

Bacterial cell differentiation enables population level survival strategies

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ABSTRACT Clonal reproduction of unicellular organisms ensures the stable inheritance of genetic information. However, this means of reproduction lacks an intrinsic basis for genetic variation, other than spontaneous mutation and horizontal gene transfer. To make up for this lack of genetic variation, many unicellular organisms undergo the process of cell differentiation to achieve phenotypic heterogeneity within isogenic populations. Cell differentiation is either an inducible or obligate program. Induced cell differentiation can occur as a response to a stimulus, such as starvation or host cell invasion, or it can be a stochastic process. In contrast, obligate cell differentiation is hardwired into the organism's life cycle. Whether induced or obligate, bacterial cell differentiation requires the activation of a signal transduction pathway that initiates a global change in gene expression and ultimately results in a morphological change. While cell differentiation is considered a hallmark in the development of multicellular organisms, many unicellular bacteria utilize this process to implement survival strategies. In this review, we describe well-characterized cell differentiation programs to highlight three main survival strategies used by bacteria capable of differentiation: (i) environmental adaptation, (ii) division of labor, and (iii) bet-hedging.

KEYWORDS cell differentiation, bet-hedging, division of labor, *Caulobacter crescentus*, *Bacillus subtilis*, *Myxococcus xanthus*, signal transduction

Cell differentiation is a biological process used by multiple bacterial species to implement individual and population-level survival strategies. In this review, we discuss how the process of cell differentiation allows bacteria to implement (i) environmental adaptation, (ii) division of labor, and (iii) bet-hedging strategies.

- i. Many bacteria respond to environmental changes by differentiating into new cell types that are better suited to their new conditions. Environmental adaptation is implemented at both the individual level through cell-autonomous signaling pathways and at the population level through cell-cell communication (1, 2).
- ii. Division of labor is a population-level survival strategy in which different cell types within a population perform distinct tasks that benefit the population as a whole. This strategy requires individual cells within the population to differentiate into specialized cell types, capable of performing specific tasks. While specialization often comes at a cost to individual cells, this price is outweighed by the increased fitness experienced by the population as a whole (3).
- iii. Bet-hedging is a population-level survival strategy that entails the coexistence of multiple cell types, even some maladapted to the current environmental condition. Bet-hedging lowers the population-level risk by spreading it across multiple different cell types. This strategy is particularly useful to bacteria that experience sudden, unpredictable environmental changes. In this instance, a

Editor Marcio Rodrigues, Instituto Carlos Chagas, Curitiba, Brazil

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The authors declare no conflict of interest.

See the funding table on p. 10.

Published 21 May 2024

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fraction of the population that was maladapted to the previous condition might be well suited for survival under a new, unanticipated condition (4).

The process of cell differentiation can either be induced or obligate. Induced cell differentiation occurs either stochastically or in response to a stimulus, while obligate cell differentiation is hardwired into the bacteria's life cycle. *Bacillus subtilis* and *Myxococcus xanthus* undergo induced cell differentiation from dividing cells to metabolically inactive spores upon sensing nutrient depletion (1, 2), while uropathogenic *Escherichia coli* differentiate into coccoid and filamentous morphologies upon host cell invasion (5). *B. subtilis* has been observed to stochastically switch from singlet motile cells to non-motile chaining cells under nutrient-rich conditions (6). In contrast, the aquatic bacterium, *Caulobacter crescentus*, undergoes obligate cell differentiation from a motile swarmer cell to a stationary stalked cell with each cell cycle. While induced cell differentiation is aptly suited for environmental adaptation, *Caulobacter* has been shown to respond to environmental cues by modulating the timing of certain cell cycle events (7–10). Bacteria that undergo either induced or obligate cell differentiation can utilize division of labor and/or bet-hedging strategies. This review will mostly focus on cell differentiation programs in relationship to survival strategies. Further details on each differentiation program can be found in the reviews cited throughout each section.

