Review

Coordinated Cell Death in Isogenic Bacterial Populations: Sacrificing Some for the Benefit of Many?

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Abstract

Antibiotics are classically perceived as biological weapons that bacteria produce to hold their ground against competing species in their natural habitat. But in the context of multicellular differentiation processes, antimicrobial compounds sometimes also play a role in intraspecies competition, resulting in the death of a sub-population of genetically identical siblings for the benefit of the population. Such a strategy is based on the diversification and hence phenotypic heterogeneity of an isogenic bacterial population. This review article will address three such phenomena. In *Bacillus subtilis*, cannibalism is a differentiation strategy that enhances biofilm formation, prolongs or potentially even prevents full commitment to endospore formation under starvation conditions, and protects cells within the biofilm against competing species. The nutrients released by lysed cells can be used by the toxin producers, thereby delaying the full activation of the master regulator of sporulation. A related strategy is associated with the initiation of competence development under nutrient excess in *Streptococcus pneumoniae*. This process, termed fratricide, causes allolysis in a sub-population and is thought to enhance genetic diversity within the species. In *Myxococcus xanthus*, a large fraction of the population undergoes programmed cell death during the formation of fruiting bodies. This sacrifice ensures the survival of the sporulating sub-population by providing nutrients and hence energy to complete this differentiation process. The biological relevance and underlying regulatory mechanisms of these three processes will be discussed in order to extract common features of such strategies. Moreover, open questions and future challenges will be addressed.

Introduction

In their natural habitat, bacteria constantly fight for their survival in the face of growth-limiting resources and competition with rivaling species occupying the same ecological niche. One aspect of this biological warfare is the production of antibiotics, secondary metabolites aimed at suppressing the growth or even killing competing microorganisms [1–4]. Not surprisingly, bacteria have developed numerous mechanisms to sense threatening compounds and respond appropriately. Upon stimulus perception, signal transduction systems usually induce gene expression to initiate defense mechanisms, thus ensuring survival [5–8]. Production of and defense against antibiotics are therefore crucial elements of the biological repertoire that bacteria rely on to establish and defend themselves in their territory [9]. But this relatively narrow perspective precludes a number of additional and important properties of antibiotics: There is increasing evidence that at sub-lethal concentrations, antibiotics play an important role as signaling molecules, for example, to induce and coordinate differentiation programs in bacterial communities. In addition, some antimicrobial compounds do not exclusively target hostile competitors but are instead also—sometimes even preferentially—active against the producer species itself and closely related bacteria [10–16].

What could be the biological relevance of producing such self-threatening toxins and how do bacteria control their lethal activity to avoid eradicating themselves? The best-understood examples of antimicrobial compounds that act against the producing organism itself are so-called toxin–antitoxin (TA) systems. TA systems were first identified on plasmids and described as “addiction modules” to ensure plasmid maintenance (reviewed, e.g., in Ref. [17]). Later, such systems were also found on numerous bacterial chromosomes and being responsible for bacterial programmed cell death (PCD) in the context of population traits. The *mazEF*

system of *Escherichia coli* is particularly well studied. This stress-induced "suicide module" triggers PCD to eradicate damaged cells and ultimately ensure the survival of the population in the presence of diverse stress conditions (reviewed in Refs. [18,19]). TA systems are widespread in gram-negative and gram-positive bacteria and, for example, play an important role in the context of biofilm formation in *Staphylococcus aureus* (reviewed in Refs. [20,21]). Irrespective of the exact nature of the toxin, the overall logic of how such TA systems work is comparably similar: the genes encoding the toxin and the antitoxin are normally encoded in an operon and constantly expressed under normal growth conditions. While the toxin is a stable gene product, the antitoxin is not. This causes no harm to the cell as long as constant expression of the operon ensures resupply of the antitoxin: the cells survive. But as soon as the expression of the operon or translation of its mRNA comes to a halt, for example, in response to stress conditions, the difference in protein stability causes an increasing imbalance between the stable toxin and the vanishing antitoxin, ultimately unleashing the lethal activity of the toxin, resulting in PCD.

TA system-mediated PCD has already been covered by a number of excellent and recent reviews (see citations above) and shall therefore not be considered further for this review. Instead, we will exclusively focus on three different phenomena that are associated with multicellular differentiation. (i) At the onset of stationary phase, a sub-population of the gram-positive model organism *Bacillus subtilis* produces cannibalism toxins that kill the non-producing sub-fraction to feed on them and hence delay the commitment for sporulation [22]. (ii) During competence development of *Streptococcus pneumoniae*, pneumococci induce the production of peptide antibiotics, specifically targeting non-competent siblings to enhance the genetic diversity throughout the population in a process termed fratricide [23]. (iii) In *Myxococcus xanthus*, almost the entire population undergoes PCD during fruiting body formation. An extensive "altruistic autolysis" of vegetative cells ensures the survival of a small sub-population to complete their process of sporulation [24,25].

In this review, we will summarize and compare these three processes with respect to their physiological relevance and the underlying regulation in both the producer and the susceptible sub-populations. We will also point out unaddressed and open questions in this field to highlight future research directions.

Coordinated Cell Death and the Role of Phenotypic Heterogeneity

The physiological relevance of such unusual processes can only be understood in the context of bacterial multicellularity and phenotypic heterogeneity: This phenomenon describes the separation of an isogenic population into phenotypically distinguishable sub-populations, each of which is characterized by a specific gene expression profile [26,27]. These diversification processes are common for multicellular traits such as bacterial biofilms [28,29].

Discussing the underlying principles that cause phenotypic heterogeneity goes beyond the scope of this review, and readers are referred to a number of excellent articles on this topic [30–35]. But with regard to its physiological relevance, two distinct evolutionary strategies are generally accepted as biological benefits that ultimately select for phenotypically heterogeneous traits in a population; division-of-labor or bet-hedging.

In the case of *division-of-labor*, a beneficial compound (e.g., a secreted molecule) is produced only by a small part of the population. By making this common good available to the entire population, this reduces the overall production costs without abandoning the advantages. An example of this strategy is the allocation of the chelating agent pyoverdine by a sub-population of *Pseudomonas putida* [36].

Bet-hedging describes a population strategy that aims at minimizing the risks of extinction in fluctuating environments. By splitting the population into sub-populations, each following its own survival strategy, chances are maximized that at least one sub-population will be adequately adapted to any given change in an uncertain future. This strategy deliberately takes a partial extinction of the population into account, to ensure survival of the species. The different cell types that emerge for *B. subtilis* during stationary phase adaption represent one of the best-investigated examples of such a strategy.

In light of these definitions, all three cases of coordinated cell death—cannibalism, fratricide, and altruistic autolysis—could be viewed as extreme examples of division-of-labor, in which the providing sub-population is sacrificed for the benefits of the receiving sub-population.

Cannibalism in *B. subtilis*

Upon starvation, the gram-positive model organism *B. subtilis* initiates a complex differentiation program to adjust and prepare the population to deteriorating growth conditions in fluctuating environments. This bet-hedging strategy leads to the diversification into distinct sub-populations of specialized cell types that try to extract different types of nutrients from the environment [37–39]. The underlying differentiation process is orchestrated in response to numerous extra- and intracellular cues by a complex regulatory network that serves as a check-and-balance systems to monitor the energy level of the cell [40,41]. Ultimately, when faced with severe nutrient limitation for prolonged times, this network activates Spo0A, the master regulator of sporulation, biofilm formation, and also cannibalism [37–39]. This activation is a gradual process that is determined by increasing the active, that is, phosphorylated fraction of this key regulator, Spo0A-P [42]. Of the approx. 120 genes that are

controlled by Spo0A, those involved in biofilm formation and cannibalism toxin production harbor high-affinity Spo0A binding sites that only require low levels of Spo0A-P, while genes that are directly involved in the process of spore formation, such as *spo0IIG* or *spoIIA*, depend on high Spo0A-P levels. [43,44]. Once the latter are induced, *B. subtilis* will ultimately form dormant endospores that are highly resistant and can outlast harsh environmental conditions virtually "forever" [45–47].

In fluctuating environments, where nutrient supply is unpredictable, a premature decision to sporulation could become a fatal dead end trap. The sporulation process is energy demanding, needs several hours for completion, and is at a certain stage irreversible. Continuing to form dormant spores under improving nutrient conditions, with surrounding competitors directly resuming vegetative growth, could easily lead to outgrowth of the spore formers [48]. As a consequence, sporulation is rather considered as a strategy of last resort and *B. subtilis* delays full commitment as long as possible via pursuing a number of alternative differentiation processes [37,49].

One of the last delay strategies is called cannibalism, during which one sub-population of *B. subtilis*, the "cannibals," produce peptide toxins and the corresponding resistance mechanisms. In contrast, the non-producers, which lack this auto-immunity, are killed through lysis. As a result, nutrients are released on which the toxin-producing cannibals can feast on and pursue vegetative growth. The phenomenon was initially identified through the discovery of two early and low Spo0A-P level-dependent genes that are highly expressed at onset of stationary phase. These were then shown to encode the sporulation delaying protein (SDP) and the sporulation killing factor (SKF) [22] (Fig. 1). When nutrients become limiting, *B. subtilis* divides into sub-populations, distinguishable by the broadly heterogeneous distribution of Spo0A-P levels [50,51]. Those cells first reaching a critical, yet low, Spo0A-P level (referred to as Spo0A-ON from now on and in Fig. 1) then activate the cannibalism toxins loci *skfABCDEFG* and *sdpABCRI* among other earliest sporulation genes.

