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Jan-Willem Veening,[1,3] Wiep Klaas Smits,[2,3] and Oscar P. Kuipers[3]

[1]Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle upon Tyne NE2 4HH, United Kingdom; email: j.w.veening@ncl.ac.uk

[2]Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139; email: smitswk@mit.edu

[3]Molecular Genetics Group, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, 9751 NN Haren, The Netherlands; email: o.p.kuipers@rug.nl

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Bacillus subtilis, competence, sporulation, AND gate, phenotypic variation, synthetic biology

Abstract

Clonal populations of microbial cells often show a high degree of phenotypic variability under homogeneous conditions. Stochastic fluctuations in the cellular components that determine cellular states can cause two distinct subpopulations, a property called bistability. Phenotypic heterogeneity can be readily obtained by interlinking multiple gene regulatory pathways, effectively resulting in a genetic logic-AND gate. Although switching between states can occur within the cells' lifetime, cells can also pass their cellular state over to the next generation by a mechanism known as epigenetic inheritance and thus perpetuate the phenotypic state. Importantly, heterogeneous populations can demonstrate increased fitness compared with homogeneous populations. This suggests that microbial cells employ bet-hedging strategies to maximize survival. Here, we discuss the possible roles of interlinked bistable networks, epigenetic inheritance, and bet-hedging in bacteria.

Contents

PHENOTYPIC VARIATION AND ITS ORIGINS

Bacterial growth is traditionally viewed as the result of (symmetrical) cell division yielding siblings that are genetically identical. Consequently, the results from reporter studies such as those employing *lacZ* have traditionally been interpreted using the assumption that all cells in a culture behave in an identical manner. However, it has long been recognized that within isogenic populations, bacterial cells can display various phenotypes. This microbial cell individuality or phenotypic variation is receiving increased attention because of its relevance for cellular differentiation and implications for the treatment of bacterial infections (92). Phenotypic variation is a widespread phenomenon in the bacterial realm. Some of the well-characterized examples include the lysis-lysogeny switch of phage lambda, lactose utilization and chemotaxis in *Escherichia coli*, phase variation in a number of pathogens, and cellular differentiation in *Bacillus subtilis* (for recent reviews see References 11, 25, and 92). Strikingly, many documented cases of phenotypic variability relate to responses to environmental stresses, suggesting that phenotypic variation aids in the survival of cells under adverse conditions and therefore may be an evolvable trait. The potential function of phenotypic variation as a bet-hedging strategy is further elaborated upon in other parts of this review.

Various different mechanisms are involved in phenotypic variation. Phenotypic differences can be due to mutation, variations in the microenvironment, mutation, phase variation, cell cycle, and the wiring of the network that governs a specific stress response (11, 92). The focus of this review is on the role of phenotypic variability that results from amplified noise in gene expression.

***lacZ*:** β-galactosidase, traditional reporter that cleaves colorless X-gal, resulting in bright blue products

Bet-hedging: a risk spreading strategy to diversify phenotypes with the aim to increase fitness in temporally variable conditions

NETWORK TOPOLOGY

As early as 1961, Monod and Jacob postulated that the differences in the response of individual cells to a stimulus could in theory be explained by the architecture of the underlying gene regulatory network (66). However, their hypotheses could not be experimentally addressed until the development of single-cell techniques and were not computationally tractable until recently. Considering the importance of this type of mechanism in generating phenotypic variation (92), it is discussed in more detail below.

Noise, Hysteresis, and Bistability

In biological systems, signals are never discrete because of random fluctuations in the biochemical reactions in the cell. This stochastic variation is called noise and is a key determinant

of phenotypic variation (49, 81, 85). Noise is predicted to be most dominant when the number of molecules involved is small (finite number effect). Experimental verification of this notion came from fluorescent reporter studies (28, 78, 98). This effect is notable for two reasons. First, transcription and translation are thought to generally involve relatively small numbers of molecules compared with, for instance, the numbers of molecules participating in protein-protein interactions. Second, when not activated, transcription factors are usually in low abundance. Moreover, many stress responses are accompanied by a reduction in general transcriptional and/or translational efficiency (38). This potentially leads to an induction of phenotypic variation under these conditions. Generating variable phenotypes may be beneficial for the survival of populations under adverse conditions, and stimulating noisy expression might be an elegant way of achieving this (72).

Noise can be exploited under certain conditions to generate phenotypic heterogeneity. For example, noise in the regulatory cascade that governs the chemotactic response of *E. coli* results in behavioral individuality with respect to the rotational direction of the flagella (54). When a noisy signal is amplified by net positive feedback, gene expression levels can be further bifurcated and this situation deserves special attention. In the presence of positive feedback, a graded response (i.e., with intermediate levels of expression) can be converted to a binary response, in which cells express a certain gene at high or low levels (13). At the population level, this switch-like behavior can result in a bimodal distribution in gene expression because some cells switch, whereas others do not. This type of gene expression pattern is commonly referred to as bistability (25, 92).

In physics, multistationarity describes a network that has more than one stable state. Extending this to biology, it means that a gene regulatory network potentially exhibits two (or more) discrete levels of gene expression (a high state and a low state). Bistability describes a parameter regime in which a dynamic system can rest in either of two stable states. Analogous to the previous definition, it refers to conditions under which cells can be in a high-expressing or low-expressing state for biological systems. Multistationarity at the cellular level is an intuitive explanation for population bistability; hence, the terms are frequently used interchangeably. Although most biological systems that demonstrate population bistability involve noise amplified by some form of net positive feedback, they are not necessarily bistable in a deterministic sense (95).

The requirements for a gene network to exhibit multistationarity have been explored in detail (29, 92). In summary, the system needs to display nonlinear kinetics in addition to positive feedback. For transcriptional regulators, nonlinearity can be the result of multimerization, cooperative binding to target sequences on the DNA, or phosphorylation of certain amino acid residues. In many cases nonlinearity is evident as a sharp increase in the expression of a downstream target gene above a certain threshold level of the regulator. Only networks that include an even number of negative-feedback loops and/or any number of positive-feedback loops are capable of causing multistationarity (8). Experimentally, some bistable gene expression patterns rely on positive feedback as well as double-negative feedback (toggle switch) (92 and references therein). However, positive feedback in itself is no guarantee for bistability (29), and bistability is also possible when based on other types of network architecture (8) or mechanisms such as multisite phosphorylation (55, 77).

A common feature of bistability is hysteresis (74). Hysteresis refers to the situation in which the transition from one state to the other requires an induction (or relief of induction) greater than that for the reverse transition. This imposes memory-like characteristics onto the network (see also Epigenetic Inheritance of Phenotypic Variation, below), making the response of cells dependent on their recent history. Hysteresis in biological systems can reside, for instance, in the stability of one of the proteins involved. When Novick & Weiner (76) described the all-or-none enzyme induction

Multistationarity: multiple stationary stable states within a (genetic) network between which switching is possible

Bistable: a network with two steady states, or two distinguishable phenotypes within a clonal population

Sporulation: a developmental process ultimately resulting in the formation of a highly resistant (endo)spore

Competence: the ability to take up DNA from the environment and stably maintain its information in the genome

in lactose utilization they noted that at near-threshold concentrations of inducer the population of *E. coli* cells segregated into two subpopulations, which is now regarded as one of the earliest examples of bistability. Subsequent experiments revealed that the history of the inoculum influenced the fraction of cells in each subpopulation (23). The hysteretic behavior of the multistable lactose utilization network is a result of the stability and abundance of the lactose permease (79, 107). Hysteresis can act as a buffer, reducing accidental switching between states due to minor perturbations (1, 16).

Although bistable systems are in principle reversible, the time required for a cell to revert to the initial state (escape time) may exceed the duration of the experiment or even the lifetime of the organism. Moreover, phenotypic switches can be rendered unidirectional by downstream signaling events. For instance, the bistable switch governing sporulation in *B. subtilis* becomes irreversible after its earliest stages owing to an orchestrated sequence of events (26).