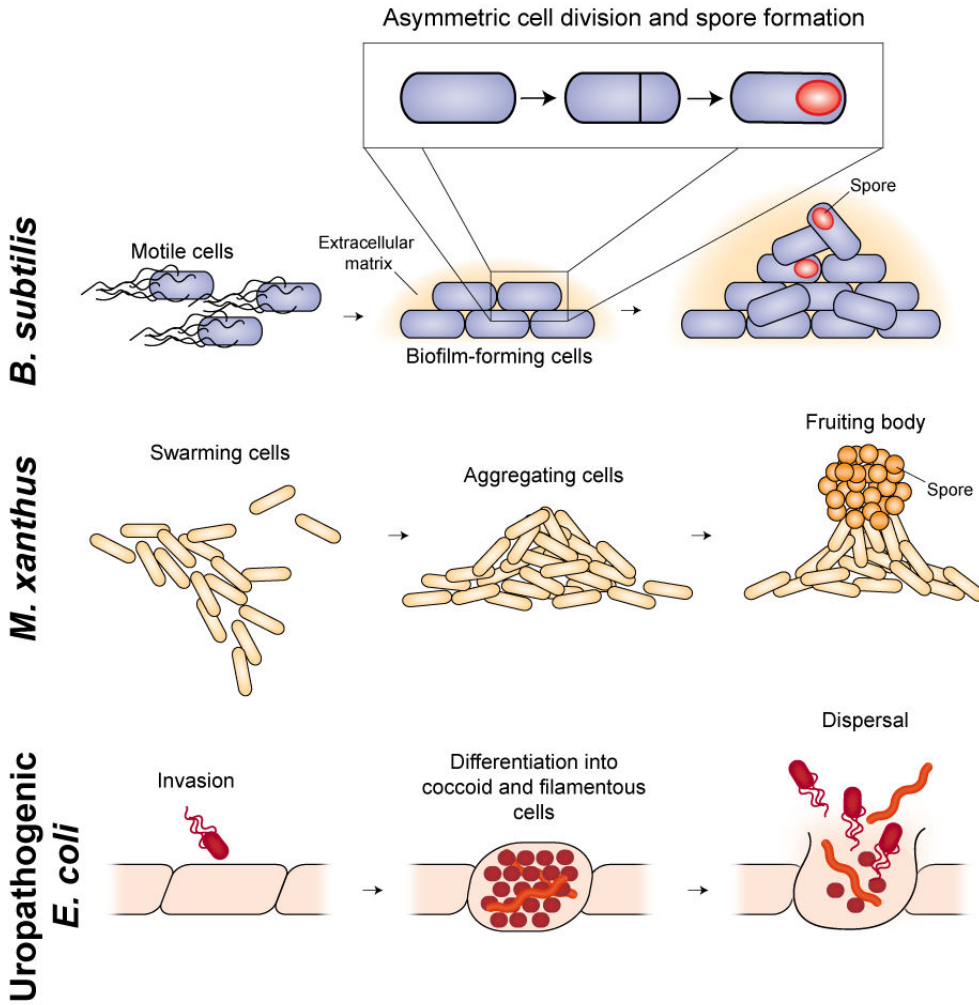
INDUCED CELL DIFFERENTIATION FACILITATES ENVIRONMENTAL ADAPTATION

Cell differentiation has been independently employed as a strategy to withstand environmental adversity by both the gram-positive bacterium, *B. subtilis*, and the gram-negative bacterium, *M. xanthus*. Both species form metabolically inactive spores that are protected by a thick coat of proteins and polysaccharides, allowing them to withstand harsh environmental conditions such as starvation, drought, and extreme temperatures (Fig. 1) (2, 11, 12). *B. subtilis* spore formation requires sequential asymmetric cell division and cell differentiation. This developmental program is initiated by phosphorylation and activation of the master transcription factor, Spo0A (13). Phosphorylation of Spo0A is driven by five histidine kinases, KinA–KinE, which respond to various stimuli (14, 15). Upon compartmentalization, large and small daughter cells initiate distinct gene expression programs, specific to each cell fate (11, 16). At a defined point in this developmental program, the large daughter cell engulfs the small daughter cell, allowing the small cell to fully develop into a spore within the cytoplasm of the large cell (11, 17).

Spo0A phosphorylation is responsible for initiation of both biofilm formation and sporulation survival strategies. Biofilm formation is a strategy used by many bacteria to increase tolerance to chemical and physical stressors as well as to exchange metabolites and other common goods between cells. Moderate levels of phosphorylated Spo0A (Spo0A~P) leads to matrix secretion and biofilm formation, while high levels of Spo0A~P is required for sporulation (13, 18). Activation of one or more of the Kin histidine kinases leads to Spo0A phosphorylation either directly or indirectly by a phosphorelay through the response regulator, Spo0F, and the phosphotransfer protein, Spo0B (Fig. 2) (14). KinA and KinB have been previously identified as the main kinases responsible for sporulation, while KinC and KinD have been linked to biofilm formation (14). Analysis of purified proteins revealed that KinA is a more efficient kinase than either KinC or KinD *in vitro* (14, 19). Therefore, activation of specific Kin kinases tunes the level of Spo0A~P to initiate differentiation into either biofilm-forming or spore-forming cell fates.