The eight genes comprising the *skf* operon are co-transcribed as one 6.1-kb mRNA and encode a typical antimicrobial peptide (AMP) locus [52] (Fig. 1). The first gene, *skfA*, encodes the 56-amino acid long pro-AMP, which is post-translationally modified by SkfB, a radical *S*-adenosyl-methionine enzyme [53,54]. The resulting pre-SkfA is further processed to its active state by the putative thioredoxin oxidoreductase SkfH [55]. The putative transmembrane protein SkfC is predicted to be involved in the circulation process, prior or during the

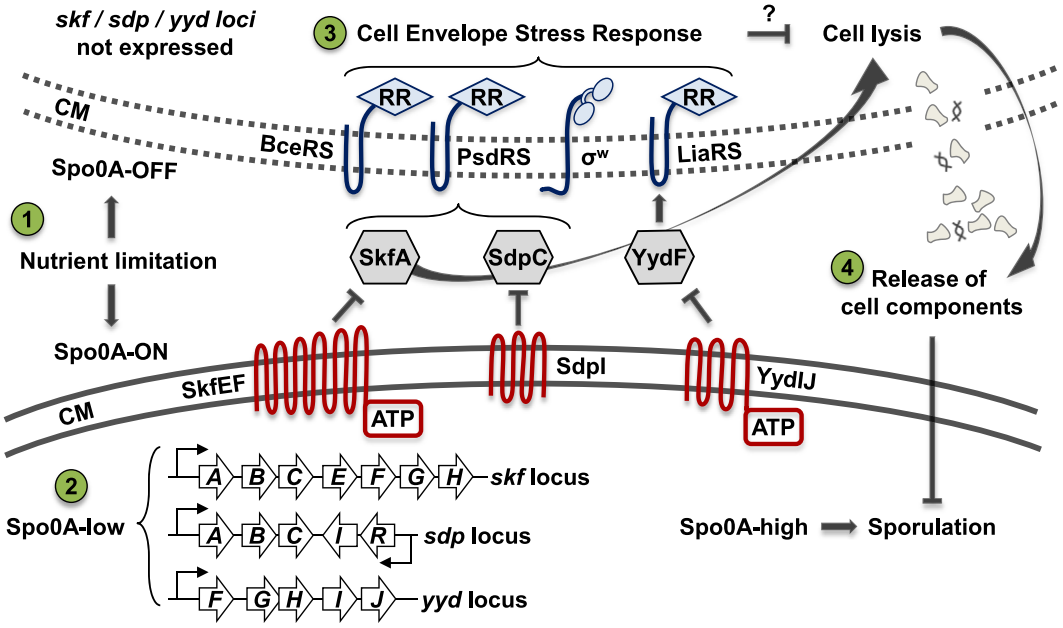

Fig. 1. Concept of cannibalism in *B. subtilis*. Upon nutrient limitation, *B. subtilis* divides into two sub-population differing in their activity of the master regulator Spo0A (1). At increasing levels of Spo0A-P ("Spo0A-ON" cells), the production of the cannibalism toxins loci *skf* and *sdp* is initiated, together with *yyd* (2). The machinery for modification, processing and autoimmunity (black) are co-transcribed for each AMP. The susceptible Spo0A-OFF sub-population lacks any necessary immunity proteins specific to the cannibalism toxins and is solely capable to respond via launching a general CESR (red, 3). The presence of SkfA and SdpC activates the Bce and Psd TCS and triggers the σ^W regulon (opening curly bracket). The YydF peptide is perceived by the LiaRS TCS. Spo0A-ON cells ultimately feed on the nutrients released by their siblings to maintain low levels of Spo0A (4). In case of prolonged nutrient limiting conditions and exceeding Spo0A levels, cells eventually commit to the process of spore formation. T-bars, inhibition; CM, cytoplasmic membrane.

transport across the cytoplasmic membrane. SkfA export is presumably mediated by the ABC-transporter SkfEF, which also provides immunity against the mature SKF toxin [22]. The physiological role of SkfG awaits future clarification. Regulation of the *skf* locus is directly dependent on low Spo0A-P levels, since the *skf* promoter exhibits high affinity for the master regulator [43].

The initial observation of an accelerated sporulation in an *skf* mutant strain leads to the hypothesis that production of the SKF toxin serves as a mechanism to delay sporulation. This was supported by co-cultivation experiments using an *skf* knockout strain and the wild type, demonstrating that a loss of *skf* leads to a decrease in cell count at the onset of stationary phase and that spore formation was increased 10-fold compared to the wild type [22].

In contrast to directly controlling *skf* expression, Spo0A only plays an indirect role for regulating the *sdp* locus. During vegetative growth, the global transition-state regulator AbrB represses *sdp* activity. But with the transition to stationary phase, the *abrB* gene itself is repressed by increasing Spo0A-P levels, ultimately leading to the depletion of the AbrB protein, thus allowing *sdp* expression [56]. Upon transcription of the *sdpABC* operon, the toxin SdpC is post-translationally modified by SdpAB to its active 63 amino acid long form. In addition to its function as a cannibalism toxin, SdpC also acts as a signaling molecule that induces the expression of the cognate autoimmunity gene *spdI* and its repressor gene *sdpR*. An autoregulatory feedback loop then ensures that Spo0A-ON cells that produce the toxin are also protected from its damaging activity: SdpC serves as ligand that binds SdpI, thereby causing a conformational change in the latter that titrates and thereby inactivates the autorepressor SpdR. This results in an increased production of SdpI and SdpR. As long as free SdpC toxin prevails in the extracellular space, this feedback loop remains intact. SdpI binds SdpC and subsequently sequesters SdpR. But as soon as the toxin concentration decreases, free SdpR repressor starts accumulating in the cell and finally shuts down *sdpIR* expression. Spo0A-OFF cells do not induce SdpC production and hence are susceptible to the toxin [56,57]. A detailed study on the mode-of-action of SdpC has revealed that this cationic AMP collapses the proton motive force and induces autolysis in *B. subtilis* [58].

While the term "cannibalism" emphasizes the action of both toxins against *B. subtilis* itself, a number of studies indicate a more versatile physiological role of the cannibalism toxins. Lamsa *et al*. [58] demonstrated that SDP inhibits the growth of a variety of Firmicutes bacteria, including different *Bacillus* spp. and *Staphylococcus epidermidis*. Competition experiments between differentiating biofilms of *B. subtilis* and *Bacillus simplex* demonstrated that the cannibalism toxins of *B. subtilis*—together and cooperatively with surfactin—inhibit the growth and biofilm formation of *B. simplex* at concentrations that are tolerated by *B. subtilis* biofilms [59]. These findings are in line with a previous report demonstrating an important role of cannibalism in biofilm development for *B. subtilis* itself. Indeed, the matrix-producing subpopulation within the biofilm is identical to the cannibals, and both traits are simultaneously triggered by surfactin [39] in good agreement with the above study. SDP and SKF therefore seem to play multiple roles in both intra- and interspecies competition, with the ultimate goal to ensure survival of *B. subtilis* population in biofilm communities.

Cannibalism toxins have also been shown to trigger the cell envelope stress response (CESR) in *B. subtilis* [60]. Maintenance of envelope integrity is orchestrated by the two-component systems BceRS, PsdRS, and LiaRS, and extracytoplasmic function sigma factors [61] (Fig. 1). It is attractive to postulate that especially in the Spo0A-OFF sub-population, which fails to produce any of the SKF- or SDP-specific immunity proteins, the envelope stress caused by the action of these cannibalism toxins results in launching a general CESR when faced with these toxins. Planctonic cultures indeed intrinsically activate the BceR- and PsdR-dependent target promoters at the onset of stationary phase. This induction depends on the presence of both cannibalism toxins. However, the physiological significance of launching the general CESR remains to be discovered, since none of the systems provide any detectable resistance against cannibalism toxins [60].

In addition to the known cannibalism toxins, *B. subtilis* also produces other AMPs, including the YydF peptide (Fig. 1). The *yyd* locus was initially identified in a transposon mutagenesis study, aimed at identifying genes that intrinsically activate LiaR-dependent gene expression [62]. Mutants carrying different transposon insertions in the *yydIJ* genes, encoding an ABC transporter, showed elevated P_{lial} promoter activity. The *lial* gene is co-transcribed with *liaH* and reassembles the only target of the LiaR response regulator in *B. subtilis* [63,64]. Recently, the biosynthesis and antimicrobial activity of YydF have been demonstrated [65]. Ongoing follow-up investigations in our laboratory on the mode-of-action of YydF verified that this AMP indeed triggers the Lia system both intrinsically in stationary phase and when externally applied.

Taken together, *B. subtilis* initiates an Spo0A-dependent differentiation program upon starvation that leads to a bifurcation of the population. While the Spo0A-ON sub-population produces the SKF and SDP cannibalism toxins, the Spo0A-OFF cells serves as a sacrifice that is lysed, thereby releasing nutrients that can delay the commitment to sporulation in the toxin-producing Spo0A-ON sub-population. The susceptible Spo0A-OFF cells launch a general CESR that may play a role in preventing cell lysis. While very attractive, this

hypothesis still awaits verification, particularly at single cell level, as discussed at the end of this review.

