

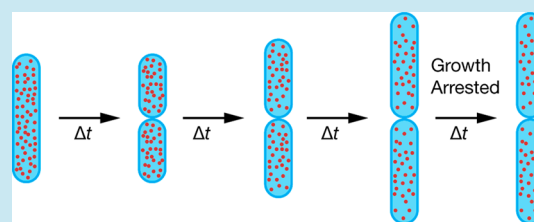
Survival of Phenotypic Information during Cellular Growth Transitions

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Supporting Information

ABSTRACT: Phenotypic memory can predispose cells to physiological outcomes, contribute to heterogeneity in cellular populations, and allow computation of environmental features, such as nutrient gradients. In bacteria and synthetic circuits in general, memory can often be set by protein concentrations: because of the relative stability of proteins, the degradation rate is often dominated by the growth rate, and inheritance is a significant factor. Cells can then be primed to respond to events that recur with frequencies faster than the time to eliminate proteins. Protein memory can be extended if cells reach extremely low growth rates or no growth. Here we characterize the necessary time scales for different quantities of protein memory, measured as relative entropy (Kullback–Leibler divergence), for a variety of cellular growth arrest transition dynamics. We identify a critical manifold in relative protein degradation/growth arrest time scales where information is, in principle, preserved indefinitely because proteins are trapped at a concentration determined by the competing time scales as long as nongrowth-mediated protein degradation is negligible. We next asked what characteristics of growth arrest dynamics and initial protein distributions best preserve or eliminate information about previous environments. We identified that sharp growth arrest transitions with skewed initial protein distributions optimize flexibility, with information preservation and minimal cost of residual protein. As a result, a nearly memoryless regime, corresponding to a form of bet-hedging, may be an optimal strategy for storage of information by protein concentrations in growth-arrested cells.



KEYWORDS: cellular computation, phenotypic memory, growth, information, relative entropy, bet-hedging

Computation depends on memory,^{1,2} and the ability to clear previous memory storage imposes a lower limit on the energetic costs of computation (Landauer's Principle).^{3,4} Memory effects have been explored in biological computation as well.^{2,5} In dividing cells from bacteria to metazoa, phenotypic memory has been well-established as an important factor in both natural and synthetic contexts,^{6–11} and strategies for engineering it with gene circuits have been developed.¹² While memory can arise from a variety of sources,¹³ one important mechanism is via inheritance of protein concentrations. Most naturally occurring proteins in bacteria, and synthetic networks lacking specific degradation tags in any cells, can have a strong effect on memory because protein degradation is dominated by dilution from growth, and growth rate can have a global effect on gene expression.¹⁴ Expression levels of proteins have been shown to affect the dynamics of bacterial signaling in some contexts^{13,15} but not others.^{16,17} Expression costs in natural and synthetic circuits can also create a growth feedback loop where expression or production of a toxic agent creates a metastable or bistable population growth dynamic with different growth rates between subpopulations of cells.^{6,14,18,19} In nonlaboratory environments, the effects of growth arrest on synthetic circuits may be an important factor; determining how to engineer appropriate generational phenotypic information transfer is an important challenge.

Many classic²⁰ and modern^{21–23} studies in bacteria have characterized mechanisms and characteristics of growth arrest and modulation of proliferation in bacteria.²⁴ As bacterial cells spend most of their time in a growth-arrested state, the residual protein content from previous environments may have a strong effect on resumption of growth. The well-known stochasticity of gene expression in single cells, combined with a dynamic loss of growth-mediated dilution during the transition to growth arrest, creates the possibility of a distribution of memory levels in growth arrested populations. As cells enter growth arrest, fluctuations in protein concentrations could become frustrated, quenched at levels far from the previous steady state. Thus, in common with kinetic trapping in protein assembly,²⁵ multi-allelic optimization in evolution,^{26,27} and glass formation in physics,²⁸ competing dynamics stand to lock transient fluctuations in place. This effect then leads to random configurations with potentially diverse phenotypes on resumption of growth, similar to cases of bet-hedging studied in the past.^{29,30} Such an effect could have implications for computation in living matter, and stands to have important

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practical consequences in the evolution and synthetic biology of gene networks.

