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MicroReview

Bistability in bacteria

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Summary

Gene expression in bacteria is traditionally studied from the average behaviour of cells in a population, which has led to the assumption that under a particular set of conditions all cells express genes in an approximately uniform manner. The advent of methods for visualizing gene expression in individual cells reveals, however, that populations of genetically identical bacteria are sometimes heterogeneous, with certain genes being expressed in a non-uniform manner across the population. In some cases, heterogeneity is manifested by the bifurcation into distinct subpopulations, and we adopt the common usage, referring to this phenomenon as bistability. Here we consider four cases of bistability, three from *Bacillus subtilis* and one from *Escherichia coli*, with an emphasis on random switching mechanisms that generate alternative cell states and the biological significance of phenotypic heterogeneity. A review describing additional examples of bistability in bacteria has been published recently.

The world inhabited by bacteria and other microorganisms is perilous. These tiny, single-cell creatures must cope with the vicissitudes of an environment that undergoes perpetual alterations in temperature, salinity, osmolarity, pH, and the availability of nutrients and in which they are challenged by antibiotics, bacteriophage, mutagens, toxins, radiation, and a variety of other adverse agents and conditions. What strategies do microorganisms, and bacteria in particular (the focus of this review), employ to optimize their chances of coping and competing in an ever changing environment? One well-known strategy called the stress response involves the activation of specific sets of genes in response to particular environmental conditions (Storz and Hengge-Aronis, 2000). Thus, when temperature increases abruptly or a readily metabolized source of carbon or nitrogen becomes scarce, bacteria sense the change in their environment and respond by switching on the expression of specialized genes that help the cells adapt to the adverse circumstances. The often unstated assumption in considering such phenomena is that cells respond in a more-or-less uniform manner to the inducing signal, because the techniques classically used in these studies determine average values across an entire population. In part because of the increased use of methods like flow cytometry and fluorescence microscopy, several examples have recently emerged of non-uniform gene expression in clonal populations of bacteria. Here we must define some useful terms. Clonal populations of bacteria exhibit unimodal variation in the expression of a given gene, due to random fluctuations in the rates of synthesis and degradation of the cognate gene product. This is usually referred to as 'noise' and we will employ this usage. As we shall see, noise is important, sometimes giving rise to a second type of variation that is non-unimodal. In this type, the population bifurcates into subpopulations, leaving the intermediate states relatively unoccupied. We will adopt the common usage, referring to this phenotypic phenomenon as 'bistability'. As bistability occurs in populations of genetically identical cells, grown in homogeneous and identical environments (e.g. in liquid media in well-stirred flasks), it must arise stochastically at the cellular level. In other words, the choice of which individual cells exhibit altered gene expression is random.

Bistability is exemplified in *Escherichia coli* by persister cells and in *Bacillus subtilis* by genetic competence, spore formation/cannibalism, and swimming/chaining, all of which will be described below. Bistability arises from unimodal noise in the expression of a master regulatory gene. If a cell passes a threshold in expression of this gene, the quantitative change becomes qualitative and a new pattern of gene expression emerges: the population bifurcates into coexisting cell types. Bistability implies that a regulatory system can switch between two alternative

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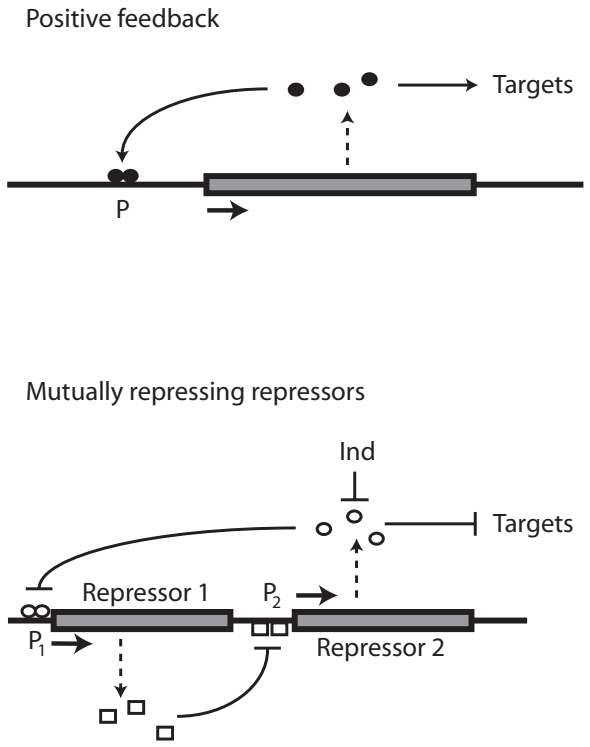