Fratricide in *S. pneumoniae*

S. pneumoniae is not only the causative agent of pneumonia but also an important model organism in bacterial genetics. In his famous experiments, Griffith [66] demonstrated in 1928 that the ability to synthesis a capsule and hence become virulent can be transferred from heat-inactivated "smooth" strains to living but avirulent "rough" strains of *S. pneumoniae*. This initial observation was then taken up and painstakingly expanded by Avery *et al*. [67], who ultimately demonstrated for the very first time that DNA (and not proteins or lipids) is the "transforming principle." These hallmark studies not only paved the way for identifying the crucial role of DNA in heredity but also identified the process of transformation, that is, the uptake of free DNA from the environment. *S. pneumoniae* is therefore the first of an ever-increasing number of bacteria that can become naturally competent for genetic transformation. Fratricide is initiated along with the development of natural competence: non-competent siblings are challenged with killing factors that ultimately lead to allolysis, cell death through cell-lysis either directly or *in trans* (Fig. 2) [68,69].

S. pneumoniae enters the competent state under favorable growth conditions during exponential growth, in a process that is governed by quorum sensing [16,70,71]. The rapid increase in cell density leads to an extracellular accumulation of the quorum sensing signal, the *competence-stimulating peptide* (CSP). Once it exceeds a threshold concentration, it activates the ComD histidine kinase, leading to its autophosphorylation. Upon phospho-transfer, its cognate response regulator, ComE, becomes activated and regulates about 20 early competence (*com*) genes [70,72,73]. Among these are two identical copies of *comX*, which encode alternative sigma factors that activate the transcription of the late *com* genes [74] (Fig. 2). In total, the CSP-responsive regulon in *S. pneumoniae* comprises about 120 genes, of which only 22 are necessary for developing natural competence [23,75,76]. This suggests that the majority of the *com* system is involved in additional cellular processes beyond the machinery required for DNA uptake and recombination [16].

Fratricide is expressed both in a ComX-dependent and -independent manner. The ComX-dependent genes encode the murein hydrolases CbpD, the autolysin LytA as well as the two peptide bacteriocin CibAB [23,68,77]. In addition, the non-CSP-regulated lysozyme LytC is suggested to contribute to fratricide

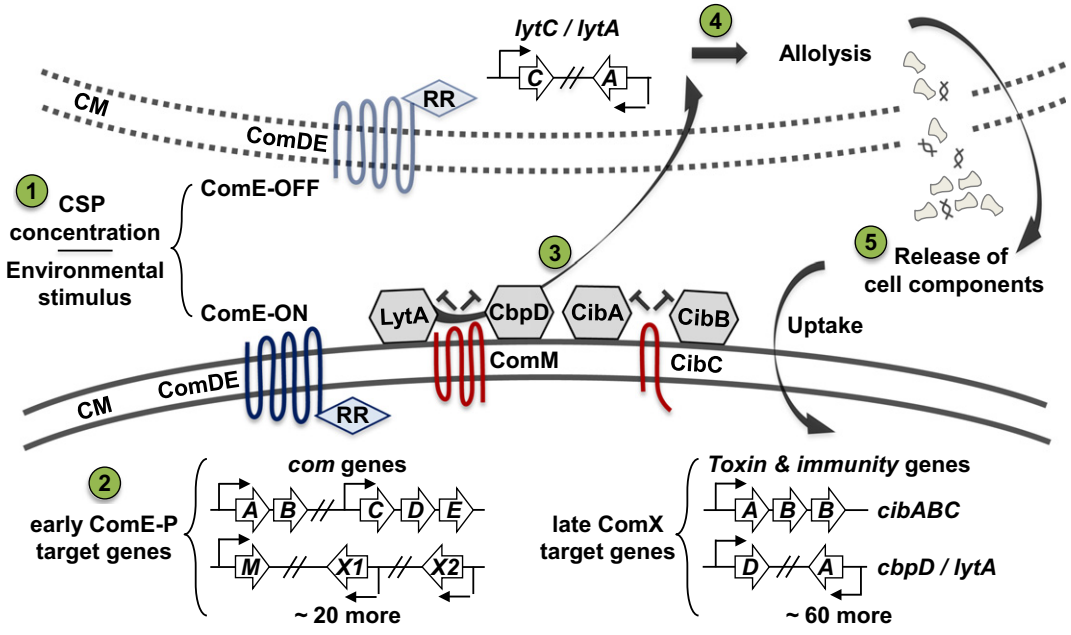

Fig. 2. Concept of fratricide in *S. pneumoniae*. Under favorable growth conditions and upon reaching a critical threshold concentration of quorum sensing-dependent CSP, *S. pneumoniae* becomes naturally competent (1). Upon activation and autoregulatory stimulation of the ComDE (red)-dependent genes in the competent sub-population, the competence σ factor ComX is activated and initiates fratricide (2): the two-peptide bacteriocin CibAB and the murein hydrolase CbpD are produced (3). Auto-immunity is ensured by CibC and ComM (black, T-bars). As a consequence, the non-competent, ComE-OFF siblings are killed by allolysis, either directly or via LytA (4). Cellular components are released and potentially enhance the genetic diversity of the population, for example, by facilitating the exchange of virulence factors (5). T-bars, inhibition; CM, cytoplasmic membrane.

[78] (Fig. 2). Experimentally, fratricide is monitored in liquid culture based on the release of chromosomal DNA and cytoplasmic β-galactosidase, as well as the ability for clumping [79–81]. Clumping was already observed more than four decades ago under mild acidic conditions and linked to competence development. Later, it was demonstrated that this behavior relies on the release of chromosomal DNA [23,82]. Fratricide is also detectable on solid blood agar plates, where lysis can be followed by the release of pneumolysin, a cytolytic virulence factor [68].

The contribution and impact of the individual fratricide toxins depends on the assay applied [16]. CbpD plays only a minor role in fratricide development on plates, but abolishes the effect of clumping and has a strong effect on DNA and β-galactosidase release in liquid cultures [23,68,77]. The opposite effects are observed for CibAB, since inactivation leads to the loss of pneumolysin release on plates, while clumping remains un-effected [23,68]. In contrast, both lytic enzymes, LytA and LytC, are absolutely required, since inactivation results in a strong and assay-independent reduction of fratricide.

The lysis of non-competent cells by the competent cells is termed *allolysis* and requires cell-to-cell contact [68]. In the absence of the lytic enzymes, CibAB cannot provoke allolysis alone, suggesting that CibAB only supports cell lysis by inserting into the membrane of non-competent siblings and de-energizing them, thereby increasing their susceptibility toward lysis [16,23,68]. CibAB is co-transcribed with a putative transmembrane protein, CibC, which is implicated in CibAB immunity, since its inactivation increases susceptibility to allolysis [68]. Protection against self-lysis of the competent subpopulation is ensured by ComM, a CSP-responsive early *com* gene [23] (Fig. 2). Overexpression of ComM results in growth inhibition and severe morphological defects. During competence, accumulation of ComM is prevented through its processing—and hence inactivation—by an intra-membrane protease [83]. But the mechanism of how this membrane protein confers resistance is still unknown.

The physiological role of fratricide is still not fully understood. It seems to play a role in enhancing the genetic diversity throughout the population by providing extracellular DNA for uptake. By the targeted elimination of non-competent cells, the exchange of genetic material could be promoted [23]. Fratricide seems to be particularly important for efficient gene transfer between pneumococci in biofilms, where it is important for the active acquisition of homologous donor DNA under natural conditions [84]. An antibiotic resistance marker was transferred much more efficiently from neighboring cells than from the growth medium. Under biofilm conditions, efficient lysis of target cells requires CbpD and LytC, while the major autolysin LytA does not seem to be important for fratricide in the biofilm environment [84]. Another hypothesis along those lines suggests that *S. pneumoniae* triggers fratricide in order to release potential cytoplasmic virulence factors and inflammatory mediators from the non-competent cells, as part of the infection process within the host [16,23].

PCD in *M. xanthus*

M. xanthus is a soil-dwelling gram-negative δ-proteobacterium with a very unusual life style [85]. It is a social predator that hunts in swarming packs and feeds on the lysed remains of other bacteria and fungi serving as prey [86–88]. These joint attacks are coordinated at the population level and require efficient contact-dependent cell-to-cell communication [89]. When faced with nutrient-limiting conditions, *M. xanthus* cells aggregate and ultimately develop into fruiting bodies. These beautifully shaped, mushroom-like multicellular structures are a prerequisite for forming reproductive myxospores [90]. All of these different behaviors are embedded in a complex regulatory network that orchestrates this spatio-temporal developmental program through strictly coordinated cascades of gene expression [87,91].

Fruiting body formation requires a solid surface to allow motility and reaching a critical threshold of population density to initiate the cascade of inter- and intra-signaling [87,92]. As a result of this regulation, approximately 80% of the vegetative, non-sporulating cells altruistically initiate PCD during this stage in the life cycle [93]. This type of profound self-commitment carried out by such a large portion of the population is tightly regulated and only pursued upon successfully passing multiple checkpoints [87,89].

