RodA-PBP2 complex. Nat Commun. 2023;14(1):5151. https://doi.org/10.1038/s41467-023-40483-8

42. Banzhaf M, van den Berg van Saparoea B, Terrak M, Fraipont C, Egan A, Philippe J, et al. Cooperativity of peptidoglycan synthases active in bacterial cell elongation. Mol Microbiol. 2012;85(1):179–94. https://doi.org/10.1111/j.1365-2958.2012.08103.x

43. Ikebe R, Kuwabara Y, Chikada T, Niki H, Shiomi D. The periplasmic disordered domain of RodZ promotes its self-interaction in *Escherichia coli*. Genes Cells. 2018;23(4):307–17. https://doi.org/10.1111/gtc.12572

44. Bendezú FO, Hale CA, Bernhardt TG, de Boer PA. RodZ (YfgA) is required for proper assembly of the MreB actin cytoskeleton and cell shape in *E. coli*. EMBO J. 2009;28(3):193–204. https://doi.org/10.1038/emboj.2008.264

45. Bratton BP, Shaevitz JW, Gitai Z, Morgenstein RM. MreB polymers and curvature localization are enhanced by RodZ and predict *E. coli*'s cylindrical uniformity. Nat Commun. 2018;9(1):2797. https://doi.org/10.1038/s41467-018-05186-5

46. Morgenstein RM, Bratton BP, Nguyen JP, Ouzounov N, Shaevitz JW, Gitai Z. RodZ links MreB to cell wall synthesis to mediate MreB rotation and robust morphogenesis. Proc Natl Acad Sci U S A. 2015;112(40):12510–5. https://doi.org/10.1073/pnas.1509610112

47. Mahone CR, Goley ED. Bacterial cell division at a glance. J Cell Sci. 2020;133(7):jcs237057. https://doi.org/10.1242/jcs.237057

48. Hajduk IV, Rodrigues CDA, Harry EJ. Connecting the dots of the bacterial cell cycle: Coordinating chromosome replication and segregation with cell division. Semin Cell Dev Biol. 2016;53:2–9. https://doi.org/10.1016/j.semcdb.2015.11.012

49. McQuillen R, Xiao J. Insights into the structure, function, and dynamics of the bacterial cytokinetic FtsZ-ring. Annu Rev Biophys. 2020;49(1):309–41. https://doi.org/10.1146/annurev-biophys-121219-081703

50. Ithurbide S, Gribaldo S, Albers SV, Pende N. Spotlight on FtsZ-based cell division in Archaea. Trends Microbiol. 2022;30(7):665–78. https://doi.org/10.1016/j.tim.2022.01.005

51. TerBush AD, Yoshida Y, Osteryoung KW. FtsZ in chloroplast division: Structure, function and evolution. Curr Opin Cell Biol. 2013;25(4):461–70. https://doi.org/10.1016/j.ceb.2013.04.006

52. Lutkenhaus JF, Wolf-Watz H, Donachie WD. Organization of genes in the *ftsA-envA* region of the *Escherichia coli* genetic map and identification of a new *fts* locus (*ftsZ*). J Bacteriol. 1980;142(2):615–20. https://doi.org/10.1128/jb.142.2.615-620.1980

53. Bi EF, Lutkenhaus J. FtsZ ring structure associated with division in *Escherichia coli*. Nature. 1991;354(6349):161–4. https://doi.org/10.1038/354161a0

54. Bramhill D, Thompson CM. GTP-dependent polymerization of *Escherichia coli* FtsZ protein to form tubules. Proc Natl Acad Sci U S A. 1994;91(13):5813–7. https://doi.org/10.1073/pnas.91.13.5813

55. de Boer P, Crossley R, Rothfield L. The essential bacterial cell-division protein FtsZ is a GTPase. Nature. 1992;359(6392):254–6. https://doi.org/10.1038/359254a0

56. Erickson HP, Taylor DW, Taylor KA, Bramhill D. Bacterial cell division protein FtsZ assembles into protofilament sheets and minirings, structural homologs of tubulin polymers. Proc Natl Acad Sci U S A. 1996;93(1):519–23. https://doi.org/10.1073/pnas.93.1.519

57. Löwe J, Amos LA. Crystal structure of the bacterial cell-division protein FtsZ. Nature. 1998;391(6663):203–6. https://doi.org/10.1038/34472

58. Mukherjee A, Lutkenhaus J. Dynamic assembly of FtsZ regulated by GTP hydrolysis. EMBO J. 1998;17(2):462–9. https://doi.org/10.1093/emboj/17.2.462

59. Scheffers DJ, de Wit JG, den Blaauwen T, Driessen AJ. GTP hydrolysis of cell division protein FtsZ: Evidence that the active site is formed by the association of monomers. Biochemistry. 2002;41(2):521–9. https://doi.org/10.1021/bi011370i

60. Fu G, Huang T, Buss J, Coltharp C, Hensel Z, Xiao J. *In vivo* structure of the *E. coli* FtsZ-ring revealed by photoactivated localization microscopy (PALM). PLoS One. 2010;5(9):e12682. https://doi.org/10.1371/journal.pone.0012680

61. Holden SJ, Pengo T, Meibom KL, Fernandez Fernandez C, Collier J, Manley S. High throughput 3D super-resolution microscopy reveals *Caulobacter crescentus in vivo* Z-ring organization. Proc Natl Acad Sci U S A. 2014;111(12):4566–71. https://doi.org/10.1073/pnas.1313368111

62. Lyu Z, Coltharp C, Yang X, Xiao J. Influence of FtsZ GTPase activity and concentration on nanoscale Z-ring structure *in vivo* revealed by three-dimensional Superresolution imaging. Biopolymers. 2016;105(10):725–34. https://doi.org/10.1002/bip.22895

63. Bisson-Filho AW, Hsu YP, Squyres GR, Kuru E, Wu F, Jukes C, et al. Treadmilling by FtsZ filaments drives peptidoglycan synthesis and bacterial cell division. Science. 2017;355(6326):739–43. https://doi.org/10.1126/science.aak9973

64. Yang X, Lyu Z, Miguel A, McQuillen R, Huang KC, Xiao J. GTPase activity-coupled treadmilling of the bacterial tubulin FtsZ organizes septal cell wall synthesis. Science. 2017;355(6326):744–7. https://doi.org/10.1126/science.aak9995

65. Merino-Salomón A, Babl L, Schwille P. Self-organized protein patterns: The MinCDE and ParABS systems. Curr Opin Cell Biol. 2021;72:106–15. https://doi.org/10.1016/j.ceb.2021.07.001

66. Burby PE, Simmons LA. Regulation of cell division in bacteria by monitoring genome integrity and DNA replication status. J Bacteriol. 2020;202(2):e00408–19. https://doi.org/10.1128/jb.00408-19

67. Wang M, Fang C, Ma B, Luo X, Hou Z. Regulation of cytokinesis: FtsZ and its accessory proteins. Curr Genet. 2020;66(1):43–9. https://doi.org/10.1007/s00294-019-01005-6

68. Huang KH, Durand-Heredia J, Janakiraman A. FtsZ ring stability: Of bundles, tubules, crosslinks, and curves. J Bacteriol. 2013;195(9):1859–68. https://doi.org/10.1128/jb.02157-12

69. Dajkovic A, Pichoff S, Lutkenhaus J, Wirtz D. Cross-linking FtsZ polymers into coherent Z rings. Mol Microbiol. 2010;78(3):651–68. https://doi.org/10.1111/j.1365-2958.2010.07352.x

70. Gueiros-Filho FJ, Losick R. A widely conserved bacterial cell division protein that promotes assembly of the tubulin-like protein FtsZ. Genes Dev. 2002;16(19):2544–56. https://doi.org/10.1101/gad.1014102

71. Durand-Heredia JM, Yu HH, De Carlo S, Lesser CF, Janakiraman A. Identification and characterization of ZapC, a stabilizer of the FtsZ ring in *Escherichia coli*. J Bacteriol. 2011;193(6):1405–13. https://doi.org/10.1128/jb.01258-10

72. Ebersbach G, Galli E, Møller-Jensen J, Löwe J, Gerdes K. Novel coiled-coil cell division factor ZapB stimulates Z ring assembly and cell division. Mol Microbiol. 2008;68(3):720–35. https://doi.org/10.1111/j.1365-2958.2008.06190.x

73. Hale CA, Shiomi D, Liu B, Bernhardt TG, Margolin W, Niki H, et al. Identification of *Escherichia coli* ZapC (YcbW) as a component of the division apparatus that binds and bundles FtsZ polymers. J Bacteriol. 2011;193(6):1393–404. https://doi.org/10.1128/jb.01245-10

74. Durand-Heredia J, Rivkin E, Fan G, Morales J, Janakiraman A. Identification of ZapD as a cell division factor that promotes the assembly of FtsZ in *Escherichia coli*. J Bacteriol. 2012;194(12):3189–98. https://doi.org/10.1128/jb.00176-12

75. Marteyn BS, Karimova G, Fenton AK, Gazi AD, West N, Touqui L, et al. ZapE is a novel cell division protein interacting with FtsZ and modulating the Z-ring dynamics. mBio. 2014;5(2):e00022–14. https://doi.org/10.1128/mBio.00022-14

76. DiBiasio EC, Dickinson RA, Trebino CE, Ferreira CN, Morrison JJ, Camberg JL. The stress-active cell division protein ZapE alters FtsZ filament architecture to facilitate division in *Escherichia coli*. Front Microbiol. 2021;12:733085. https://doi.org/10.3389/fmicb.2021.733085

77. Naha A, Haeusser DP, Margolin W. Anchors: A way for FtsZ filaments to stay membrane bound. Mol Microbiol. 2023;120(4):525–38. https://doi.org/10.1111/mmi.15067

78. Pichoff S, Lutkenhaus J. Unique and overlapping roles for ZipA and FtsA in septal ring assembly in *Escherichia coli*. EMBO J. 2002;21(4):685–93. https://doi.org/10.1093/emboj/21.4.685

79. Pichoff S, Lutkenhaus J. Tethering the Z ring to the membrane through a conserved membrane targeting sequence in FtsA. Mol Microbiol. 2005;55(6):1722–34. https://doi.org/10.1111/j.1365-2958.2005.04522.x

80. Pichoff S, Lutkenhaus J. Identification of a region of FtsA required for interaction with FtsZ. Mol Microbiol. 2007;64(4):1129–38. https://doi.org/10.1111/j.1365-2958.2007.05735.x

81. Szwedziak P, Wang Q, Freund SM, Löwe J. FtsA forms actin-like protofilaments. EMBO J. 2012;31(10):2249–60. https://doi.org/10.1038/emboj.2012.76

82. Haney SA, Glasfeld E, Hale C, Keeney D, He Z, de Boer P. Genetic analysis of the *Escherichia coli* FtsZ. ZipA interaction in the yeast two-hybrid system. Characterization of FtsZ residues essential for the interactions with ZipA and with FtsA. J Biol Chem. 2001;276(15):11980–7. https://doi.org/10.1074/jbc.M009810200

83. Liu Z, Mukherjee A, Lutkenhaus J. Recruitment of ZipA to the division site by interaction with FtsZ. Mol Microbiol. 1999;31(6):1853–61. https://doi.org/10.1046/j.1365-2958.1999.01322.x