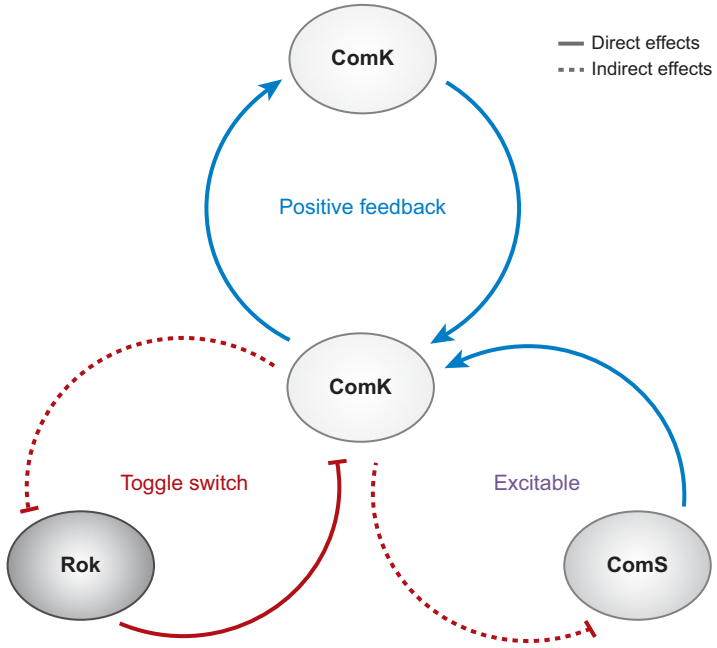

Figure 1

Regulatory elements featured in current models on competence development in *Bacillus subtilis*. A highly simplified representation of the three core elements of the competence regulatory network: (*a*) ComK autostimulation is responsible for a positive-feedback loop required for a bimodal expression pattern, (*b*) a putative toggle switch is dispensable for a bimodal expression pattern, and (*c*) interlinked positive- and negative-feedback loops that result in excitable behavior are implicated in the temporal nature of competence. Solid lines represent direct or well-characterized interactions; dotted lines represent putative or indirect effects.

COMPETENCE FOR GENETIC TRANSFORMATION IN *BACILLUS SUBTILIS*

To further explore general mechanisms by which phenotypic variation can arise, we discuss one of the best-understood naturally occurring bistable systems in bacteria: competence development in *B. subtilis*. The first evidence for the existence of subpopulations in a competent culture of *B. subtilis* came from elegant experiments that demonstrate biosynthetic latency of competent cells (17, 41, 73). Subsequently, the expression of the key regulator of competence development, ComK, was limited to the competent fraction of the culture (42).

ComK is a multimeric transcription factor that is necessary and sufficient to activate the expression of all genes that encode the DNA uptake and integration machinery by binding to a consensus motif in the target promoters (44). Key features of the complex regulatory network that controls ComK levels are transcriptional regulation at the *comK* promoter and proteolytic degradation of ComK protein (44). ComK stimulates its own expression by reversing the effects of at least two repressors, one of them named Rok (for repressor of *comK*), establishing a positive-feedback loop (91). Additionally, ComK is believed to repress transcription of *rok*. This interaction forms a putative toggle switch. Proteolytic degradation of ComK is antagonized by the anti-adaptor protein ComS, which is required for the initiation of competence. Evidence suggests an indirect negative-feedback loop, as overproduction of ComK inhibits ComS expression (95). The features described above are summarized in **Figure 1**, and they all form modules that are potentially involved in phenotypic variation.

ComK autostimulation is necessary and can be sufficient to establish a bimodal expression pattern (61, 90), but it is independent of Rok, excluding a toggle switch–like mechanism. The transition between low- and high-expressing states was attributed to stochastic fluctuations in conjunction with the positive-feedback loop that would amplify the signal as the concentrations of ComK exceed a certain threshold (103). The role of noise was experimentally addressed in two studies. Süel and coworkers averaged out the noise of multiple cells by depleting cells for *ftsW*, which is required for septation. An analysis of competence development under those conditions revealed that the chance of initiation of competence was greatly reduced (96). In a more direct approach, Maamar and coworkers (62) adopted a method derived from Elowitz et al. (28) to show that intrinsic noise in *comK* expression selects cells for competence. Reducing intrinsic noise, by increasing transcriptional efficiency and reducing translational efficiency, caused significantly less cells to enter competence. Their findings are consistent with another report that demonstrated significant variation in basal promoter activity of *comK* (57). Because ComK is responsible for the activation of the late competence genes (such as *comG*), intrinsic noise in *comK* expression results in pathway-specific extrinsic noise in *comG* expression.

Competence is a transient process; under laboratory conditions it is limited to several hours in stationary growth phase or until cells are resuspended in fresh growth medium. Although the molecular mechanisms responsible for escape from the competent state remain elusive, mathematical modeling has recently shed some light on potential mechanisms and has led to the development of two predominant models. Both models share the notion that noise is amplified by the ComK autostimulatory loop. In the bistable model, intrinsic noise of *comK* expression (57, 62) is critical for the switching of cells from the noncompetent to the competent state. In the excitable model, the source of the noise that triggers the excursion from the vegetative state remains undefined (95, 96). Although both models can result in a bimodal distribution at the population level (as both involve stochastic switching), only the first model is bistable in a deterministic sense. The excitable model generates a bimodal gene expression pattern because the transition to the high-expressing state is fast compared with the slowly acting negative-feedback loop, but the high expression level does not represent a stable state (95).

Excitable: a transient excursion from a stable state leading to the expression of a phenotype in a limited period of time

Both models offer a different explanation for the temporal nature of competence. In the bistable model, two mechanisms are at play. First, cells can revert from the high-expressing to the vegetative state by stochastic transitions. Second, the basal promoter activity of *comK*, as measured by the number of mRNA molecules per cell, is greatly reduced in stationary growth phase (57, 62). This causes a window of opportunity for cells to switch to the competent state and generates conditions under which the saturated proteolytic complex reduces ComK levels enough to escape the competent state. The validity of this hypothesis was confirmed through mathematical modeling (62).

The excitable model offers an attractive hypothesis for the limited time span during which cells are competent for DNA uptake. In contrast to the bistable model, the competent state is not stable owing to the action of a slowly acting negative-feedback loop. As a result cells will always return to the vegetative and stable state. The model makes some predictions about the dynamics of the competence network that are experimentally addressed using time-lapse fluorescent microscopy (96).

The elegance of the excitable model has attracted a lot of attention, as it resembles the dynamics of oscillatory systems such as cell cycle and circadian rhythms. However, it fails to couple back to the observations made in single-cell analyses of competent cultures that demonstrate a limited time frame during which competence occurs in a culture and does not take the observed decrease in basal *comK* transcription into account. Although certain features of

Epigenetic inheritance (EI): inheritance of a phenotype from one generation to the next that does not depend on changes in DNA sequence

the two models are not reconcilable, it is possible that both mechanisms occur in nature under different conditions, for example, the timescale on which they occur could vary. Moreover, it has been suggested that stochastic activation of *comK* in combination with positive feedback could result in a bimodal expression pattern, even in the absence of bistability in the deterministic sense (9, 50). It is a challenge for future investigators to address these unanswered questions.

PROSPECTS OF USING BISTABLE SWITCHES FOR BIOTECHNOLOGY AND SYNTHETIC BIOLOGY

Construction of synthetic genetic circuits using naturally occurring cellular components in living cells allows them to be tested separately from the context of other physiological processes. Synthetic switches are operational in prokaryotic and mammalian cells and valuable for gaining insight into naturally occurring genetic circuitries (45, 46). Synthetic biology also allows the creation of entirely new, or rerouted, networks, such as toggle switches, oscillatory networks, and even synthetic multicellular clocks based on quorum sensing (10, 27, 34, 36). Some of these findings made it to patents (37), showing the realistic prospect of industrial utilization of engineered circuitries leading to phenotypic variation.

Combinatorial promoter design also is effective for engineering noisy gene expression (71), and various successful examples of combinatorial promoter design have been published (24, 43). Global transcription machinery engineering (gTME) is a compatible strategy for improving metabolic engineering efforts. Instead of direct enzyme or metabolic pathway engineering, gTME reprograms the transcription machinery, resulting for example in increased ethanol tolerance and production in yeast (7). This method could be well combined with the strategies outlined above to engineer novel regulatory circuits.