While the Kin histidine kinases have been extensively studied, little is presently known about the specific stimuli that activate these kinases. Previous studies have suggested that the sporulation-associated kinases, KinA and KinB, likely respond to changes in growth rate or respiration, rather than to external environmental stimuli (20, 21). In contrast, the biofilm-associated kinases, KinC and KinD, likely respond to *B.*

Induced differentiation



Obligate differentiation

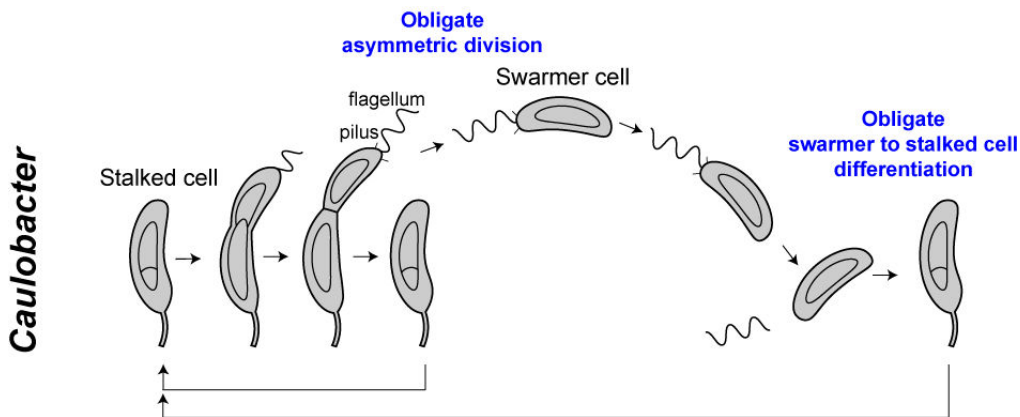


FIG 1 Examples of induced and obligate bacterial cell differentiation. Many bacteria undergo either induced or obligate cell differentiation. Induced cell differentiation is initiated either in response to a stimulus or stochastically. Obligate cell differentiation is hardwired into the organism’s life cycle. Under starvation conditions, *B. subtilis* cells differentiate into (Continued on next page)

FIG 1 (Continued)

biofilm-forming cells and metabolically inactive spores. Sporulation requires a coordinated process of asymmetric cell division and spore formation. *M. xanthus* bacteria also form spores upon nutrient depletion. Swarming *M. xanthus* cells differentiate into aggregating cells that form a fruiting body consisting of spores. *M. xanthus* sporulation does not require cell division, but instead entails the complete remodeling of the cytoskeleton and cell wall. Uropathogenic *E. coli* differentiate into coccoid and filamentous morphologies upon host cell invasion. When infected cells rupture, released bacteria are able to invade neighboring cells, resulting in infection persistence. *Caulobacter crescentus* is an aquatic bacterium that undergoes obligate differentiation from a replication-incompetent motile swarmer cell to a replicating stationary stalked cell, with each cell cycle. Asymmetric division and cell differentiation are both obligate events, hardwired into *Caulobacter's* cell cycle.

subtilis-secreted substances such as matrix components and surfactants (2, 22). These studies suggest that activation of the Kin kinases might be secondary to the molecular pathways responsible for directly sensing changes in environmental conditions. Further investigation into the mechanisms involved in perceiving environmental cues and relaying information to the Kin kinases is required to fully understand how *B. subtilis* differentiation programs are initiated in response to environmental changes. The ability of *B. subtilis* to differentiate into biofilm-forming and spore-forming cells in response to environmental changes ultimately allows the bacteria to survive under extreme conditions.

In contrast to *B. subtilis*, spore formation in *M. xanthus* does not involve cell division. Instead, individual rod-shaped cells completely remodel their cytoskeleton and secrete coat components in order to differentiate into spherical spores (12). Under nutrient-rich, vegetative conditions, cells swarm radially outward, collectively feeding on prey bacteria (1). Upon nutrient depletion, cells undergo a highly coordinated process of aggregation, fruiting body formation, and sporulation that ultimately allows the species to persist through different environmental conditions (Fig. 1) (1, 3, 23). It has been shown that the developmental program responsible for fruiting body formation initiates in reaction to an intracellular accumulation of the stringent response alarmone (p)ppgpp (24, 25). Therefore, similar to *B. subtilis* sporulation, the molecular pathway responsible for *M. xanthus* differentiation senses a starvation-induced change in cell physiology rather than nutrient limitation itself. Subsequent to (p)ppgpp synthesis, cells synthesize and release a quorum-sensing A-signal required for cell aggregation (26). Once the A-signal surpasses a threshold level, cells re-direct their motility, aggregating into a protruding mound that develops into a large (~0.08 mm) structure that is the fruiting body (Fig. 1) (27). Within the fruiting body, only ~1%–15% of cells differentiate into spores, while ~80% undergo programmed cell death, and ~5% remain as non-aggregating peripheral rod cells (28, 29). It has been speculated that this large-scale cell death is an example of bacterial altruism (28). Transition from a population of vegetative, predatory cells to a fruiting body consisting of spores requires initiation of an intricate molecular program acting at the individual and population levels. This coordinated program of cell aggregation, fruiting body formation, and sporulation ultimately allows *M. xanthus* to survive through periods of harsh environmental conditions.