84. RayChaudhuri D. ZipA is a MAP–Tau homolog and is essential for structural integrity of the cytokinetic FtsZ ring during bacterial cell division. EMBO J. 1999;18(9):2372–83–83. https://doi.org/10.1093/emboj/18.9.2372

85. Duman R, Ishikawa S, Celik I, Strahl H, Ogasawara N, Troc P. Structural and genetic analyses reveal the protein SepF as a new membrane anchor for the Z ring. Proc Natl Acad Sci U S A. 2013;110(48):4601–10. https://doi.org/10.1073/pnas.1313978110

86. Ishikawa S, Kawai Y, Hiramatsu K, Kuwano M, Ogasawara N. A new FtsZ-interacting protein, YlmF, complements the activity of FtsA during progression of cell division in *Bacillus subtilis*. Mol Microbiol. 2006;60(6):1364–80. https://doi.org/10.1111/j.1365-2958.2006.05184.x

87. Gola S, Munder T, Casonato S, Manganelli R, Vicente M. The essential role of SepF in mycobacterial division. Mol Microbiol. 2015;97(3):560–76. https://doi.org/10.1111/mmi.13050

88. Sogues A, Martinez M, Gaday Q, Ben Assaya M, Graña M, Voegele A. Essential dynamic interdependence of FtsZ and SepF for Z-ring and septum formation in *Corynebacterium glutamicum*. Nat Commun. 2020;11(1):1641. https://doi.org/10.1038/s41467-020-15490-8

89. Berezuk AM, Roach EJ, Seidel L, Lo RY, Khursigara CM. FtsA G50E mutant suppresses the essential requirement for FtsK during bacterial cell division in *Escherichia coli*. Can J Microbiol. 2020;66(4):313–27. https://doi.org/10.1139/cjm-2019-0493

90. Du S, Henke W, Pichoff S, Lutkenhaus J. How FtsEX localizes to the Z ring and interacts with FtsA to regulate cell division. Mol Microbiol. 2019;112(3):881–95. https://doi.org/10.1111/mmi.14324

91. Baranova N, Radler P, Hernández-Rocamora VM, Alfonso C, López-Pelegrín M, Rivas G, et al. Diffusion and capture permits dynamic coupling between treadmilling FtsZ filaments and cell division proteins. Nat Microbiol. 2020;5(3):407–17. https://doi.org/10.1038/s41564-019-0657-5

92. Busiek KK, Margolin W. A role for FtsA in SPOR-independent localization of the essential *Escherichia coli* cell division protein FtsN. Mol Microbiol. 2014;92(6):1212–26. https://doi.org/10.1111/mmi.12623

93. Pazos M, Peters K, Casanova M, Palacios P, VanNieuwenhze M, Breukink E, et al. Z-ring membrane anchors associate with cell wall synthases to initiate bacterial cell division. Nat Commun. 2018;9(1):5090. https://doi.org/10.1038/s41467-018-07559-2

94. Crozat E, Grainge I. FtsK DNA translocase: The fast motor that knows where it's going. ChemBioChem. 2010;11(16):2232–43. https://doi.org/10.1002/cbic.201000347

95. Chan H, Mohamed AMT, Grainge I, Rodrigues CDA. FtsK and SpoIIIE, coordinators of chromosome segregation and envelope remodeling in bacteria. Trends Microbiol. 2022;30(5):480–94. https://doi.org/10.1016/j.tim.2021.10.002

96. Liu G, Draper GC, Donachie WD. FtsK is a bifunctional protein involved in cell division and chromosome localization in *Escherichia coli*. Mol Microbiol. 1998;29(3):893–903. https://doi.org/10.1046/j.1365-2958.1998.00986.x

97. Du S, Pichoff S, Lutkenhaus J. Roles of ATP hydrolysis by FtsEX and interaction with FtsA in regulation of septal peptidoglycan synthesis and hydrolysis. mBio. 2020;11(4). https://doi.org/10.1128/mBio.01247-20

98. Yang DC, Peters NT, Parzych KR, Uehara T, Markovski M, Bernhardt TG. An ATP-binding cassette transporter-like complex governs cell-wall hydrolysis at the bacterial cytokinetic ring. Proc Natl Acad Sci U S A. 2011;108(45):E1052–60. https://doi.org/10.1073/pnas.1107780108

99. Uehara T, Parzych KR, Dinh T, Bernhardt TG. Daughter cell separation is controlled by cytokinetic ring-activated cell wall hydrolysis. EMBO J. 2010;29(8):1412–22. https://doi.org/10.1038/emboj.2010.36

100. Fraipont C, Alexeeva S, Wolf B, van der Ploeg R, Schloesser M, den Blaauwen T, et al. The integral membrane FtsW protein and peptidoglycan synthase PBP3 form a subcomplex in *Escherichia coli*. Microbiology. 2011;157(Pt 1):251–9. https://doi.org/10.1099/mic.0.040071-0

101. Park KT, Du S, Lutkenhaus J. Essential role for FtsL in activation of septal peptidoglycan synthesis. mBio. 2020;11(6):e03012–20. https://doi.org/10.1128/mBio.03012-20

102. Boes A, Olatunji S, Breukink E, Terrak M. Regulation of the peptidoglycan polymerase activity of PBP1b by antagonist actions of the core divisome proteins FtsBLQ and FtsN. mBio. 2019;10(1):e01912–18. https://doi.org/10.1128/mBio.01912-18

103. Boes A, Kerff F, Herman R, Touze T, Breukink E, Terrak M. The bacterial cell division protein fragment (E)FtsN binds to and activates the major peptidoglycan synthase PBP1b. J Biol Chem. 2020;295(52):18256–65. https://doi.org/10.1074/jbc.RA120.015951

104. Liu B, Persons L, Lee L, de Boer PAJ. Roles for both FtsA and the FtsBLQ subcomplex in FtsN-stimulated cell constriction in *Escherichia coli*. Mol Microbiol. 2015;95(6):945–70. https://doi.org/10.1111/mmi.12906

105. Müller P, Ewers C, Bertsche U, Anstett M, Kallis T, Breukink E, et al. The essential cell division protein FtsN interacts with the murein (peptidoglycan) synthase PBP1B in *Escherichia coli*. J Biol Chem. 2007;282(50):36394–402. https://doi.org/10.1074/jbc.M706390200

106. Kawalek A, Wawrzyniak P, Bartosik AA, Jagura-Burdzy G. Rules and exceptions: The role of chromosomal ParB in DNA segregation and other cellular processes. Microorganisms. 2020;8(1). https://doi.org/10.3390/microorganisms8010105

107. Jalal ASB, Le TBK. Bacterial chromosome segregation by the ParABS system. Open Biol. 2020;10(6):200097. https://doi.org/10.1098/rsob.200097

108. Gruber S, Errington J. Recruitment of condensin to replication origin regions by ParB/SpoOJ promotes chromosome segregation in *B. subtilis*. Cell. 2009;137(4):685–96. https://doi.org/10.1016/j.cell.2009.02.035

109. Nolivos S, Sherratt D. The bacterial chromosome: Architecture and action of bacterial SMC and SMC-like complexes. FEMS Microbiol Rev. 2014;38(3):380–92. https://doi.org/10.1111/1574-6976.12045

110. Uhlmann F. A unified model for cohesin function in sister chromatid cohesion and chromatin loop formation. Mol Cell. 2025;85(6):1058–71. https://doi.org/10.1016/j.molcel.2025.02.005

111. Hiraga S, Niki H, Ogura T, Ichinose C, Mori H, Ezaki B, et al. Chromosome partitioning in *Escherichia coli*: Novel mutants producing anucleate cells. J Bacteriol. 1989;171(3):1496–505. https://doi.org/10.1128/jb.171.3.1496-1505.1989

112. Yamanaka K, Ogura T, Niki H, Hiraga S. Identification of two new genes, *mukE* and *mukF*, involved in chromosome partitioning in *Escherichia coli*. Mol Gen Genet. 1996;250(3):241–51. https://doi.org/10.1007/bf02174381

113. Espéli O, Borne R, Dupaigne P, Thiel A, Gigant E, Mercier R, et al. A MatP-divisome interaction coordinates chromosome segregation with cell division in *E. coli*. EMBO J. 2012;31(14):3198–211. https://doi.org/10.1038/emboj.2012.128

114. Männik J, Castillo DE, Yang D, Siopsis G, Männik J. The role of MatP, ZapA and ZapB in chromosomal organization and dynamics in *Escherichia coli*. Nucleic Acids Res. 2016;44(3):1216–26. https://doi.org/10.1093/nar/gkv1484

115. Mercier R, Petit MA, Schbath S, Robin S, El Karoui M, Boccard F, et al. The MatP/*matS* site-specific system organizes the terminus region of the *E. coli* chromosome into a macrodomain. Cell. 2008;135(3):475–85. https://doi.org/10.1016/j.cell.2008.08.031

116. Nolivos S, Upton AL, Badrinarayanan A, Müller J, Zawadzka K, Wiktor J, et al. MatP regulates the coordinated action of topoisomerase IV and MukBEF in chromosome segregation. Nat Commun. 2016;7:10466. https://doi.org/10.1038/ncomms10466

117. Hirsch J, Klostermeier D. What makes a type IIA topoisomerase a gyrase or a Topo IV? Nucleic Acids Res. 2021;49(11):6027–42. https://doi.org/10.1093/nar/gkab270

118. Duprey A, Groisman EA. The regulation of DNA supercoiling across evolution. Protein Sci. 2021;30(10):2042–56. https://doi.org/10.1002/pro.4171

119. Yang DC, Blair KM, Salama NR. Staying in shape: The impact of cell shape on bacterial survival in diverse environments. Microbiol Mol Biol Rev. 2016;80(1):187–203. https://doi.org/10.1128/mmbr.00031-15

120. Young KD. Bacterial morphology: Why have different shapes? Curr Opin Microbiol. 2007;10(6):596–600. https://doi.org/10.1016/j.mib.2007.09.009

121. Young KD. The selective value of bacterial shape. Microbiol Mol Biol Rev. 2006;70(3):660–703. https://doi.org/10.1128/mmbr.00001-06

122. Pinho MG, Kjos M, Veening JW. How to get (a)round: Mechanisms controlling growth and division of coccoid bacteria. Nat Rev Microbiol. 2013;11(9):601–14. https://doi.org/10.1038/nrmicro3088

123. Ramos-León F, Ramamurthi KS. How do spherical bacteria regulate cell division? Biochem Soc Trans. 2025;53(2):447–60. https://doi.org/10.1042/bst20240956

124. Reichmann NT, Tavares AC, Saraiva BM, Jousselin A, Reed P, Pereira AR, et al. SEDS-bPBP pairs direct lateral and septal peptidoglycan synthesis in *Staphylococcus aureus*. Nat Microbiol. 2019;4(8):1368–77. https://doi.org/10.1038/s41564-019-0437-2

125. Costa SF, Saraiva BM, Veiga H, Marques LB, Schäper S, Sporniak M, et al. The role of GpsB in *Staphylococcus aureus* cell morphogenesis. mBio. 2024;15(3):e03235–23. https://doi.org/10.1128/mbio.03235-23

126. Cameron TA, Zupan JR, Zambryski PC. The essential features and modes of bacterial polar growth. Trends Microbiol. 2015;23(6):347–53. https://doi.org/10.1016/j.tim.2015.01.003

