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Bacterial Dormancy: How to Decide When to Wake Up

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Many bacteria form metabolically inactive spores to survive harsh conditions. But how do spores decide to germinate when sensing of the environment is hampered by their inactivity? A new study shows how phenotypic variation leads to stochastic germination of spores.

Natural environments are ever-changing and often hostile. To survive periods of adverse conditions many bacteria switch to a metabolically inactive, dormant state [1]. One of the best-studied types of dormancy is sporulation [2]. Spores are physiologically distinct from vegetative cells; they are highly resistant to stresses, such as antibiotics and heat, and can survive for years in wait for better times. Once growth-permissive conditions return, the spores germinate and reinitiate growth [3]. But how do spores decide when the time is right to germinate? One strategy is to initiate germination when positive signals are detected in the environment. For some species, adding specific stimuli to the growth medium can indeed induce germination [3]. However, the metabolic inactivity of spores makes it unlikely that they can sense all possible growth-permissive conditions. Exclusively relying on the ability to sense environmental changes to trigger germination would thus lead to missed opportunities for regrowth. An alternative strategy is stochastic germination [4,5]. In this scenario, spores 'wake up' at random; if the environmental conditions are still hostile, the germinated cell will eventually die. But if conditions allow for growth, the cell can repopulate the habitat. Using such a bet-hedging strategy, the population as a whole can directly take advantage of favorable conditions without the need for any individual cell to sense its environment [4–6]. Such stochastic germination has been observed for non-spore-forming dormant cells of, among others, *Escherichia coli* and *Mycobacterium smegmatis* [7,8], and has also been suggested to occur at low frequencies for spores of *Bacillus subtilis* [9]. However, very little is known about how stochastic germination works in spore-forming bacteria. In this issue of *Current Biology*, Sturm and Dworkin show that *B. subtilis* spores germinate stochastically as a consequence of variation in the expression level of a transcription factor involved in spore assembly [10].

Spore formation in *B. subtilis* is a highly regulated and complex developmental process whereby an asymmetric cell division gives rise to a metabolically inactive spore [2]. These spores contain germination receptors that will induce germination upon stimulation with high concentrations of certain nutrients [3]. However, even in the absence of any known inducing factor, *B. subtilis* spores have been reported to germinate at low frequency [9]. Sturm and Dworkin studied this spontaneous germination by using a growth medium that supports growth, but does not contain any known inducing factors [10] (Figure 1). When spores were plated on this medium a small but measurable number of spores still germinated. The frequency of germination was about four orders of magnitude lower than that observed when spores were plated on a medium containing the inducer alanine. The total number of germinated spores increased linearly with time over a period of 100 days, suggesting that spores germinate stochastically at a (near) constant rate [10].

The spontaneous germination observed in these experiments is most likely the result of phenotypic variation between spores and not a result of mutations: when spontaneously germinated spores were put through three more rounds of sporulation and germination, the frequency of spontaneous germination did not change [10]. So what is the cause of this phenotypic variation? One possibility is heterogeneity in the number of germination receptors, which has previously been linked to differences in germination time during induced germination [11]. However, strains lacking one or more of the germination receptors showed identical frequencies of germination in the absence of inducers, disproving a significant role for these receptors in spontaneous germination [10]. Instead, the authors hypothesized that differences in the spore coat cause differences in germination times. The proteinaceous spore coat plays an important role in the resistance against environmental stressors [12]. A large number of the genes involved in the assembly of the spore coat are under the control of the GerE transcription factor, and thus variation in GerE levels could affect germination frequency. Using a fluorescent reporter the authors found that there is a large variation in *gerE* gene expression between cells [10]. Furthermore, the expression level of *gerE* inversely correlated with the frequency of spontaneous germination: cells with the lowest expression of *gerE* had the highest frequency of spontaneous sporulation. In line with these results, it was found that a *gerE* knockout mutant had a significantly increased rate of spontaneous germination [10].