Pathogenic bacteria use cell differentiation to facilitate their survival inside infected hosts (5). Upon invasion of bladder epithelial cells, uropathogenic *Escherichia coli* (UPEC) transition from rod to coccoid morphology (5). In addition, a small subset of UPEC cells adopt a long, filamentous morphology that hinders phagocytosis, allowing these cells to evade the host immune system (Fig. 1) (30, 31). Infected epithelial cells eventually rupture, releasing bacteria into the extracellular space. The released bacteria are able to invade neighboring epithelial cells, leading to persistence of the infection (Fig. 1) (5). Many other pathogenic bacteria differentiate into filamentous cell morphologies upon host infection, including *Mycobacterium tuberculosis* and *Salmonella typhimurium* (32, 33). Understanding the signaling pathways associated with these differentiation programs can provide putative targets for decreasing the virulence of these bacterial pathogens.

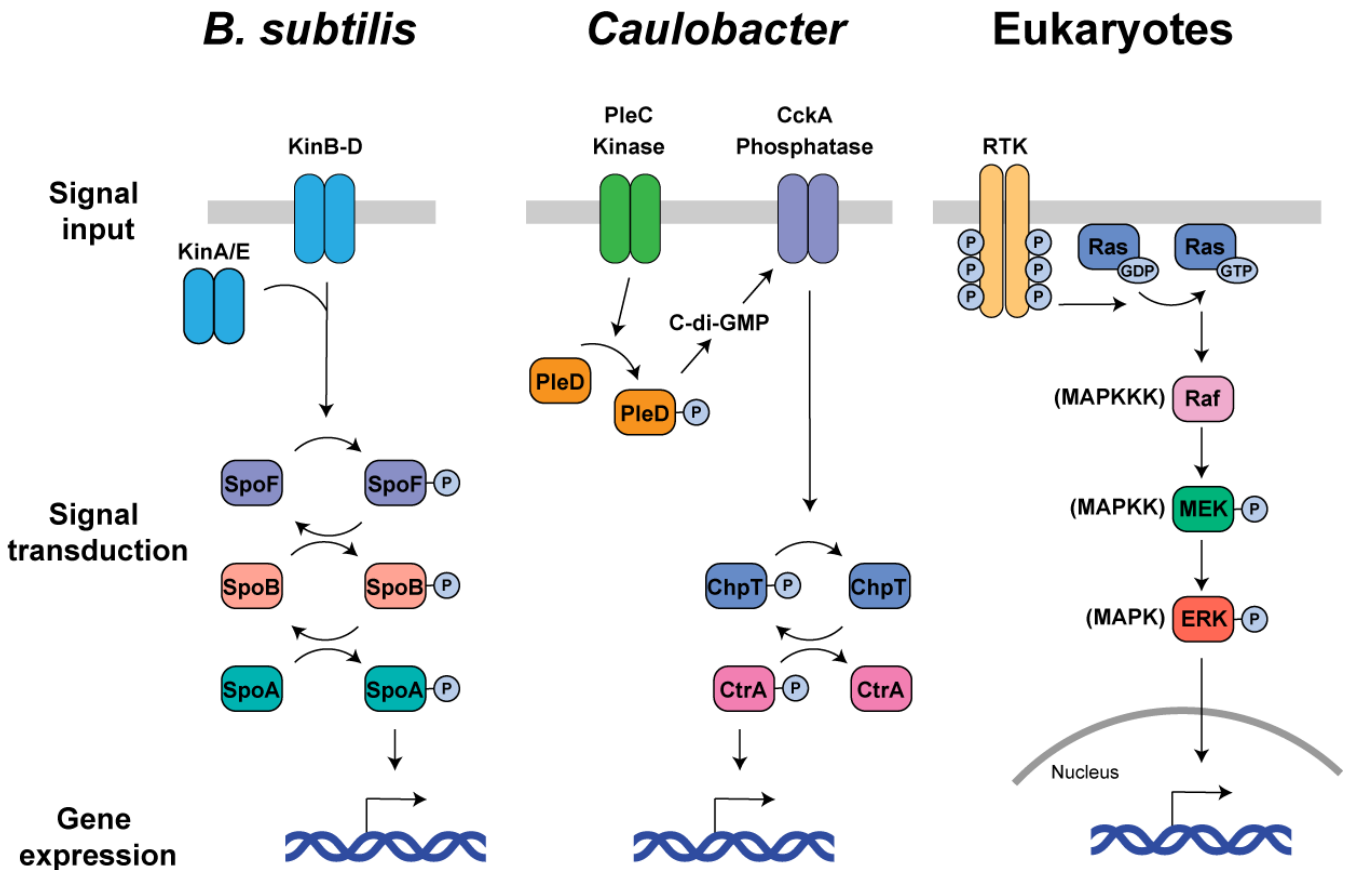


FIG 2 The basic framework of signal transduction pathways is broadly conserved. Signal transduction is required in cells across all kingdoms in order to integrate external environmental and internal physiological signals into biological outcomes such as cell differentiation, cell cycle progression, and cellular adaptation. *B. subtilis* and *Caulobacter* use two-component systems to integrate signals into the decision of whether or not to differentiate. These signaling pathways are built into complex molecular networks that are not depicted in their entirety here. Receptor tyrosine kinase (RTK)/Ras/mitogen-activated protein kinase (MAPK) signaling pathways are an example of eukaryotic signaling pathways that integrate signals into cell fate and cell proliferation decisions. While the specific proteins and their interactions are not conserved between bacterial and eukaryotic signal transduction pathways, the basic framework of these pathways shares many similarities.

PHENOTYPIC HETEROGENEITY ENABLES DIVISION OF LABOR AND BET-HEDGING STRATEGIES

While the process of induced cell differentiation is especially suited for environmental adaptation, species that employ this type of cell differentiation are not precluded from utilizing division of labor or bet-hedging strategies. It has been proposed that *B. subtilis* and *M. xanthus* use both of these population-level survival strategies (3, 4). Division of labor involves distinct cell types performing specialized tasks that benefit the population as a whole. These tasks are divided among individual cells in a way that bears a population-level fitness advantage. Therefore, bacteria are able to bypass the evolutionary pressure put on individual cells by prioritizing the success of the population. In a system in which individual cells expend resources to produce public goods or services, one can imagine that