127. Zupan JR, Grangeon R, Robalino-Espinosa JS, Garnica N, Zambryski P. GROWTH POLE RING protein forms a 200-nm-diameter ring structure essential for polar growth and rod shape in *Agrobacterium tumefaciens*. Proc Natl Acad Sci U S A. 2019;116(22):10962–7. https://doi.org/10.1073/pnas.1905900116

128. Kang CM, Nyayapathy S, Lee JY, Suh JW, Husson RN. Wag31, a homologue of the cell division protein DivIVA, regulates growth, morphology and polar cell wall synthesis in mycobacteria. Microbiology. 2008;154(Pt 3):725–35. https://doi.org/10.1099/mic.0.2007/014076-0

129. Woldemeskel SA, Goley ED. Shapeshifting to survive: Shape determination and regulation in *Caulobacter crescentus*. Trends Microbiol. 2017;25(8):673–87. https://doi.org/10.1016/j.tim.2017.03.006

130. Figge RM, Divakaruni AV, Gober JW. MreB, the cell shape-determining bacterial actin homologue, co-ordinates cell wall morphogenesis in *Caulobacter crescentus*. Mol Microbiol. 2004;51(5):1321–32. https://doi.org/10.1111/j.1365-2958.2003.03936.x

131. Ausmees N, Kuhn JR, Jacobs-Wagner C. The bacterial cytoskeleton: An intermediate filament-like function in cell shape. Cell. 2003;115(6):705–13. https://doi.org/10.1016/s0092-8674(03)00935-8

132. Cabeen MT, Charbon G, Vollmer W, Born P, Ausmees N, Weibel DB, et al. Bacterial cell curvature through mechanical control of cell growth. EMBO J. 2009;28(9):1208–19. https://doi.org/10.1038/emboj.2009.61

133. Bartlett TM, Bratton BP, Duvshani A, Miguel A, Sheng Y, Martin NR, et al. A periplasmic polymer curves *Vibrio cholerae* and promotes pathogenesis. Cell. 2017;168(1-2):172–85.e15. https://doi.org/10.1016/j.cell.2016.12.019

134. Cheng T, Boneca IG. The shapeshifting *Helicobacter pylori*: From a corkscrew to a ball. Mol Microbiol. 2024;121(2):260–74. https://doi.org/10.1111/mmi.15218

135. San Martin F, Fule L, Iraola G, Buschiazzo A, Picardeau M. Diving into the complexity of the spirochetal endoflagellum. Trends Microbiol. 2023;31(3):294–307. https://doi.org/10.1016/j.tim.2022.09.010

136. Motaleb MA, Corum L, Bono JL, Elias AF, Rosa P, Samuels DS, et al. *Borrelia burgdorferi* periplasmic flagella have both skeletal and motility functions. Proc Natl Acad Sci U S A. 2000;97(20):10899–904. https://doi.org/10.1073/pnas.200221797

137. Caccamo PD, Brun YV. The molecular basis of noncanonical bacterial morphology. Trends Microbiol. 2018;26(3):191–208. https://doi.org/10.1016/j.tim.2017.09.012

138. Govers SK, Jacobs-Wagner C. *Caulobacter crescentus*: Model system extraordinaire. Curr Biol. 2020;30(19):R1151–R8. https://doi.org/10.1016/j.cub.2020.07.033

139. Armitage JP, Berry RM. Assembly and dynamics of the bacterial flagellum. Annu Rev Microbiol. 2020;74:181–200. https://doi.org/10.1146/annurev-micro-090816-093411

140. Shanmugasundarasamy T, Karaiyagowder Govindarajan D, Kandaswamy K. A review on pilus assembly mechanisms in Gram-positive and Gram-negative bacteria. Cell Surf. 2022;8:100077. https://doi.org/10.1016/j.tcsw.2022.100077

141. Barrows JM, Goley ED. Synchronized swarmers and sticky stalks: *Caulobacter crescentus* as a model for bacterial cell biology. J Bacteriol. 2023;205(2):e0038422. https://doi.org/10.1128/jb.00384-22

142. Baranowski C, Rego EH, Rubin Eric J. The dream of a mycobacterium. Microbiol Spectr. 2019;7(2):10.1128/microbiolspec.gpp3–0008–2018. https://doi.org/10.1128/microbiolspec.gpp3-0008-2018

143. Shen JP, Chou CF. Morphological plasticity of bacteria-Open questions. Biomicrofluidics. 2016;10(3):031501. https://doi.org/10.1063/1.4953660

144. Justice SS, Harrison A, Becknell B, Mason KM. Bacterial differentiation, development, and disease: Mechanisms for survival. FEMS Microbiol Lett. 2014;360(1):1–8. https://doi.org/10.1111/1574-6968.12602

145. Errington J, Wu LJ. Cell cycle machinery in *Bacillus subtilis*. Subcell Biochem. 2017;84:67–101. https://doi.org/10.1007/978-3-319-53047-5_3

146. Flärdh K, Buttner MJ. *Streptomyces* morphogenetics: Dissecting differentiation in a filamentous bacterium. Nat Rev Microbiol. 2009;7(1):36–49. https://doi.org/10.1038/nrmicro1968

147. Persat A, Stone HA, Gitai Z. The curved shape of *Caulobacter crescentus* enhances surface colonization in flow. Nat Commun. 2014;5:3824. https://doi.org/10.1038/ncomms4824

148. Gonin M, Quardokus EM, O'Donnol D, Maddock J, Brun YV. Regulation of stalk elongation by phosphate in *Caulobacter crescentus*. J Bacteriol. 2000;182(2):337–47. https://doi.org/10.1128/jb.182.2.337-347.2000

149. Greenberg EP, Canale-Parola E. Relationship between cell coiling and motility of spirochetes in viscous environments. J Bacteriol. 1977;131(3):960–9. https://doi.org/10.1128/jb.131.3.960-969.1977

150. Jeltsch A. Oxygen, epigenetic signaling, and the evolution of early life. Trends Biochem Sci. 2013;38(4):172–6. https://doi.org/10.1016/j.tibs.2013.02.001

151. Shapiro JA. Thinking about bacterial populations as multicellular organisms. Annu Rev Microbiol. 1998;52:81–104. https://doi.org/10.1146/annurev.micro.52.1.81

152. Shapiro JA. Bacteria as multicellular organisms. Scientific American. 1988;258(6):82–9.

153. Claessen D, Rozen DE, Kuipers OP, Søgaard-Andersen L, van Wezel GP. Bacterial solutions to multicellularity: A tale of biofilms, filaments and fruiting bodies. Nat Rev Microbiol. 2014;12(2):115–24. https://doi.org/10.1038/nrmicro3178

154. Lyons NA, Kolter R. On the evolution of bacterial multicellularity. Curr Opin Microbiol. 2015;24:21–8. https://doi.org/10.1016/j.mib.2014.12.007

155. Grosberg RK, Strathmann RR. The evolution of multicellularity: A minor major transition? Annu Rev Ecol Evol Syst. 2007;38:621–54. https://doi.org/10.1146/annurev.ecolsys.36.102403.114735

156. Rebolleda-Gómez M, Travisano M. The cost of being big: Local competition, importance of dispersal, and experimental evolution of reversal to unicellularity. Am Nat. 2018;192(6):731–44. https://doi.org/10.1086/700095

157. Stoy KS, Ratcliff WC. Uncovering the hidden complexity of multicellular magnetotactic bacteria. PLoS Biol. 2024;22(7):e3002695. https://doi.org/10.1371/journal.pbio.3002695

158. Herrero A, Stavans J, Flores E. The multicellular nature of filamentous heterocyst-forming cyanobacteria. FEMS Microbiol Rev. 2016;40(6):831–54. https://doi.org/10.1093/femsre/fuw029

159. Bush MJ, Casu B, Schlimpert S. Dividing lines: Compartmentalisation and division in *Streptomyces*. Curr Opin Microbiol. 2025;85:102611. https://doi.org/10.1016/j.mib.2025.102611

160. Penesyan A, Paulsen IT, Kjelleberg S, Gillings MR. Three faces of biofilms: A microbial lifestyle, a nascent multicellular organism, and an incubator for diversity. NPJ Biofilms Microbiomes. 2021;7(1):80. https://doi.org/10.1038/s41522-021-00251-2

161. Vlamakis H, Chai Y, Beauregard P, Losick R, Kolter R. Sticking together: Building a biofilm the *Bacillus subtilis* way. Nat Rev Microbiol. 2013;11(3):157–68. https://doi.org/10.1038/nrmicro2960

162. Kroos L, Wall D, Islam ST, Whitworth DE, Muñoz-Dorado J, Higgs PI, et al. Milestones in the development of *Myxococcus xanthus* as a model multicellular bacterium. J Bacteriol. 2025;207(7):e00071–25. https://doi.org/10.1128/jb.00071-25

163. Muñoz-Dorado J, Marcos-Torres FJ, García-Bravo E, Moraleda-Muñoz A, Pérez J. Myxobacteria: Moving, killing, feeding, and surviving together. Front Microbiol. 2016;7:781. https://doi.org/10.3389/fmicb.2016.00781

164. Herrou J, Murat D, Mignot T. Gear up! An overview of the molecular equipment used by *Myxococcus* to move, kill, and divide in prey colonies. Curr Opin Microbiol. 2024;80:102492. https://doi.org/10.1016/j.mib.2024.102492

165. Gmiter D, Kaca W. Into the understanding the multicellular lifestyle of *Proteus mirabilis* on solid surfaces. Front Cell Infect Microbiol. 2022;12:864305. https://doi.org/10.3389/fcimb.2022.864305

166. Herrero A. Distinct cell division features in *Anabaena*, a multicellular cyanobacterium. J Bacteriol. 2025;207(7):e00032–25. https://doi.org/10.1128/jb.00032-25

167. Traxler MF, Rozen DE. Ecological drivers of division of labour in *Streptomyces*. Curr Opin Microbiol. 2022;67:102148. https://doi.org/10.1016/j.mib.2022.102148

168. Wildermuth H. Development and organization of the aerial mycelium in *Streptomyces coelicolor*. Microbiology. 1970;60(1):43–50. https://doi.org/10.1099/00221287-60-1-43

169. Liu S, Feng X, Zhang H, Li P, Yang B, Gu Q. Decoding bacterial communication: Intracellular signal transduction, quorum sensing, and cross-kingdom interactions. Microbiol Res. 2025;292:127995. https://doi.org/10.1016/j.micres.2024.127995

170. Wall D. Molecular recognition in myxobacterial outer membrane exchange: Functional, social and evolutionary implications. Mol Microbiol. 2014;91(2):209–20. https://doi.org/10.1111/mmi.12450

171. Wall D. Kin recognition in bacteria. Annu Rev Microbiol. 2016;70:143–60. https://doi.org/10.1146/annurev-micro-102215-095325

172. Yaniv K, Gibbs KA. Kin recognition systems and their role in multicellular behaviors. Annu Rev Microbiol. 2025;79:475–95. https://doi.org/10.1146/annurev-micro-051724-092527

173. Gibbs KA, Urbanowski ML, Greenberg EP. Genetic determinants of self identity and social recognition in bacteria. Science. 2008;321(5886):256–9. https://doi.org/10.1126/science.1160033

174. Wenren LM, Sullivan NL, Cardarelli L, Septer AN, Gibbs KA. Two independent pathways for self-recognition in *Proteus mirabilis* are linked by type VI-dependent export. mBio. 2013;4(4):e00374–13. https://doi.org/10.1128/mBio.00374-13

175. Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Klenk H-P, et al. Taxonomy, physiology, and natural products of *Actinobacteria*. Microbiol Mol Biol Rev. 2016;80(1):1–43. https://doi.org/doi:10.1128/mmbr.00019-15

176. Hazarika SN, Thakur D. Chapter 21 - Actinobacteria. In: Amaresan N, Senthil Kumar M, Annapurna K, Kumar K, Sankaranarayanan A, editors. Beneficial Microbes in Agro-Ecology: Academic Press; 2020. p. 443–76.