Together, these data show that variation in the time at which germination occurs is, at least in part, a consequence of variation in the expression level of the GerE transcription factor. Yet there remain some unanswered questions. The central role of GerE in spore coat assembly makes it likely that spontaneous germination is related to variation in coat structure or composition. However, at the moment there is no

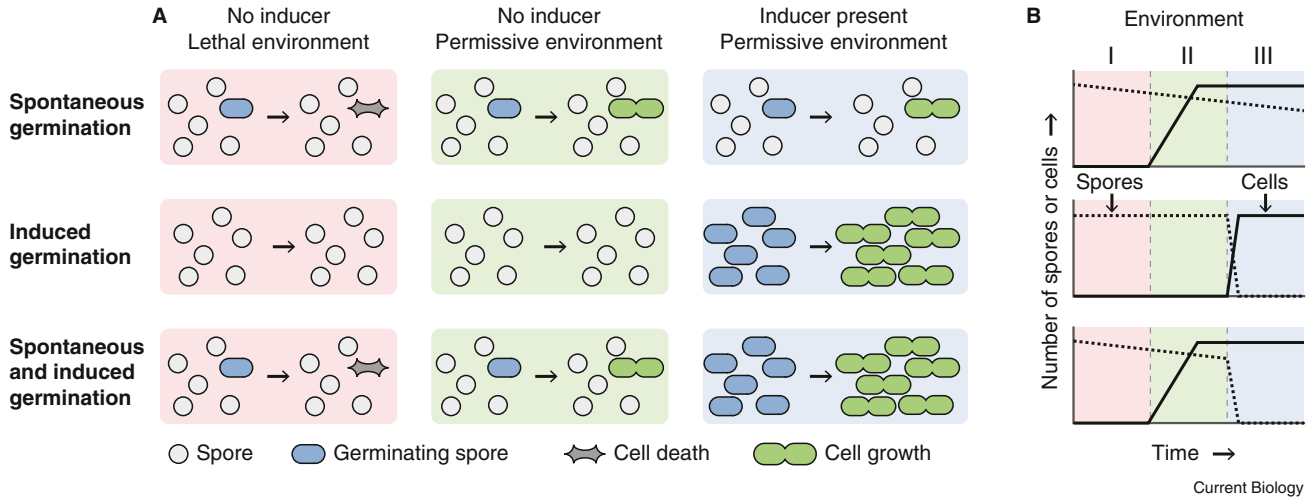

Figure 1. Overview of germination strategies.
Three germination strategies are shown: spontaneous germination (top row), induced germination (middle), and a combination of spontaneous and induced germination (bottom). (A) The fate of germinating spores is shown for three environments: a lethal environment (left column), a growth-permissive environment lacking germination inducers (middle) and a growth-permissive environment containing inducers (right). (B) Temporal evolution of the number of spores (dashed line) and vegetative cells (solid line) for the three different germination strategies. The environment switches with time between the three environments shown in (A), from lethal (I, pink shading), to growth permissive (II, green) to growth permissive including germination inducer (III, blue).

conclusive evidence for this and it cannot be ruled out that GerE affects spontaneous germination via other, spore-coat-independent, routes. Furthermore, it is unclear if variation in *gerE* expression levels is the sole cause of variation in germination timing. Spore formation is controlled by a series of complex regulatory networks and it is likely that variation in other components also leads to physiological differences affecting spore germination [2,3]. Finally, it is still an open question how variation in *gerE* expression gives rise to a constant rate of germination per unit of time. The complex regulatory network of which GerE is a member makes answering these questions challenging, but also offers many interesting opportunities for further investigation using theoretical and experimental approaches.