cells that do not act cooperatively can exploit the efforts of the surrounding cells. The emergence of exploitative cells may be less prevalent in monocultures where cells are considered to have a “common goal.” While this review focuses on isogenic populations, cooperation has been documented in multi-species bacterial communities. Therefore, division of labor might be a survival strategy that is employed by both clonal populations and multi-species communities (34, 35).

B. subtilis biofilms comprise a consortium of matrix-secreting cells, exoprotease-producing cells, sporulating cells, motile cells, and competent cells, capable of acquiring new DNA (2). Matrix-secreting cells provide a service to the population by producing and secreting matrix components that are required for biofilm integrity. Exoprotease-producing cells secrete proteases that degrade proteins into peptides which can be taken up by cells within the biofilm (36). This type of specialization comes at a cost as specialized cells must expend energy to provide these services. This energetic cost is outweighed by the emergent benefits that cooperation provides to all the cells within the population. In isogenic populations, spores also provide a service as their presence helps to ensure the long-term success of the population. *M. xanthus* spores and non-sporulating cells have been observed to participate in a seemingly one-sided cooperation. In fruiting bodies, aggregating cells form a stalk-like structure that props up the spores. Placement of spores at the end of this cell-made stalk structure has been proposed to aid in spore dispersal (37). Therefore, seemingly altruistic cooperation between various cell types brings forth an increased probability of success for the entire population.

In addition to division of labor, bacteria utilize bet-hedging as another population-level survival strategy. Bet-hedging is a strategy that lowers the population-level risk by spreading it across multiple cell types, even some that are maladapted to the current environmental condition (4). While sporulation is initiated in response to starvation conditions, only a small fraction of *B. subtilis* cells enter the spore-forming cell fate (38, 39). To deter neighboring cells from differentiating, sporulating cells release signaling factors that not only prevent nearby cells from initiating sporulation, but also cause cell lysis. Metabolites from lysed cells are used by their siblings to continue to grow and divide, in a process described as “cannibalism” (40). These dividing cells are poised to initiate rapid growth in case of nutrient influx (41). Conversely, dormant spores are able to persist through prolonged harsh conditions, continuing the genetic legacy of the population. In this way, the population sets itself up for optimal success in the event of any unforeseen changes in nutrient availability.