177. El-Naggar NE-A. Chapter 11 - *Streptomyces*-based cell factories for production of biomolecules and bioactive metabolites. In: Singh V, editor. Microbial Cell Factories Engineering for Production of Biomolecules: Academic Press; 2021. p. 183–234.

178. Seipke RF, Kaltenpoth M, Hutchings MI. *Streptomyces* as symbionts: An emerging and widespread theme? FEMS Microbiol Rev. 2012;36(4):862–76. https://doi.org/10.1111/j.1574-6976.2011.00313.x

179. Loria R, Coombs J, Yoshida M, Kers J, Bukhalid R. A paucity of bacterial root diseases: *Streptomyces* succeeds where others fail. Physiol Mol Plant Pathol. 2003;62(2):65–72. https://doi.org/10.1016/S0885-5765(03)00041-9

180. Quintana ET, Wierzbicka K, Mackiewicz P, Osman A, Fahal AH, Hamid ME, et al. *Streptomyces sudanensis* sp. nov., a new pathogen isolated from patients with actinomycetoma. Antonie Van Leeuwenhoek. 2008;93(3):305–13. https://doi.org/10.1007/s10482-007-9205-z

181. Chater KF, Biró S, Lee KJ, Palmer T, Schrempf H. The complex extracellular biology of *Streptomyces*. FEMS Microbiol Rev. 2010;34(2):171–98. https://doi.org/10.1111/j.1574-6976.2009.00206.x

182. Morin LMC, Dekoninck K, Sridhar V, Disney-McKeethen S, Proctor T, Eng AY, et al. Why do filamentous Actinomycetota produce such a vast array of specialized metabolites? Annu Rev Microbiol. 2025;79:753–72. https://doi.org/10.1146/annurev-micro-060424-051257

183. Schlimpert S, Elliot MA. The best of both worlds-*Streptomyces coelicolor* and *Streptomyces venezuelae* as model species for studying antibiotic production and bacterial multicellular development. J Bacteriol. 2023;205(7):e0015323. https://doi.org/10.1128/jb.00153-23

184. Nikolaidis M, Hesketh A, Frangou N, Mossialos D, Van de Peer Y, Oliver SG, et al. A panoramic view of the genomic landscape of the genus *Streptomyces*. Microb Genom. 2023;9(6). https://doi.org/10.1099/mgen.0.001028

185. Zaburannyi N, Rabyk M, Ostash B, Fedorenko V, Luzhetskyy A. Insights into naturally minimised *Streptomyces albus* J1074 genome. BMC Genomics. 2014;15:97. https://doi.org/10.1186/1471-2164-15-97

186. Wood V, Gwilliam R, Rajandream MA, Lyne M, Lyne R, Stewart A, et al. The genome sequence of *Schizosaccharomyces pombe*. Nature. 2002;415(6874):871–80. https://doi.org/10.1038/nature724

187. Bentley SD, Chater KF, Cerdeño-Tárraga AM, Challis GL, Thomson NR, James KD, et al. Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). Nature. 2002;417(6885):141–7. https://doi.org/10.1038/417141a

188. Lin YS, Kieser HM, Hopwood DA, Chen CW. The chromosomal DNA of *Streptomyces lividans* 66 is linear. Mol Microbiol. 1993;10(5):923–33. https://doi.org/10.1111/j.1365-2958.1993.tb00964.x

189. Bao K, Cohen SN. Terminal proteins essential for the replication of linear plasmids and chromosomes in *Streptomyces*. Genes Dev. 2001;15(12):1518–27. https://doi.org/10.1101/gad.896201

190. Huang CH, Lin YS, Yang YL, Huang SW, Chen CW. The telomeres of *Streptomyces* chromosomes contain conserved palindromic sequences with potential to form complex secondary structures. Mol Microbiol. 1998;28(5):905–16. https://doi.org/10.1046/j.1365-2958.1998.00856.x

191. Hopwood DA. Soil to genomics: The *Streptomyces* chromosome. Annu Rev Genet. 2006;40:1–23. https://doi.org/10.1146/annurev.genet.40.110405.090639

192. Bury-Moné S, Thibessard A, Lioy VS, Leblond P. Dynamics of the *Streptomyces* chromosome: Chance and necessity. Trends Genet. 2023;39(11):873–87. https://doi.org/10.1016/j.tig.2023.07.008

193. Alam K, Mazumder A, Sikdar S, Zhao Y-M, Hao J, Song C, et al. *Streptomyces*: The biofactory of secondary metabolites. Front Microbiol. 2022;13:968053. https://doi.org/10.3389/fmicb.2022.968053

194. Nguyen P-T, Ly L-P, Le M-T, Vuong B-N, Phan P-L, Nguyen H-T. Unlocking *Streptomyces* biosynthetic gene clusters: Bioelicitors, co-culture, and beyond. Syst Microbiol Biomanuf. 2025;6:1. https://doi.org/10.1007/s43393-025-00397-6

195. Schatz A, Bugle E, Waksman SA. Streptomycin, a substance exhibiting antibiotic activity against Gram-positive and Gram-negative bacteria. Proc Soc Exp Biol Med. 1944;55(1):66–9. https://doi.org/10.3181/00379727-55-14461

196. Belknap KC, Park CJ, Barth BM, Andam CP. Genome mining of biosynthetic and chemotherapeutic gene clusters in *Streptomyces* bacteria. Sci Rep. 2020;10(1):2003. https://doi.org/10.1038/s41598-020-58904-9

197. Bobek J, Šmídová K, Čihák M. A Waking review: Old and novel insights into the spore germination in *Streptomyces*. Front Microbiol. 2017;8:2205. https://doi.org/10.3389/fmicb.2017.02205

198. Mikulík K, Bobek J, Bezoušková S, Benada O, Kofroňová O. Expression of proteins and protein kinase activity during germination of aerial spores of *Streptomyces granaticolor*. Biochem Biophys Res Commun. 2002;299(2):335–42. https://doi.org/10.1016/S0006-291X(02)02606-2

199. Strakova E, Bobek J, Zikova A, Rehulka P, Benada O, Rehulkova H, et al. Systems insight into the spore germination of *Streptomyces coelicolor*. J Proteome Res. 2013;12(1):525–36. https://doi.org/10.1021/pr300980v

200. Rueda B, Miguélez EM, Hardisson C, Manzanal MB. Changes in glycogen and trehalose content of *Streptomyces brasiliensis* hyphae during growth in liquid cultures under sporulating and non-sporulating conditions. FEMS Microbiol Lett. 2001;194(2):181–5. https://doi.org/10.1111/j.1574-6968.2001.tb09466.x

201. De Jong W, Manteca A, Sanchez J, Bucca G, Smith CP, Dijkhuizen L, et al. NepA is a structural cell wall protein involved in maintenance of spore dormancy in *Streptomyces coelicolor*. Mol Microbiol. 2009;71(6):1591–603. https://doi.org/10.1111/j.1365-2958.2009.06633.x

202. Haiser HJ, Yousef MR, Elliot MA. Cell wall hydrolases affect germination, vegetative growth, and sporulation in *Streptomyces coelicolor*. J Bacteriol. 2009;191(21):6501–12. https://doi.org/10.1128/JB.00767-09

203. Sexton DL, Herlihey FA, Brott AS, Crisante DA, Shepherdson E, Clarke AJ, et al. Roles of LysM and LytM domains in resuscitation-promoting factor (Rpf) activity and Rpf-mediated peptidoglycan cleavage and dormant spore reactivation. J Biol Chem. 2020;295(27):9171–82. https://doi.org/10.1074/jbc.RA120.013994

204. Sexton DL, St-Onge RJ, Haiser HJ, Yousef MR, Brady L, Gao C, et al. Resuscitation-promoting factors are cell wall-lytic enzymes with important roles in the germination and growth of *Streptomyces coelicolor*. J Bacteriol. 2015;197(5):848–60. https://doi.org/10.1128/jb.02464-14

205. Derouaux A, Halici S, Nothaft H, Neutelings T, Moutzourelis G, Dusart J, et al. Deletion of a cyclic AMP receptor protein homologue diminishes germination and affects morphological development of *Streptomyces coelicolor*. J Bacteriol. 2004;186(6):1893–7. https://doi.org/10.1128/jb.186.6.1893-1897.2004

206. Piette A, Derouaux A, Gerkens P, Noens EE, Mazzucchelli G, Vion S, et al. From dormant to germinating spores of *Streptomyces coelicolor* A3(2): New perspectives from the *crp* null mutant. J Proteome Res. 2005;4(5):1699–708. https://doi.org/10.1021/pr050155b

207. St-Onge RJ, Haiser HJ, Yousef MR, Sherwood E, Tschowri N, Al-Bassam M, et al. Nucleotide second messenger-mediated regulation of a muralytic enzyme in *Streptomyces*. Mol Microbiol. 2015;96(4):779–95. https://doi.org/10.1111/mmi.12971

208. Flärdh K. Essential role of DivIVA in polar growth and morphogenesis in *Streptomyces coelicolor* A3(2). Mol Microbiol. 2003;49(6):1523–36. https://doi.org/10.1046/j.1365-2958.2003.03660.x

209. Flärdh K. Growth polarity and cell division in *Streptomyces*. Curr Opin Microbiol. 2003;6(6):564–71. https://doi.org/10.1016/j.mib.2003.10.011

210. Flärdh K, Richards DM, Hempel AM, Howard M, Buttner MJ. Regulation of apical growth and hyphal branching in *Streptomyces*. Curr Opin Microbiol. 2012;15(6):737–43. https://doi.org/10.1016/j.mib.2012.10.012

211. Hempel AM, Wang SB, Letek M, Gil JA, Flärdh K. Assemblies of DivIVA mark sites for hyphal branching and can establish new zones of cell wall growth in *Streptomyces coelicolor*. J Bacteriol. 2008;190(22):7579–83. https://doi.org/10.1128/jb.00839-08

212. Richards DM, Hempel AM, Flärdh K, Buttner MJ, Howard M. Mechanistic basis of branch-site selection in filamentous bacteria. PLoS Comput Biol. 2012;8(3):e1002423. https://doi.org/10.1371/journal.pcbi.1002423

213. Zambri MP, Williams MA, Elliot MA. How *Streptomyces* thrive: Advancing our understanding of classical development and uncovering new behaviors. Adv Microb Physiol. 2022;80:203–36. https://doi.org/10.1016/bs.ampbs.2022.01.004

214. Bush MJ, Tschowri N, Schlimpert S, Flärdh K, Buttner MJ. c-di-GMP signalling and the regulation of developmental transitions in streptomycetes. Nat Rev Microbiol. 2015;13(12):749–60. https://doi.org/10.1038/nrmicro3546