Fig. 1. Two network configurations that lead to bistable expression. The first involves a positive transcriptional autoregulatory loop together with cooperativity in promoter activation (Becskei *et al*., 2001). The second involves two mutually repressing repressors (Gardner *et al*., 2000). An inducer antagonizes the action of repressor 2, throwing the switch in the direction of derepression of target genes under the control of repressor 2. See text for discussion. Open symbols denote repressors and closed circles denote activators.

states but cannot rest at intermediate states. Two mechanisms have been proposed to drive this kind of bifurcation (reviewed in Ferrell, 2002) (Fig. 1). The first mechanism requires that the master gene is positively autoregulated and that it responds to itself non-linearly. Non-linearity makes the response hypersensitive to changes in regulator concentration. Cells with more than a threshold amount of the regulator are driven to accumulate even more, due to the positive autoregulation. The expression of downstream genes is then altered and the population bifurcates. A second mechanism (Fig. 1) requires the presence of a pair of mutually repressing repressors. If R_2 is inactivated (perhaps because an inducer, Ind is added) then R_1 is produced, shutting off synthesis of R_2. Note that this is equivalent to positive autoregulation, because the increase in R_1 results in even more R_1 production. If R_2 represses a set of downstream genes, these will now be expressed. Remarkably, if Ind is withdrawn, the system will tend to remain in the state in which R_1 is ON and R_2 is OFF. If only a portion of the population was altered before the inducer was withdrawn, perhaps because the concentration of Ind was low, the two cell types will coexist, until random fluctuations of repressor concentration flips the switch. These two schemes have been implemented by combining regulatory elements *in vivo* in *E. coli* (Gardner *et al*., 2000) and in budding yeast (Becskei *et al*., 2001) and the predicted bistability has been observed. A classic example of a naturally occurring bistable switch of the second type is the double-negative regulatory circuit (involving the mutually repressing repressors CI and Cro) that governs the alternative lysogenic and lytic states of phage lambda (Ptashne, 2004).

It is easy to imagine how bistability might be advantageous, allowing cells to hedge their bets so that a few cells enter a state that would be better adapted to one circumstance or another should that circumstance arise. Cells cannot tell the future, but heterogeneity of this type may allow them to specialize in advance of changed circumstances. In some cases the heterogeneity may not be strictly constitutive, but may instead arise when stress is more likely, such as when nutrients become limiting. Spore formation and genetic competence are examples of this sort of behaviour.

We are concerned here with cells in a clonal population, and the survival of their shared genome will be maximized by these strategies. The penalty for throwing off specialized cell types that might not be needed is outweighed by the presence of some cells in the population that are already prepared for changed circumstances should they arise. This is an issue of resource allocation, and evolutionary experience dictates the optimal proportion of alternative cell types. A recent theoretical analysis suggests that bistability is an optimal strategy for coping with infrequent changes in the environment whereas responding only after a change is sensed is preferred when cells must deal with frequent fluctuations in environmental conditions (Kussell and Leibler, 2005).

Bistable switches are epigenetic in that they are not mediated by genetic changes. Thus, we draw a distinction here between bistability, the subject of this review, and another kind of heterogeneity-generating process known as phase variation that does involve reversible alterations to the genome. A classic example of phase variation is the generation of cells of *Salmonella typhimurium* expressing one or the other of two alternative alleles of the gene for the flagellum protein flagellin. Switching between the expression of one gene or the other is governed by a chromosomal inversion that occurs spontaneously and reversibly (Zieg *et al*., 1977). A mixed population of cells of the pathogen is thought to improve the chances for survival in a host that has generated antibodies to one or the other form of the surface displayed flagellin protein. Yet another kind of phase variation involves genes known as contingency loci that spontaneously switch between functional and non-functional states (Moxon *et al*., 1994). Switching in contingency loci may occur by a process known as

slipped strand mispairing, which reversibly generates single base pair insertions and deletions. Phase variation and contingency loci are outside the scope of this review, and except for one example will not be considered further.

