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Non-genetic diversity shapes infectious capacity and host resistance

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The spontaneous generation of distinct phenotypes within a clonal population of cells allows for both bet-hedging at the population level and the division of labor among subpopulations. This is emerging as an important theme in bacterial pathogenesis, because bacterial pathogens exhibit phenotypic heterogeneity with respect to characteristics that impact virulence. The phenomenon of persister cells and models of *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) pathogenesis illustrate the importance of non-genetic diversity in the disease process. Such heterogeneity can arise from specific genetic architectures amplifying stochastic fluctuations in factors affecting gene expression, and this also drives variation in eukaryotic cells. Thus reproducible variation in both host and pathogen processes affects the outcome of infection.

Diversification and disease

Recent advances in technologies that permit high-resolution analysis of events in single cells have revealed remarkable non-genetic diversity in populations of cells that were previously considered to be largely homogeneous (Figure 1). Much of the observed diversity is currently attributed to stochastic variation, or noise, in the biochemical reactions of gene expression. Experiments using engineered *Escherichia coli* strains demonstrate that gene expression can vary substantially both between identical promoters within a single cell and among isogenic cells within a population [1], supporting the hypothesis that noise can give rise to substantial diversity.

The revelation that a given set of environmental conditions frequently gives rise to a range of cellular states is changing the way we view and study biology. Variation is more frequently recognized as an important biological phenomenon. In some cases, variation in the copy number of particular molecules results in strikingly different cell fates. This is best documented in prokaryotes, where noise plus specific genetic circuits that produce net positive feedback can give rise to multiple stable and distinct phenotypes within a clonal population (Figure 2). When two stable phenotypes are present the situation is referred to as bistability and the genetic architecture producing such a distribution of phenotypes is termed a bistable switch [2,3]. Many prokaryotic bistable switches have been described, and the mechanisms are diverse [4–9].

To be successful, pathogenic microbes must either evade or exploit the immune system of their host. Disease progression can involve sequential colonization of different host compartments, and the immune response is a kinetic process that radically alters conditions within infected tissues, as does antimicrobial therapy. In addition, many pathogens must live outside the host for varying lengths of time. Thus, pathogenic microbes must transition between diverse environments and endure a variety of stresses. Deterministic adaptation, whereby microbes sense environmental cues and respond accordingly, is a common solution to this problem. However, a pre-existing mixture of distinct physiologies that confer situation-specific selective advantages could be more adaptive than sensing and responding under some circumstances, and phenotypic heterogeneity provides another solution to the problem of survival in fluctuating environments. The ability to generate multiple phenotypes reproducibly from the same genotype allows for both bet-hedging and the division of labor within populations, while preserving the genotype of the clone. In bet-hedging scenarios, microbes differentiate into multiple phenotypes that improve the fitness of the clone across environments. Labor division allows multiple roles to be fulfilled simultaneously, each by a different subgroup of cells.

The study of phenotypic variation in the host is challenging. Subtle gradients in nutrient availability, moisture, or toxins, instead of stochastic variation of intracellular biochemical reactions, could trigger differential gene expression patterns in bacteria that are close together within tissues. What is clear is that medically important microbial characteristics, such as the expression of virulence factors, vary stochastically *in vitro* (e.g., for *Pseudomonas aeruginosa* [10], *Vibrio cholerae* [7], and *S.* Typhimurium [11]), suggesting the possibility that this occurs *in vivo* as well. Here we discuss the role of stochastically formed bacterial subpopulations in three disease scenarios. (i) Many bacterial species generate small subpopulations of transiently antibiotic-tolerant cells known as persisters, which could contribute to the problem of persistent bacterial infection. (ii) The presence of both cytotoxic and non-cytotoxic subpopulations of *Salmonella* in the gut tissue may contribute to systemic bacterial dissemination. (iii) Differential expression of *Salmonella* genes conferring motility and invasion generates subpopulations that play distinct roles during colitis. Mammalian cells in culture also display stochastic variation in characteristics that impact disease, such as the activation

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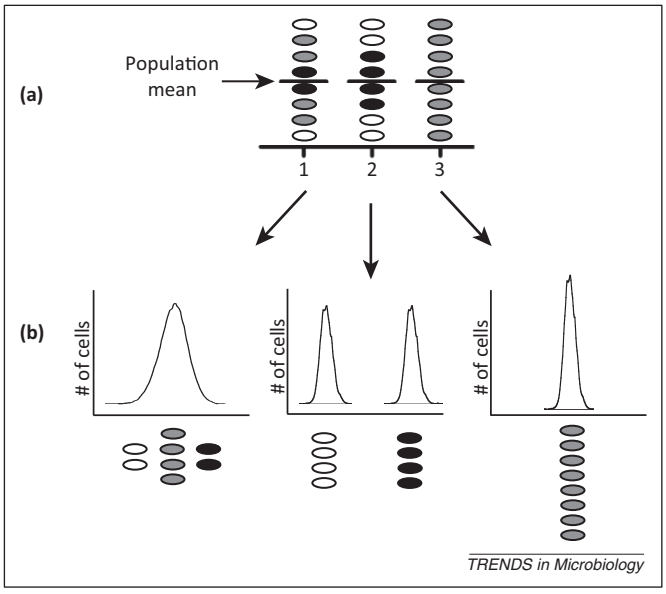

Figure 1. Surprises lurk within averaged data sets. Colors represent cells with low (white), medium (gray) and high (black) levels of the measured parameter. **(a)** Bulk culture experimental techniques such as Western blots and β-galactosidase assays measure the average of a parameter across an entire population, but equal means may characterize very different populations as indicated by the different shading. **(b)** Single-cell analysis techniques such as flow cytometry or microscopy generate a more complete picture of the diversity that may be concealed by averaging.

of cell death pathways. Thus, evidence that phenotypic heterogeneity drives processes that impact disease is accumulating.