An additional question concerns the possible biological function of spontaneous germination. One appealing and likely explanation is that spontaneous germination serves as a bet-hedging strategy. By having a small number of cells always germinating, the population as a whole can directly profit from favorable environmental changes, without the need for a costly sensing apparatus [4,6,7]. It is hard to demonstrate conclusively that any behavior is the result of adaptive evolution. However, there is one aspect of this hypothesis that can be tested: if spontaneous germination is the result of positive selection, its properties must be under genetic control. It turns out that this is indeed the case. *gerE* is regulated by the mother-cell-specific transcription factor σ^K, which is the product of a disrupted gene: its coding sequence is split into two parts separated by the ~48 kb long *skin* element [13]. To obtain a fully functional σ^K, this *skin* element has to be excised from the genome [13]. The authors used a mutant called *skinless* that lacks this element. As a genomic rearrangement is not necessary for σ^K expression in this mutant, σ^K is expressed earlier in spore development and as a result, *gerE* expression levels were observed to decrease. The lower expression of *gerE* in turn leads to an increase in the rate of spontaneous germination [10].

The rate of spontaneous germination is thus affected by genetic changes. However, to successfully implement a bet-hedging strategy this rate has to be optimized. If it is too low, there will be periods when there are no germinated cells around to reinitiate growth. If it is too high, the number of spores will quickly decrease with time and the population risks going extinct during prolonged periods of harsh conditions. For the strain of *B. subtilis* used in this study, the observed germination frequency seems to be right in the middle: for a population of 1 billion spores (the number typically found in 1 g of soil), 10,000 spores would germinate each day and there would still be plenty of spores present, even after 100 years [10].

Taken together, this work gives a clear picture of how variation in the expression of a transcription factor leads to variation in the time at which germination occurs. As a result, spores germinate stochastically with a constant rate, allowing for instant repopulation of the habitat when environmental conditions allow for it. This regrowth does not require communication or coordination between cells. Rather, it could simply be a consequence of the proliferation of a spontaneously germinated spore that happened to 'wake up' during a time of favorable conditions. However, previous work suggests that cells could influence each other's decision to germinate. During growth and germination, *B. subtilis* releases peptidoglycan into the environment [14]. Degradation products of this peptidoglycan can induce germination in *B. subtilis* [14], raising the question of whether successful regrowth of stochastically germinated spores could cause other spores to germinate via induced mechanisms. Whether such feedback mechanisms indeed exist,

however, needs to be investigated in future work.

Many bacteria besides *B. subtilis* form spores, some of which are important sources of food spoilage and human disease [1,15]. This raises the question of whether bet-hedging strategies based on stochastic germination times are also used by other species. The observation of similar rates of spontaneous germination in three other *Bacillus* species suggests that this might indeed be the case [10], although more work is required to assess the generality of this mechanism.

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Evolutionary Ecology: Insect Mothers Control Their Egg Colours

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Animal egg coloration has long provided a valuable testing ground for evolutionary ideas. A new study shows that female stink bugs can flexibly control the colour of their eggs depending on the prevailing conditions, including for protection from ultraviolet light.

Adaptive coloration in animals has a long and rich history of study, stemming back to many of the first evolutionary biologists [1]. Ever since, it has been an important area for testing theories of adaptation, behaviour and ecology. Of this, the study of animal egg colours has played an important role [2,3], with suggested functions ranging from camouflage, warning signals, thermoregulation, brood parasitism, to even sexual signalling [4]. However, much of this work has focussed on a few select groups (especially birds), whereas the possible adaptive function of egg coloration elsewhere has been comparatively neglected. Furthermore, most research has explicitly or implicitly investigated the evolution and function of egg colours over multiple generations, or simply as correlated with traits such as parental condition. In contrast, we know little about how mothers may directly control egg colour depending on prevailing or predicted environmental conditions. However, a new study in *Current Biology* by Abram *et al.* [5] shows not only that egg coloration in an insect seems to be adaptive in protecting embryos from harmful ultraviolet (UV) light, but also that mothers can selectively control egg appearance depending on where the eggs are laid, and hence risk of UV exposure.

Abram *et al.* [5] investigated egg coloration in a stink bug (*Podisus maculiventris*), in which egg clusters vary in appearance from pale yellow to dark brown or black. They made a number of important findings regarding how the colour of eggs arises. First, females tend to lay darker coloured eggs when offered substrates that were dark, and lighter