relevant' stimulation. With holding and walking having sensory input working synergistically, this approach may have the most potent calming effect. It would be great to have more integrated studies examining multiple calming options and limits along with real-time data.

As Esposito *et al.* [3] suggest, one area of possible future interest is that the monitoring of infant response could also be used as a type of diagnosis. Somatosensory and proprioceptive inputs are needed for the calming response. The calming response provides a metric of how those function in an infant. Some individuals with neurological disorders, including autism, have been reported to have problems with body adjustment during holding [16]. Thus, it would be useful if early infant physiological reactions could be evaluated in relationship to later neurological profiles. With such information in hand, in the future, one might use carrying response as an additional early diagnostic tool.

Babies have been crying for thousands of years. They may also have been responding to holding and carrying by calming down and helping with their transport for thousands of years. In short, they have been giving us a clear communication signal for a long time. Whether we as parents knew this consciously may not matter as transport was helped and possible predator detection was reduced. Additional bonding may also have occurred. Kids have long accused parents of not 'getting' them; however, the study by Esposito *et al.* [3] may allow us to cross at least one example of enduring parental ignorance off the list.

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Microbial Evolution: Regulatory Design Prevents Cancer-like Overgrowths

Mutant lineages may cause cancer-like overgrowths in microbial populations. Theory predicts that microbial regulatory controls may be designed to limit the origin and competitive potential of rogue lineages. A new study shows how a *Salmonella* species protects itself against overgrowths.

Steven A. Frank

Cancer occurs when a subpopulation of cells grows faster than normal. Such overgrowth can reduce the efficiency and survival of the overall cellular population that makes up the individual. In microbes, a mutant that consumes resources faster and less efficiently may grow faster [1]. Fast, inefficient growth allows a mutant to outcompete its neighbors and spread, reducing the overall efficiency of the local population of cells. In this regard, rapidly growing and inefficient microbial mutants resemble cancer. However, simply equating overly rapid growth in microbes to cancer is, by itself, not very interesting. The interesting aspect arises when we consider the possible consequences for the design of regulatory control systems in microbes. In mammals, cells and tissues are protected against uncontrolled growth by numerous regulatory systems that monitor mutations and provide checks and balances on cell cycle progression, such as the *p53* network [2]. I previously suggested that microbial regulatory control might also be designed to protect against the emergence of rogue lineages, so that mutations that enhance growth and lower efficiency become less likely to spread [3]. However, there was no clear evidence for such controls, perhaps because it is not usual to interpret microbial biology in terms of age-related diseases such as cancer. A new study by Diard *et al.* [4] now shows how regulatory design in a bacterium controls rapidly growing mutant lineages.

The bacterium *Salmonella enterica* serovar Typhimurium (*S. typhimurium*) secretes a virulence factor (T1) that induces gut inflammation in its

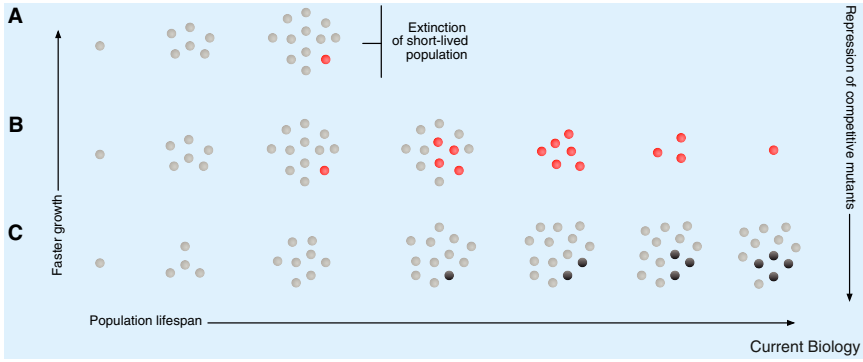

Figure 1. Repression of competitive mutants in microbial populations.