Two different events trigger the earliest stages of *Myxococcus* fruiting body formation: nutrient depletion and high cell density. In bacteria, cell density-dependent processes are usually coordinated through quorum sensing signals, such as signaling peptides (common in gram-positive bacteria) or homoserine-lactones (the typical gram-negative quorum sensing molecules). These signaling molecules are produced and secreted by all cells of the population and thereby accumulate in the medium of a growing population until a critical threshold concentration is reached. This will then be perceived by suitable sensor proteins and transduced to the inside to initiate gene expression programs, often resulting in a synchronization of gene expression patterns between the cells of a bacterial population. In the case of *Myxococcus* development, the chemical nature of the quorum-sensing-like molecule, termed A-signal, that initiates the differentiation process is unknown. Its production depends on the *asgA*, *asgB*, and *asgC* gene loci [94,95], which encode regulatory functions (summarized in Ref. [96]). These depend on the stringent response. The latter is initiated upon amino acid starvation and perceived based on the occurrence of uncharged tRNAs during translation. In response, the ribosome-associated protein RelA mediates the formation of the second messenger

guanosine tetra- (and penta-) phosphate ((p)ppGpp), an "alarmone" that in response activates amino acid biosynthesis and reduces rRNA and tRNA synthesis. In *Myxococcus,* these two events, nutrient depletion and high cell density, trigger the early stages of fruiting body formation (Fig. 3) [96]. The intracellular response is mediated by the sensor histidine kinase SasS [89,97,98]. A-signaling, together with accumulation of (p)ppGpp leads to transcription of FruA [99,100]. This master regulator then induces the contact-dependent C-signaling pathway (Fig. 3), which requires a processed product of the *csgA* gene, termed p17 [89]. This C-signal is an outer membrane-attached protein facing the extracellular space that is recognized by a yet unidentified p17-receptor. Thus, transmission and coordination requires direct cell-to-cell contact [101]. Upon close contact, activated FruA cells proceed on the path toward sporulation via activating the *dev* operon and regulate aggregation by targeting the *frz* locus [102–108].

A fruiting body consists of about 10^5 cells, but only about 10% of the population undergoes the process of sporulation under laboratory conditions [92]. A small part of the population remains outside the complex and is referred to as peripheral rods. This specialized cell type is well adapted for nutrient-limiting conditions and resembles persistent cells [109–111]. Development of peripheral rods could be explained by either the lack of C-signaling through insufficient cell-to-cell contact or an insufficient accumulation of the transcriptional regulators required for activation of FruA [106,112]. Remarkably, the vast majority of cells within the fruiting body initiate PCD [93]. This sacrifice is thought to be dedicated to the spore-forming subpopulation to provide sufficient resources for completing their differentiation program under conditions of severe nutrient limitations [87].

To date, two strategies are proposed addressing the mechanism of PCD. On the one hand, production of autocides, a composition of fatty acids and phosphatidyl ethanolamines, leads to destabilization of the cell membrane ultimately causing cell lysis [113,114]. The second strategy comprises MazF and MrpC (Fig. 3) [93,115], a typical TA system as described at the beginning of this review. During vegetative growth, MazF–MrpC are negatively regulated via a cascade of protein kinases, which comprise Pkn8 and Pkn14 [115]. Transition to fruiting body formation leads to modification of MrpC by the ATP-dependent protease LonD, which liberates MrpC from the repression by the

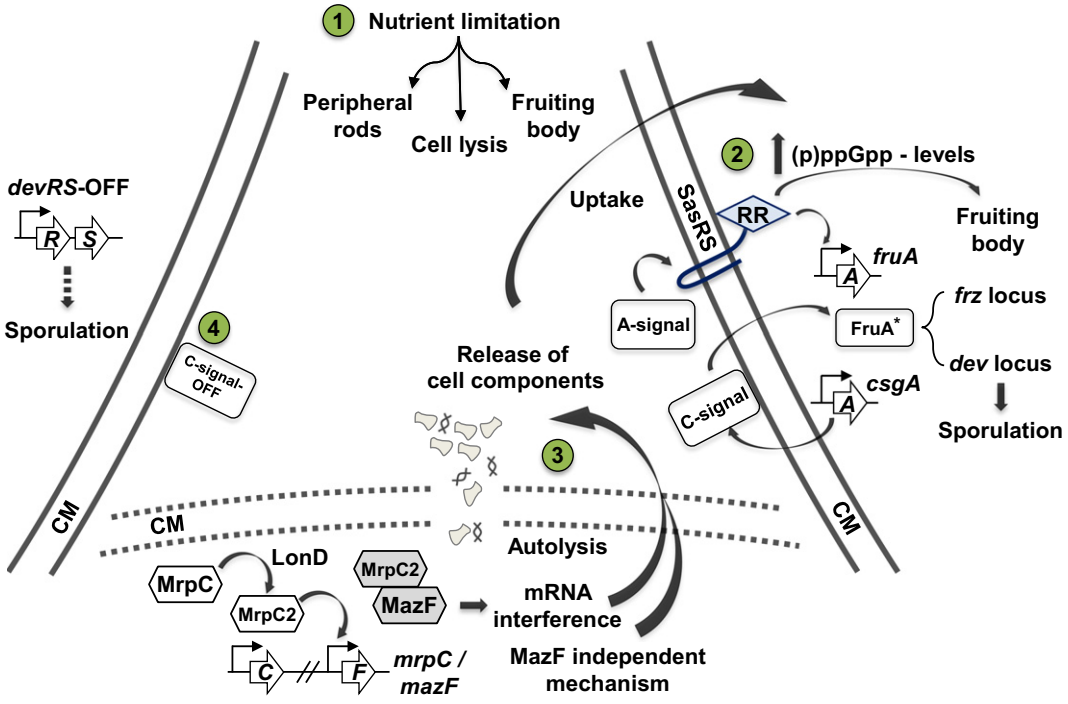

Fig. 3. Concept of PCD in *M. xanthus*. Nutrient limitation triggers fruiting body formation in *M. xanthus* (1). The environmental cues are integrated by the A- and C-signaling pathways (hexagons). The cellular response is mediated by the sensor histidine kinase SasS (red), which—in combination with accumulated (p)ppGpp levels—activates the master regulator FruA (2). This sub-population is dedicated to aggregation and ultimately spore formation, a process controlled by the gene products of the *dev* operon and the *frz* locus, respectively. A large proportion of cells within the fruiting body commit PCD and thereby releases nutrients for the sporulating sub-population (3). Autolysis of the sacrificed cells is mediated by the TA system MazF–MrpC or the accumulation of autocides (see text for details). Outside of the fruiting bodies, peripheral rods fail to induce C-signaling and take over the role of persister cells (4). CM, cytoplasmic membrane.

Pkn8–Pkn14 cascade [115,116]. Finally, MrpC regulates MazF production. This endonuclease causes severe cleavage of cellular mRNAs, thereby potentially triggering autolysis via the induction of multiple autolytic enzymes [115] (Fig. 3).

The concept of sacrificing a significant percentage of the population to allow a subpopulation to complete sporulation has also been described for streptomycetes. During their differentiation cycle, these filamentous gram-positive bacteria switch their growth and life style from a primary, substrate mycelium, which displays primary metabolism to a secondary, aerial mycelium that switches to secondary metabolism. Ultimately, the terminal so-called sporogenic cells will be transformed into large chains of spores in a complex differentiation process [117,118]. In the course of this process, the mycelium undergoes two waves of PCD: The first affects certain hyphae segments of the substrate mycelium. Later in the process, the innermost part of the aerial mycelium suffers a second round of PCD, which is thought to provide the uppermost, sporogenic cells with enough nutrients to finish the process of sporulation [119,120].

Coordinated Cell Death: Altruistic Suicide or Social Killing?

Bacteria are constantly faced with changes of abiotic as well as biotic factors in their environment. Appropriate responses allow them to actively participate in, adjust, and also contribute to their habitat. Antibiotics are but one example of such an exchange between a cell and its surrounding. While antibiotics are produced by bacteria to outlast the "chemical warfare" with competitors, some antimicrobial compounds show a surprisingly narrow specificity and seem to be particularly active against the producing species themselves. Here, we have addressed this phenomenon by focusing on compounds involved in developmental processes that could be regarded as "social killing": antimicrobial compounds that are produced by one sub-population to kill another subpopulation with the ultimate goal of generating a benefit for the whole population.

In *B. subtilis* cannibalism, the two corresponding toxins, SDP and SKF, are proposed to ultimately delay the committing and hence irreversible process of sporulation. The advantage of this sacrifice of one subpopulation is that it extends the time that the starving population can remain in a "transition state," which enables the cells to still respond to their environment, for example, in case that nutrients do become available after a period of famine. This logic is in line with viewing sporulation as a strategy of last resort that a population only embarks on, if all else—including killing some of their siblings—fails [37,49].

Such a delay is physiologically highly relevant, since the process of endospore formation is time- and energy-consuming, and might turn into a selective disadvantage against other microorganisms competing for the same ecological niche, in case that nutrients become available again. Once committed, sporulation proceeds irrespective of environmental changes, even if those would be beneficial for vegetative cells. As a consequence, sporulating cells would suddenly find themselves surrounded by growing and dividing competitors that feast on external nutrients and ultimately outgrow *B. subtilis*. In light of this threat, production of SDP and SKF could provide an internal source of nutrients that ensures that *B. subtilis* remains responsive to its environment for a longer period of time.

But the tight link between cannibalism and biofilm formation suggests another, more subtle role of cannibalism: SDP and SKF might provide *B. subtilis* populations with a mechanism to identify "kin" cells that contribute to the extracellular matrix and discriminate them from and thereby also simultaneously removing non-producing cheaters that would otherwise benefit from this public good without contributing to it (discussed recently in Ref. [121]). The cannibalism toxins would therefore have a dual role as both signals of kin discrimination (input) and also effectors by eliminating non-resistant, non-producing cheaters within a biofilm (output). In addition, the observed activity of cannibalism toxins against other Firmicutes species indicates that SDP and SKF might also function as "ordinary" AMPs that suppress the growth of competitors and protect the biofilm community. Clearly, we have just begun to grasp the complexity of the cannibalism strategy (see next section for open questions).