Although we confine ourselves to bacteria in this review, mechanisms resembling bistable switches are also found in eukaryotes, including metazoans. Thus, during development the nematode *Caenorhabditis elegans* generates bilaterally symmetric gustatory neurones known as ASE *left* and ASE *right* that express genes for alternative taste receptors. A double-negative feedback loop that can be stably maintained in one state or another dictates whether a common precursor cell will acquire the *left* or *right* fate (Johnston *et al*., 2005). Upstream signals dictate whether the switch is thrown in the direction of the *left* cell fate on the left side of the body or in the direction of the *right* cell fate on the other side of the body. For other examples of bistability in higher organisms see review by Ferrell (2002). A review describing examples of bistability in bacteria, other than those discussed below, has been published recently (Smits *et al*., 2006).

Persister cells and antibiotic resistance

A frequent challenge to life in the microbial work is chemical warfare among competing species. Thus, one bacterium produces one or more antimicrobial agents that kill or block the growth of other competing species in its vicinity. The producing bacterium avoids suicide by having a resistance mechanism for protecting itself from the antibiotic, such as an export pump, an altered antibiotic target, or an enzyme that chemically inactivates the antibiotic inside the cell. But sensitive species of bacteria are not helpless in the face of this warfare. They can mutate to resistance to the antibiotic (for example, in the case of rifampicin by producing a mutant form of RNA polymerase that resists the action of the antibiotic) or can acquire such resistance by the lateral transfer of genes that confer resistance.

It has long been recognized, however, that some bacteria avoid killing by certain antibiotics without mutation. For example, when *Staphyloccocus aureus* is challenged with penicillin, a small number of bacteria survive the antibiotic treatment and resume growth when the antibiotic is removed (Bigger, 1944). When retreated with penicillin, the bacteria are found to be just as sensitive as the original cells but once again a small number of cells survive a second round of drug challenge. The surviving cells are known as persisters. Important insights into the persister state have come from studies with *E. coli* for which it has been possible to isolate mutants known as *hip* that exhibit an increased abundance of cells in the persister state (Moyed and Bertrand, 1983). *hip* mutants are experimentally useful, because they raise the frequency of persisters. Importantly, experiments with *hip* mutants have revealed that a small number of cells in the population spontaneously enter (and later exit) a state of no or slow growth and do so prior to drug treatment (Balaban *et al*., 2004). These experiments were based on the use of a microfluidic device in which it is possible to follow the growth (or lack thereof) of individual cells arrayed in channels whose narrow diameters allow propagation in only one dimension. Thus, the persister state represents a condition of reduced growth that is entered into spontaneously. Because killing by ampicillin depends on active cell wall growth, cells in the persister state avoid death. If the drug is absent when the persister cells resume growth, they escape the drug treatment. Thus, persistence is an epigenetic phenomenon in which *E. coli* copes with antibiotics by phenotypic heterogeneity, spontaneously throwing off non- or slow-growing cells that can survive drug treatment. The cost of generating non-growing cells is outweighed by the benefit to the species because survival of the shared genome is enhanced.

Two types of persisters were detected. Type I persisters were generated during stationary phase. In contrast, Type II persisters were produced continuously during growth. Simple mathematical models satisfactorily describe both types and rest on the assumption that switching between the normal and the persister states occurs at constant rates in each direction (Balaban *et al*., 2004).

A further important insight into the nature of the persister state has come from the work of Keren *et al*. (2004), who devised a procedure for isolating persister cells and showed that the cells preferentially express the genes for toxin–antitoxin modules. The authors argue that toxin expression inhibits macromolecular synthesis, such as translation, thereby protecting the cells from the damaging effects of antibiotics that target the ribosome, or that otherwise target cells in a growth-dependent manner. Overexpression of toxin genes, such as *relE* and *hipA*, increased the number of persisters while deletion of the *hipBA* toxin–antitoxin module decreased the number of persisters during stationary phase. It is intriguing that the involvement of the toxin–antitoxin genes implies the existence of a positive feedback loop operating at both the transcriptional and post-transcriptional levels. As is usual for such systems, the antitoxin (HipB) not only antagonizes the action of the toxin (HipA), but also serves as a transcriptional repressor of *hipAB* and the antitoxin is expected to be unstable compared with the toxin. A stochastic decrease of the antitoxin concentration in a given cell, or an increase in the toxin, will therefore lead to slowed translation, to increased *hipAB* transcription and to an enhanced imbalance between the accumulation of toxin and antitoxin. The result will be growth arrest: a switch to the persister state. The constant rate of switching from the normal to the persister state would therefore