Population lifespan determines whether or not natural selection favors mechanisms to repress competitive mutants. (A) Short population lifespan occurs when an infection within a host lasts a short time or when a food resource dries up quickly. With a short lifespan, microbial populations are favored to grow quickly. A costly mechanism to repress competitive mutants would have little benefit, because the population tends to die off before rapidly growing mutants can spread. (B) In longer-lived populations, rapidly growing competitive mutants (red) can overgrow the population, potentially causing the population to crash. In Diard *et al*. [4], the competitive mutants (T1^m) overgrow a population with only secretors (T1+) and no non-secretors (T1-). (C) A mechanism to repress competitive mutants (black) may cause the population to grow more slowly than otherwise possible, but provides a benefit by slowing mutant overgrowth. Diard *et al*. [4] suggest that S. *typhimurium* produces a higher frequency of non-secretors than is optimal for fast grow, because the non-secretors also function to outcompete mutants that would otherwise cause overgrowth and a population crash. In general, any mechanism may potentially be favored if it slows the origin of new competitive mutants or slows the growth of overly competitive mutants, even if those repressive mechanisms impose a cost on the rate of growth in the absence of competition [3,6].

mammalian host. *S. typhimurium* requires the host inflammatory response to outcompete resident commensal bacteria [5]. The new study of Diard *et al.* [4] now reveals a cooperative strategy in *S. typhimurium* (Figure 1). Diard *et al.* [4] show that a genetically identical clone splits into cells that secrete the factor ($T1^+$) and those that don't ($T1^-$). These different phenotypes arise from alternative stable states of gene expression from the same genotype. In addition, mutants that never secrete inevitably appear. The non-secreting mutants ($T1^m$) and the non-secreting phenotype of the initial invaders ($T1^-$) grow faster than the secretors ($T1^+$). Apparently, the cost of secretion slows growth. In the absence of $T1^-$, the mutant $T1^m$ outcompetes $T1^+$. Thus, if the initial secreting invaders do not contain non-secretors, then non-secreting mutants inevitably arise and overgrow the initial population. As the non-secreting mutants take over, the bacteria no longer produce the virulence factor that is essential for outcompeting other commensals, and the population crashes. In effect, the mutant overgrowth kills off the population from which it arose, just as a cancerous overgrowth kills off the individual. Alternatively, when $T1^-$ non-secretors arise in the initial clone, the population dynamics are different. As the initial invading clone splits into $T1^+$ and $T1^-$, the non-secreting phenotypes grow faster but the overall clone maintains a sufficient fraction of secretors to suppress commensal competitors and continue the infection. With a balance of slow growing $T1^+$ and fast growing $T1^-$, the initial genotype can slow the growth of the $T1^m$ mutant non-secretors by the superior competitive ability of the $T1^-$ fraction of the original genotype.

The alternative expression of $T1^+$ and $T1^-$ in genetically identical clones suppresses overgrowth by the inevitable mutations that would otherwise lead to non-secreting rogue lineages. Diard *et al.* [4] suggest that the switch regulating alternative virulence factor expression may be the result of evolutionary pressure to protect against potential mutant overgrowth. Alternatively, the particular pattern of alternative expression for $T1^+$ and $T1^-$ may simply maximize the clonal growth of the initial genotype. Perhaps only a fraction of cells must secrete the virulence factor to achieve the concentration needed for most efficient invasion and reproduction. If so, then suppression of competing mutant lineages would be a consequence of maximizing clonal growth rather than a regulatory design feature to suppress rogue lineages. My own theoretical work shows that pressure from mutant lineages can significantly alter the evolutionary design of regulatory control to suppress mutant overgrowth [3,6]. Diard *et al.* [4] draw a similar conclusion from their theoretical models based on the case of *S. typhimurium*. Those models suggest that the particular controls of virulence factor expression in *S. typhimurium* are partly tuned to suppress mutant growth rather than merely to maximize clonal growth. In general, it seems likely that the regulatory design features of microbes to suppress cancer-like overgrowths will turn out to have much wider scope than the particular dynamics of virulence factor secretion [3,6].

Overgrowth and 'Old Age'

Levin and Bull [7] noted that some infections, such as HIV, often form large, long-lived populations within hosts. In microbes, age is measured as the number of generations since the initial infection and establishment of the population. As in bacteria, rapidly growing virus mutants will outcompete their neighbors and rise in frequency, so the rapidly growing mutant viruses are also like a cancer. Overgrowth arises simply as the inevitable degradation of a population by mutation and internal competition [8]. Pathogen populations may often suffer age-related fitness declines: the force of natural selection "is inevitably greatest for traits that influence new rather than old infections because all infections pass through a new stage, but many fail to reach 'old age'" [9].