With regard to the two major strategies the underlay phenotypic heterogeneity [34,35], cannibalism seems to best represent an extreme case of division-of-labor (as stated above), since the Spo0A-OFF cells are sacrificed and seem to only play a role as a source of nutrients for the Spo0A-ON fraction. It is not possible to envision any circumstances for Spo0A-OFF cells that would provide them with a selective advantage over Spo0A-ON cells. Hence, the prerequisites for bet-hedging do not seem to be fulfilled [35].

In contrast, PCD of *M. xanthus* in the course of fruiting body development has been evidently demonstrated [25] and its physiological role seems to be clear: the death of a majority of cells within fruiting bodies provides those cells undergoing sporulation with enough energy to complete the process. This sacrifice is therefore necessary to ensure survival of the population during starvation [90]. And the same interpretation seems to hold true for the comparable PCD during sporulation in streptomycetes. While both types of PCD and cannibalism are all associated with sporulation during stationary phase upon severe nutrient limitation, the latter seems to have little else in common with the PCD of myxobacteria and streptomycetes.

The physiological role of fratricide differs in many respects from both of these two phenomena. Since competence for genetic transformation in *S. pneumoniae* is reached in exponential phase, nutrient limitation does not seem to play a role for sacrificing a sub-population. Moreover, fratricide is not linked to a differentiation process but instead to quorum sensing-dependent development of genetic competence, the ability to take up free DNA from the environment. While initial studies were performed in liquid cultures or on solid agar plates [79–81], follow-up work indicated that fratricide-induced allolysis during competence provides a significant fitness advantage for pneumococcal cells exchanging genetic material in biofilms [84]. Most recently, fratricide toxins have been implicated in being important for the competition that determines the success in host colonization and persistence. In this "owner–intruder asymmetry," a disproportionate success rate of the initial resident "owner" over the "intruder" was observed [122]. Among others, this competitive advantage of the residents could be associated with the competence-induced bacteriocins A and B (CibAB) implicated in fratricide.

Open Questions and Future Directions

While the physiological role of both PCD and fratricide seem to become clearer, cannibalism, in particular, still holds more questions than it offers answers. In fact, the only detailed follow-up studies on this phenomenon solely addressed the biosynthetic pathway and mechanism-of-action of the two cannibalism toxins SDP and SKF [55,58]. The true nature and relevance of cannibalism itself has been virtually untouched since the initial discovery [22]. While the CibAB toxin loci and the MazF toxin are widely distributed, the two AMPs originally associated with cannibalism—SDP and SKF—as well as homologs to the corresponding genetic loci are hardly found outside *B. subtilis*. According to the Microbesonline database, the *skf* locus is only present in *Paenibacillus larvae* (out of 52 different *Bacillus* spp. genomes currently represented in the database) and not found outside this genus. The *sdpABC* operons is only conserved in *Bacillus clausii* and the *Bacillus cereus* plasmid pBC239. This very narrow distribution of the two cannibalism toxin loci indicates a very unique role of this process in differentiation of *B. subtilis*. Remarkably, the only significant hit outside the genus *Bacillus* is found in *M. xanthus* (MXAN_6613–6615).

For cannibalism, the current hypothesis demands that Spo0A-OFF cells should be susceptible to SDP/SKF-dependent killing and hence lysis, while Spo0A-ON cells, which activate the production of cannibalism toxins, should (i) be immune to SDP/SKF-dependent killing and (ii) be able delay sporulation by resuming growth after lysis of Spo0A-OFF cells.

While all results summarized in this review are consistent with the physiological role and social function attributed to cannibalism, a direct demonstration is still missing. Approaching such hypotheses will require single-cell data. While the study of microcolonies in microfluidic devices has already been used for a number of important studies on phenotypic heterogeneity in *B. subtilis* [123,124], it has so far not been applied to address the role of cannibalism. This experimental approach would be crucial to establish a direct correlation between activation of Spo0A and production of cannibalism toxins. If the producing subpopulation indeed feeds on the released nutrients of the killed siblings cells, differences in the morphology and maybe even altered growth behavior could be expected.

Such studies would also be crucial to understand the physiological link between CESR and cannibalism, since induction of the CESR does not seem to provide any fitness advantage to the population, at least on bulk level ([60], unpublished data). Approaching this question would require correlating the induction of CESR with both the production of cannibalism toxins and the activation of Spo0A. The most obvious hypothesis is that induction of CESR systems occurs in Spo0A-OFF cells that do not produce SDP and SKF and hence also lack autoimmunity against these AMPs. However, it is also possible that the intrinsic activation of CESR systems occurs in the producers themselves, either due to a temporal delay between production and autoimmunity development, or because SKF and SDP exceed concentrations that the self-protection machinery can handle alone.

Outlook

Given the huge implications that coordinated cell death should have on understanding bacterial physiology and differentiation, it is surprising that the concepts of cannibalism, fratricide or PCD—all identified over 15 years ago—have not been found or studied outside the originally described microorganisms. So far, all three remain isolated phenotypes associated with only a single organism each. While fratricide is clearly a mechanism that seems to be widespread in streptococci, the cannibalism loci of *B. subtilis* and the PCD-related functions in *M. xanthus* seem to be almost restricted to these organisms, indicative of a very organism-specific function. But a lack of sequence conservation of these specific systems does not rule out a much wider distribution of comparable mechanisms mediating coordinated cell death in other microorganisms. But identifying such mechanisms will first require a shift in perception of microorganisms: altruistic death and killing only make sense in the context of appreciating bacteria as multicellular organisms that live in diversified and highly differentiated communities consisting of

different sub-populations with distinct functions. It seems that this journey has just begun.

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Abbreviations used:
TA, toxin–antitoxin; PCD, programmed cell death; SDP, sporulation delaying protein; SKF, sporulation killing factor; AMP, antimicrobial peptide; CESR, cell envelope stress response; CSP, competence-stimulating peptide.

References

[1] T.L. Czárán, R.F. Hoekstra, L. Pagie, Chemical warfare between microbes promotes biodiversity, Proc. Natl. Acad. Sci. 99 (2002) 786–790, https://doi.org/10.1073/pnas.012399899.

[2] B. Kerr, M.A. Riley, M.W. Feldman, B.J.M. Bohannan, Local dispersal promotes biodiversity in a real-life game of rock–paper–scissors, Nature. 418 (2002) 171–174, https://doi.org/10.1038/nature00823.

[3] N. Narisawa, S. Haruta, H. Arai, M. Ishii, Y. Igarashi, Coexistence of antibiotic-producing and antibiotic-sensitive bacteria in biofilms is mediated by resistant bacteria, Appl. Environ. Microbiol. 74 (2008) 3887–3894, https://doi.org/10.1128/AEM.02497-07.

[4] K.R. Foster, T. Bell, Competition, not cooperation, dominates interactions among culturable microbial species, Curr. Biol. 22 (2012) 1845–1850, https://doi.org/10.1016/j.cub.2012.08.005.

[5] K. Stephenson, J.A. Hoch, Virulence- and antibiotic resistance-associated two-component signal transduction systems of gram-positive pathogenic bacteria as targets for antimicrobial therapy, Pharmacol. Ther. 93 (2002) 293–305, https://doi.org/10.1016/S0163-7258(02)00198-5.

[6] A.M. Stock, I.B. Zhulin, Two-component signal transduction: a special issue in the *Journal of Bacteriology*, J. Bacteriol. 199 (2017)https://doi.org/10.1128/JB.00443-17.

[7] S. Jordan, M.I. Hutchings, T. Mascher, Cell envelope stress response in gram-positive bacteria, FEMS Microbiol. Rev. 32 (2008) 107–146, https://doi.org/10.1111/j.1574-6976.2007.00091.x.

[8] R.L. Guest, T.L. Raivio, Role of the gram-negative envelope stress response in the presence of antimicrobial agents, Trends Microbiol. 24 (2016) 377–390, https://doi.org/10.1016/j.tim.2016.03.001.

[9] M.A. Bauer, K. Kainz, D. Carmona-Gutierrez, F. Madeo, Microbial wars: competition in ecological niches and within the microbiome, Microb. Cell. 5 (2018) 215–219, https://doi.org/10.15698/mic2018.05.628.

[10] G. Yim, H.H. Wang, J. Davies, Antibiotics as signalling molecules, Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 362 (2007) 1195–1200, https://doi.org/10.1098/rstb.2007.2044.

[11] D. Romero, M.F. Traxler, D. Lopez, R. Kolter, Antibiotics as signal molecules, Chem. Rev. 111 (2011) 5492–5505, https://doi.org/10.1021/cr2000509.

[12] A. Fajardo, J.L. Martínez, Antibiotics as signals that trigger specific bacterial responses, Curr. Opin. Microbiol. 11 (2008) 161–167, https://doi.org/10.1016/j.mib.2008.02.006.

[13] C.D. Nadell, J.B. Xavier, K.R. Foster, The sociobiology of biofilms, FEMS Microbiol. Rev. 33 (2009) 206–224, https://doi.org/10.1111/j.1574-6976.2008.00150.x.

[14] M.I. Abrudan, F. Smakman, A.J. Grimbergen, S. Westhoff, E.L. Miller, G.P. van Wezel, et al., Socially mediated induction and suppression of antibiosis during bacterial coexistence, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 11054–11059, https://doi.org/10.1073/pnas.1504076112.

[15] J.E. González-Pastor, Cannibalism: a social behavior in sporulating *Bacillus subtilis*, FEMS Microbiol. Rev. 35 (2011) 415–424, https://doi.org/10.1111/j.1574-6976.2010.00253.x.