215. Hopwood DA, Wildermuth H, Palmer HM. Mutants of *Streptomyces coelicolor* defective in sporulation. J Gen Microbiol. 1970;61(3):397–408. https://doi.org/10.1099/00221287-61-3-397

216. Merrick MJ. A morphological and genetic mapping study of bald colony mutants of *Streptomyces coelicolor*. J Gen Microbiol. 1976;96(2):299–315. https://doi.org/10.1099/00221287-96-2-299

217. Hopwood DA, Glauert AM. Electron microscope observations on the surface structures of *Streptomyces violaceoruber*. J Gen Microbiol. 1961;26:325–30. https://doi.org/10.1099/00221287-26-2-325

218. Wildermuth H, Wehrli E, Horne RW. The surface structure of spores and aerial mycelium in *Streptomyces coelicolor*. J Ultrastruct Res. 1971;35(1):168–80. https://doi.org/10.1016/s0022-5320(71)80149-1

219. Elliot MA, Karoonuthaisiri N, Huang J, Bibb MJ, Cohen SN, Kao CM, et al. The chaplins: A family of hydrophobic cell-surface proteins involved in aerial mycelium formation in *Streptomyces coelicolor*. Genes Dev. 2003;17(14):1727–40. https://doi.org/10.1101/gad.264403

220. Claessen D, Wösten HAB, Keulen Gv, Faber OG, Alves AMCR, Meijer WG, et al. Two novel homologous proteins of *Streptomyces coelicolor* and *Streptomyces lividans* are involved in the formation of the rodlet layer and mediate attachment to a hydrophobic surface. Mol Microbiol. 2002;44(6):1483–92. https://doi.org/10.1046/j.1365-2958.2002.02980.x

221. Guijarro J, Santamaria R, Schauer A, Losick R. Promoter determining the timing and spatial localization of transcription of a cloned *Streptomyces coelicolor* gene encoding a spore-associated polypeptide. J Bacteriol. 1988;170(4):1895–901. https://doi.org/10.1128/jb.170.4.1895-1901.1988

222. Willey J, Schwedock J, Losick R. Multiple extracellular signals govern the production of a morphogenetic protein involved in aerial mycelium formation by *Streptomyces coelicolor*. Genes Dev. 1993;7(5):895–903. https://doi.org/10.1101/gad.7.5.895

223. Kodani S, Hudson ME, Durrant MC, Buttner MJ, Nodwell JR, Willey JM. The SapB morphogen is a lantibiotic-like peptide derived from the product of the developmental gene *ramS* in *Streptomyces coelicolor*. Proc Natl Acad Sci U S A. 2004;101(31):11448–53. https://doi.org/10.1073/pnas.0404220101

224. Claessen D, Rink R, de Jong W, Siebring J, de Vreugd P, Boersma FG, et al. A novel class of secreted hydrophobic proteins is involved in aerial hyphae formation in *Streptomyces coelicolor* by forming amyloid-like fibrils. Genes Dev. 2003;17(14):1714–26. https://doi.org/10.1101/gad.264303

225. Bokhove M, Claessen D, de Jong W, Dijkhuizen L, Boekema EJ, Oostergetel GT. Chaplins of *Streptomyces coelicolor* self-assemble into two distinct functional amyloids. J Struct Biol. 2013;184(2):301–9. https://doi.org/10.1016/j.jsb.2013.08.013

226. Claessen D, de Jong W, Dijkhuizen L, Wösten HA. Regulation of *Streptomyces* development: Reach for the sky! Trends Microbiol. 2006;14(7):313–9. https://doi.org/10.1016/j.tim.2006.05.008

227. Claessen D, Stokroos I, Deelstra HJ, Penninga NA, Bormann C, Salas JA, et al. The formation of the rodlet layer of streptomycetes is the result of the interplay between rodlins and chaplins. Mol Microbiol. 2004;53(2):433–43. https://doi.org/10.1111/j.1365-2958.2004.04143.x

228. Bibb MJ, Domonkos A, Chandra G, Buttner MJ. Expression of the chaplin and rodlin hydrophobic sheath proteins in *Streptomyces venezuelae* is controlled by σ(BldN) and a cognate anti-sigma factor, RsbN. Mol Microbiol. 2012;84(6):1033–49. https://doi.org/10.1111/j.1365-2958.2012.08070.x

229. den Hengst CD, Tran NT, Bibb MJ, Chandra G, Leskiw BK, Buttner MJ. Genes essential for morphological development and antibiotic production in *Streptomyces coelicolor* are targets of BldD during vegetative growth. Mol Microbiol. 2010;78(2):361–79. https://doi.org/10.1111/j.1365-2958.2010.07338.x

230. Elliot MA, Bibb MJ, Buttner MJ, Leskiw BK. BldD is a direct regulator of key developmental genes in *Streptomyces coelicolor* A3(2). Mol Microbiol. 2001;40(1):257–69. https://doi.org/10.1046/j.1365-2958.2001.02387.x

231. Bibb MJ, Molle V, Buttner MJ. sigma(BldN), an extracytoplasmic function RNA polymerase sigma factor required for aerial mycelium formation in *Streptomyces coelicolor* A3(2). J Bacteriol. 2000;182(16):4606–16. https://doi.org/10.1128/jb.182.16.4606-4616.2000

232. Molle V, Buttner MJ. Different alleles of the response regulator gene *bldM* arrest *Streptomyces coelicolor* development at distinct stages. Mol Microbiol. 2000;36(6):1265–78. https://doi.org/10.1046/j.1365-2958.2000.01977.x

233. Bush MJ, Chandra G, Al-Bassam MM, Findlay KC, Buttner MJ. BldC delays entry into development to produce a sustained period of vegetative growth in *Streptomyces venezuelae*. mBio. 2019;10(1):e02812–18. https://doi.org/10.1128/mBio.02812-18

234. Al-Bassam MM, Bibb MJ, Bush MJ, Chandra G, Buttner MJ. Response regulator heterodimer formation controls a key stage in *Streptomyces* development. PLoS Genet. 2014;10(8):e1004554. https://doi.org/10.1371/journal.pgen.1004554

235. Aínsa JA, Parry HD, Chater KF. A response regulator-like protein that functions at an intermediate stage of sporulation in *Streptomyces coelicolor* A3(2). Mol Microbiol. 1999;34(3):607–19. https://doi.org/10.1046/j.1365-2958.1999.01630.x

236. Chater KF, Bruton CJ, Plaskitt KA, Buttner MJ, Méndez C, Helmann JD. The developmental fate of *S. coelicolor* hyphae depends upon a gene product homologous with the motility sigma factor of *B. subtilis*. Cell. 1989;59(1):133–43. https://doi.org/10.1016/0092-8674(89)90876-3

237. Ausmees N, Wahlstedt H, Bagchi S, Elliot MA, Buttner MJ, Flardh K. SmeA, a small membrane protein with multiple functions in *Streptomyces* sporulation including targeting of a SpoIIIE/FtsK-like protein to cell division septa. Mol Microbiol. 2007;65:1458–73. https://doi.org/10.1111/j.1365-2958.2007.05877.x

238. Kelemen GH, Brian P, Flärdh K, Chamberlin L, Chater KF, Buttner MJ. Developmental regulation of transcription of *whiE*, a locus specifying the polyketide spore pigment in *Streptomyces coelicolor*. J Bacteriol. 1998;180(9):2515–21. https://doi.org/10.1128/JB.180.9.2515-2521.1998

239. Gallagher KA, Schumacher MA, Bush MJ, Bibb MJ, Chandra G, Holmes NA, et al. c-di-GMP arms an anti-σ to control progression of multicellular differentiation in *Streptomyces*. Mol Cell. 2020;77(3):586–99.e6. https://doi.org/10.1016/j.molcel.2019.11.006

240. Ryding NJ, Kelemen GH, Whatling CA, Flärdh K, Buttner MJ, Chater KF. A developmentally regulated gene encoding a repressor-like protein is essential for sporulation in *Streptomyces coelicolor* A3(2). Mol Microbiol. 1998;29(1):343–57. https://doi.org/10.1046/j.1365-2958.1998.00939.x

241. Aínsa JA, Ryding NJ, Hartley N, Findlay KC, Bruton CJ, Chater KF. WhiA, a protein of unknown function conserved among gram-positive bacteria, is essential for sporulation in *Streptomyces coelicolor* A3(2). J Bacteriol. 2000;182(19):5470–8. https://doi.org/10.1128/jb.182.19.5470-5478.2000

242. Bush MJ, Chandra G, Bibb MJ, Findlay KC, Buttner MJ. Genome-wide chromatin immunoprecipitation sequencing analysis shows that WhiB is a transcription factor that cocontrols its regulon with WhiA to initiate developmental cell division in *Streptomyces*. mBio. 2016;7(2):e00523–16. https://doi.org/10.1128/mbio.00523-16

243. Bush MJ, Chandra G, Findlay KC, Buttner MJ. Multi-layered inhibition of *Streptomyces* development: BldO is a dedicated repressor of *whiB*. Mol Microbiol. 2017;104(5):700–11. https://doi.org/10.1111/mmi.13663

244. Flärdh K, Findlay KC, Chater KF. Association of early sporulation genes with suggested developmental decision points in *Streptomyces coelicolor* A3(2). Microbiology. 1999;145(9):2229–43. https://doi.org/10.1099/00221287-145-9-2229

245. Davis NK, Chater KF. The *Streptomyces coelicolor whiB* gene encodes a small transcription factor-like protein dispensable for growth but essential for sporulation. Mol Gen Genet. 1992;232(3):351–8. https://doi.org/10.1007/bf00266237

246. Bagchi S, Tomenius H, Belova LM, Ausmees N. Intermediate filament-like proteins in bacteria and a cytoskeletal function in *Streptomyces*. Mol Microbiol. 2008;70(4):1037–50. https://doi.org/10.1111/j.1365-2958.2008.06473.x

247. Higo A, Horinouchi S, Ohnishi Y. Strict regulation of morphological differentiation and secondary metabolism by a positive feedback loop between two global regulators AdpA and BldA in *Streptomyces griseus*. Mol Microbiol. 2011;81(6):1607–22. https://doi.org/10.1111/j.1365-2958.2011.07795.x

248. Higo A, Hara H, Horinouchi S, Ohnishi Y. Genome-wide distribution of AdpA, a global regulator for secondary metabolism and morphological differentiation in *Streptomyces*, revealed the extent and complexity of the AdpA regulatory network. DNA Res. 2012;19(3):259–74. https://doi.org/10.1093/dnares/dss010

249. Ohnishi Y, Yamazaki H, Kato JY, Tomono A, Horinouchi S. AdpA, a central transcriptional regulator in the A-factor regulatory cascade that leads to morphological development and secondary metabolism in *Streptomyces griseus*. Biosci Biotechnol Biochem. 2005;69(3):431–9. https://doi.org/10.1271/bbb.69.431

250. Chandra G, Chater KF. Developmental biology of *Streptomyces* from the perspective of 100 actinobacterial genome sequences. FEMS Microbiol Rev. 2014;38(3):345–79. https://doi.org/10.1111/1574-6976.12047

251. Tschowri N. Cyclic dinucleotide-controlled regulatory pathways in *Streptomyces* Species. J Bacteriol. 2016;198(1):47–54. https://doi.org/10.1128/jb.00423-15