Bet-hedging is also observed under nutrient-rich conditions. Exponentially growing *B. subtilis* populations consist of singlet motile cells and chains of non-motile cells joined at the poles by extracellular matrix. Motile cells are thought of as foragers, able to seek new nutrient sources, while non-motile chains take full advantage of the current niche (6). Non-motile chains also serve as starting points for biofilm formation, should environmental conditions change (42). Entry into the chaining cell fate is a stochastic process, dictated at its core by just two proteins, the SinR transcriptional repressor and its antagonist, SinI. When free and active, SinR binds to and represses the transcription of genes related to the chaining phenotype. SinR repressor activity is inhibited by direct binding by SinI. Small fluctuations in the relative abundance of SinR and SinI proteins are enough to drive stochastic entry into a SinI dominant state, corresponding with the chaining phenotype (43, 44). Interestingly, the reverse switch from chaining to motile state is not stochastic and relies on a molecular feedback loop that encodes a generational memory, committing cells to the chaining phenotype for a stereotypical number of generations before disassembly into singlet motile cells (45). Constant switching between these two cell types allows *B. subtilis* populations to simultaneously explore and colonize new habitats while being poised to initiate biofilm formation in the event of any unforeseen environmental changes.

How are different gene expression programs initiated in a population of cells that are experiencing relatively the same environment? While heterogeneous micro-environments caused by nutrient and oxygen gradients can explain some level of phenotypic heterogeneity, it has been proposed that many bacteria utilize noise to generate mutually exclusive phenotypic states (46). Positive and negative feedback loops enable small amounts of noise to amplify into large changes in magnitude, resulting in activation or repression of a bistable switch. Such feedback loops within differentiation programs have been proposed to give rise to multiple cell types within isogenic populations (46, 47). As discussed previously, activation of one or many of the

KinA–E kinases leads to phosphorylation of the master regulator, Spo0A, responsible for sporulation. In addition, Spo0A~P itself participates in feedback loops that breed further Spo0A synthesis and phosphorylation. For example, Spo0A~P stimulates transcription of both its own gene and that of the upstream response regulator, Spo0F (Fig. 2) (48, 49). While starvation induces Spo0A phosphorylation, it curiously also leads to the synthesis of phosphatases that act on Spo0A~P and Spo0F~P. It has been shown that dephosphorylation of Spo0A~P and Spo0F~P is critical for garnering phenotypic bistability (38, 50, 51). In addition to activating developmental programs associated with biofilm formation and spore formation, Spo0A~P inhibits transcription of the *fla/che* operon, required for the motile cell fate (52). In this way, small amounts of noise in the Spo0A signaling pathway give rise to motile, biofilm-forming, and spore-forming cells, within the same population.

OBLIGATE DIFFERENTIATION ALLOWS FOR DIVISION OF LABOR AND BET-HEDGING STRATEGIES

Unlike induced cell differentiation, obligate differentiation is hardwired into an organism's life cycle. The aquatic bacterium, *Caulobacter crescentus*, is studied as a model bacterium that undergoes obligate cell differentiation and asymmetric cell division with each cell cycle (53). Motile, replication-incompetent swarmer cells undergo differentiation into sessile, replicating stalked cells (Fig. 1). This developmental program involves shedding of the polar flagellum, pili retraction, degradation of the chemotaxis machinery, initiation of genome replication, and biogenesis of the stalk appendage (54). These developmental events result from an intricate signaling cascade, initiated by activation of the PleC histidine kinase (Fig. 2) (55–57). PleC phosphorylates the diguanylate cyclase, PleD, allowing for rapid synthesis of the small molecule, cyclic di-GMP (c-di-GMP) (58). This rapid increase in intracellular c-di-GMP concentration leads to dephosphorylation and degradation of the master transcription factor, CtrA (Fig. 2) (59, 60). As phosphorylated CtrA binds to the origin of replication, thereby inhibiting replication initiation, CtrA dephosphorylation and degradation is required to initiate chromosome replication (61). c-di-GMP synthesis additionally triggers the histidine kinase, ShkA, prompting the onset of the stalk biogenesis program (62). Therefore, activation of the PleC kinase initiates an intricate signaling cascade that ultimately results in swarmer to stalked cell differentiation (55, 57).

Conversely, *Caulobacter* asymmetric cell division involves phosphorylation and activation of CtrA in pre-divisional cells (59, 63, 64). Once phosphorylated and active, CtrA promotes the transcription of over 90 developmental genes, many involved in the development of the new flagellar pole (65). Distinct biomolecular condensates positioned at the stalked and flagellar poles allow asymmetric localization of cellular components prior to cell division (66–69). *Caulobacter's* bi-phasic cell cycle gives rise to two distinct sub-populations: planktonic swarmer cells and stationary stalked and pre-divisional cells. This bi-phasic cell cycle allows for both division of labor and bet-hedging strategies. Division of labor is achieved by stationary cells performing the tasks of proliferation and biofilm formation, while swarmer cells are responsible for dispersal. While this specialization may seem trivial as many other species of bacteria are able to simultaneously disperse and replicate, this division of labor brings forth an emergent advantage for *Caulobacter*. In low-nutrient environments, bacteria must be prudent in their resource allocation. Halting replication allows swarmer cells to persist through various conditions and disperse to new habitats prior to committing to genome replication and matrix secretion, which is required for biofilm formation. In addition, biofilm formation and proliferation exclusively by stalked and pre-divisional cells maintains the integrity of already established communities. These established communities serve as proliferation centers whereby newly born swarmer cells disperse.