[16] J.-P. Claverys, L.S. Håvarstein, Cannibalism and fratricide: mechanisms and raisons d'être, Nat. Rev. Microbiol. 5 (2007) 219–229, https://doi.org/10.1038/nrmicro1613.

[17] H. Engelberg-Kulka, G. Glaser, Addiction modules and programmed cell death and antideath in bacterial cultures, Annu. Rev. Microbiol. 53 (1999) 43–70, https://doi.org/10.1146/annurev.micro.53.1.43.

[18] H. Engelberg-Kulka, S. Amitai, I. Kolodkin-Gal, R. Hazan, Bacterial programmed cell death and multicellular behavior in bacteria, PLoS Genet. 2 (2006), e135. https://doi.org/10.1371/journal.pgen.0020135.

[19] B.C.M. Ramisetty, B. Natarajan, R.S. Santhosh, mazEF-mediated programmed cell death in bacteria: "what is this?", Crit. Rev. Microbiol. 41 (2015) 89–100, https://doi.org/10.3109/1040841X.2013.804030.

[20] C.F. Schuster, R. Bertram, Toxin–antitoxin systems of *Staphylococcus aureus*, Toxins (Basel) 8 (2016) 140, https://doi.org/10.3390/toxins8050140.

[21] D.E. Moormeier, K.W. Bayles, *Staphylococcus aureus* biofilm: a complex developmental organism, Mol. Microbiol. 104 (2017) 365–376, https://doi.org/10.1111/mmi.13634.

[22] J.E. González-Pastor, E.C. Hobbs, R. Losick, Cannibalism by sporulating bacteria, Science. 301 (2003) 510–513, https://doi.org/10.1126/science.1086462.

[23] L.S. Håvarstein, B. Martin, O. Johnsborg, C. Granadel, J.-P. Claverys, New insights into the pneumococcal fratricide: relationship to clumping and identification of a novel immunity factor, Mol. Microbiol. 59 (2006) 1297-1037, https://doi.org/10.1111/j.1365-2958.2005.05021.x.

[24] J.W. Wireman, M. Dworkin, Morphogenesis and developmental interactions in myxobacteria, Science. 189 (1975) 516–523.

[25] J.W. Wireman, M. Dworkin, Developmentally induced autolysis during fruiting body formation by *Myxococcus xanthus*, J. Bacteriol. 129 (1977) 798–802.
[26] M. Ackermann, A functional perspective on phenotypic heterogeneity in microorganisms, Nat. Rev. Microbiol. 13 (2015) 497–508, https://doi.org/10.1038/nrmicro3491.
[27] M.B. Elowitz, A.J. Levine, E.D. Siggia, P.S. Swain, Stochastic gene expression in a single cell, Science. 297 (2002) 1183–1186, https://doi.org/10.1126/science.1070919.
[28] D. Claessen, D.E. Rozen, O.P. Kuipers, L. Søgaard-Andersen, G.P. van Wezel, Bacterial solutions to multicellularity: a tale of biofilms, filaments and fruiting bodies, Nat. Rev. Microbiol. 12 (2014) 115–124, https://doi.org/10.1038/nrmicro3178.
[29] H. Vlamakis, C. Aguilar, R. Losick, R. Kolter, Control of cell fate by the formation of an architecturally complex bacterial community, Genes Dev. 22 (2008) 945–953, https://doi.org/10.1101/gad.1645008.
[30] S.A. West, G.A. Cooper, Division of labour in microorganisms: an evolutionary perspective, Nat. Rev. Microbiol. 14 (2016) 716–723, https://doi.org/10.1038/nrmicro.2016.111.
[31] D. Huh, J. Paulsson, Non-genetic heterogeneity from stochastic partitioning at cell division, Nat. Genet. 43 (2011) 95–100, https://doi.org/10.1038/ng.729.
[32] N.Q. Balaban, J. Merrin, R. Chait, L. Kowalik, S. Leibler, Bacterial persistence as a phenotypic switch, Science. 305 (2004) 1622–1625, https://doi.org/10.1126/science.1099390.
[33] N. Nikolic, T. Barner, M. Ackermann, Analysis of fluorescent reporters indicates heterogeneity in glucose uptake and utilization in clonal bacterial populations, BMC Microbiol. 13 (2013) 258, https://doi.org/10.1186/1471-2180-13-258.
[34] A. Dal Co, C. Brannon, M. Ackermann, Division of labor in bacteria, Elife. 7 (2018), 497. https://doi.org/10.7554/eLife.38578.
[35] J.-W. Veening, W.K. Smits, O.P. Kuipers, Bistability, epigenetics, and bet-hedging in bacteria, Annu. Rev. Microbiol. 62 (2008) 193–210, https://doi.org/10.1146/annurev.micro.62.081307.163002.
[36] F. Becker, K. Wienand, M. Lechner, E. Frey, H. Jung, Interactions mediated by a public good transiently increase cooperativity in growing *Pseudomonas putida* metapopulations, Sci. Rep. 8 (2018), 4093. https://doi.org/10.1038/s41598-018-22306-9.
[37] D. Lopez, R. Kolter, Extracellular signals that define distinct and coexisting cell fates in *Bacillus subtilis*, FEMS Microbiol. Rev. 34 (2010) 134–149, https://doi.org/10.1111/j.1574-6976.2009.00199.x.
[38] T. Msadek, When the going gets tough: survival strategies and environmental signaling networks in *Bacillus subtilis*, Trends Microbiol. 7 (1999) 201–207.
[39] D. Lopez, H. Vlamakis, R. Kolter, Generation of multiple cell types in *Bacillus subtilis*, FEMS Microbiol. Rev. 33 (2009) 152–163, https://doi.org/10.1111/j.1574-6976.2008.00148.x.
[40] D. Burbulys, K.A. Trach, J.A. Hoch, Initiation of sporulation in *B. subtilis* is controlled by a multicomponent phosphorelay, Cell. 64 (1991) 545–552.
[41] M. Jiang, W. Shao, M. Perego, J.A. Hoch, Multiple histidine kinases regulate entry into stationary phase and sporulation in *Bacillus subtilis*, Mol. Microbiol. 38 (2000) 535–542.
[42] M. Fujita, R. Losick, Evidence that entry into sporulation in *Bacillus subtilis* is governed by a gradual increase in the level and activity of the master regulator Spo0A, Genes Dev. 19 (2005) 2236–2244, https://doi.org/10.1101/gad.1335705.
[43] M. Fujita, J.E. González-Pastor, R. Losick, High- and low-threshold genes in the Spo0A regulon of *Bacillus subtilis*, J. Bacteriol. 187 (2005) 1357–1368, https://doi.org/10.1128/JB.187.4.1357-1368.2005.
[44] J.-W. Veening, L.W. Hamoen, O.P. Kuipers, Phosphatases modulate the bistable sporulation gene expression pattern in *Bacillus subtilis*, Mol. Microbiol. 56 (2005) 1481–1494, https://doi.org/10.1111/j.1365-2958.2005.04659.x.
[45] R.J. Cano, M.K. Borucki, Revival and identification of bacterial spores in 25- to 40-million-year-old Dominican amber, Science. 268 (1995) 1060–1064.
[46] R.H. Vreeland, W.D. Rosenzweig, D.W. Powers, Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal, Nature. 407 (2000) 897–900, https://doi.org/10.1038/35038060.
[47] J. Narula, S.N. Devi, M. Fujita, O.A. Igoshin, Ultrasensitivity of the *Bacillus subtilis* sporulation decision, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) E3513–E3522, https://doi.org/10.1073/pnas.1213974109.
[48] A.D. Grossman, Genetic networks controlling the initiation of sporulation and the development of genetic competence in *Bacillus subtilis*, Annu. Rev. Genet. 29 (1995) 477–508, https://doi.org/10.1146/annurev.genet.29.1.477.
[49] M.D. Yudkin, J. Clarkson, Differential gene expression in genetically identical sister cells: the initiation of sporulation in *Bacillus subtilis*, Mol. Microbiol. 56 (2005) 578–589, https://doi.org/10.1111/j.1365-2958.2005.04594.x.
[50] I.G. de Jong, J.-W. Veening, O.P. Kuipers, Heterochronic phosphorelay gene expression as a source of heterogeneity in *Bacillus subtilis* spore formation, J. Bacteriol. 192 (2010) 2053–2067, https://doi.org/10.1128/JB.01484-09.
[51] A. Chastanet, D. Vitkup, G.-C. Yuan, T.M. Norman, J.S. Liu, R.M. Losick, Broadly heterogeneous activation of the master regulator for sporulation in *Bacillus subtilis*, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 8486–8491, https://doi.org/10.1073/pnas.1002499107.
[52] P. Nicolas, U. Mäder, E. Dervyn, T. Rochat, A. Leduc, N. Pigeonneau, et al., Condition-dependent transcriptome reveals high-level regulatory architecture in *Bacillus subtilis*, Science. 335 (2012) 1103–1106, https://doi.org/10.1126/science.1206848.
[53] L. Flühe, O. Burghaus, B.M. Wieckowski, T.W. Giessen, U. Linne, M.A. Marahiel, Two [4Fe–4S] clusters containing radical SAM enzyme SkfB catalyze thioether bond formation during the maturation of the sporulation killing factor, J. Am. Chem. Soc. 135 (2013) 959–962, https://doi.org/10.1021/ja310542g.
[54] T.G. Pérez Morales, T.D. Ho, W.-T. Liu, P.C. Dorrestein, C.D. Ellermeier, Production of the cannibalism toxin SDP is a multistep process that requires SdpA and SdpB, J. Bacteriol. 195 (2013) 3244–3251, https://doi.org/10.1128/JB.00407-13.
[55] W.T. Liu, Y.L. Yang, Y. Xu, A. Lamsa, N.M. Haste, J.Y. Yang, et al., Imaging mass spectrometry of intraspecies metabolic exchange revealed the cannibalistic factors of *Bacillus subtilis*, Proc. Natl. Acad. Sci. 107 (2010) 16286–16290, https://doi.org/10.1073/pnas.1008368107.
[56] C.D. Ellermeier, E.C. Hobbs, J.E. González-Pastor, R. Losick, A three-protein signaling pathway governing immunity to a bacterial cannibalism toxin, Cell. 124 (2006) 549–559, https://doi.org/10.1016/j.cell.2005.11.041.
[57] T.L. Povolotsky, E. Orlova, D.G. Tamang, M.H. Saier, Defense against cannibalism: the SdpI family of bacterial immunity/signal transduction proteins, J. Membr. Biol. 235 (2010) 145–162, https://doi.org/10.1007/s00232-010-9260-7.