To explain age-related decline in relation to rogue mutants, I introduced a simple model focused on microbial resource uptake and metabolic rate [6]. The key trade-off between rate and yield arises from the inevitable tendency for faster cellular growth to reduce the efficiency at which cells convert resources into biomass [1]. The overgrowth by competitive rogue lineages with relatively high metabolic rate has two consequences: first, the spread of mutant lineages causes populations to suffer an age-related decline in reproductive efficiency and fitness; second, the longer each population tends to survive, the greater

the selective pressure from the inevitable emergence of rogue lineages. Increased selective pressure from descendant rogue lineages causes an evolutionary response favoring the initial colonizers to grow faster, with lower efficiency and lower overall fitness. One can understand this process by focusing on the initial colonizer of a new resource patch. The longer the resource patch lasts, the more intense becomes the competition from rogue lineages. As the potential for competition rises, selection favors an increase in the initial growth rate so that the non-mutated descendants of the colonizer can compete with the rogue descendants. This theory and the model presented by Diard *et al.* [4] support their view that the regulatory control of the $T1^{+}/T1^{-}$ split is partly influenced by the advantage gained from suppressing rogue lineages.

Regulatory Controls of Overgrowth

Apart from the split between expressors and non-expressors of a virulence factor, are there other ways in which regulatory controls could reduce the rate at which rogue lineages arise? In mammals, numerous tumor suppressors prevent acceleration of the cell cycle, stop the cell cycle in response to mutational damage, or cause the cells to commit suicide in response to internal signs of abnormal function [2]. In microbes, overgrowth may depend primarily on ramping up the rate of resource acquisition or reducing the expression of costly secretions, although cell cycle regulators may also play a role. There are at present no obvious general principles known about how microbes may protect themselves against rogue lineages.

Two approaches may be useful. First, as noted in the previous section, population lifespan may be important, because long-lived populations suffer a much greater risk of rogue lineages than do short-lived populations. To test the importance of population lifespan, one could compare modes of regulatory control between short-lived and long-lived populations. Second, *a priori* theory can predict the kinds of traits that may evolve to protect against rogue lineages. For example, changes in metabolic rate and efficiency play a key role in competition between lineages. In laboratory cultures, intense competition often leads to gene duplications of receptors involved in resource uptake or in key regulators of resource metabolism [10,11]. Such gene duplications are likely to increase growth rate, but perhaps come at a cost in terms of efficiency of resource use. Thus, gene duplications may be a key type of mutational change with regard to cellular competition in microbial populations. If so, then a faster gene duplication rate may provide a benefit by allowing a rapid increase in competitive potential, but may impose a cost by increasing the rate at which rogue lineages arise in long-lived populations. To control the origin of rogue mutants in long-lived populations, genomic mechanisms may arise to reduce the duplication rate.

Duplication is just one example of how genomes might alter the rate at which rogue lineages arise and become suppressed, but a similar logic applies to other potential mechanisms. There must be numerous other aspects of metabolic regulation or virulence factor secretion that influence the growth rate versus the efficiency of resource usage [1,12]. The issue here concerns how those aspects of regulatory control may be designed so that mutations that enhance growth and lower efficiency become less likely to occur [3,4]. For example, additional feedback controls may stabilize growth rate and efficiency near favored levels, such that mutational perturbations have less effect. If so, then the design of regulatory controls over microbial metabolism may be strongly influenced by the evolutionary pressure to protect against the mutational origin of highly competitive rogue lineages.

Mutation is sometimes thought to be too weak a force to favor complex regulatory design features that protect against perturbations [13]. However, when one considers large, long-lived cellular populations, growth altering mutations become inevitable and have powerful selective consequences. Protection against somatic mutations and cancer are widely accepted as playing a key role in the design of mammalian cells [2]. So, I am arguing that the same processes could also strongly influence the design of microbial cells, particularly aspects of metabolic regulation. Protection against rogue lineages follows the classic arguments about the declining force of selection with age that have been used to explain senescence [14]. The force of natural selection is strongest at birth or, in our case, at the initiation of a local microbial population. The force of selection then declines with the age of the population because, at each succeeding age, a declining fraction of populations remains alive.