Here we characterize conditions that affect the accumulation of long-lived proteins during growth arrest and analyze their implications for survival of information during the growth arrest transition. We hypothesized that the competing time scales of protein dilution and growth arrest would dominate the level of phenotypic molecular memory from the previous environment. Mathematical models demonstrate the existence of a critical time scale of growth arrest, below which memory is an important factor and beyond which it is effectively erased. Considering the unpredictable nature of future environments and potential differential fitness effects of surviving proteins, we determined the optimally flexible memory level of cells (i.e., highest information transfer with the least mean residual protein) based on timing and cooperativity of the growth arrest rate. As a result of these analyses, we arrive at two novel conclusions about computation and memory in growing cells with long-lived components. First, there is a parameter manifold that distinguishes high-memory and memoryless regimes depending on the suddenness of the growth arrest transition. Second, an almost-memoryless regime represents a flexible phenotype where residual information from the previous environment can be retained, but easily discarded, depending on the phenotypic needs in the future environment. The flexible phenotype can arise if the growth arrest transition is sufficiently switchlike and the initial distribution of protein sufficiently skewed. This result suggests a strategy for encoding flexible synthetic phenotypic memory by designing programmed cellular growth arrest with appropriate parameters, which may be useful for synthetic biology in contexts outside of the laboratory.³¹

We begin with a simple mean-field mathematical model of protein concentration, $\langle p \rangle$, in the presence of competing time scales of first-order loss and growth arrest, cellular growth being driven by extrinsic (i.e., non- p) stresses. While protein synthesis is capable of continuing after growth arrest in bacteria,³² many naturally occurring gene expression events are halted as part of the regulated transition into growth arrest.^{21,33} We therefore restrict consideration to the case where synthesis has already stopped, and ask what effect remaining growth has on protein concentrations and information transfer across growth arrest transitions. For this protein (or ensemble of proteins degraded at identical rates), we have

$$\frac{dp}{dt} = -[V_{d0}g(t) + \delta]p(t) \quad (1)$$

where V_{d0} represents the initial degradation rate (the growth rate of the cell in the previous, faster-growing environment), $g \in [0,1]$ represents a decreasing function of time, and δ represents the rate of growth-independent protein degradation, absent active enzymatic degradation of p , $\delta \ll V_{d0} p(t)$. In principle, g could fluctuate nonmonotonically between $[0,1]$ to represent arbitrary dynamical changes from alterations in protein synthesis and degradation as well, but here we analyze the dynamics of entering a longer-lived growth arrest state.

We have some initial protein concentration, $p(0) = p_0$. The goal is to identify the possible outcomes of protein concentrations at various time scales. Experiments have shown that batch cultures of *Escherichia coli* can stop growing at a variety of speeds, depending on the stressor.²¹ Growth rates of single cells undergoing growth arrest appear to be dominated by the extrinsic conditions as well,³² suggesting that a

deterministic forcing function is an acceptable approximation. To capture a wide variety of deterministic growth transition dynamics, let us exploit the convenient Hill function: $g(t) = \frac{q^{-n}}{q^{-n} + t^n}$. Here parameter q represents the halfway point of growth arrest, and n determines the sharpness of the loss rate (Figure 1a). Then the solution to eq 1 is, in the most general case, of the form