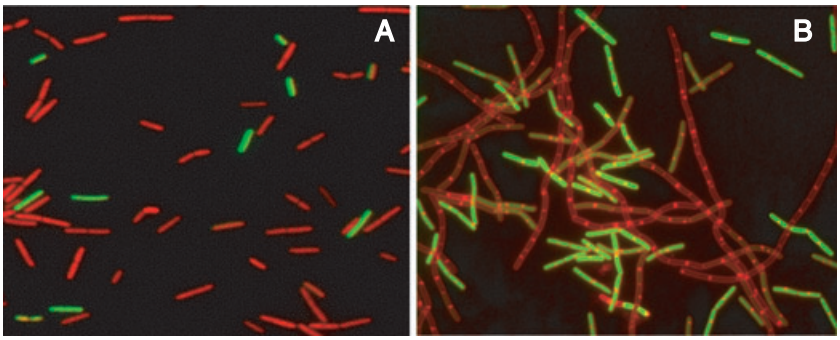

Fig. 2. Bistability in *B. subtilis*.
A. The cells contained a fusion of the gene for the GFP fused to *comK* and the native *comK* promoter. Competent cells in stationary phase were visualized by the green fluorescence. RNA and DNA were stained with propidium iodide (red) to visualize the non-competent cells.
B. The cells contained a fusion of the gene for the GFP to the gene (*hag*) for flagellin, which is under the control of σ^D. Cells that exhibited green fluorescence were singlets and doublets of motile cells that were active for σ^D (σ^D-ON). Cells were treated with the vital membrane stain FM4-64 (red) to visualize non-motile, chains of unseparated cells in which σ^D was inactive (σ^D-OFF). The cells were mutant for *swrA*, which in its wild type state biases bistability in the direction of σ^D-ON (see the text for an explanation).

correspond to the probability that the ratio of toxin to antitoxin will exceed a threshold value.

Genetic competence

A classic and well-understood example of bistability is entry into the state of genetic competence in *B. subtilis*. Certain kinds of bacteria can take up naked DNA from the environment and permanently incorporate the DNA into their genome by recombination, a genetically programmed process known as transformation that requires the synthesis of DNA transport and recombination proteins (Chen *et al*., 2005). When these proteins are produced, and the cells can be transformed, the cells are referred to as being in a state of 'competence.' In *B. subtilis*, competence occurs naturally as a culture enters stationary phase and, remarkably, the transport proteins are synthesized in only about 10% of the cells. These cells are not genetically distinct; when competent or non-competent cells from the same culture are returned to the initial conditions, again 10% express competence in stationary phase. Thus, the bifurcation of stationary phase cells into a mixture of competent and non-competent cells is an example of bistability.

The master regulator for competence is the transcriptional activator ComK, which drives the expression of all the downstream DNA transport genes (van Sinderen *et al*., 1995). ComK is also a positive autoregulator, which is directly required for transcription of its own gene (van Sinderen and Venema, 1994). What is more, ComK binds to the *comK* promoter (P_{comK}) as a dimer of dimers, and transcription is therefore expected to be hypersensitive to the concentration of ComK (Hamoen *et al*., 1998). These properties therefore satisfy the two conditions noted above that can enable bistability: positive autoregulation and non-linearity. In fact, two recent studies have shown that the bimodal distribution of fluorescence intensity from a fusion of the green fluorescent protein (GFP) to ComK as cells enter stationary phase (Fig. 2A) is dependent on the positive autoregulatory loop involving ComK binding to P_{comK} (Maamar and Dubnau, 2005; Smits *et al*., 2005).