If the death rate of populations is low, then most populations do survive to the later ages at which rogue lineages impose a cost. That strong force of selection favors changes in regulatory design that protect against competition from rogue lineages. Such changes may impose a cost at earlier ages when no competition occurs. For example, simply increasing the rate of resource acquisition at early ages above that favored by selection at early ages provides greater competitive ability against the later, inevitable rogue lineages. With regard to regulatory design, extra feedback control to stabilize metabolic rates against mutational perturbations may be costly. Or genomic changes to protect against the sort of gene duplications that lead to rogue lineages may be associated with reduced efficiency in normal regulation or replication.

The costs of reduced efficiency at early ages may be offset by the benefits of protections against rogue lineages at later ages. Such pleiotropic tradeoffs between earlier and later ages strongly influence the evolutionary design of traits that control age-related performance and the age-related decline of senescence [15,16]. My point is that many evolutionary aspects of aging in multicellular organisms also apply to the traits of microbes when considered in terms of the lifespan of multicellular microbial populations [17].

For example, environmental conditions in microbial populations may typically change from the time of initial colonization to later population ages. The microbes may themselves alter the environment through their metabolic activities. If population lifespan tends to be short, then the force of selection becomes weak as the environment changes, and one expects relatively poor adaptation of the microbes to the changing conditions. By contrast, in long-lived populations one expects enhanced adaptations to changing conditions, even if the improvements in

coping with the changes at later ages impose a cost in efficiency at earlier ages.

The ability to cope with age-related environmental changes may arise through adaptation of fixed characteristics, such as the kinetic properties of an enzyme, or by conditional adjustments in traits as the environment changes. Conditional adjustments have the benefit of tracking environmental changes. But conditional adjustments also have the costs associated with the need for enhanced sensors of environmental change and enhanced regulatory controls to alter traits in response to the changed environment. Thus, the machinery of conditional adjustment probably also reduces efficiency at earlier ages compared with a simpler system designed with respect to the typical environment at early ages.

The topic leaves many open questions. Is regulatory control or genomic architecture in microbes partly designed to protect against competition from rogue mutant lineages? Do long-lived populations have enhanced protections compared with short-lived populations? How much cost in terms of reduced short-term efficiency arises from protections against rogue lineages that arise later in the lifespan of populations? How much of regulatory design is influenced by age-related changes in microbial populations?

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Chemical Communication: A Jewel Sheds Light on Signal Evolution

When others show sexy tails or sing elaborate songs, many animals use the language of chemistry to attract potential mates. A study provides insights into the evolutionary conundrum of how new chemical signals can evolve in an established communication system.

Jean-Marc Lassance[1] and Christer Löfstedt[2]

The chemical senses — smell and taste — have evolved in a wide range of organisms from bacteria to vertebrates and have enabled them to sense the surrounding environment and benefit from information carried by external chemical cues [1]. While organisms can perceive only a fraction of the chemical compounds present in their surroundings, they also contribute to the global olfactory cacophony by — intentionally or not — emitting molecules, such as waste products or biosynthetic by-products [2]. Urine, for example, appears to be an endless source of chemical signals in mammals [3]. Chemical cues convey information to those who can detect them, which can have great fitness consequences for both the emitter and the receiver and can provide the bases for the evolution of communication systems [4]. In a recent study in *Nature*, Niehuis *et al.* [5] show how a new chemical cue evolved and was integrated into the communication system of the jewel wasp *Nasonia vitripennis*.

Sexual reproduction usually entails the encounter of the sexes, and the use of sex pheromones — chemical signals that mediate interactions between individuals of the same species — is commonplace. Sex pheromones can be used to mediate attraction, to trigger sophisticated courtship displays and acceptance of the suitor or to repel competitors and incompatible mates. For example, the (*Z*7,*Z*11)-heptacosadiene produced by *Drosophila melanogaster* females acts as an aphrodisiac on conspecific males but deters males of other fruitfly species [6]. Accordingly, the emergence of new species is often accompanied by a divergence in the pheromones used by the forming species [7,8]. Since the first pheromone was identified in the silkworm *Bombyx mori* in 1959 [9], a tremendous diversity of chemical signals have been identified, many of them in insects. How the evolutionary diversification of sexual communication signals can take place poses a conundrum: normally, selections would act against senders