CELL AGE AND ITS ROLE IN PHENOTYPIC VARIATION

Although aging has already been described to cause phenotypic variability in yeast (4), *Caulobacter crescentus* was the first bacterium for which aging was demonstrated (3). It was found that the reproductive output of cells decreased with age. Asymmetric division is a hallmark of the life cycle of this bacterium, and these observations are therefore consistent with the hypothesis that mortality requires asymmetry (80).

In many other prokaryotes, however, cell division leads to two visibly identical daughter cells, and as a result, they have been regarded as nonsenescent. Yet, the subcellular localization of a set of proteins may distinguish old and new poles in morphologically symmetrical bacteria. By following single *E. coli* cells through several rounds of cell division, Stewart and coworkers showed that growth rate inversely correlates to cell pole age, demonstrating that aging is not limited to organisms with asymmetric division (94). It was recently found that aggregated proteins and chaperones preferentially accumulate at the old cell pole (59), reminiscent of the situation in yeast in which oxidatively damaged proteins accumulate in the mother cell (4).

Recently, time-lapse microscopy has been used to follow the growth, division, and cellular differentiation of individual cells of *B. subtilis* (104), an organism that is well known for asymmetric division prior to the formation of an endospore. The study revealed that *B. subtilis*, like *E. coli* and *C. crescentus*, suffers from aging but that spore formation is not biased toward either the old or the new cell pole (104). Interestingly, the magnitude of this aging effect is nearly identical to that seen in *E. coli* and *C. crescentus*.

EPIGENETIC INHERITANCE OF PHENOTYPIC VARIATION

Epigenetic inheritance (EI) (or non-Mendelian inheritance) is the passage of cellular states from one generation to the next, without alterations

of the genome (48). The classic example of EI is the stable transfer of a phenotype by modifications to the DNA such as methylation (19). This modification can be stable over multiple rounds of cell division but it does not involve actual changes in the DNA sequence of the organism. Other epigenetic phenomena include prions, genomic imprinting, and histone modification (19 and references therein).

It has been proposed that autophosphorylating kinases have the potential to store memory. In this scenario, a specific stimulus activates the kinase, and because of its autocatalytic properties the kinase stays in its active state, regardless of the presence or absence of the stimulus (60). As a result, the progeny of cells in the ON-state will also be in the ON-state because the activated kinase is passed on to the offspring. Using artificial bistable gene regulatory circuits in both *E. coli* and *Saccharomyces cerevisiae*, autostimulatory regulation systems can function as memory devices in microorganisms (13, 36).

EI of phenotypic variation can also be based on the transfer of active transcriptional regulators during cell division via positive feedback (18, 60, 84). When cells divide, not only DNA but also cellular factors such as proteins and RNA are partitioned, and importantly, this can dictate future life-history decisions of the new offspring. Valuable knowledge on the molecular mechanism responsible for EI and the minimal requirements to generate stable inheritance of phenotypic variation is arising from studies using well-defined artificial gene networks (6, 51). The simplest network that demonstrates EI is one in which a positive regulator autostimulates its own promoter upon stimulation by an exogenous signal. Once activated, the positive feedback of the system will ensure high intracellular levels of the positive regulator, regardless of the absence or presence of the signal. In such a system, the degradation rate of the regulator and the growth rate of the cell are determining factors of the stability of the memory response (6).

An example of a simple (but general) network motif that putatively generates EI is depicted in **Figure 2**. A number of requirements need to be met before EI can occur. The network should show two stable steady states (activator OFF and activator ON). This depends on activator production/decay rate and growth rate, and activator production should be cooperative (6). In addition to this, the basal activator levels should be at a level lower than required to autoactivate its own synthesis; otherwise cells will always be in the ON state. Furthermore, once the system is activated, activator levels should be high enough to drive its own expression; if not, cells will quickly switch back from the ON to the OFF state and EI cannot be established. Even cell fates driven by a semistable stochastic switch with reduced positive feedback inherit epigenetically. This is likely caused by initial bursts of activator protein in the mother cell, which maintains at high levels through multiple rounds of division (51). Two examples of the significance of EI of phenotypic variation in bacteria are discussed below. Other instances, primarily from eukaryotes, fall outside the scope of this review.

Memory Within the *lac* Operon

As discussed above, bistable systems depend on some form of positive feedback within the gene network. The first epigenetic system described in bacteria is the *lac* operon of *E. coli* (76). The genes that encode the proteins required for the uptake and utilization of lactose are induced in the presence of the gratuitous (nonmetabolizable) lactose analogue, isopropyl-D-thio-β-galactopyranoside (IPTG). At high IPTG concentrations the *lac* operon is fully derepressed and cells highly express the IPTG permease protein and thus remain highly activated. At low concentrations, however, cells that were previously uninduced and do not have any permease in their membranes do not respond to the low level of IPTG and remain in the OFF state. Cells that were previously induced and still have some permease are activated by the low level of IPTG and remain in the ON state. Reculturing of single cells results in populations that either give high or low *lac* expression (70 and references therein). This phenomenon

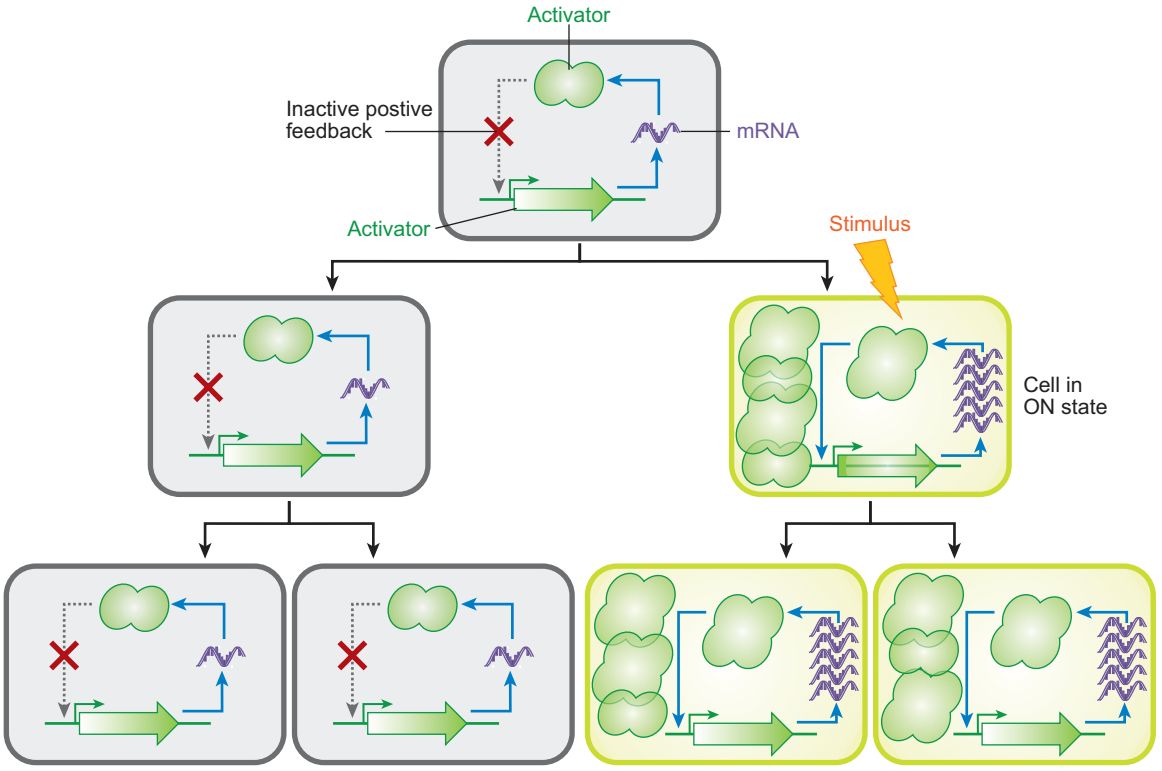

Figure 2

Epigenetic inheritance by positive feedback. A basal level of activator protein and mRNA (*single helix*) is always present regardless of the absence of stimulus (*lightning symbol*). However, this basal level is insufficient to activate the positive-feedback loop (*red X*) and activator protein levels remain low. When the signal is present, however (which might be caused by noise), activator protein multimerizes and stimulates its own expression, resulting in high concentrations of activator, and in this example, high activator concentrations induce multimerization. Because of the positive-feedback loop, intracellular activator concentrations remain above the threshold required to stimulate transcription and cells remain in the ON state (*green cells*) for multiple generations even in the absence of stimulus. Cell growth and division can dilute activator, but as long as the concentrations remain high enough to drive promoter firing, cells will remain in the ON state.