[58] A. Lamsa, W.-T. Liu, P.C. Dorrestein, K. Pogliano, The *Bacillus subtilis* cannibalism toxin SDP collapses the proton motive force and induces autolysis, Mol. Microbiol. 84 (2012) 486–500, https://doi.org/10.1111/j.1365-2958.2012.08038.x.

[59] G. Rosenberg, N. Steinberg, Y. Oppenheimer-Shaanan, T. Olender, S. Doron, J. Ben-Ari, et al., Not so simple, not so subtle: the interspecies competition between *Bacillus simplex* and *Bacillus subtilis* and its impact on the evolution of biofilms, NPJ Biofilms Microbiomes 2 (2016), 15027. https://doi.org/10.1038/npjbiofilms.2015.27.

[60] C. Höfler, J. Heckmann, A. Fritsch, P. Popp, S. Gebhard, G. Fritz, et al., Cannibalism stress response in *Bacillus subtilis*, Microbiology. 162 (2016) 164–176, https://doi.org/10.1099/mic.0.000176.

[61] J. Radeck, G. Fritz, T. Mascher, The cell envelope stress response of *Bacillus subtilis*: from static signaling devices to dynamic regulatory network, Curr. Genet. 63 (2016) 79–90, https://doi.org/10.1007/s00294-016-0624-0.

[62] B.G. Butcher, Y.-P. Lin, J.D. Helmann, The *yydFGHIJ* operon of *Bacillus subtilis* encodes a peptide that induces the LiaRS two-component system, J. Bacteriol. 189 (2007) 8616–8625, https://doi.org/10.1128/JB.01181-07.

[63] T. Mascher, N.G. Margulis, T. Wang, R.W. Ye, J.D. Helmann, Cell wall stress responses in *Bacillus subtilis*: the regulatory network of the bacitracin stimulon, Mol. Microbiol. 50 (2003) 1591–1604.

[64] T. Mascher, S.L. Zimmer, T.-A. Smith, J.D. Helmann, Antibiotic-inducible promoter regulated by the cell envelope stress-sensing two-component system LiaRS of *Bacillus subtilis*, Antimicrob. Agents Chemother. 48 (2004) 2888–2896, https://doi.org/10.1128/AAC.48.8.2888-2896.2004.

[65] A. Benjdia, A. Guillot, P. Ruffié, J. Leprince, O. Berteau, Post-translational modification of ribosomally synthesized peptides by a radical SAM epimerase in *Bacillus subtilis*, Nat. Chem. (2017)https://doi.org/10.1038/nchem.2714.

[66] F. Griffith, The significance of pneumococcal types, J. Hyg. (Lond) 27 (1928) 113–159.

[67] O.T. Avery, C.M. Macleod, M. McCarty, Studies on the chemical nature of the substance inducing transformation of pneumococcal types, J. Exp. Med. 79 (1944) 137–158.

[68] S. Guiral, T.J. Mitchell, B. Martin, J.-P. Claverys, Competence-programmed predation of noncompetent cells in the human pathogen *Streptococcus pneumoniae*: genetic requirements, Proc. Natl. Acad. Sci. 102 (2005) 8710–8715, https://doi.org/10.1073/pnas.0500879102.

[69] M.S. Gilmore, W. Haas, The selective advantage of microbial fratricide, Proc. Natl. Acad. Sci. 102 (2005) 8401–8402, https://doi.org/10.1073/pnas.0503828102.

[70] L.S. Havarstein, P. Gaustad, I.F. Nes, D.A. Morrison, Identification of the streptococcal competence-pheromone receptor, Mol. Microbiol. 21 (1996) 863–869.

[71] R. PAKULA, W. WALCZAK, On the nature of competence of transformable streptococci, J. Gen. Microbiol. 31 (1963) 125–133, https://doi.org/10.1099/00221287-31-1-125.

[72] L.S. Havarstein, G. Coomaraswamy, D.A. Morrison, An unmodified heptadecapeptide pheromone induces competence for genetic transformation in *Streptococcus pneumoniae*, Proc. Natl. Acad. Sci. 92 (1995) 11140–11144, https://doi.org/10.1073/pnas.92.24.11140.

[73] E.V. Pestova, L.S. Havarstein, D.A. Morrison, Regulation of competence for genetic transformation in *Streptococcus pneumoniae* by an auto-induced peptide pheromone and a two-component regulatory system, Mol. Microbiol. 21 (1996) 853–862.

[74] P. Luo, D.A. Morrison, Transient association of an alternative sigma factor, ComX, with RNA polymerase during the period of competence for genetic transformation in *Streptococcus pneumoniae*, J. Bacteriol. 185 (2003) 349–358, https://doi.org/10.1128/JB.185.1.349-358.2003.

[75] A. Dagkessamanskaia, M. Moscoso, V. Hénard, S. Guiral, K. Overweg, M. Reuter, et al., Interconnection of competence, stress and CiaR regulons in *Streptococcus pneumoniae*: competence triggers stationary phase autolysis of ciaR mutant cells, Mol. Microbiol. 51 (2004) 1071–1086, https://doi.org/10.1111/j.1365-2958.2003.03892.x.

[76] S.N. Peterson, C.K. Sung, R. Cline, B.V. Desai, E.C. Snesrud, P. Luo, et al., Identification of competence pheromone responsive genes in *Streptococcus pneumoniae* by use of DNA microarrays, Mol. Microbiol. 51 (2004) 1051–1070.

[77] L. Kausmally, O. Johnsborg, M. Lunde, E. Knutsen, L.S. Håvarstein, Choline-binding protein D (CbpD) in *Streptococcus pneumoniae* is essential for competence-induced cell lysis, J. Bacteriol. 187 (2005) 4338–4345, https://doi.org/10.1128/JB.187.13.4338-4345.2005.

[78] P. García, M. Paz González, E. García, J.L. García, R. López, The molecular characterization of the first autolytic lysozyme of *Streptococcus pneumoniae* reveals evolutionary mobile domains, Mol. Microbiol. 33 (1999) 128–138.

[79] H. Steinmoen, E. Knutsen, L.S. Håvarstein, Induction of natural competence in *Streptococcus pneumoniae* triggers lysis and DNA release from a subfraction of the cell population, Proc. Natl. Acad. Sci. 99 (2002) 7681–7686, https://doi.org/10.1073/pnas.112464599.

[80] H. Steinmoen, A. Teigen, L.S. Håvarstein, Competence-induced cells of *Streptococcus pneumoniae* lyse competence-deficient cells of the same strain during cocultivation, J. Bacteriol. 185 (2003) 7176–7183, https://doi.org/10.1128/JB.185.24.7176-7183.2003.

[81] M. Moscoso, J.-P. Claverys, Release of DNA into the medium by competent *Streptococcus pneumoniae*: kinetics, mechanism and stability of the liberated DNA, Mol. Microbiol. 54 (2004) 783–794, https://doi.org/10.1111/j.1365-2958.2004.04305.x.

[82] A. Tomasz, E. Zanati, Appearance of a protein "agglutinin" on the spheroplast membrane of pneumococci during induction of competence, J. Bacteriol. 105 (1971) 1213–1215.

[83] D. Straume, G.A. Stamsås, Z. Salehian, L.S. Håvarstein, Overexpression of the fratricide immunity protein ComM leads to growth inhibition and morphological abnormalities in *Streptococcus pneumoniae*, Microbiology (Reading, Engl.) 163 (2017) 9–21, https://doi.org/10.1099/mic.0.000402.

[84] H. Wei, L.S. Håvarstein, Fratricide is essential for efficient gene transfer between pneumococci in biofilms, Appl. Environ. Microbiol. 78 (2012) 5897–5905, https://doi.org/10.1128/AEM.01343-12.

[85] H. Reichenbach, The ecology of the myxobacteria, Environ. Microbiol. 1 (1999) 15–21, https://doi.org/10.1046/j.1462-2920.1999.00016.x.

[86] H. Oetker, Untersuchungen über die Ernährung einiger Myxobakterien, Arch. Mikrobiol. 19 (1953) 206–246, https://doi.org/10.1007/BF00446400.

[87] J. Muñoz-Dorado, F.J. Marcos-Torres, E. García-Bravo, A. Moraleda-Muñoz, J. Pérez, Myxobacteria: moving, killing, feeding, and surviving together, Front. Microbiol. 7 (2016) 781-18, https://doi.org/10.3389/fmicb.2016.00781.