The regulation of *comK* expression is complex (Fig. 3). Briefly, any ComK synthesized during exponential growth is rapidly degraded. A quorum-sensing mechanism involving the interaction of an extracellular pheromone with a membrane-embedded histidine kinase initiates a phosphorylation cascade that leads to the synthesis of ComS, a small protein that turns off the degradation of ComK as

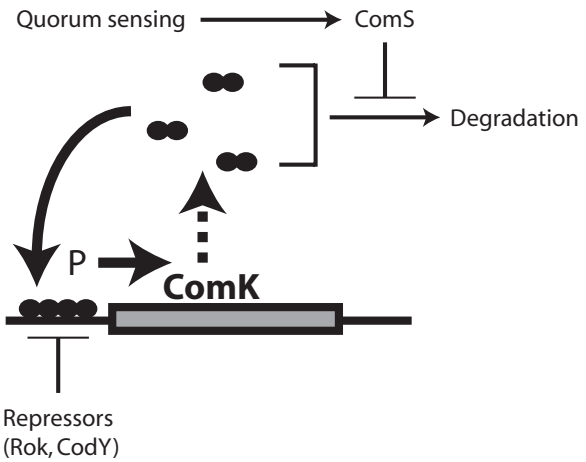

Fig. 3. Regulation of competence. ComK, the master regulator of competence-specific transcription, is required for the transcription of its own gene. This positive autoregulatory loop is central to the regulation of competence. ComK binds to the *comK* promoter as a dimer of dimers. In addition to ComK, several other proteins act at the promoter of *comK*, including the repressors Rok and CodY. Quorum sensing leads to the synthesis of ComS, which in turn protects ComK from degradation. The elements operating when ComK synthesis is turned on are emphasized.

cells approach stationary phase (Magnuson *et al*., 1994; Turgay *et al*., 1998). At least three repressors and one activator act at P_{comK}, and ComK antagonizes the action of one or more of these repressors, establishing the positive loop (Serror and Sonenshein, 1996; Hamoen *et al*., 2000; 2003; Hoa *et al*., 2002; Keren *et al*., 2004; W.K. Smits, T.T. Hoa and D. Dubnau, 2006, unpublished). It is proposed that noise in the expression of *comK* selects cells for competence. Due to noise, the concentration of ComK exceeds a threshold in some cells, activating the positive loop and driving these cells into the competent state. The autoregulatory switch governing competence can therefore be thought of as being poised on a knife-edge such that small, noise-driven variations among cells can determine whether or not the switch tips in the direction of a self-reinforcing cycle of ComK accumulation.

ComK exerts a negative effect on the expression of *comS*, acting either directly or indirectly (Hahn *et al*., 1994). It has been proposed based on experiments with fluorescence microscopy, that this negative feedback loop governs the escape from the competent state; a decrease in the ComS content of the competent cells would lead to increased degradation of ComK (Suel *et al*., 2006). The same study presents an interesting mathematical model of competence regulation as an excitable gene regulatory circuit with two feedback loops: the positive loop involving ComK acting at P_{comK} and this negative one involving ComK acting at P_{comS}. It is proposed that cells can spontaneously switch from the competent to the non-competent state and vice versa, although how this occurs and the source of the noise that is responsible for the transitions are not yet clear.

Two kinds of noise have been identified: intrinsic and extrinsic (Swain *et al*., 2002). The ComK-associated noise may be an intrinsic feature of P_{comK}, reflecting stochastic variation in the firing of the promoter. Alternatively, the noise may be extrinsic, determined by cell-to-cell variation in the total activity of the proteins that act at P_{comK} or determine the degradation rate of ComK. Although the real situation may involve both intrinsic and extrinsic contributions to noise in *comK* expression, it is conceptually useful to consider the two extreme possibilities. Let us first consider what may take place if the determinative noise is entirely intrinsic. In this case, the complex pathways that regulate ComK degradation and feed into P_{comK} via repressors may function only to govern the all-over probability that cells will switch to the competent state. In this scenario, repression at P_{comK} is lifted in all the cells and sufficient ComS is present to prevent the degradation of ComK in the entire population when it enters stationary phase. When this happens, the switch frequency rises from near zero during exponential phase to a value that results in 10% competent cells. The choice of which cells become competent depends not on cell-to-cell variation in these extrinsic factors, but rather on the intrinsic noise at P_{comK}. Due to this noise, a critical level in the concentration of ComK is exceeded in about 10% of the cells throwing the switch, so that these cells become competent. Although, as cells enter stationary phase, the basal level of transcription from P_{comK} may be adjusted upward due to the lifting of repression, this will simply shift the initial distribution in *comK* expression to a higher average. Still, it is the noise at P_{comK} that selects cells for competence. In fact, experimental inactivation of the repressors *rok* or *codY* that act at P_{com}, results in a larger fraction of cells achieving competence after stationary phase is reached, although competence expression remains bistable (Maamar and Dubnau, 2005). This model explains why some cells become competent before others. However, a critical question remains. Why do all the cells not eventually become competent? One possibility is that some mechanism limits the 'window of opportunity' for the selection process to be successful. In fact, as cells enter stationary phase, the rate of transcription from P_{comK} decreases dramatically in competent cells and even the low basal level of transcription in non-competent cells is shut off (H. Maamar, A. Raj and D.D., unpublished). Perhaps this transcriptional shutdown represents the closing of the postulated window.