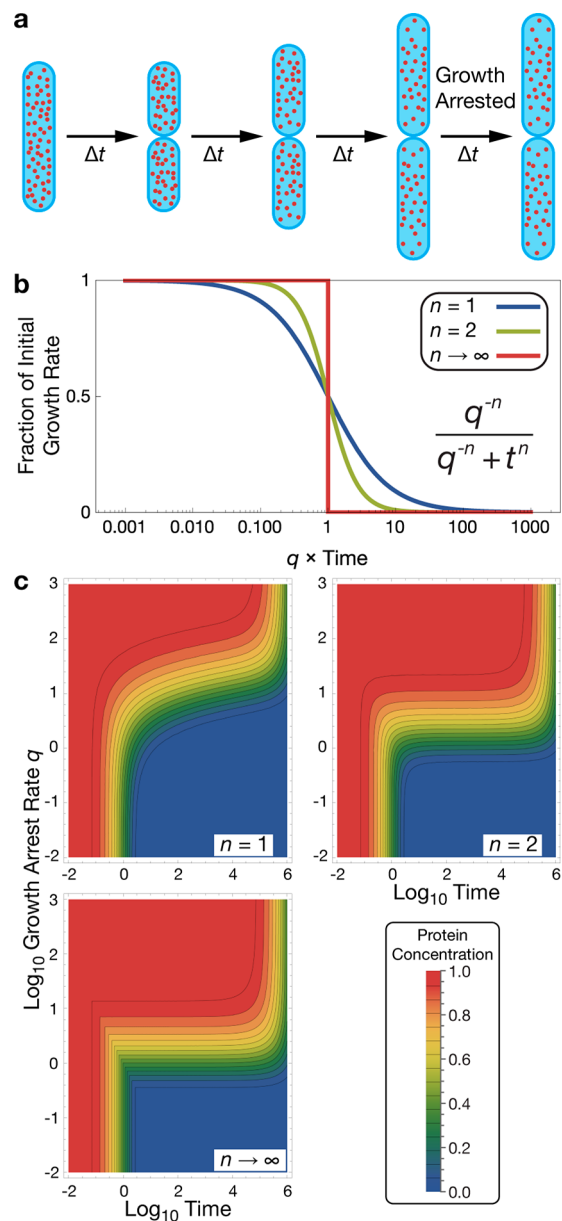


Figure 1. Cellular growth arrest traps long-lived molecules at concentrations strongly dependent on initial conditions. (a) Illustration of the growth arrest process quenching cytoplasmic protein degradation at present levels. In the model, the cell has already stopped producing more of the protein (red), and subsequent growth lowers its cytoplasmic concentration until growth has ceased. (b) We consider three models of growth arrest with Hill functions: gradual arrest ($n = 1$), steeper arrest ($n = 2$), and instantaneous arrest ($n \rightarrow \infty$, a Heaviside step function). (c) Resulting predicted mean-field protein dynamics for the three arrest models at various arrest rates q . Protein concentrations represent fraction of initial concentration, p_0 . Parameter $\delta = 10^{-6}$ for these plots.

$$p(t) = p_0 \exp\left(-t\left[\delta + V_{d0} {}_2F_1\left(1, \frac{1}{n}; 1 + \frac{1}{n}; -(qt)^n\right)\right]\right) \quad (2)$$

with $n, q > 0$, where ${}_2F_1$ is the ordinary hypergeometric function. Three cases of n are informative for intuitive purposes: $n = 1$, $n = 2$, and $n \rightarrow \infty$ (Figure 1a). They correspond to gradual loss of growth, intermediate steepness of growth, and a fast switch between the highest growth rate and complete growth arrest. These cases also simplify eq 2, creating familiar closed-form functions:

$$p(t) = \begin{cases} p_0 e^{-\delta t} (1 + qt)^{-V_{d0}/q} & n = 1 \\ p_0 e^{-[\delta t + V_{d0} \arctan(qt)/q]} & n = 2 \\ p_0 e^{-[\delta t + V_{d0}(t + (1/q - t)\theta(t - 1/q))]} & n \rightarrow \infty \end{cases} \quad (3)$$

where θ is the Heaviside step function. We can see by inspection of eq 3 and of their nondimensionalized graphs (Figure 1) that there is a distinct growth arrest rate regime where the concentration of p is frozen in arbitrary concentrations that are sensitive to the rate of arrest before eventual growth-independent protein loss has a significant effect (Figure 1b–d). (In the interest of studying time scales on which residual protein concentrations are capable of persisting in growth arrested cells, hereafter we assume that nongrowth-mediated protein degradation is negligible: $\delta \rightarrow 0$). On that time scale, more gradual growth arrest transitions soften the edges of the high-memory regime. Generally, as the transition becomes less sharp, the quenching parameter q must be larger for memory to form, corresponding to a shorter time to reach the growth-mediated protein half-life. In terms of growth arrest physiology, this means that a gradual downregulation of growth-limiting factors in response to stress will reduce memory compared to more extreme events.

What are realistic parameters that could result in controllable protein memory? That is, given a known concentration of initial protein, what arrest parameters are necessary to attain either effective memory extinction or residual protein at a certain concentration?