252. Gallagher KA, Tschowri N, Brennan RG, Schumacher MA, Buttner MJ. How c-di-GMP controls progression through the *Streptomyces* life cycle. Curr Opin Microbiol. 2024;80:102516. https://doi.org/10.1016/j.mib.2024.102516

253. Hunt AC, Servín-González L, Kelemen GH, Buttner MJ. The *bldC* developmental locus of *Streptomyces coelicolor* encodes a member of a family of small DNA-binding proteins related to the DNA-binding domains of the MerR family. J Bacteriol. 2005;187(2):716–28. https://doi.org/10.1128/jb.187.2.716-728.2005

254. Elliot MA, Flärdh K. Streptomycete Spores. Encyclopedia of Life Sciences2020. p. 1–11.

255. Schwedock J, McCormick JR, Angert ER, Nodwell JR, Losick R. Assembly of the cell division protein FtsZ into ladder-like structures in the aerial hyphae of *Streptomyces coelicolor*. Mol Microbiol. 1997;25(5):847–58. https://doi.org/10.1111/j.1365-2958.1997.mmi507.x

256. Ensign JC. Formation, properties, and germination of actinomycete spores. Annu Rev Microbiol. 1978;32:185–219. https://doi.org/10.1146/annurev.mi.32.100178.001153

257. Blanco G, Brian P, Pereda A, Méndez C, Salas JA, Chater KF. Hybridization and DNA sequence analyses suggest an early evolutionary divergence of related biosynthetic gene sets encoding polyketide antibiotics and spore pigments in *Streptomyces* spp. Gene. 1993;130(1):107–16. https://doi.org/10.1016/0378-1119(93)90352-4

258. Novakova R, Bistakova J, Kormanec J. Characterization of the polyketide spore pigment cluster *whiESa* in *Streptomyces aureofaciens* CCM3239. Arch Microbiol. 2004;182(5):388–95. https://doi.org/10.1007/s00203-004-0720-2

259. Burger A, Sichler K, Kelemen G, Buttner M, Wohlleben W. Identification and characterization of the *mre* gene region of *Streptomyces coelicolor* A3(2). Mol Gen Genet. 2000;263(6):1053–60. https://doi.org/10.1007/s004380050034

260. Kleinschnitz EM, Heichlinger A, Schirner K, Winkler J, Latus A, Maldener I, et al. Proteins encoded by the *mre* gene cluster in *Streptomyces coelicolor* A3(2) cooperate in spore wall synthesis. Mol Microbiol. 2011;79(5):1367–79. https://doi.org/10.1111/j.1365-2958.2010.07529.x

261. Vollmer B, Steblau N, Ladwig N, Mayer C, Macek B, Mitousis L, et al. Role of the *Streptomyces* spore wall synthesizing complex SSSC in differentiation of *Streptomyces coelicolor* A3(2). Int J Med Microbiol. 2019;309(6):151327. https://doi.org/10.1016/j.ijmm.2019.07.001

262. Heichlinger A, Ammelburg M, Kleinschnitz EM, Latus A, Maldener I, Flärdh K, et al. The MreB-like protein Mbl of *Streptomyces coelicolor* A3(2) depends on MreB for proper localization and contributes to spore wall synthesis. J Bacteriol. 2011;193(7):1533–42. https://doi.org/10.1128/jb.01100-10

263. Sigle S, Steblau N, Wohlleben W, Muth G. Polydiglycosylphosphate transferase PdtA (SCO2578) of *Streptomyces coelicolor* A3(2) is crucial for proper sporulation and apical tip extension under stress conditions. Appl Environ Microbiol. 2016;82(18):5661–72. https://doi.org/10.1128/aem.01425-16

264. Mazza P, Noens EE, Schirner K, Grantcharova N, Mommaas AM, Koerten HK, et al. MreB of *Streptomyces coelicolor* is not essential for vegetative growth but is required for the integrity of aerial hyphae and spores. Mol Microbiol. 2006;60(4):838–52. https://doi.org/10.1111/j.1365-2958.2006.05134.x

265. Ladwig N, Franz-Wachtel M, Hezel F, Soufi B, Macek B, Wohlleben W, et al. Control of morphological differentiation of *Streptomyces coelicolor* A3(2) by phosphorylation of MreC and PBP2. PLoS One. 2015;10(4):e0125425. https://doi.org/10.1371/journal.pone.0125425

266. Ramijan K, Ultee E, Willemse J, Zhang Z, Wondergem JAJ, van der Meij A, et al. Stress-induced formation of cell wall-deficient cells in filamentous actinomycetes. Nat Commun. 2018;9(1):5164. https://doi.org/10.1038/s41467-018-07560-9

267. Errington J, Mickiewicz K, Kawai Y, Wu LJ. L-form bacteria, chronic diseases and the origins of life. Philos Trans R Soc Lond B Biol Sci. 2016;371(1707). https://doi.org/10.1098/rstb.2015.0494

268. Jones SE, Ho L, Rees CA, Hill JE, Nodwell JR, Elliot MA. *Streptomyces* exploration is triggered by fungal interactions and volatile signals. Elife. 2017;6:e21738. https://doi.org/10.7554/eLife.21738

269. Shepherdson EMF, Elliot MA. Redefining development in *Streptomyces venezuelae*: Integrating exploration into the classical sporulating life cycle. mBio. 2024;15(4):e0242423. https://doi.org/10.1128/mbio.02424-23

270. Shepherdson EMF, Elliot MA. Cryptic specialized metabolites drive *Streptomyces* exploration and provide a competitive advantage during growth with other microbes. Proc Natl Acad Sci U S A. 2022;119(40):e2211052119. https://doi.org/10.1073/pnas.2211052119

271. Shepherdson EMF, Netzker T, Stoyanov Y, Elliot MA. Exploratory growth in *Streptomyces venezuelae* involves a unique transcriptional program, enhanced oxidative stress response, and profound acceleration in response to glycerol. J Bacteriol. 2022;204(4):e0062321. https://doi.org/10.1128/jb.00623-21

272. Jones SE, Pham CA, Zambri MP, McKillip J, Carlson EE, Elliot MA. *Streptomyces* volatile compounds influence exploration and microbial community dynamics by altering iron availability. mBio. 2019;10(2):e00171–19. https://doi.org/10.1128/mBio.00171-19

273. Zambri MP, Baglio CR, Irazoki O, Jones SE, Garner EC, Cava F, et al. *Streptomyces* uses both polar and dispersed cell wall synthesis during exploratory growth. Nat Microbiol. 2025;10(9):2245–56. https://doi.org/10.1038/s41564-025-02080-x

274. Norte DM, Avitia-Dominguez LA, Rozen DE. Evolution and ecology of *Streptomyces*. Annu Rev Microbiol. 2025;79:383–403. https://doi.org/10.1146/annurev-micro-051524-032254

275. Jakimowicz D, van Wezel GP. Cell division and DNA segregation in *Streptomyces*: How to build a septum in the middle of nowhere? Mol Microbiol. 2012;85(3):393–404. https://doi.org/10.1111/j.1365-2958.2012.08107.x

276. Hopwood DA. Phase-contrast observations on *Streptomyces coelicolor*. J Gen Microbiol. 1960;22(1):295–302. https://doi.org/10.1099/00221287-22-1-295

277. Chater KF. Taking a genetic scalpel to the *Streptomyces* colony. Microbiology. 1998;144(6):1465–78. https://doi.org/10.1099/00221287-144-6-1465

278. Hoskisson PA, Barona-Gómez F, Rozen DE. Phenotypic heterogeneity in *Streptomyces* colonies. Curr Opin Microbiol. 2024;78:102448. https://doi.org/10.1016/j.mib.2024.102448

279. Zacharia Vineetha M, Ra Y, Sue C, Alcala E, Reaso Jewel N, Ruzin Steven E, et al. Genetic network architecture and environmental cues drive spatial organization of phenotypic division of labor in *Streptomyces coelicolor*. mBio. 2021;12(3):e00794–21. https://doi.org/10.1128/mbio.00794-21

280. Zhang Z, Du C, de Barsy F, Liem M, Liakopoulos A, van Wezel GP, et al. Antibiotic production in *Streptomyces* is organized by a division of labor through terminal genomic differentiation. Sci Adv. 2020;6(3):eaay5781. https://doi.org/10.1126/sciadv.aay5781

281. Howell M, Brown PJB. Building the bacterial cell wall at the pole. Curr Opin Microbiol. 2016;34:53–9. https://doi.org/10.1016/j.mib.2016.07.021

282. Flärdh K. Cell polarity and the control of apical growth in *Streptomyces*. Curr Opin Microbiol. 2010;13(6):758–65. https://doi.org/10.1016/j.mib.2010.10.002

283. Singh Mavi P, Flärdh K. A histidine pseudokinase modulates polar growth and cell shape in *Streptomyces venezuelae*. Commun Biol. 2026;9(1):345. https://doi.org/10.1038/s42003-026-09620-z

284. Hammond LR, White ML, Eswara PJ. ¡vIVA la DivIVA! J Bacteriol. 2019;201(21):e00245–19. https://doi.org/10.1128/jb.00245-19

285. Kaval KG, Halbedel S. Architecturally the same, but playing a different game: The diverse species-specific roles of DivIVA proteins. Virulence. 2012;3(4):406–7. https://doi.org/10.4161/viru.20747

286. Halbedel S, Lewis RJ. Structural basis for interaction of DivIVA/GpsB proteins with their ligands. Mol Microbiol. 2019;111(6):1404–15. https://doi.org/10.1111/mmi.14244

287. Oliva MA, Halbedel S, Freund SM, Dutow P, Leonard TA, Veprintsev DB, et al. Features critical for membrane binding revealed by DivIVA crystal structure. EMBO J. 2010;29(12):1988–2001. https://doi.org/10.1038/emboj.2010.99

288. Jurásek M, Flärdh K, Vácha R. Effect of membrane composition on DivIVA-membrane interaction. Biochim Biophys Acta - Biomembr. 2020;1862(8):183144. https://doi.org/10.1016/j.bbamem.2019.183144

289. Harry EJ, Lewis PJ. Early targeting of Min proteins to the cell poles in germinated spores of *Bacillus subtilis*: Evidence for division apparatus-independent recruitment of Min proteins to the division site. Mol Microbiol. 2003;47(1):37–48. https://doi.org/10.1046/j.1365-2958.2003.03253.x

290. Lenarcic R, Halbedel S, Visser L, Shaw M, Wu LJ, Errington J, et al. Localisation of DivIVA by targeting to negatively curved membranes. EMBO J. 2009;28(15):2272–82. https://doi.org/10.1038/emboj.2009.129

291. Renner LD, Eswaramoorthy P, Ramamurthi KS, Weibel DB. Studying biomolecule localization by engineering bacterial cell wall curvature. PLoS One. 2013;8(12):e84143. https://doi.org/10.1371/journal.pone.0084143

292. Eswaramoorthy P, Erb ML, Gregory JA, Silverman J, Pogliano K, Pogliano J, et al. Cellular architecture mediates DivIVA ultrastructure and regulates min activity in *Bacillus subtilis*. mBio. 2011;2(6). https://doi.org/10.1128/mBio.00257-11