In a second model, cells may be selected for competence by extrinsic noise: cell-to-cell variation in the levels of repressor proteins and/or those that regulate the degradation of ComK. In other words, the joint probability that these factors allow 10% of the cells to exceed a threshold level of ComK synthesis would be determinative. Although the intrinsic noise hypothesis would clearly be simpler to study and to model, there are insufficient data to distinguish between these possibilities.

Finally, it is interesting to note that in several wild strains of *B. subtilis* the proportion of cells in the population that enter the competence state is considerably lower than the 10% observed with laboratory strains (~1%) (H. Maamar, J. Hahn and D.D., unpublished). Evidently, enhanced levels of competence were selected in the history of the domestication of *B. subtilis* for use in experimental studies. It would be interesting to know precisely what feature(s) of laboratory strains causes the positive feedback loop to fire with greater probability than in wild strains. We will encounter a second example of bistability below in which the proportion of two cell types has been influenced by domestication.

Sporulation and cannibalism

Another example of bistability in *B. subtilis* is entry into sporulation, an elaborate developmental process that culminates in the formation of a dormant cell type known as the spore that is able to resist environmental extremes.

The master regulator for spore formation is Spo0A, a response regulator whose activity is governed by phosphorylation via a multicomponent phosphorelay (Burbulys *et al*., 1991). Spo0A is activated under conditions in which cells are limited for nutrients, but as demonstrated over a decade ago by flow cytometry some cells in a population of nutrient-limited cells activate the master regulator (Spo0A-ON cells) and some do not (Spo0A-OFF cells) (Chung *et al*., 1994). That this population heterogeneity arises under conditions that trigger sporulation was confirmed recently by fluorescence microscopy with cells harbouring a fusion of the gene for GFP to a promoter under the control of Spo0A (Gonzalez-Pastor *et al*., 2003). Evidently, then, nutrient limitation is a prerequisite for entry into sporulation, but activation of Spo0A is additionally subject to a bistable switch. Spo0A is subject to additional modes of regulation that are beyond the scope of this review (Fujita and Losick, 2003; 2005).

What is the basis for the bistable switch? A recent study has shown that the bimodal distribution of fluorescence from a fusion of the gene for GFP to a gene under Spo0A control is dependent upon a positive feedback loop involving both the transcription of the gene for Spo0A and the phosphorylation of the response regulator (Veening *et al*., 2005). At the heart of this unusually complicated feedback loop is the relay that governs the phosphorylation of Spo0A (Burbulys *et al*., 1991). Phosphoryl groups are fed into the relay by a series of histidine kinases [most notably KinA, KinB and KinC (Fujita and Losick, 2005)] that catalyse phosphorylation of the relay protein Spo0F (Burbulys *et al*., 1991). Phosphoryl groups from Spo0F~P are transferred to a second relay protein Spo0B, which, in turn, transfers the phosphoryl groups to Spo0A. Next, Spo0A~P stimulates the synthesis of the alternative sigma factor σ^H by an indirect pathway involving a repressor (AbrB) of the gene for σ^H. Finally, σ^H stimulates the transcription of the genes for KinA, Spo0F and for Spo0A itself. Thus, Spo0A~P sets up a self-reinforcing cycle that stimulates both its synthesis and phosphorylation. Presumably, the positive autoregulatory loop is close to the tipping point such that fluctuations in one or more components of the loop due to noise determine whether or not a self-reinforcing cycle of Spo0A~P accumulation is triggered in any particular cell. As in the case of competence, the precise source of this noise is not known.