In the discrete growth rate switch after the growth arrest transition ($n \rightarrow \infty$), we arrive at the frozen protein concentration $p^* = p_0 e^{-V_{d0}/q}$. For the case of $n = 2$, we similarly have $p^* = p_0 e^{-\pi V_{d0}/(2q)}$. Therefore, parameters selecting for sufficiently large p^* can be clearly chosen for $n \geq 2$. There is no mathematically clear threshold for the frozen concentrations with $n = 1$ because gradual loss of protein concentration continues indefinitely. We can get an estimate for when each case is approaching a high-memory state by taking the logarithmic sensitivity of $p(t)$ to t (Figure S1):

$$L(p(t), t) = \frac{\partial \ln p(t)}{\partial \ln t} = \frac{t}{p(t)} \frac{\partial p(t)}{\partial t} = -\frac{V_{d0} t}{1 + (qt)^n} \quad (4)$$

For $q \rightarrow 0^+$, $L \rightarrow -\infty$ with t (memoryless regime). For $q \rightarrow \infty$, $L \approx 0$ for all t (“perfect” memory regime). The transition between the regimes occurs when $q^n t^{n-1} \approx V_{d0}$. Here, there is an initial transient with an eventual slowing down of protein dynamic sensitivity, settling to a sensitivity near the ratio V_{d0}/q in the case of $n = 1$ (Figure S1a). When this loss rate is lower than the fluctuation rate of the environment, phenotypic memory is a significant factor. This result is also precisely the

sensitivity of frozen protein concentrations to changes when the instantaneous switch to growth arrest occurs, q , in the case of $n \rightarrow \infty$.

Then for a given characteristic time scale τ , the threshold for the high memory regime is approximated by

$$\frac{V_{d0}}{q} = p(\tau) \quad (5)$$

and we can find it precisely only with implicit equations. For instance, in the case of $n \rightarrow \infty$, and assuming $\tau > 1/q$, we have $q = V_{d0}/W(p_0)$ where W is the product logarithm, or the Lambert W function: the inverse of $f(W) = We^W$.

Another way to view the kinetics of protein survival is to consider a constant number of proteins, n_p , in an initial cell volume Ω_0 , and follow the (deterministic) growth of cell volume. Mathematically, this is equivalent to eq 2, with $\delta = 0$ and protein concentration as

$$p(t) = p_0 / \Omega(t) \quad (6)$$

Consider p_0 to be a random variable in an initial distribution, $P(p_0)$, that is well-characterized, parametric, and has a multiplicative scaling rule. Because the time evolution of the system is deterministic, we can exploit multiplicative scaling to analytically derive time evolution of the distribution and its entropy. For example, if the protein is initially in a gamma distribution, $p_0 \sim \text{Gamma}(\alpha, \beta)$ and $kp_0 \sim \text{Gamma}(\alpha, k\beta)$. The distribution over time is then simply $p(t) \sim \text{Gamma}(\alpha, \beta/\Omega(t))$ (Figure 2). This is a biologically relevant model of protein concentrations because, in many parameter regimes, protein distributions in bacteria^{34–36} and other organisms³⁷ are well-approximated by gamma distributions (though other parametrizations, such as log-normal and Fréchet distributions, have been suggested as well³⁸). We now explore the implications of various growth arrest rates assuming an initial gamma distribution.

We can compute the relative entropy, or Kullback–Leibler divergence,³⁹ without the need for special functions in the entropy term itself after cancellation of terms, and avoiding some of the problems given by the standard entropy in a continuous probability density function. The divergence is asymmetric:

$$D(P(p_0) \| P(p_0/\Omega(t))) = \int_{p_0} P(p_0) \log_2 \left(\frac{P(p_0)}{P(p_0/\Omega(t))} \right) dp_0$$

In words, this equation gives the divergence of information over time, in units of bits. In practice, the divergence is often symmetrized, but that is unnecessary because we are concerned with irreversible divergence from an initial condition with time. For brevity, we refer to the divergence at a given time as $D(t)$.