Bet-hedging: persister cells

Antibiotic treatment of sensitive bacterial cultures often uncovers a small subpopulation of cells with greater tolerance to antibiotics; these have been termed persisters [12]. Persisters are poorly understood and potentially very important because subsequent outgrowth of this subpopulation may explain the failure to completely sterilize bacterial infections in some patients [13].

The progeny of persisters are as susceptible to antibiotics as the parent strain, indicating that persistence is not genetically determined. However, the propensity to form persisters is genetically regulated because mutations that increase the size of the persister subpopulation have been reported in many bacterial species [14]. A clear, unified mechanism of persistence has not emerged from the data, indicating that many distinct mechanisms may exist by which antibiotic-sensitive cells can gain short-lived, reversible tolerance, often to multiple antibiotics [14].

Persisters are often functionally characterized as dormant or slow-growing subpopulations [15]. Antimicrobial strategies designed specifically to eliminate persisters focus upon minimizing heterogeneity by 'waking up' the dormant subpopulation to some extent, thus rendering the entire population uniformly susceptible to antimicrobial therapy. Re-establishing the proton motive force (PMF) in populations of *E. coli* and *Staphylococcus aureus* persistent to aminoglycosides resensitized the cells to the antibiotics [16]. PMF, which is required for aminoglycoside uptake, was restored by the addition of specific metabolites. Interestingly, addition of the metabolites did not restore normal growth; instead, the persisters entered an energized but non-dividing state. Kim *et al.* [17] used high-throughput chemical screening to identify a compound (C10) that does not alter the growth of *E. coli* when used alone, but potentiates killing of persisters by both ampicillin and norfloxacin. The lag time for persisters to resume growth after antibiotic treatment was considerably shortened in the presence of C10, suggesting that this compound sensitizes persisters to antibiotics by stimulating a return to normal growth physiology.

Thus, persisters are an example of a bet-hedging strategy hinging upon spontaneously generated phenotypic subpopulations. The presence of a few antibiotic-tolerant, metabolically-inactive cells spreads risk, positioning the population as a whole both to replicate vigorously in the short-term and survive threatening conditions should they arise.

Division of labor: *Salmonella* infection paradigms

Heterogeneous gene expression by *S.* Typhimurium also impacts pathogenesis. This organism lives freely within

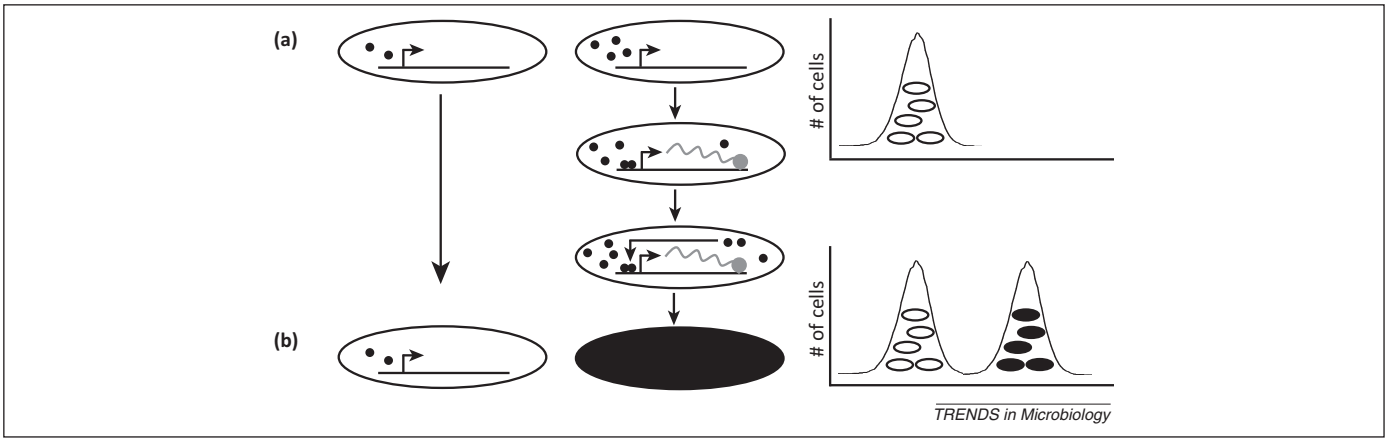

Figure 2. Noise and bistable gene expression circuits. The biochemical reactions of gene expression are subject to a degree of randomness or stochasticity [47]. **(a)** A modest degree of population diversity in the concentration of factors that activate or repress gene expression (represented by black closed circles), especially when such factors are in low abundance, together with alternating permissive and non-permissive promoter DNA conformations, can lead to transcriptional bursting in a subset of cells. Above a critical threshold of activation, positive feedback and non-linear kinetics (such as dimerization of a regulator, shown) can accelerate gene expression, bifurcating a unimodal distribution into a bistable gene expression pattern **(b)** where intermediate levels of expression are rare events. This combination of noise, net positive feedback, and non-linear kinetics has been documented in many bistable switches found in nature [2]; however, the mechanistic details of bistable switches are variable and may be exceedingly complex. Multiple positive feedback loops, or even numbers of negative feedback loops (thus producing net positive feedback), may participate in the circuit, which can include many layers of regulation from transcriptional to post-translational.