A further feature of the positive feedback loop is the action of aspartyl phosphatases that drain phosphoryl groups from the relay, such as RapA, which dephosphorylates Spo0F~P, and Spo0E, which acts on Spo0A~P itself. A recent study indicates that RapA acts to stably maintain Spo0A in the OFF state in cells that have not activated the response regulator because in mutant bacteria lacking the phosphatase a high proportion of the cell population eventually becomes activated for Spo0A (Veening *et al*., 2005).

What is the biological significance of subjecting entry into sporulation to bistability? One possible explanation comes from the fact that spore formation is an energy-intensive process that becomes irreversible at an early stage. Thus, if the nutrient scarcity that triggers the activation of Spo0A in a population of cells proves to be fleeting, cells that have not entered the pathway to sporulate (Spo0A-OFF cells) will be able to rapidly resume growth when nutrients become available again. Studies of cells under conditions of high cell-population density reinforce the view that a mixed population of Spo0A-ON and Spo0A-OFF cells is a mechanism to cope with uncertainty in the future availability of nutrients. Clonal colonies of *B. subtilis* cells are observed to exhibit a behaviour referred to as cannibalism in which the Spo0A-ON cells in the population trigger the lysis of non-sporulating siblings (Spo0A-OFF cells) via the elaboration of a killing factor and a toxin (Gonzalez-Pastor *et al*., 2003). Nutrients released from the non-sporulating siblings arrest or slow further progression into sporulation by the Spo0A-ON cells, impeding those cells from becoming committed to spore formation. Cannibalism is therefore a delaying tactic that helps the population to certify that lack of nutrients is not a fleeting condition. According to this view, the cost of fratricide is off set by the advantage of delaying commitment for as long as possible.

Swimming and chaining

Certain kinds of cell population heterogeneity, such as that seen during entry into sporulation, arise under conditions in which stress becomes likely, such as when nutrients become limiting. In contrast, a recent study reveals a striking example of cell population heterogeneity that occurs under apparently steady-state conditions during the exponential phase of growth in rich medium. It is an old observation in the *B. subtilis* field that a population of rapidly dividing cells consists of a mixture of long chains of bacteria that have completed cytokinesis but have not separated from each other together with singlets and doublets of actively swimming cells (although as cells enter stationary phase the population becomes highly enriched for swimming cells) (Fig. 2B). The master regulator for swimming and for cell separation in *B. subtilis* is the alternative sigma factor σ^D. The σ^D factor directs the transcription of genes involved in flagellum biosynthesis and motility as well as genes for autolysins, enzymes that degrade the cell wall linking newly divided cells to each other (Mirel and Chamberlin, 1989; Margot *et al*., 1999). A recent study employing a fusion of the gene for GFP to a promoter under the control of σ^D reveals that cells at the mid-exponential phase of growth are a mixed population in which σ^D is active (σ^D-ON cells, the swimming cells) and cells in which the alternative sigma factor is inactive (σ^D-

OFF cells, the long chains of non-motile cells) (Kearns and Losick, 2005). Evidently, then, σ^D is subject to a bistable switch that operates under steady-state conditions. Unlike the examples of ComK and Spo0A, the switch governing the activity of σ^D does not operate at the level of the transcription of the gene for the alternative master regulator. Rather, evidence points to an as yet to be elucidated mechanism possibly operating at the level of the activity or stability of the protein.

What is the biological significance of bistability under conditions when cells are not being subjected to stress? An appealing interpretation is that the chains of σ^D-OFF cells are sessile cells that are optimized to exploit the particular microniche that the population currently occupies whereas the σ^D-ON cells are nomadic cells that are optimized to seek nutrients in new niches. If so, then a striking parallel exists between *B. subtilis* and the unrelated, dimorphic bacterium *Caulobacter crescentus*, which generates a mixed population of swimming (nomadic) cells and stalked (sessile) cells (Skerker and Laub, 2004). In contrast to *B. subtilis*, however, in which cell population heterogeneity arises stochastically, the generation of nomadic and sessile cells by *Caulobacter* is hard-wired into the bacterium's cell cycle, occurring obligatorily during each round of division. If these interpretations are correct, then convergent evolution has endowed *B. subtilis* and *Caulobacter* with similar strategies for optimizing their chances for survival in the face of uncertainty about the future but by radically different mechanisms.