For the gamma distribution we have a divergence of

$$D(t) = (\alpha_0 - \alpha(t))\psi(\alpha_0) - \log_2 \left(\frac{\Gamma(\alpha(t))}{\Gamma(\alpha_0)} \right) + \alpha(t) \log_2 \left(\frac{\beta}{\beta/\Omega(t)} \right) + \alpha_0 \frac{\beta/\Omega(t) - \beta}{\beta} \quad (7)$$

where the 0 subscript indicates the initial condition, ψ is the digamma function, and Γ is the gamma function.⁴⁰ Because α is constant with time, and canceling the β terms, eq 7 simplifies to

$$D(t) = \alpha(\log_2(\Omega(t)) + \Omega(t)^{-1} - 1) \quad (8)$$

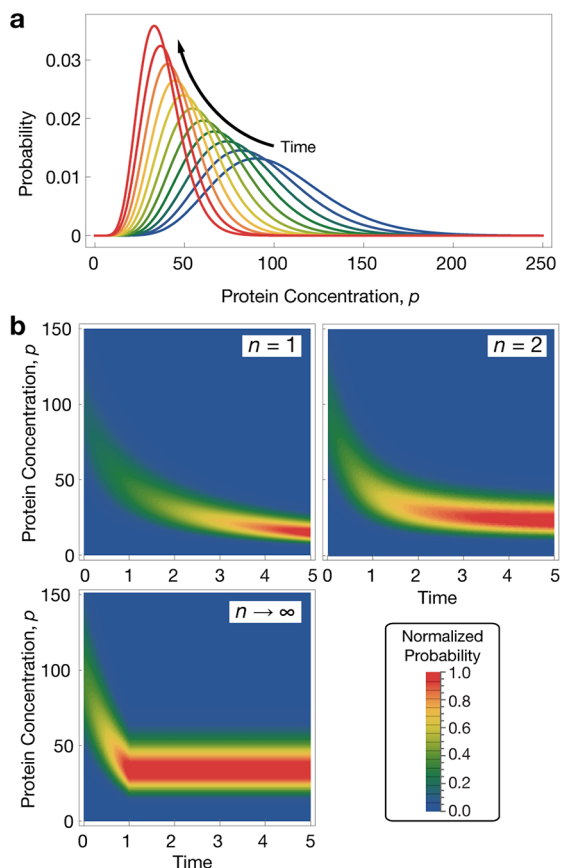


Figure 2. Analytical prediction of exact time evolution of a gamma-distributed protein in an infinite, deterministically growth arresting population. (a) Example of the distribution evolving over time. Parameters: $\alpha = 10$, $\beta = 10$, $V_{d0} = 1$, $n \rightarrow \infty$, $q = 1$, $\delta = 0$. (b) Comparison of probability distribution dynamics for different orders (n) of growth arrest. All other parameters are the same as in panel a. Probabilities are normalized so that red is the maximum probability over all time, and blue is the minimum.

The three specific growth models considered above ($n = 1$, $n = 2$, $n \rightarrow \infty$) give the following solutions:

$$D(t) = \begin{cases} \alpha(\log_2((1 + qt)^{V_{d0}/q}) + (1 + qt)^{-V_{d0}/q} - 1), & n = 1 \\ \alpha \left(\frac{V_{d0} \arctan(qt)}{q \ln 2} + e^{-V_{d0} \arctan(qt)/q} - 1 \right), & n = 2 \\ \alpha \left(\frac{V_{d0}}{\ln 2} \left[t + \left(\frac{1}{q} - t \right) \theta \left(t - \frac{1}{q} \right) \right] + e^{-V_{d0} [t + (1/q - t) \theta(t - 1/q)]} - 1 \right), & n \rightarrow \infty \end{cases} \quad (9)$$

Various limits make eq 9 more intuitive. For example, choosing time units of $V_{d0}/\ln 2 = 1$, for $n \rightarrow \infty$ and $t > 1/q$, we get $D = \alpha \left(\frac{1}{q} + 2 e^{-1/q} - 1 \right)$, and so for slow (later) growth arrest, the divergence is approximately α/q bits while for fast (immediate) growth arrest it approaches 0. Figure S2 graphs the divergence for a selection of parameter values.