is called all-or-none enzyme induction (76) and is indicative of the presence of two coexisting subpopulations. The permease plays a pivotal role and constitutes the positive-feedback loop in this system: High permease levels keep the levels of intracellular IPTG high, thus inducing permease gene expression. Importantly, under low inducer conditions, either the ON or OFF state can be epigenetically inherited by the offspring through multiple rounds of growth and division. In this situation, the physiological state of the offspring is a reflection of the past state of its ancestor. A possible explanation for such a positive-feedback loop in the *lac* operon is that in the presence of (metabolizable) lactose, the *E. coli* population can quickly drain the sugar pool even when the sugar concentration starts to decrease (18).

IPTG: isopropyl-D-thio-β-galactopyranoside

Sporulation in *B. subtilis*

Sporulation of *B. subtilis* has been described as a bistable process because two distinct subpopulations can be distinguished within an isogenic population of stationary-phase cells: sporulating and nonsporulating cells (reviewed in References 25 and 92). Initiation of sporulation is driven by the master sporulation regulator Spo0A. A basal level of Spo0A is always present, and upon specific environmental

signals such as high cell density and nutrient deprivation, Spo0A is phosphorylated and directly activates expression of more than 100 genes, including its own gene (31, 65). Sporulation bistability is not a simple ON/OFF switch, because the levels of Spo0A∼P increase gradually after activation (32). Recent research has shown that although the positive feedback of Spo0A∼P on *spo0A* transcription plays an important role in the distribution of cellular states (31, 101), it is not critical in establishing sporulation bistability (104). Rather it seems that the activity of the phosphorelay dictates sporulation bistability because cells constructed to express a mutant form of Spo0A (Sad67) (47) that does not require activation no longer show bistability (104).

A recent study using time-lapse microscopy found a strong correlation between cell lineage and the decision to sporulate or not sporulate (104). Close relatives often demonstrate a similar phenotype (to either sporulate or not sporulate). Phylogenetic reconstruction of sporulating microcolonies using parsimony analyses showed that the decision to sporulate could often be traced back more than two generations before the actual appearance of the phenotype (**Figure 3**). This finding indicates that the signal to sporulate already occurs during the logarithmic growth phase and is epigenetically passed on. Again, an important role for the sporulation phosphorelay was identified for this epigenetic effect (104), indicating that bistability is a prerequisite for EI of the sporulation signal.

The putative benefits of EI within a sporulating population are complex. For cold-shock adaptation in bacteria, cells pretreated by a mild cold shock memorize this stress and are better prepared for a harsher cold shock, which would otherwise be lethal (40). In analogy to this, it can be envisaged that propagation of the sporulation signal from the mother cell to its descendants helps the progeny to be prepared for potential nutritional limitations in the future in such a way that they can rapidly respond

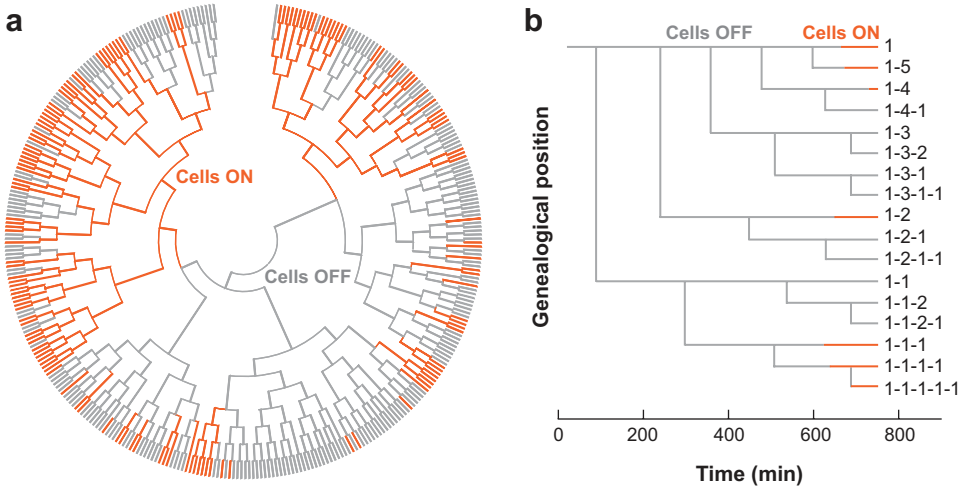

Figure 3

Lineage reconstruction to plot cell fate distributions within isogenic populations. (*a*) Parsimony reconstruction of the sporulation signal within a *Bacillus subtilis* microcolony. Every node in the radial tree represents one cell division event. Every endpoint in the tree represents one offspring cell. Orange tips are cells that have activated Spo0A. Parsimony reconstruction shows the first appearance of a mother cell that creates offspring of mostly cells in the ON state (*orange lines*). Figure from Reference 104. (*b*) Family tree of *Saccharomyces cerevisiae* harboring an artificial bistable switch. Gray lines indicate cells in the OFF state, whereas orange lines represent cells after they have switched to the ON state. In this genealogy graph, in contrast to panel *a*, line length is a direct measure of time. Figure from Reference 51.

and commit to spore formation when required. Alternatively, EI may serve to coordinate multicellular behavior (104), a process which in *B. subtilis* is also dictated by Spo0A (5).

CRIF: *cis*-regulatory input function

Altruism: a behavior that decreases the fitness of the altruistic individual while benefiting others

GENETIC LOGIC-AND GATES

Often, transcription of a gene is regulated by more than one regulator (input). The way these inputs control the transcription rate (output) is described by the *cis*-regulatory input function (CRIF) (64). CRIFs can often be described by Boolean-type functions such as logic-AND gates and logic-OR gates (64 and references therein). Synthetic logic-AND gates can be exploited to program specific responses of cells (75). If one of the inputs of a CRIF is heterogeneous and the target gene is under control of a logic-AND gate, then by definition the output is also heterogeneous.

A number of studies recognize that certain genes of one pathway are heterogeneously expressed because their regulation is interlinked with another (bistable) network through a logic-AND gate. The use of an AND gate system is a simple strategy to generate phenotypic variability without the necessity to create complex switches with multiple steady states (**Figure 4*a***). Here we consider a few examples in which heterogeneity in gene expression can be ascribed to the logic of the underlying circuitry. We discuss the putative physiological relevance of the observed heterogeneity as a result of the AND circuit.

Heterogeneity in Exoprotease and Biofilm Matrix Production

Recently, it was found that high expression of *aprE* (subtilisin) and *bpr* (bacillopeptidase), two important extracellular proteases (exoproteases) of *B. subtilis*, is limited to only a small part of the population (**Figure 4*b***) (102). Exoprotease production has been described as a survival strategy under nutrient-limiting conditions, and these enzymes act as scavenging proteins that degrade (large) proteins into smaller fragments that can be subsequently taken up as a new nutrient source (68). Studies using wild *B. subtilis* strains also indicate a role for exoproteases during biofilm formation (53, 105).

Expression of both *aprE* and *bpr* is under the control of the DegS-DegU two-component system (68). To activate *aprE* gene expression, DegU needs to be phosphorylated by the DegS sensor protein (69). In addition, *aprE* is under direct negative control of at least three other transcriptional regulators (AbrB, SinR, and ScoC), all of which are under direct or indirect negative control by the key sporulation regulator, Spo0A∼P (31). The result of this intertwinement with the sporulation pathways is that *aprE* will only be derepressed in a subpopulation when nutrients become limiting. Together, the *aprE* gene regulatory network acts as a logic-AND circuit in which a threshold level of dimerized DegU∼P and Spo0A∼P is integrated to activate gene expression (102) (**Figure 4*c***).