Bet-hedging is built into the *Caulobacter* cell cycle as the two cell types are specifically suited to withstand distinct challenges. For example, stalked cells are particularly suited to withstand phage infection as they lack flagella and pili, known sites of phage

attachment (70, 71). Additionally, they possess an outer lipopolysaccharide capsule that covers and obscures the proteinaceous S-layer, another phage attachment site (72). In contrast, only swarmer cells are able to enter the persister cell fate, in which cells experience increased antibiotic resistance (73). *Caulobacter's* continual cycles of colonization and dispersal provide an additional form of bet-hedging. The constant establishment of new communities diminishes the population-level impact associated with the destruction of previously established communities. Utilization of these two strategies, division of labor and bet-hedging, enables *Caulobacter* to thrive in fluctuating, low-nutrient environments such as fresh-water lakes and streams (74). This bi-phasic lifestyle is not only employed by bacteria. Some eukaryotic organisms have convergently evolved life cycles with obligate free-living and sessile phases, including sea jellies and colonial tunicates. Interestingly, these metazoans live in ocean environments where nutrient availability is often low and inconsistent (75, 76).

While obligate cell differentiation is encoded in the *Caulobacter* cell cycle, the process of cell differentiation can be either stimulated or stalled, depending on environmental cues. Multiple studies have reported that the process of swarmer to stalked cell differentiation can be stimulated by surface attachment. In this instance, signal transduction through either the polar pili or flagellum initiates cell differentiation (7, 8, 10). It has also been reported that under starvation conditions, differentiating swarmer cells initiate stalk biogenesis but halt stalk elongation, resulting in a stunted stalk appendage. These cells fail to initiate chromosome replication, not fully committing to the stalked cell fate (9, 77). Therefore, while cell differentiation is obligate to the *Caulobacter* cell cycle, timing of this differentiation event is modulated in response to various environmental cues. In this way, *Caulobacter* is capable of environmental adaptation by tuning the duration of specific cell cycle events.

Conservation among cell differentiation signaling pathways

Both induced and obligate cell differentiation processes enable employment of population-level survival strategies. While activation of these two types of differentiation programs differ, all cell differentiation programs require initiation of a signal transduction pathway. Two-component systems are responsible for most signal transduction in prokaryotes. They consist of a histidine kinase that either phosphorylates or dephosphorylates a response regulator, that then either directly or indirectly enacts a change in gene expression (78, 79). In *B. subtilis*, differentiation into biofilm-forming or spore-forming cells occurs as a result of activation of one or more of the Kin histidine kinases (Fig. 2). Increase in phosphorylation of the master regulator, Spo0A, either directly or indirectly by the Kin kinases, enacts changes in gene expression that result in cell differentiation (14, 15). Obligate swarmer to stalked cell differentiation in *Caulobacter* results from activation of the histidine kinase, PleC, which phosphorylates the response regulator, PleD (55–57). Phosphorylated PleD synthesizes c-di-GMP, indirectly leading to dephosphorylation and degradation of the master regulator, CtrA (Fig. 2) (59, 60). This signaling pathway ultimately results in a change in gene expression, initiation of chromosome replication, and stalk biogenesis.

While two-component systems are found rarely in eukaryotes (although there are reported examples in plants [80]), the basic framework of many eukaryotic signaling pathways shares similarities with those of bacteria. For example, once activated, receptor tyrosine kinase (RTK)/Ras/mitogen-activated protein kinase (MAPK) pathways transmit a signal through a series of protein-protein interactions and post-translational modifications, ultimately resulting in a change in gene expression (81, 82). Typically, when an RTK is bound to a ligand, it undergoes transphosphorylation, in which each dimer subunit phosphorylates tyrosine residues on the other subunit. The resulting multi-phosphorylated protein domain enables docking of signaling proteins which leads to conversion of Ras-GDP to Ras-GTP. Active Ras-GTP stimulates a cascade of MAPK activation and phosphorylation events that results in a change in gene expression (Fig. 2) (83). RTK/Ras/MAPK signaling pathways are used by many eukaryotic cell types to control

cell proliferation and cell differentiation (84). While the individual proteins involved in eukaryotic signaling pathways are not homologous to those found in bacterial signaling pathways, the basic framework of many prokaryotic and eukaryotic signaling pathways is quite similar.