293. Marston AL, Errington J. Selection of the midcell division site in *Bacillus subtilis* through MinD-dependent polar localization and activation of MinC. Mol Microbiol. 1999;33(1):84–96. https://doi.org/10.1046/j.1365-2958.1999.01450.x

294. Thomaides HB, Freeman M, El Karoui M, Errington J. Division site selection protein DivIVA of *Bacillus subtilis* has a second distinct function in chromosome segregation during sporulation. Genes Dev. 2001;15(13):1662–73. https://doi.org/10.1101/gad.197501

295. Donovan C, Sieger B, Krämer R, Bramkamp M. A synthetic *Escherichia coli* system identifies a conserved origin tethering factor in Actinobacteria. Mol Microbiol. 2012;84(1):105–16. https://doi.org/10.1111/j.1365-2958.2012.08011.x

296. Ginda K, Bezulska M, Ziółkiewicz M, Dziadek J, Zakrzewska-Czerwińska J, Jakimowicz D. ParA of *Mycobacterium smegmatis* co-ordinates chromosome segregation with the cell cycle and interacts with the polar growth determinant DivIVA. Mol Microbiol. 2013;87(5):998–1012. https://doi.org/10.1111/mmi.12146

297. Matusiak I, Strzałka A, Wadach P, Gongerowska-Jac M, Szwajczak E, Szydłowska-Helbrych A, et al. The interplay between the polar growth determinant DivIVA, the segregation protein ParA, and their novel interaction partner PapM controls the *Mycobacterium smegmatis* cell cycle by modulation of DivIVA subcellular distribution. Microbiol Spectr. 2023;11(6):e01752–23. https://doi.org/10.1128/spectrum.01752-23

298. Jani C, Eoh H, Lee JJ, Hamasha K, Sahana MB, Han J-S, et al. Regulation of polar peptidoglycan biosynthesis by Wag31 phosphorylation in mycobacteria. BMC Microbiol. 2010;10(1):327. https://doi.org/10.1186/1471-2180-10-327

299. Kang CM, Abbott DW, Park ST, Dascher CC, Cantley LC, Husson RN. The *Mycobacterium tuberculosis* serine/threonine kinases PknA and PknB: Substrate identification and regulation of cell shape. Genes Dev. 2005;19(14):1692–704. https://doi.org/10.1101/gad.1311105

300. Meniche X, Otten R, Siegrist MS, Baer CE, Murphy KC, Bertozzi CR, et al. Subpolar addition of new cell wall is directed by DivIVA in mycobacteria. Proc Natl Acad Sci U S A. 2014;111(31):E3243–51. https://doi.org/10.1073/pnas.1402158111

301. Letek M, Ordóñez E, Vaquera J, Margolin W, Flärdh K, Mateos LM, et al. DivIVA is required for polar growth in the MreB-lacking rod-shaped actinomycete *Corynebacterium glutamicum*. J Bacteriol. 2008;190(9):3283–92. https://doi.org/10.1128/jb.01934-07

302. Ramos A, Honrubia MP, Valbuena N, Vaquera J, Mateos LM, Gil JA. Involvement of DivIVA in the morphology of the rod-shaped actinomycete *Brevibacterium lactofermentum*. Microbiology. 2003;149(Pt 12):3531–42. https://doi.org/10.1099/mic.0.26653-0

303. Nguyen L, Scherr N, Gatfield J, Walburger A, Pieters J, Thompson CJ. Antigen 84, an effector of pleiomorphism in *Mycobacterium smegmatis*. J Bacteriol. 2007;189(21):7896–910. https://doi.org/10.1128/jb.00726-07

304. Hempel AM, Cantlay S, Molle V, Wang SB, Naldrett MJ, Parker JL, et al. The Ser/Thr protein kinase AfsK regulates polar growth and hyphal branching in the filamentous bacteria *Streptomyces*. Proc Natl Acad Sci U S A. 2012;109(35):E2371–9. https://doi.org/10.1073/pnas.1207409109

305. Passot FM, Cantlay S, Flärdh K. Protein phosphatase SppA regulates apical growth and dephosphorylates cell polarity determinant DivIVA in *Streptomyces coelicolor*. Mol Microbiol. 2022;117(2):411–28. https://doi.org/10.1111/mmi.14856

306. Holmes NA, Walshaw J, Leggett RM, Thibessard A, Dalton KA, Gillespie MD, et al. Coiled-coil protein Scy is a key component of a multiprotein assembly controlling polarized growth in *Streptomyces*. Proc Natl Acad Sci U S A. 2013;110(5):E397–406. https://doi.org/10.1073/pnas.1210657110

307. Walshaw J, Gillespie MD, Kelemen GH. A novel coiled-coil repeat variant in a class of bacterial cytoskeletal proteins. J Struct Biol. 2010;170(2):202–15. https://doi.org/10.1016/j.jsb.2010.02.008

308. Fuchino K, Bagchi S, Cantlay S, Sandblad L, Wu D, Bergman J, et al. Dynamic gradients of an intermediate filament-like cytoskeleton are recruited by a polarity landmark during apical growth. Proc Natl Acad Sci U S A. 2013;110(21):E1889–97. https://doi.org/10.1073/pnas.1305358110

309. Fröjd MJ, Flärdh K. Apical assemblies of intermediate filament-like protein FilP are highly dynamic and affect polar growth determinant DivIVA in *Streptomyces venezuelae*. Mol Microbiol. 2019;112(1):47–61. https://doi.org/10.1111/mmi.14253

310. McCormick JR. Cell division is dispensable but not irrelevant in *Streptomyces*. Curr Opin Microbiol. 2009;12(6):689–98. https://doi.org/10.1016/j.mib.2009.10.004

311. McCormick JR, Su EP, Driks A, Losick R. Growth and viability of *Streptomyces coelicolor* mutant for the cell division gene *ftsZ*. Mol Microbiol. 1994;14(2):243–54. https://doi.org/10.1111/j.1365-2958.1994.tb01285.x

312. Santos-Beneit F, Roberts DM, Cantlay S, McCormick JR, Errington J. A mechanism for FtsZ-independent proliferation in *Streptomyces*. Nat Commun. 2017;8(1):1378. https://doi.org/10.1038/s41467-017-01596-z

313. Bush MJ, Gallagher KA, Chandra G, Findlay KC, Schlimpert S. Hyphal compartmentalization and sporulation in *Streptomyces* require the conserved cell division protein SepX. Nat Commun. 2022;13(1):71. https://doi.org/10.1038/s41467-021-27638-1

314. Bennett JA, Aimino RM, McCormick JR. *Streptomyces coelicolor* genes *ftsL* and *divIC* play a role in cell division but are dispensable for colony formation. J Bacteriol. 2007;189(24):8982–92. https://doi.org/10.1128/jb.01303-07

315. Cantlay S, Sen BC, Flärdh K, McCormick JR. Influence of core divisome proteins on cell division in *Streptomyces venezuelae* ATCC 10712. Microbiology. 2021;167(2):001015. https://doi.org/10.1099/mic.0.001015

316. McCormick JR, Losick R. Cell division gene *ftsQ* is required for efficient sporulation but not growth and viability in *Streptomyces coelicolor* A3(2). J Bacteriol. 1996;178(17):5295–301. https://doi.org/10.1128/jb.178.17.5295-5301.1996

317. Mistry BV, 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. 2008;190(16):5555–66. https://doi.org/10.1128/jb.00398-08

318. Wang L, Yu Y, He X, Zhou X, Deng Z, Chater KF, et al. Role of an FtsK-like protein in genetic stability in *Streptomyces coelicolor* A3(2). J Bacteriol. 2007;189(6):2310–8. https://doi.org/10.1128/jb.01660-06

319. Keijser BJ, Noens EE, Kraal B, Koerten HK, van Wezel GP. The *Streptomyces coelicolor ssgB* gene is required for early stages of sporulation. FEMS Microbiol Lett. 2003;225(1):59–67. https://doi.org/10.1016/s0378-1097(03)00481-6

320. Noens EE, Mersinias V, Traag BA, Smith CP, Koerten HK, van Wezel GP. SsgA-like proteins determine the fate of peptidoglycan during sporulation of *Streptomyces coelicolor*. Mol Microbiol. 2005;58(4):929–44. https://doi.org/10.1111/j.1365-2958.2005.04883.x

321. van Wezel GP, van der Meulen J, Kawamoto S, Luiten RG, Koerten HK, Kraal B. *ssgA* is essential for sporulation of *Streptomyces coelicolor* A3(2) and affects hyphal development by stimulating septum formation. J Bacteriol. 2000;182(20):5653–62. https://doi.org/10.1128/jb.182.20.5653-5662.2000

322. Jiang H, Kendrick KE. Characterization of *ssfR* and *ssgA*, two genes involved in sporulation of *Streptomyces griseus*. J Bacteriol. 2000;182(19):5521–9. https://doi.org/10.1128/jb.182.19.5521-5529.2000

323. Willemse J, Borst JW, De Waal E, Bisseling T, Van Wezel GP. Positive control of cell division: FtsZ is recruited by SsgB during sporulation of *Streptomyces*. Genes Dev. 2011;25(1):89–99. https://doi.org/10.1101/gad.600211

324. Zhang L, Willemse J, Claessen D, van Wezel GP. SepG coordinates sporulation-specific cell division and nucleoid organization in *Streptomyces coelicolor*. Open Biol. 2016;6(4):150164. https://doi.org/10.1098/rsob.150164

325. Ramos-León F, Bush MJ, Sallmen JW, Chandra G, Richardson J, Findlay KC, et al. A conserved cell division protein directly regulates FtsZ dynamics in filamentous and unicellular actinobacteria. Elife. 2021;10:e63387. https://doi.org/10.7554/eLife.63387

326. Del Sol R, Pitman A, Herron P, Dyson P. The product of a developmental gene, *crgA*, that coordinates reproductive growth in *Streptomyces* belongs to a novel family of small actinomycete-specific proteins. J Bacteriol. 2003;185(22):6678–85. https://doi.org/10.1128/jb.185.22.6678-6685.2003

327. Del Sol R, Mullins JG, Grantcharova N, Flärdh K, Dyson P. Influence of CrgA on assembly of the cell division protein FtsZ during development of *Streptomyces coelicolor*. J Bacteriol. 2006;188(4):1540–50. https://doi.org/10.1128/jb.188.4.1540-1550.2006

328. White ML, Eswara PJ. *ylm* has more than a (Z anchor) ring to it! J Bacteriol. 2021;203(3):e00460–20. https://doi.org/10.1128/jb.00460-20

329. Singh JK, Makde RD, Kumar V, Panda D. SepF increases the assembly and bundling of FtsZ polymers and stabilizes FtsZ protofilaments by binding along its length. J Biol Chem. 2008;283(45):31116–24. https://doi.org/10.1074/jbc.M805910200

330. Gündoğdu ME, Kawai Y, Pavlendova N, Ogasawara N, Errington J, Scheffers DJ, et al. Large ring polymers align FtsZ polymers for normal septum formation. EMBO J. 2011;30(3):617–26. https://doi.org/10.1038/emboj.2010.345

331. Hamoen L, Meile J, Jong W, Noirot P, Errington J. SepF, a novel FtsZ-interacting protein required for a late step in cell division. Mol Microbiol. 2006;59(3):989–99. https://doi.org/10.1111/j.1365-2958.2005.04987.x