It has been hypothesized that cells that produce and secrete these proteases help not only themselves, but all clonal cells within the local growth medium. This might be regarded as a simple form of altruism. One explanation for altruism is when the cooperation is directed toward individual cells that are genetically similar (kin selection) (63). Heterogeneity in gene expression ensures that not all cells commence into the costly production of Bpr and AprE, but all cells within the clonal population benefit from the activity of these extracellular proteases.

Similarly, the extracellular matrix within biofilms of *B. subtilis* is produced by a small fraction of cells within the population (20, 106). Expression of the products that form the extracellular matrix (EpsA-O, YqxM, and TasA) is under direct negative control of SinR, the master biofilm regulator in *B. subtilis* (52). This regulator is antagonized by SinI, a protein under control of Spo0A. *sinI* seems to be activated by low levels of Spo0A∼P but repressed at high levels of Spo0A∼P (20), although this still awaits experimental validation. Thus, expression of *sinI* and, as a result, the genes responsible for the extracellular matrix

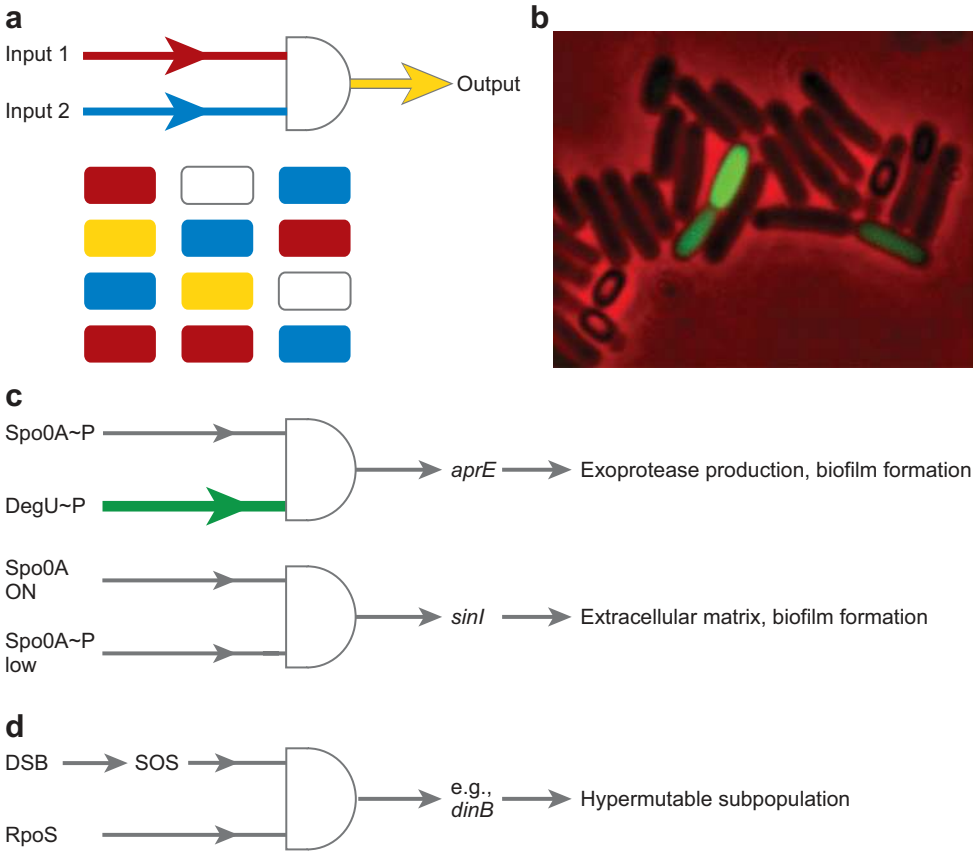

Figure 4

Naturally occurring genetic logic-AND gates. Arrows indicate positive actions. (*a*) Input 1 (*red arrow*) and input 2 (*blue arrow*) are active only in ~50% of the isogenic population (*red and blue cells*, respectively). Cells not active for input 1 or 2 are depicted in white. The output of the system (*yellow arrow*) is expressed only when both input 1 and input 2 are active within the same cell (*yellow*). As a result of this AND gate, the isogenic population of four distinguishable phenotypes can exist: white, red, blue, or yellow cells. (*b*) Heterogeneous expression of *aprE*. Expression of *aprE* is monitored by a fusion of the *aprE* promoter to *gfp*. Within stationary-phase cultures, three distinct phenotypes can readily be observed: sporulating cells, vegetative cells, and *aprE-gfp*-expressing cells. (*c*) The genetic circuit responsible for *aprE* and biofilm heterogeneity. Thick arrows indicate that the system can be overridden by (artificial) induction of the activator protein. For simplicity, we depict the effect of Spo0A~P on the multiple repressors that act on *aprE* as a single positive arrow. (*d*) SOS response and RpoS requirement for the formation of a hypermutable subpopulation in *Escherichia coli*. See text for details.

within biofilms are activated only when two conditions are met: (*a*) Spo0A needs to be activated and (*b*) Spo0A~P levels cannot be too high. Although this is not a true logic-AND circuit, the result of the network wiring is that only a small subpopulation of cells expresses *sinI*. Because the production of the extracellular matrix within biofilms is energetically costly, the division of labor might enhance the total fitness of the entire bacterial community. It will be interesting to see how this labor is divided in multispecies biofilms.

Hypermutable Subpopulations in *E. coli*

Clonal populations of cells may diverge owing to changes in their genetic makeup. The

occurrence of mutations may give certain cells a selective advantage over others, and this may cause a subpopulation to form or even take over the culture (108).

Under conditions of stress, adaptive mutagenesis (and/or stationary-phase mutagenesis) can occur (for recent review see Reference 30). In *E. coli*, adaptive mutations were associated with other, unselected mutations, indicating the existence of a hypermutable subpopulation (HMS) (100). The observed hypermutation is not caused by a stable mutator phenotype that could result from genetic differences, but reflects a transient differentiated state (39, 87).

The mechanisms involved in hypermutation include double-strand break (DSB) repair, SOS response, and a general stress response, of which the first two have a causal relationship (33). The critical factor in HMS, though not the only one, is that cells are continuously facing DNA double-strand breaks, even in the absence of external DNA-damaging agents. The induction of DSB repair is evidenced by the formation of foci of RecA protein, a key protein in the repair pathway, in a subset of cells (86, 88). DSBs can lead to induction of the SOS response, and ~1% of growing *E. coli* cells is SOS induced under steady-state conditions (82).

The switch to HMS requires an additional requirement to be satisfied, as artificially induced DSBs do not lead to HMS until cells enter stationary phase (83). At that time, the levels of the general stress sigma factor RpoS rise, and it was found that artificially inducing RpoS can lead to HMS in exponential growth phase (83). Thus, the preexisting heterogeneous input of (at least) the SOS response, together with RpoS, forms a logic-AND gate that leads to the formation of the HMS (**Figure 4*d***).

PHENOTYPIC VARIATION AS A BET-HEDGING STRATEGY

A major question that arises from the finding of population heterogeneity is, Why do bacteria display phenotypic variation? The most apparent hypothesis is that this strategy is a form of bet-hedging. Under challenging conditions, the production of offspring with variable phenotypes ensures that at least one offspring will be appropriate (fit) under a given situation (22). This is a risk-spreading or bet-hedging strategy, because not every offspring will be optimally suited for the future environment. However, the overall fitness of the genotype will increase because some offspring will have the proper adaptation. Although heterogeneity might not be ideal under homogenous, steady-state conditions, mathematical studies support the notion that in a variable environment a heterogeneous population outcompetes (or is fitter than) a homogeneous population (56, 99). Importantly, it was suggested that phenotypic variation is an evolvable trait. This was recently underscored in an elegant study on *S. cerevisiae*, in which interphenotype switching rates, like those between the two stable states of gene expression in a bistable system, are tuned to the frequency of changes in the environment (2).