CELL CYCLE-DEPENDENT ACTIVATION OF A SIGNAL PATHWAY INITIATES OBLIGATE DIFFERENTIATION

Signal transduction pathways are designed to initiate in response to a signal. So, how is obligate *Caulobacter* cell differentiation initiated in the absence of an external stimulus? The signal transduction pathway responsible for swarmer to stalked cell differentiation is initiated by PleC kinase activation (55). Therefore, PleC kinase must be activated in a cell cycle-dependent manner to ensure proper timing of swarmer to stalked cell differentiation. PleC kinase has been shown to be activated through three distinct but coordinated cell cycle-dependent events: (i) dissociation and degradation of the PodJ polar complex (57), (ii) subcellular localization of the DivK signaling protein (55), and (iii) retraction of the polar pili (56). When present, the polar scaffold protein, PodJ, localizes to the flagellar pole and inhibits PleC kinase activity (85, 86). Upon PodJ proteolysis and dissociation from the cell pole, PleC is released from the pole and freed from PodJ inhibition (57). At this time, the signaling protein, DivK, is localized to the same pole, where it allosterically binds to PleC, stimulating PleC kinase activity (55). Pili retraction at the same pole increases the PilA concentration in the inner membrane, which binds to PleC and further stimulates PleC activity (56). Therefore, at least three distinct cell cycle-dependent events coordinate the molecular signaling pathway responsible for swarmer to stalked cell differentiation with the rest of the *Caulobacter* cell cycle.

Progression through the *Caulobacter* cell cycle is dictated by seven master regulators, whose peak abundance oscillates out of phase with each other. These master regulators are responsible for initiating specific gene expression programs associated with cell cycle events such as flagellum assembly or chromosome segregation (87). Acting in concert with these core master regulators are spatially resolved signaling circuits that, once initiated, execute cell cycle events at the correct time and location. Each signaling circuit acts as a functional module that communicates with other modules both to indicate their completion and to signal for the initiation of the next module (88). The three signaling events that contribute to swarmer to stalked cell differentiation provide an example of how seemingly independent modules coordinate cell cycle progression. Prior to swarmer to stalked cell differentiation, the PodJ polar complex is dissociated from the cell pole through displacement by another polar scaffold protein, SpmX (66). At this time, PodJ is also degraded by the proteases PerP and MmpA (89). Once PodJ is removed from the cell pole, PleC is freed from kinase inhibition. Stimulation of PleC kinase activity through allosteric binding of the phosphotransfer protein, DivK, requires a high concentration of DivK at the cell pole. While DivK is abundant in swarmer cells, it is not localized to the cell pole until it is recruited by SpmX through interaction with the histidine kinase, DivJ (90, 91). In addition to PleC, DivJ also phosphorylates PleD, further ramping up the intracellular concentration of c-di-GMP (55). Therefore, PleC release from kinase inhibition and kinase stimulation depends on PodJ removal and SpmX localization at the incipient stalked pole. Lastly, the PleC kinase is further stimulated by direct binding of PilA to PleC's N-terminal transmembrane domain upon retraction of the polar pili. This additional stimulation coordinates PleC kinase activity with the deconstruction of a swarmer cell-specific polar appendage (56). Therefore, multiple signaling pathways feed into PleC kinase activation to initiate swarmer to stalked cell differentiation. Input from these three signaling pathways allows for modulation at multiple levels and coordination of swarmer to stalked cell differentiation with the rest of the cell cycle.

CONCLUSIONS

Many species of bacteria have evolved cell differentiation programs. Whether to survive the onset of harsh environmental conditions, to adapt to life inside a host, or to employ

division of labor or bet-hedging strategies, cell differentiation has been implemented by a multitude of bacterial species to excel in various niches. Both induced and obligate cell differentiation programs have come about by organizing seemingly simple two-component systems into intricate molecular networks. Cell differentiation in eukaryotes is achieved through signal transduction pathways that are divergent from those in bacteria but accomplish the same goal of coordinating signal input with differential gene expression and morphological change. In this review, we described well-characterized examples of bacterial cell differentiation. Beyond these examples, there is undoubtedly a wide array of bacterial differentiation programs that result in various cell fates, contributing to the widespread success of bacteria over billions of years.

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FUNDING

Funder	Grant(s)	Author(s)
HHS NIH National Institute of General Medical Sciences (NIGMS)	R35-GM118071	Lucy Shapiro
Chan Zuckerberg Initiative (CZI)		Lucy Shapiro

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