332. Gupta S, Banerjee SK, Chatterjee A, Sharma AK, Kundu M, Basu J. Essential protein SepF of mycobacteria interacts with FtsZ and MurG to regulate cell growth and division. Microbiology. 2015;161(8):1627–38. https://doi.org/10.1099/mic.0.000108

333. Schlimpert S, Wasserstrom S, Chandra G, Bibb MJ, Findlay KC, Flärdh K, et al. Two dynamin-like proteins stabilize FtsZ rings during *Streptomyces* sporulation. Proc Natl Acad Sci U S A. 2017;114(30):E6176–e83. https://doi.org/10.1073/pnas.1704612114

334. Wenzel M, Celik Gulsoy IN, Gao Y, Teng Z, Willemse J, Middelkamp M, et al. Control of septum thickness by the curvature of SepF polymers. Proc Natl Acad Sci U S A. 2021;118(2):e2002635118. https://doi.org/10.1073/pnas.2002635118

335. Zhang L, Willemse J, Yagüe P, de Waal E, Claessen D, van Wezel GP. The SepF-like proteins SflA and SflB prevent ectopic localization of FtsZ and DivIVA during sporulation of *Streptomyces coelicolor*. Biochem Biophys Res Commun. 2023;645:79–87. https://doi.org/10.1016/j.bbrc.2023.01.021

336. Bramkamp M. Structure and function of bacterial dynamin-like proteins. Biol Chem. 2012;393(11):1203–14. https://doi.org/10.1515/hsz-2012-0185

337. Praefcke GJK, McMahon HT. The dynamin superfamily: Universal membrane tubulation and fission molecules? Nat Rev Mol Cell Biol. 2004;5(2):133–47. https://doi.org/10.1038/nrm1313

338. Dedrick RM, Wildschutte H, McCormick JR. Genetic interactions of *smc*, *ftsK*, and *parB* genes in *Streptomyces coelicolor* and their developmental genome segregation phenotypes. J Bacteriol. 2009;191(1):320–32. https://doi.org/10.1128/jb.00858-08

339. Jakimowicz D, Chater K, Zakrzewska-Czerwínska J. The ParB protein of *Streptomyces coelicolor* A3(2) recognizes a cluster of *parS* sequences within the origin-proximal region of the linear chromosome. Mol Microbiol. 2002;45(5):1365–77. https://doi.org/10.1046/j.1365-2958.2002.03102.x

340. Donczew M, Mackiewicz P, Wróbel A, Flärdh K, Zakrzewska-Czerwińska J, Jakimowicz D. ParA and ParB coordinate chromosome segregation with cell elongation and division during *Streptomyces* sporulation. Open Biol. 2016;6(4). https://doi.org/10.1098/rsob.150263

341. Szafran MJ, Małecki T, Strzałka A, Pawlikiewicz K, Duława J, Zarek A, et al. Spatial rearrangement of the *Streptomyces venezuelae* linear chromosome during sporogenic development. Nat Commun. 2021;12(1):5222. https://doi.org/10.1038/s41467-021-25461-2

342. Pawlikiewicz K, Strzałka A, Majkowski M, Duława-Kobeluszczyk J, Szafran MJ, Jakimowicz D. SMC modulates ParB engagement in segregation complexes in *streptomyces*. Nat Commun. 2025;16(1):8999. https://doi.org/10.1038/s41467-025-64044-3

343. Salerno P, Larsson J, Bucca G, Laing E, Smith CP, Flärdh K. One of the two genes encoding nucleoid-associated HU proteins in *Streptomyces coelicolor* is developmentally regulated and specifically involved in spore maturation. J Bacteriol. 2009;191(21):6489–500. https://doi.org/10.1128/JB.00709-09

344. Strzałka A, Mikołajczyk J, Kowalska K, Skurczyński M, Holmes NA, Jakimowicz D. The role of two major nucleoid-associated proteins in *Streptomyces*, HupA and HupS, in stress survival and gene expression regulation. Microb Cell Fact. 2024;23(1):275. https://doi.org/10.1186/s12934-024-02549-0

345. Cantu Morin LM, Dekoninck K, Min K-Y, Traxler MF. The accessory protein CvnF8 modulates histidine kinase activity in an actinobacterial G protein system in *Streptomyces coelicolor*. mBio. 2026;0(0):e00774–26. https://doi.org/10.1128/mbio.00774-26

346. Komatsu M, Takano H, Hiratsuka T, Ishigaki Y, Shimada K, Beppu T, et al. Proteins encoded by the conservon of *Streptomyces coelicolor* A3(2) comprise a membrane-associated heterocomplex that resembles eukaryotic G protein-coupled regulatory system. Mol Microbiol. 2006;62(6):1534–46. https://doi.org/10.1111/j.1365-2958.2006.05461.x

347. Wuichet K, Søgaard-Andersen L. Evolution and diversity of the Ras superfamily of small GTPases in prokaryotes. Genome Biol Evol. 2014;7(1):57–70. https://doi.org/10.1093/gbe/evu264

348. Cherfils J, Zeghouf M. Regulation of small GTPases by GEFs, GAPs, and GDIs. Physiol Rev. 2013;93(1):269–309. https://doi.org/10.1152/physrev.00003.2012

349. Hartzell P, Kaiser D. Function of MglA, a 22-kilodalton protein essential for gliding in *Myxococcus xanthus*. J Bacteriol. 1991;173(23):7615–24. https://doi.org/10.1128/jb.173.23.7615-7624.1991

350. Leonardy S, Miertzschke M, Bulyha I, Sperling E, Wittinghofer A, Søgaard-Andersen L. Regulation of dynamic polarity switching in bacteria by a Ras-like G-protein and its cognate GAP. EMBO J. 2010;29(14):2276–89. https://doi.org/10.1038/emboj.2010.114

351. Dinet C, Mignot T. Unorthodox regulation of the MglA Ras-like GTPase controlling polarity in *Myxococcus xanthus*. FEBS Lett. 2023;597(6):850–64. https://doi.org/10.1002/1873-3468.14565

352. Mayer C, Takiff H. The molecular genetics of fluoroquinolone resistance in *Mycobacterium tuberculosis*. Microbiol Spectr. 2014;2(4):Mgm2–0009–2013. https://doi.org/10.1128/microbiolspec.MGM2-0009-2013

353. Zhu B, Cen Z, Chen Y, Shang K, Zhai J, Han M, et al. α-Pyrone mediates quorum sensing through the conservon system in *Nocardiopsis* sp. Microbiol Res. 2024;285:127767. https://doi.org/10.1016/j.micres.2024.127767

354. Komatsu M, Kuwahara Y, Hiroishi A, Hosono K, Beppu T, Ueda K. Cloning of the conserved regulatory operon by its aerial mycelium-inducing activity in an *amfR* mutant of *Streptomyces griseus*. Gene. 2003;306:79–89. https://doi.org/10.1016/s0378-1119(03)00405-0

355. Takano H, Hashimoto K, Yamamoto Y, Beppu T, Ueda K. Pleiotropic effect of a null mutation in the *cvn1* conservon of *Streptomyces coelicolor* A3(2). Gene. 2011;477(1-2):12–8. https://doi.org/10.1016/j.gene.2011.01.005

356. Bonet B, Ra Y, Cantu Morin LM, Soto Bustos J, Livny J, Traxler MF. The *cvn8* conservon system is a global regulator of specialized metabolism in *Streptomyces coelicolor* during interspecies interactions. mSystems. 2021;6(5):e0028121. https://doi.org/10.1128/mSystems.00281-21

357. Gomez-Escribano JP, Holmes NA, Schlimpert S, Bibb MJ, Chandra G, Wilkinson B, et al. *Streptomyces venezuelae* NRRL B-65442: Genome sequence of a model strain used to study morphological differentiation in filamentous actinobacteria. J Ind Microbiol Biotechnol. 2021;48(9-10):kuab035. https://doi.org/10.1093/jimb/kuab035

358. Huang Y, Shen Q, Xu H, Huang L, Xiang S, Li P, et al. *Mycobacterium smegmatis* MfpC is a GEF that regulates *mfpA* translationally to alter the fluoroquinolone efficacy. Commun Biol. 2024;7(1):1035. https://doi.org/10.1038/s42003-024-06737-x

359. Huang Y, Yan S, Li Y, Ai X, Yu X, Ge Y, et al. *Mycobacterium* fluoroquinolone resistance protein D (MfpD), a GTPase-activating protein of GTPase MfpB, is involved in fluoroquinolones potency. Microbiol Spectr. 2022;10(6):e0209822. https://doi.org/10.1128/spectrum.02098-22

360. Brand A, Gow NAR. Mechanisms of hypha orientation of fungi. Curr Opin Microbiol. 2009;12(4):350–7. https://doi.org/10.1016/j.mib.2009.05.007

361. Nguyen T, Goley ED. The polar landscape of bacterial pathogens and predators. Curr Opin Microbiol. 2026;93:102793. https://doi.org/10.1016/j.mib.2026.102793

362. Bowman GR, Lyuksyutova AI, Shapiro L. Bacterial polarity. Curr Opin Cell Biol. 2011;23(1):71–7. https://doi.org/10.1016/j.ceb.2010.10.013

363. Treuner-Lange A, Søgaard-Andersen L. Regulation of cell polarity in bacteria. J Cell Biol. 2014;206(1):7–17. https://doi.org/10.1083/jcb.201403136

364. Miertzschke M, Koerner C, Vetter IR, Keilberg D, Hot E, Leonardy S, et al. Structural analysis of the Ras-like G protein MglA and its cognate GAP MglB and implications for bacterial polarity. EMBO J. 2011;30(20):4185–97. https://doi.org/10.1038/emboj.2011.291

365. Zhang Y, Franco M, Ducret A, Mignot T. A bacterial Ras-like small GTP-binding protein and its cognate GAP establish a dynamic spatial polarity axis to control directed motility. PLoS Biol. 2010;8(7):e1000430. https://doi.org/10.1371/journal.pbio.1000430

366. Pende N, Sogues A, Megrian D, Sartori-Rupp A, England P, Palabikyan H. SepF is the FtsZ anchor in archaea, with features of an ancestral cell division system. Nat Commun. 2021;12(1):3214. https://doi.org/10.1038/s41467-021-23099-8

367. Huang T-W, Hsu C-C, Yang H-Y, Chen CW. Topoisomerase IV is required for partitioning of circular chromosomes but not linear chromosomes in *Streptomyces*. Nucleic Acids Res. 2013;41(22):10403–13. https://doi.org/10.1093/nar/gkt757

Printed by Media-Tryck, Lund 2026 NORDIC SWAN ECOLABEL 3041 0903

Thinking Like *Streptomyces*

FROM A SINGLE GROWING TIP TO A COMMUNITY TAKING SHAPE

I spent most of my PhD in the dark, alone with streptomycetes, patiently watching them grow, divide, and take shape. Before this sounds too dramatic, by "in the dark" I of course mean the microscope room. There, I learned that *Streptomyces* mycelia, like people, are shaped by coordination within a community, and that faithful cell division relies on every step being carefully coordinated, much as life's important milestones depend on careful preparation and effective execution. Every so often in the dim microscope room, a result would appear that made the room feel a little brighter..

LUND UNIVERSITY

Lund University
Faculty of Science
Department of Biology

ISBN 978-91-90202-65-4