Experimental evidence for the benefits of phenotypic variation is limited. In yeast, clonal populations with increased variability in stress resistance are more successful than strains with limited variability under conditions of stress (15). Moreover, heterogeneous populations of yeast outcompete homogenous populations under cadmium stress conditions (89).

Bacterial Persistence

Originally identified in 1944 (14) persistence is one of the best-documented examples of a bacterial bet-hedging strategy (for a recent review see 58). Persister cells are not simply antibiotic resistant but rather reflect a transient growth arrested state. Persister cells can be grown to form a population that once again consists of antibiotic-sensitive cells and a small subpopulation of persisters (67). The switch from normal growth to persistence and vice versa is stochastic and epigenetic in nature (12). At least in *Mycobacterium tuberculosis*, the regulation of persistence appears to involve noise in gene expression amplified by positive feedback (97). Persistence is a form of bet-hedging as it ensures survival during catastrophes (56). In addition,

Adaptive mutagenesis: describes a set of conditions under which mutations appear to occur more often when selective pressure is present than when not

HMS: hypermutable subpopulation

DSB: double-strand break

Persistence: the phenomenon of the existence of a small subpopulation of cells that do not grow compared with the rest of the isogenic culture, and as a result are antibiotic resistant

persistence of a subpopulation of cells might indirectly benefit other cells in a population as the growth-arrested cells do not compete for limited resources (35). Recent mathematical modeling suggests that bacterial persistence can be regarded as a social trait and can be influenced by kin selection (35).

Sporulation Bistability as a Bet-Hedging Strategy

Recently, quantitative time-lapse microscopy was used to generate lineage and cell fate maps of single *B. subtilis* cells growing out to a sporulating microcolony (**Figure 3**). The study demonstrated that under these conditions *B. subtilis* employs a bet-hedging strategy whereby some cells sporulate and others utilize alternative metabolites to continue growth (and can putatively pursue other survival tactics) (104).

For individual cells the benefit of sporulation is clear; spores are resistant to various environmental conditions and can ensure the preservation of the clonal lineage, whereas vegetative cells could not. In the laboratory strain, however, a significant fraction of cells do not use the remaining energy sources for sporulation but rather delay spore formation or avoid it. The potential advantage for these cells is twofold. First, these cells increase in number and may sporulate later using nutrients released by cells that have lysed. This resource use, termed cannibalism or fratricide, has been demonstrated in a number of studies (21 and references therein). Second, these cells are capable of rapidly resuming growth in the event of a new flux of nutrients. In contrast, cells that have sporulated are committed to a long-term process of spore formation and subsequent germination. Each of these paths is a form of specialization that increases efficiency in one area at the expense of the other.

OUTLOOK

The strategies and mechanisms discussed in this review are not limited to the microorganisms mentioned here. Many other bacterial and fungal species display phenotypic variation that may reflect a form of bet-hedging (see 11, 25, 92, 93 and references therein). These include processes that affect intra- or interspecies competition, as well as host-pathogen interactions, such as mucoidy and cytotoxicity of *Pseudomonas aeruginosa* and bacteriocin production in *E. coli*. A major challenge for future research will be to assess the effects of variable phenotypes on the interactions between organisms under steady-state and fluctuating conditions. This finding(s) may shed light on the pressures responsible for the evolution of genetic networks that directly or indirectly result in population multistability.

SUMMARY POINTS

1. Research increasingly acknowledges the presence and importance of cell-to-cell variability for the perpetuation of clonal populations.
2. Multistability is a ubiquitous feature of bacteria involving many different processes.
3. Phenotypic variable populations show increased fitness compared with homogeneous populations under fluctuating environments.
4. Genetic logic-AND gates are common network motifs in bacteria to generate heterogeneity.
5. Cell states can be passed on from one generation to the next via EI and this process might be important in bacterial development.
6. Synthetic biology and qualitative analyses of network motifs are promising for biotechnological and medical applications.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. Acar M, Becskei A, van Oudenaarden A. 2005. Enhancement of cellular memory by reducing stochastic transitions. *Nature* 435:228–32
2. **Acar M, Mettetal JT, van Oudenaarden A. 2008. Stochastic switching as a survival strategy in fluctuating environments. *Nat. Genet.* 40:471–75**
3. Ackermann M, Stearns SC, Jenal U. 2003. Senescence in a bacterium with asymmetric division. *Science* 300:1920
4. Aguilaniu H, Gustafsson L, Rigoulet M, Nystrom T. 2003. Asymmetric inheritance of oxidatively damaged proteins during cytokinesis. *Science* 299:1751–53
5. Aguilar C, Vlamakis H, Losick R, Kolter R. 2007. Thinking about *Bacillus subtilis* as a multicellular organism. *Curr. Opin. Microbiol.* 10:638–43
6. Ajo-Franklin CM, Drubin DA, Eskin JA, Gee EP, Landgraf D, et al. 2007. Rational design of memory in eukaryotic cells. *Genes Dev.* 21:2271–76
7. Alper H, Moxley J, Nevoigt E, Fink GR, Stephanopoulos G. 2006. Engineering yeast transcription machinery for improved ethanol tolerance and production. *Science* 314:1565–68
8. Angeli D, Ferrell JE Jr, Sontag ED. 2004. Detection of multistability, bifurcations, and hysteresis in a large class of biological positive-feedback systems. *Proc. Natl. Acad. Sci. USA* 101:1822–27
9. Artyomov MN, Das J, Kardar M, Chakraborty AK. 2007. Purely stochastic binary decisions in cell signaling models without underlying deterministic bistabilities. *Proc. Natl. Acad. Sci. USA* 104:18958–63
10. Atkinson MR, Savageau MA, Myers JT, Ninfa AJ. 2003. Development of genetic circuitry exhibiting toggle switch or oscillatory behavior in *Escherichia coli*. *Cell* 113:597–607
11. Avery SV. 2006. Microbial cell individuality and the underlying sources of heterogeneity. *Nat. Rev. Microbiol.* 4:577–87
12. Balaban NQ, Merrin J, Chait R, Kowalik L, Leibler S. 2004. Bacterial persistence as a phenotypic switch. *Science* 305:1622–25
13. Becskei A, Seraphin B, Serrano L. 2001. Positive feedback in eukaryotic gene networks: cell differentiation by graded to binary response conversion. *EMBO J.* 20:2528–35
14. Bigger JW. 1944. Treatment of *Staphylococcal* infections with penicillin by intermittent sterilisation. *Lancet* 244:497–500
15. Bishop AL, Rab FA, Sumner ER, Avery SV. 2007. Phenotypic heterogeneity can enhance rare-cell survival in 'stress-sensitive' yeast populations. *Mol. Microbiol.* 63:507–20
16. Bren A, Eisenbach M. 2001. Changing the direction of flagellar rotation in bacteria by modulating the ratio between the rotational states of the switch protein FliM. *J. Mol. Biol.* 312:699–709
17. Cahn FH, Fox MS. 1968. Fractionation of transformable bacteria from competent cultures of *Bacillus subtilis* on renografin gradients. *J. Bacteriol.* 95:867–75
18. Casadesus J, D'Ari R. 2002. Memory in bacteria and phage. *Bioessays* 24:512–18
19. Casadesus J, Low D. 2006. Epigenetic gene regulation in the bacterial world. *Microbiol. Mol. Biol. Rev.* 70:830–56