[88] B.N. Singh, Myxobacteria in soils and composts; their distribution, number and lytic action on bacteria, J. Gen.

Microbiol. 1 (1947) 1–10, https://doi.org/10.1099/00221287-1-1-1.
[89] D. Kaiser, Signaling in myxobacteria, Annu. Rev. Microbiol. 58 (2004) 75–98, https://doi.org/10.1146/annurev.micro.58.030603.123620.
[90] L.J. Shimkets, Intercellular signaling during fruiting-body development of *Myxococcus xanthus*, Annu. Rev. Microbiol. 53 (1999) 525–549, https://doi.org/10.1146/annurev.micro.53.1.525.
[91] L. Søgaard-Andersen, M. Overgaard, S. Lobedanz, E. Ellehauge, L. Jelsbak, A.A. Rasmussen, Coupling gene expression and multicellular morphogenesis during fruiting body formation in *Myxococcus xanthus*, Mol. Microbiol. 48 (2003) 1–8.
[92] S.K. Kim, D. Kaiser, Cell motility is required for the transmission of C-factor, an intercellular signal that coordinates fruiting body morphogenesis of *Myxococcus xanthus*, Genes Dev. 4 (1990) 896–904, https://doi.org/10.1101/gad.4.6.896.
[93] T.O. Boynton, J.L. McMurry, L.J. Shimkets, Characterization of *Myxococcus xanthus* MazF and implications for a new point of regulation, Mol. Microbiol. 87 (2013) 1267–1276, https://doi.org/10.1111/mmi.12165.
[94] A. Kuspa, D. Kaiser, Genes required for developmental signalling in *Myxococcus xanthus*: three asg loci, J. Bacteriol. 171 (1989) 2762–2772.
[95] L. Plamann, Y. Li, B. Cantwell, J. Mayor, The *Myxococcus xanthus asgA* gene encodes a novel signal transduction protein required for multicellular development, J. Bacteriol. 177 (1995) 2014–2020.
[96] D.J. Bretl, J.R. Kirby, Molecular mechanisms of signaling in *Myxococcus xanthus* development, J. Mol. Biol. 428 (2016) 3805–3830, https://doi.org/10.1016/j.jmb.2016.07.008.
[97] H.B. Kaplan, A. Kuspa, D. Kaiser, Suppressors that permit A-signal-independent developmental gene expression in *Myxococcus xanthus*, J. Bacteriol. 173 (1991) 1460–1470, https://doi.org/10.1128/jb.173.4.1460-1470.1991.
[98] B. Sager, D. Kaiser, Intercellular C-signaling and the traveling waves of *Myxococcus*, Genes Dev. 8 (1994) 2793–2804, https://doi.org/10.1101/gad.8.23.2793.
[99] E. Ellehauge, M. Nørregaard-Madsen, L. Søgaard-Andersen, The FruA signal transduction protein provides a checkpoint for the temporal co-ordination of intercellular signals in *Myxococcus xanthus* development, Mol. Microbiol. 30 (1998) 807–817, https://doi.org/10.1046/j.1365-2958.1998.01113.x.
[100] M. Singer, D. Kaiser, Ectopic production of guanosine penta- and tetraphosphate can initiate early developmental gene expression in *Myxococcus xanthus*, Genes Dev. 9 (1995) 1633–1644, https://doi.org/10.1101/gad.9.13.1633.
[101] S.K. Kim, D. Kaiser, Cell alignment required in differentiation of *Myxococcus xanthus*, Science. 249 (1990) 926–928.
[102] L. Thöny-Meyer, D. Kaiser, devRS, an autoregulated and essential genetic locus for fruiting body development in *Myxococcus xanthus*, J. Bacteriol. 175 (1993) 7450–7462.
[103] B.D. Blackhart, D.R. Zusman, "Frizzy" genes of *Myxococcus xanthus* are involved in control of frequency of reversal of gliding motility, Proc. Natl. Acad. Sci. 82 (1985) 8767–8770, https://doi.org/10.1073/pnas.82.24.8767.
[104] L. Sogaard-Andersen, D. Kaiser, C factor, a cell-surface-associated intercellular signaling protein, stimulates the cytoplasmic Frz signal transduction system in *Myxococcus xanthus*, Proc. Natl. Acad. Sci. 93 (1996) 2675–2679, https://doi.org/10.1073/pnas.93.7.2675.
[105] D.C. Hagen, A.P. Bretscher, D. Kaiser, Synergism between morphogenetic mutants of *Myxococcus xanthus*, Dev. Biol. 64 (1978) 284–296, https://doi.org/10.1016/0012-1606(78)90079-9.
[106] B. Julien, A.D. Kaiser, A. Garza, Spatial control of cell differentiation in *Myxococcus xanthus*, Proc. Natl. Acad. Sci. 97 (2000) 9098–9103.
[107] L. Kroos, D. Kaiser, Expression of many developmentally regulated genes in Myxococcus depends on a sequence of cell interactions, Genes Dev. 1 (1987) 840–854.
[108] C.E. Morrison, D.R. Zusman, *Myxococcus xanthus* mutants with temperature-sensitive, stage-specific defects: evidence for independent pathways in development, J. Bacteriol. 140 (1979) 1036–1042.
[109] K.A. O'Connor, D.R. Zusman, Analysis of *Myxococcus xanthus* cell types by two-dimensional polyacrylamide gel electrophoresis, J. Bacteriol. 173 (1991) 3334–3341.
[110] K.A. O'Connor, D.R. Zusman, Behavior of peripheral rods and their role in the life cycle of *Myxococcus xanthus*, J. Bacteriol. 173 (1991) 3342–3355.
[111] K.A. O'Connor, D.R. Zusman, Development in *Myxococcus xanthus* involves differentiation into two cell types, peripheral rods and spores, J. Bacteriol. 173 (1991) 3318–3333.
[112] B. Lee, C. Holkenbrink, A. Treuner-Lange, P.I. Higgs, *Myxococcus xanthus* developmental cell fate production: heterogeneous accumulation of developmental regulatory proteins and reexamination of the role of MazF in developmental lysis, J. Bacteriol. 194 (2012) 3058–3068, https://doi.org/10.1128/JB.06756-11.
[113] M. Varon, S. Cohen, E. Rosenberg, Autocides produced by *Myxococcus xanthus*, J. Bacteriol. 160 (1984) 1146–1150.
[114] I. Gelvan, M. Varon, E. Rosenberg, Cell-density-dependent killing of *Myxococcus xanthus* by autocide AMV, J. Bacteriol. 169 (1987) 844–848, https://doi.org/10.1128/jb.169.2.844-848.1987.
[115] H. Nariya, M. Inouye, MazF, an mRNA interferase, mediates programmed cell death during multicellular *Myxococcus* development, Cell. 132 (2008) 55–66, https://doi.org/10.1016/j.cell.2007.11.044.
[116] H. Nariya, S. Inouye, A protein Ser/Thr kinase cascade negatively regulates the DNA-binding activity of MrpC, a smaller form of which may be necessary for the *Myxococcus xanthus* development, Mol. Microbiol. 60 (2006) 1205–1217, https://doi.org/10.1111/j.1365-2958.2006.05178.x.
[117] K. Flärdh, M.J. Buttner, *Streptomyces* morphogenetics: dissecting differentiation in a filamentous bacterium, Nat. Microbiol. 7 (2009) 36–49, https://doi.org/10.1038/nrmicro1968.
[118] M.J. Bush, N. Tschowri, S. Schlimpert, K. Flärdh, M.J. Buttner, c-di-GMP signalling and the regulation of developmental transitions in streptomycetes, Nat. Microbiol. 13 (2015) 749–760, https://doi.org/10.1038/nrmicro3546.
[119] P. Yagüe, M.T. López-García, B. Rioseras, J. Sánchez, A. Manteca, Pre-sporulation stages of *Streptomyces* differentiation: state-of-the-art and future perspectives, FEMS Microbiol. Lett. 342 (2013) 79–88, https://doi.org/10.1111/1574-6968.12128.
[120] T. Beites, P. Oliveira, B. Rioseras, S.D.S. Pires, R. Oliveira, P. Tamagnini, et al., *Streptomyces natalensis* programmed cell death and morphological differentiation are dependent on oxidative stress, Sci. Rep. 5 (2015), 12887. https://doi.org/10.1038/srep12887.

[121] M. Kalamara, M. Spacapan, I. Mandic-Mulec, N.R. Stanley-Wall, Social behaviours by *Bacillus subtilis*: quorum sensing, kin discrimination and beyond, Mol. Microbiol. 110 (2018) 863–878, https://doi.org/10.1111/mmi.14127.

[122] P. Shen, J.A. Lees, G.C.W. Bee, S.P. Brown, J.N. Weiser, Pneumococcal quorum sensing drives an asymmetric owner–intruder competitive strategy during carriage via the competence regulon, Nat. Microbiol. 4 (2018) 198–208, https://doi.org/10.1038/s41564-018-0314-4.

[123] T.M. Norman, N.D. Lord, J. Paulsson, R. Losick, Memory and modularity in cell-fate decision making, Nature. 503 (2013) 481–486, https://doi.org/10.1038/nature12804.

[124] M.T. Cabeen, J.R. Russell, J. Paulsson, R. Losick, Use of a microfluidic platform to uncover basic features of energy and environmental stress responses in individual cells of *Bacillus subtilis*, PLoS Genet. 13 (2017), e1006901. https://doi.org/10.1371/journal.pgen.1006901.