The shape parameter of the gamma distribution scales the divergence of information between initial and final states (eq 9). Populations undergoing sharper growth arrest transitions

that have more highly skewed distributions of proteins (i.e., smaller α) have a small divergence (e.g., less than 1 bit) for a larger part of the growth arrest rate parameter space (Figures 3,

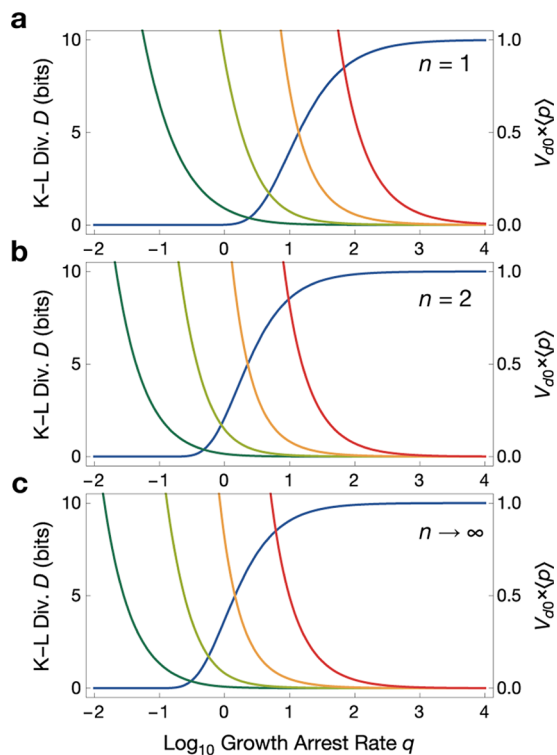


Figure 3. Divergence of information during the growth arrest process. In an infinite population, a gamma distribution of proteins relaxes for 10^3 units of time as the growth arrest rate parameter, q , is scanned. Parameter $\delta = 0$ in all cases (see main text): (blue) mean protein, $V_{d0} \times \langle p \rangle$; (green) K-L divergence, D , for $\alpha = 0.1$, $\beta = 1000$; (light green) D for $\alpha = 1$, $\beta = 100$; (orange) D for $\alpha = 10$, $\beta = 10$. (red) K-L divergence for $\alpha = 100$, $\beta = 1$. (a) $n = 1$; (b) $n = 2$; (c) $n \rightarrow \infty$.

4a). Then it should be possible to choose a shape parameter (i.e., choose a shape of the protein gamma distribution) that simultaneously minimizes the divergence and the protein concentration. Clearly, because these quantities do not have the same units, comparing them directly depends on the goal of the application: choosing a threshold amount of relative entropy and a threshold concentration of protein is necessary. For divergence threshold D^* , the shape parameter is (for $t > 1/q$ and $n \rightarrow \infty$):

$$\alpha = D^* \frac{q e^{1/q} \ln 2}{q \ln 2 + e^{1/q} (1 - q \ln 2)} \quad (10)$$

which has a nearly linear relationship with q (Figure 4b). Plotting this with the mean protein concentration parametrically over q illustrates the shape needed to attain a divergence no larger than various threshold levels ($D^* = 0.1, 1, 5$ bits; Figure 4c). Thus, distributions of cells rarely expressing a protein, but doing so in bursts, appear to maximize the flexibility of cellular populations.

We have characterized relaxation kinetics of a cellular protein distribution subject to deterministic growth-mediated dilution as growth stalls, representative of many cases in natural or synthetic systems after protein synthesis has been halted. The results predict that proteins can become trapped at an arbitrary