2. Elegant experimental study that shows how switching affects population growth.

20. Chai Y, Chu F, Kolter R, Losick R. 2007. Bistability and biofilm formation in *Bacillus subtilis*. *Mol. Microbiol.* 67:254–63
21. Claverys JP, Havarstein LS. 2007. Cannibalism and fratricide: mechanisms and raisons d'etre. *Nat. Rev. Microbiol.* 5:219–29
22. Cohen D. 1966. Optimizing reproduction in a randomly varying environment. *J. Theor. Biol.* 12:119–29
23. Cohn M, Horibata K. 1959. Analysis of the differentiation and of the heterogeneity within a population of *Escherichia coli* undergoing induced beta-galactosidase synthesis. *J. Bacteriol.* 78:613–23
24. Cox RS 3rd, Surette MG, Elowitz MB. 2007. Programming gene expression with combinatorial promoters. *Mol. Syst. Biol.* 3:145
25. Dubnau D, Losick R. 2006. Bistability in bacteria. *Mol. Microbiol.* 61:564–72
26. Dworkin J, Losick R. 2005. Developmental commitment in a bacterium. *Cell* 121:401–9
27. Elowitz MB, Leibler S. 2000. A synthetic oscillatory network of transcriptional regulators. *Nature* 403:335–38
28. Elowitz MB, Levine AJ, Siggia ED, Swain PS. 2002. Stochastic gene expression in a single cell. *Science* 297:1183–86
29. Ferrell JE Jr. 2002. Self-perpetuating states in signal transduction: positive feedback, double-negative feedback and bistability. *Curr. Opin. Cell Biol.* 14:140–48
30. Foster PL. 2007. Stress-induced mutagenesis in bacteria. *Crit. Rev. Biochem. Mol. Biol.* 42:373–97
31. Fujita M, Gonzalez-Pastor JE, Losick R. 2005. High- and low-threshold genes in the Spo0A regulon of *Bacillus subtilis*. *J. Bacteriol.* 187:1357–68
32. Fujita M, Losick R. 2005. 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.* 19:2236–44
33. Galhardo RS, Hastings PJ, Rosenberg SM. 2007. Mutation as a stress response and the regulation of evolvability. *Crit. Rev. Biochem. Mol. Biol.* 42:399–435
34. Garcia-Ojalvo J, Elowitz MB, Strogatz SH. 2004. Modeling a synthetic multicellular clock: repressilators coupled by quorum sensing. *Proc. Natl. Acad. Sci. USA* 101:10955–60
35. Gardner A, West SA, Griffin AS. 2007. Is bacterial persistence a social trait? *PLoS ONE* 2:e752
36. Gardner TS, Cantor CR, Collins JJ. 2000. Construction of a genetic toggle switch in *Escherichia coli*. *Nature* 403:339–42
37. Gardner TS, Collins JJ. 2005. Bistable genetic toggle switch. *U.S. Patent No. 6841376*
38. Gerdes K, Christensen SK, Lobner-Olesen A. 2005. Prokaryotic toxin-antitoxin stress response loci. *Nat. Rev. Microbiol.* 3:371–82
39. Godoy VG, Gizatullin FS, Fox MS. 2000. Some features of the mutability of bacteria during nonlethal selection. *Genetics* 154:49–59
40. Goldstein J, Pollitt NS, Inouye M. 1990. Major cold shock protein of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 87:283–87
41. Hadden C, Nester EW. 1968. Purification of competent cells in the *Bacillus subtilis* transformation system. *J. Bacteriol.* 95:876–85
42. Haijema BJ, Hahn J, Haynes J, Dubnau D. 2001. A ComGA-dependent checkpoint limits growth during the escape from competence. *Mol. Microbiol.* 40:52–64
43. Hammer K, Mijakovic I, Jensen PR. 2006. Synthetic promoter libraries—tuning of gene expression. *Trends Biotechnol.* 24:53–55
44. Hamoen LW, Venema G, Kuipers OP. 2003. Controlling competence in *Bacillus subtilis*: shared use of regulators. *Microbiology* 149:9–17
45. Hasty J, McMillen D, Collins JJ. 2002. Engineered gene circuits. *Nature* 420:224–30
46. Heinemann M, Panke S. 2006. Synthetic biology—putting engineering into biology. *Bioinformatics* 22:2790–99
47. Ireton K, Rudner DZ, Siranosian KJ, Grossman AD. 1993. Integration of multiple developmental signals in *Bacillus subtilis* through the Spo0A transcription factor. *Genes Dev.* 7:283–94
48. Jablonka E, Lamb MJ. 2006. *Evolution in Four Dimensions: Genetic, Epigenetic, Behavioral, and Symbolic Variation in the History of Life*. Cambridge, MA: MIT Press
49. Kærn M, Elston TC, Blake WJ, Collins JJ. 2005. Stochasticity in gene expression: from theories to phenotypes. *Nat. Rev. Genet.* 6:451–64

28. Together with Reference 78, this study pioneered the use of an in vivo method to analyze and quantify noise.

48. Thought-provoking book in which the authors argue that there is more to heredity than genes.

50. Karmakar R, Bose I. 2007. Positive feedback, stochasticity and genetic competence. *Phys. Biol.* 4:29–37
51. Kaufmann BB, Yang Q, Mettetal JT, van Oudenaarden A. 2007. Heritable stochastic switching revealed by single-cell genealogy. *PLoS Biol.* 5:e239
52. Kearns DB, Chu F, Branda SS, Kolter R, Losick R. 2005. A master regulator for biofilm formation by *Bacillus subtilis*. *Mol. Microbiol.* 55:739–49
53. Kobayashi K. 2007. Gradual activation of the response regulator DegU controls serial expression of genes for flagellum formation and biofilm formation in *Bacillus subtilis*. *Mol. Microbiol.* 66:395–409
54. Korobkova E, Emonet T, Vilar JM, Shimizu TS, Cluzel P. 2004. From molecular noise to behavioural variability in a single bacterium. *Nature* 428:574–78
55. Krishnamurthy S, Smith E, Krakauer D, Fontana W. 2007. The stochastic behavior of a molecular switching circuit with feedback. *Biol. Direct.* 2:13
56. Kussell E, Leibler S. 2005. Phenotypic diversity, population growth, and information in fluctuating environments. *Science* 309:2075–78
57. Leisner M, Stingl K, Radler JO, Maier B. 2007. Basal expression rate of *comK* sets a 'switching-window' into the K-state of *Bacillus subtilis*. *Mol. Microbiol.* 63:1806–16
58. Lewis K. 2007. Persister cells, dormancy and infectious disease. *Nat. Rev. Microbiol.* 5:48–56
59. Lindner AB, Madden R, Demarez A, Stewart EJ, Taddei F. 2008. Asymmetric segregation of protein aggregates is associated with cellular aging and rejuvenation. *Proc. Natl. Acad. Sci. USA.* 105:3076–81
60. Lisman JE. 1985. A mechanism for memory storage insensitive to molecular turnover: a bistable autophosphorylating kinase. *Proc. Natl. Acad. Sci. USA* 82:3055–57
61. Maamar H, Dubnau D. 2005. Bistability in the *Bacillus subtilis* K-state (competence) system requires a positive feedback loop. *Mol. Microbiol.* 56:615–24
62. Maamar H, Raj A, Dubnau D. 2007. Noise in gene expression determines cell fate in *Bacillus subtilis*. *Science* 317:526–29
63. Maynard Smith J. 1964. Group selection and kin selection. *Nature* 201:1145–47
64. Mayo AE, Setty Y, Shavit S, Zaslaver A, Alon U. 2006. Plasticity of the *cis*-regulatory input function of a gene. *PLoS. Biol.* 4:e45
65. Molle V, Fujita M, Jensen ST, Eichenberger P, Gonzalez-Pastor JE, et al. 2003. The Spo0A regulon of *Bacillus subtilis*. *Mol. Microbiol.* 50:1683–701
66. Monod J, Jacob F. 1961. Teleonomic mechanisms in cellular metabolism, growth, and differentiation. *Cold Spring Harb. Symp. Quant. Biol.* 26:389–401
67. Moyed HS, Broderick SH. 1986. Molecular cloning and expression of *hipA*, a gene of *Escherichia coli* K-12 that affects frequency of persistence after inhibition of murein synthesis. *J. Bacteriol.* 166:399–403
68. Msadek T. 1999. When the going gets tough: survival strategies and environmental signaling networks in *Bacillus subtilis*. *Trends Microbiol.* 7:201–7
69. Mukai K, Kawata M, Tanaka T. 1990. Isolation and phosphorylation of the *Bacillus subtilis degS* and *degU* gene products. *J. Biol. Chem.* 265:20000–6
70. Muller-Hill B. 1996. *The* lac *Operon*: *A Short History of a Genetic Paradigm*. Berlin: Walter de Gruyter
71. Murphy KF, Balazsi G, Collins JJ. 2007. Combinatorial promoter design for engineering noisy gene expression. *Proc. Natl. Acad. Sci. USA* 104:12726–31
72. Neildez-Nguyen TM, Parisot A, Vignal C, Rameau P, Stockholm D, et al. 2008. Epigenetic gene expression noise and phenotypic diversification of clonal cell populations. *Differentiation* 76:33–40
73. Nester EW, Stocker BA. 1963. Biosynthetic latency in early stages of deoxyribonucleic acid transformation in *Bacillus subtilis*. *J. Bacteriol.* 86:785–96
74. Ninfa AJ, Mayo AE. 2004. Hysteresis vs graded responses: the connections make all the difference. *Sci. STKE* 2004:e20
75. Ninfa AJ, Selinsky S, Perry N, Atkins S, Xiu SQ, et al. 2007. Using two-component systems and other bacterial regulatory factors for the fabrication of synthetic genetic devices. *Methods Enzymol.* 422:488–512
76. Novick A, Weiner M. 1957. Enzyme induction as an all-or-none phenomenon. *Proc. Natl. Acad. Sci. USA* 43:553–66
77. Ortega F, Garces JL, Mas F, Kholodenko BN, Cascante M. 2006. Bistability from double phosphorylation in signal transduction. Kinetic and structural requirements. *FEBS J.* 273:3915–26