A final twist to the swimming/chaining behaviour of *B. subtilis* is that it is not only subject to an epigenetic switch but is also influenced by a stochastic process involving reversible alterations to the DNA (Kearns *et al*., 2004). It turns out that laboratory strains are typically mutant for a novel gene called *swrA*, harbouring an A:T base pair insertion within a homopolymeric stretch of eight A:T base pairs in the *swrA* coding sequence. Wild strains, which do not harbour the frame-shift mutation, generate a strikingly higher proportion of swimming cells during mid-exponential phase growth than do laboratory strains. Evidence indicates that *swrA* is a novel regulatory gene that stimulates transcription from the promoter for a large operon containing genes involved in motility as well as the gene for σ^D itself. Thus, SwrA biases the bistable switch in the direction of σ^D-ON but is not part of the switch itself (in that cell population heterogeneity is observed both in the presence and absence of SwrA). Evidence indicates that the frame-shift mutation in *swrA* is readily reversible and that single base-pair insertions and deletions in the gene probably occur by the classical mechanism of 'slipped-strand mispairing' during DNA replication, as commonly observed in other examples of so-called contingency loci (as mentioned in the *Introduction*).

What is the biological significance of this second means for influencing the cell population heterogeneity of *B. subtilis*? It turns out that *B. subtilis* is capable of an additional mode of motility known as swarming. Swarming takes places on surfaces, where bacteria band together in groups of cells known as rafts to overcome the high viscous forces that otherwise impede surface movement. The formation of such rafts requires that a high proportion of the population is in the swimming (σ^D-ON) state and, indeed, strains (such as laboratory strains) in which *swrA* is mutant, are incapable of swarming. The same logic applies to this genetic example of stochasticity as to the epigenetic examples (bistability) that we have been considering: the ability to swarm on surfaces can be expected to be advantageous under certain environmental circumstances but not others where it might indeed be disadvantageous. Hence, the capacity to switch between a swarming state and a non-swarming state enables *B. subtilis* to hedge its bets in the face of uncertainty with regard to its future environmental circumstances.

Conclusions

The bias of classical microbiology has been to study the average behaviour of large cell populations. The recent advent of techniques that facilitate the investigation of individual cell behaviour has revealed, however, that populations of certain bacteria sometimes bifurcate into phenotypically distinct, but genetically identical, subpopulations by random switching mechanisms. Here we have reviewed four such examples of phenotypic heterogeneity from Gram-negative and Gram-positive bacteria. It is likely that such phenomena are very widespread, and we anticipate that many more examples will emerge. It may be worthwhile to systematically search for these by screening fluorescent fusions to candidate proteins for heterogeneous expression.

Presumably these switching mechanisms have evolved as a way for bacteria to be phenotypically pre-adapted to survive present or pending adverse conditions. Although from the standpoint of individual cells such behaviours may appear to be altruistic, from the viewpoint of the genome they are not, because they serve to maximize the chances of survival in clonal populations where all the cells have the same genotype.

Several examples that we have considered involve positive feedback loops, in accordance with predictions from modelling and the use of synthetic regulatory circuits, and we may anticipate that this will be a general feature of bistable systems. A vexing, but interesting question concerns the origin of the noise that drives cells into an alternative state. It is important to distinguish between this determinative noise and factors that operate across entire populations and determine the all-over switching probabil-

ity. In any given system, will the determinative noise be a property of one key regulator, or will it be the resultant of noise in the expression of several regulatory factors? Related to this question is the prediction that noise characteristics will be subject to evolutionary pressure: in other words, that the noise in expression of a regulatory gene will evolve towards some optimum, just as the average rate of expression of that gene also is subject to selective pressure. It will be interesting to pursue these questions in part by independently modifying the noise characteristics and average rates of expression of key regulatory genes.

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