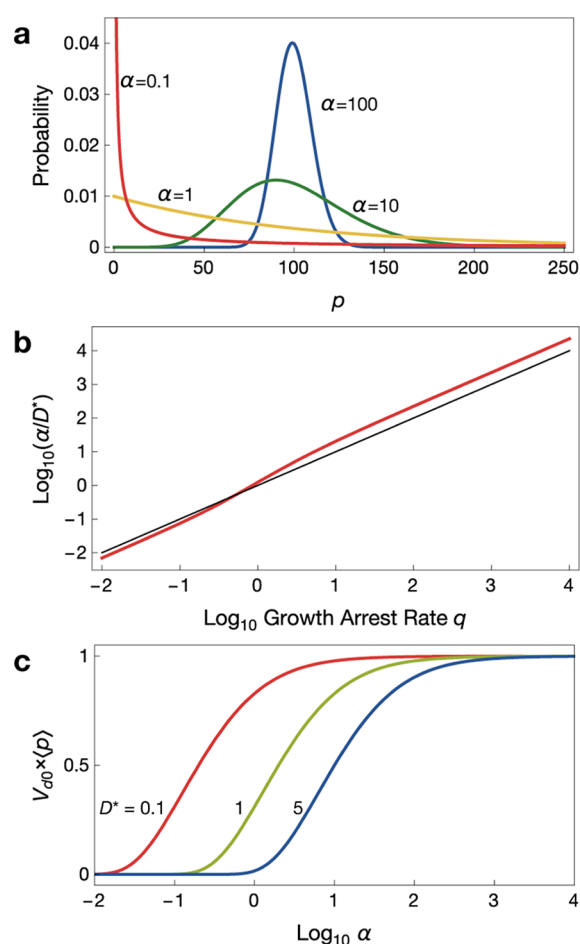


Figure 4. Distribution properties and divergence of protein concentrations during growth arrest. (a) Example distributions with identical initial mean = 100. (b) For a given growth arrest rate parameter q , the shape parameter, α , necessary for maximal divergence, D^* . Black line, $y = x$ for reference. (c) Mean protein concentration $V_{d0} \times \langle p \rangle$ and shape parameter α for various maximal divergence thresholds D^* for the case of $n \rightarrow \infty$.

concentration depending on the relative time scales of dilution and growth arrest, assuming that growth-independent protein degradation is negligible. Such an effect provides the potential for phenotypic memory, useful for creating computation in single cells across growth rate transitions, but erasable with a brief period of growth.

Our results suggest that slight cooperativity in the growth arrest rate (with a Hill coefficient ≈ 2) should be sufficient to trap protein concentrations for arbitrary amounts of time in the absence of other degradation effects.

A possible limitation of this analysis is the deterministic nature of the growth arrest transition in the models. While stress is often associated with stochastic induction of growth arrest mechanisms,^{22,41} and cell volumes can fluctuate,⁴² changes in cell volume over time show reversion to the mean,⁴³ and extrinsic stresses can simultaneously arrest cell populations.²¹ This suggests that our approximation is useful, even if it does not capture some aspects of fluctuations.

In *E. coli*, important new characteristics of the relationship between protein synthesis and growth arrest have recently been revealed. When growth arrest is strongly induced via a specific mechanism, it appears to be accompanied by a burst of expression of genes relevant to the particular stress.²¹

Furthermore, stationary phase bacteria are capable of having protein synthesis induced even after growth arrest, with resulting linear accumulation of expressed protein,³² but the patterns of what naturally present genes are subject to this phenomenon has yet to be established. Thus, it will be pressing in further studies to explore the competition between protein synthesis and degradation during growth arrest as well. While a standard entropy measure was sufficient to be revealing about information transfer in this study, cases with competing synthesis and degradation processes may require a more sophisticated information measure, such as transfer entropy,⁴⁴ to handle nonmonotonic dynamical transitions.

The different time scales of loss of protein mass and information that can be attained suggest a method for applying a cost/benefit relationship to arrive at a maximally flexible protein memory strategy, by creating skewed distributions and inducing sharp transitions to growth arrest. Systems with bursty gene expression (known to occur in many bacterial subsystems^{34,35}) in sufficiently large populations should be able to meet these criteria. Populations that use bet-hedging survival strategies³⁰ likely already conform to these requirements. Exploiting this principle could potentially be useful for programming memory without excessive cost, and for improving fitness in fluctuating environments where previous memories may no longer be beneficial. Studies have shown the importance of fitness in synthetic circuits with long memory for their evolutionary stability,⁴⁵ underscoring the potential utility of optimized protein memory.

Most studies in bet-hedging have emphasized the role of skewed phenotypic distributions in preparing for uncertain future environments.³⁰ Our results suggest that one important aspect of bet-hedging is its effect on memory: the ability of a cellular population to be responsive to the previous environment if it recurs, yet also to eliminate the residual cost if it is unnecessary. A previous study found that the *Bacillus subtilis* sporulation decision switches between memoryless and high-memory states,⁴⁶ suggesting that the strategy of minimal memory with maximal information transfer has precedence in evolution.

METHODS

Mathematical analysis and simulations were performed with Mathematica 10.2 (Wolfram Research).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssynbio.5b00229.

Figure S1, effects of changing the steepness parameter in the growth arrest rate, on protein dynamics; and Figure S2, Kullback–Leiber divergence for $n = 1, 2$ and $n \rightarrow \infty$ (PDF)

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Notes

The authors declare no competing financial interest.

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