62. Experimental study showing directly for the first time that noise in *comK* transcription determines entry into the competent state in *B. subtilis*.

78. Ozbudak EM, Thattai M, Kurtser I, Grossman AD, van Oudenaarden A. 2002. Regulation of noise in the expression of a single gene. *Nat. Genet.* 31:69–73
79. Ozbudak EM, Thattai M, Lim HN, Shraiman BI, van Oudenaarden A. 2004. Multistability in the lactose utilization network of *Escherichia coli*. *Nature* 427:737–40
80. Partridge L, Barton NH. 1993. Optimality, mutation and the evolution of ageing. *Nature* 362:305–11
81. Paulsson J. 2004. Summing up the noise in gene networks. *Nature* 427:415–18
82. Pennington JM, Rosenberg SM. 2007. Spontaneous DNA breakage in single living *Escherichia coli* cells. *Nat. Genet.* 39:797–802
83. Ponder RG, Fonville NC, Rosenberg SM. 2005. A switch from high-fidelity to error-prone DNA double-strand break repair underlies stress-induced mutation. *Mol. Cell* 19:791–804
84. Rando OJ, Verstrepen KJ. 2007. Timescales of genetic and epigenetic inheritance. *Cell* 128:655–68
85. Raser JM, O'Shea EK. 2005. Noise in gene expression: origins, consequences, and control. *Science* 309:2010–13
86. Renzette N, Gumlaw N, Nordman JT, Krieger M, Yeh SP, et al. 2005. Localization of RecA in *Escherichia coli* K-12 using RecA-GFP. *Mol. Microbiol.* 57:1074–85
87. Rosche WA, Foster PL. 1999. The role of transient hypermutators in adaptive mutation in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 96:6862–67
88. Simmons LA, Grossman AD, Walker GC. 2007. Replication is required for the RecA localization response to DNA damage in *Bacillus subtilis*. *Proc. Natl. Acad. Sci. USA* 104:1360–65
89. Smith MC, Sumner ER, Avery SV. 2007. Glutathione and Gts1p drive beneficial variability in the cadmium resistances of individual yeast cells. *Mol. Microbiol.* 66:699–712
90. Smits WK, Eschevins CC, Susanna KA, Bron S, Kuipers OP, Hamoen LW. 2005. Stripping *Bacillus*: ComK auto-stimulation is responsible for the bistable response in competence development. *Mol. Microbiol.* 56:604–14
91. Smits WK, Hoa TT, Hamoen LW, Kuipers OP, Dubnau D. 2007. Antirepression as a second mechanism of transcriptional activation by a minor groove binding protein. *Mol. Microbiol.* 64:368–81
92. Smits WK, Kuipers OP, Veening JW. 2006. Phenotypic variation in bacteria: the role of feedback regulation. *Nat. Rev. Microbiol.* 4:259–71
93. Smits WK, Veening JW, Kuipers OP. 2008. Phenotypic variation and bistable switching in bacteria. In *Bacterial Physiology: A Molecular Approach*, ed. W El-Sharoud, pp. 339–65. Berlin/Heidelberg: Springer-Verlag
94. Stewart EJ, Madden R, Paul G, Taddei F. 2005. Aging and death in an organism that reproduces by morphologically symmetric division. *PLoS Biol.* 3:e45
95. Süel GM, Garcia-Ojalvo J, Liberman LM, Elowitz MB. 2006. An excitable gene regulatory circuit induces transient cellular differentiation. *Nature* 440:545–50
96. Süel GM, Kulkarni RP, Dworkin J, Garcia-Ojalvo J, Elowitz MB. 2007. Tunability and noise dependence in differentiation dynamics. *Science* 315:1716–19
97. Sureka K, Ghosh B, Dasgupta A, Basu J, Kundu M, Bose I. 2008. Positive feedback and noise activate the stringent response regulator rel in mycobacteria. *PLoS ONE* 3:e1771
98. Swain PS, Elowitz MB, Siggia ED. 2002. Intrinsic and extrinsic contributions to stochasticity in gene expression. *Proc. Natl. Acad. Sci. USA* 99:12795–800
99. Thattai M, van Oudenaarden A. 2004. Stochastic gene expression in fluctuating environments. *Genetics* 167:523–30
100. Torkelson J, Harris RS, Lombardo MJ, Nagendran J, Thulin C, Rosenberg SM. 1997. Genome-wide hypermutation in a subpopulation of stationary-phase cells underlies recombination-dependent adaptive mutation. *EMBO J.* 16:3303–11
101. Veening JW, Hamoen LW, Kuipers OP. 2005. Phosphatases modulate the bistable sporulation gene expression pattern in *Bacillus subtilis*. *Mol. Microbiol.* 56:1481–94
102. Veening JW, Igoshin OA, Eijlander RT, Nijland R, Hamoen LW, Kuipers OP. 2008. Transient heterogeneity in extracellular protease production by *Bacillus subtilis*. *Mol. Syst. Biol.* 4:184
103. Veening JW, Smits WK, Hamoen LW, Kuipers OP. 2006. Single cell analysis of gene expression patterns of competence development and initiation of sporulation in *Bacillus subtilis* grown on chemically defined media. *J. Appl. Microbiol.* 101:531–41

78. Together with Reference 28, this study pioneered the use of an in vivo method to analyze and quantify noise.

94. Shows that even symmetrical dividing bacteria suffer from aging.

95. Provides evidence for the excitable model that dictates competence development.

104. Veening JW, Stewart EJ, Berngruber TW, Taddei F, Kuipers OP, Hamoen LW. 2008. Bet-hedging and epigenetic inheritance in bacterial cell development. *Proc. Natl. Acad. Sci. USA* 105:4393–98

105. Verhamme DT, Kiley TB, Stanley-Wall NR. 2007. DegU co-ordinates multicellular behaviour exhibited by *Bacillus subtilis*. *Mol. Microbiol.* 65:554–68

106. Vlamakis H, Aguilar C, Losick R, Kolter R. 2008. Control of cell fate by the formation of an architecturally complex bacterial community. *Genes Dev.* 22:945–53

107. Yildirim N, Santillan M, Horike D, Mackey MC. 2004. Dynamics and bistability in a reduced model of the *lac* operon. *Chaos* 14:279–92

108. Zambrano MM, Kolter R. 1996. GASPing for life in stationary phase. *Cell* 86:181–84

104. Time-lapse study that shows that sporulation in *B. subtilis* is a bet-hedging strategy and that the signal to sporulate can be epigenetically inherited.