A mathematical model for adaptive prediction of environmental changes by microorganisms

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Survival in natural habitats selects for microorganisms that are well-adapted to a wide range of conditions. Recent studies revealed that cells evolved innovative response strategies that extend beyond merely sensing a given stimulus and responding to it on encounter. A diversity of microorganisms, including *Escherichia coli*, *Vibrio cholerae*, and several yeast species, were shown to use a predictive regulation strategy that uses the appearance of one stimulus as a cue for the likely arrival of a subsequent one. A better understanding of such a predictive strategy requires elucidating the interplay between key biological and environmental forces. Here, we describe a mathematical framework to address this challenge. We base this framework on experimental systems featuring early preparation to either a stress or an exposure to improvement in the growth medium. Our model calculates the fitness advantage originating under each regulation strategy in a given habitat. We conclude that, although a predictive response strategy might by advantageous under some ecologies, its costs might exceed the benefit in others. The combined theoretical–experimental treatment presented here helps assess the potential of natural ecologies to support a predictive behavior.

adaptation | conditioning | evolution

Microorganisms are constantly faced with environmental stimuli and stresses. Over the years, cellular response to such challenges has been intensively studied in several model organisms (1–5). Prevalent response strategies follow a sense and respond logic: cells continuously monitor their environment and induce a cellular response to cope with a stimulus on encounter with it. Although evolution selects for improved sensing and response mechanisms, adaptation can also extend and result in the emergence of more sophisticated response strategies. For example, under stochastic switching, cells randomly alternate between potential cellular states. In a fluctuating environment that is hard to monitor, such a response strategy might ensure that a portion of the population is always prepared for unpredicted challenge (6–8).

Here, we focus on environments that are characterized by a stereotypical temporal order of stimuli. Previous theoretical work has suggested that such ecologies may select for organisms that use information about the natural sequence of events (8). Recent studies have revealed examples of such adaptations in model microorganisms. Tagkopoulos et al. (9) investigated the response of *Escherichia coli* to temperature elevation that is followed shortly after by a drop in oxygen availability on entry of bacteria to the host digestive tract and observed an associative anticipatory regulation pattern—each signal by itself can invoke response to both stimuli. In another study, we have shown that *E. coli* has adapted to the sequential order of exposure to different sugars along the mammalian digestive tract. Additionally, we have shown that this conditioned response entails the bacteria with fitness advantages when cells are exposed to the two sugars in their sequential natural order (10). *Vibrio cholerae*, another species that transits through the digestive tract, was also observed to exhibit an anticipatory response. Specifically, these bacteria were shown to induce genes important for the subsequent life stage outside the host during the late stages of host infection (11).

Studies on two yeast species, *Saccharomyces cerevisiae* and *Candida albicans*, have shown the potential of a predicative capacity under additional ecologies. Focusing on the shift from fermentation to respiration experienced during wine production, we have shown that *S. cerevisiae* has adapted to the typical order of stresses in this ecology (10). Recently, Rodaki et al. (12) have shown that *C. albicans*, a human pathogen, induces an oxidative and cationic stress response on encounter with glucose. This induction might reflect a protective conditioned response that cells mount when present in the blood serum to protect themselves from future encounters with the host's immune response.

These observations in diverse ecologies suggest that the recently discovered adaptive conditioning might be, in fact, ubiquitous in biology. However, although previous studies have focused on experimentally uncovering the specifics of particular examples, it is not clear a priori that a predictive behavior should always pay off. A comprehensive theoretical approach, which will weigh the cost of prediction against its benefit under various cellular systems and ecological environments, is noticeably missing. Such an approach is needed, because it can reveal the parameters that would characterize the ecologies that support conditioning and the ecologies in which this strategy would not be adaptive.

Here, we explore the potential of a predictive capacity in microorganisms by developing a mathematical framework that can estimate the advantage of this regulation strategy over the naive sense and respond strategy that is often assumed to exist. We start by experimentally exploring the fitness advantage of early preparation before two different stimuli: addition of a superior carbon source to the growth medium and exposure to a stressful environment. Next, we describe a phenomenological mathematical model that elucidates the key biological and environmental parameters that contribute to the selective advantage gained from a conditioned response strategy and the relationships between them. The economics-like model predicts a net gain in fitness because of conditioning as a balance between biological cost and benefit parameters and environmental parameters such as the regularity of the changes. Finally, we illustrate the capacity of the model as a research tool when addressing natural ecologies. We show that the model predicts that the ecology of the mammalian digestive tract favors a conditioned response in regard to sugar metabolism as was indeed observed in WT *E. coli*.

Results

Advantage of Conditioning Under Diverse Environments. Environmental changes either improve or worsen the organism's growth conditions. To explore the potential advantage of early preparation under such situations, we experimentally examined the fitness of *E. coli* in two separate setups, featuring exposure to stress or addition of a superior carbon source to the growth medium.

In our experiments, we monitored changes in the population size of two identical cultures undergoing an environmental change (Fig. 1*A*). Before the change, a conditioned culture was exposed to an inducing predictive signal to trigger early prepa-

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ration, whereas a control unconditioned culture was not exposed to the inducing signal. The fitness of early preparation is denoted by the population size ratio of a conditioned culture over the unconditioned control culture after the environmental change. Thus, under a given experimental setup, a ratio larger than one indicates that early preparation is advantageous, whereas a ratio smaller than one indicates that early preparation is actually disadvantageous. Because an adequate preparation period is likely to be required for cells to sufficiently prepare, we repeated our experiment while varying the delay time between the triggering stimuli and the environmental change.

In the first system, in which the environmental change features an improvement of growth conditions, we added lactose to *E. coli* cells initially growing on glycerol, an inferior carbon source. We reasoned that early preparation will enable conditioned cells to use the superior carbon source immediately on encounter without the typical delay time required for inducing the relevant metabolic pathway. In this setup, preparation before sugar encounter was triggered by addition of a metabolically inert artificial inducer isopropyl β-D-1-thiogalactopyranoside (IPTG). Thus, IPTG-conditioned cells preinduced the lactose operon without any energetic gain. In the second system, we explored the stress autoprotection phenotype to quantify the advantage of early preparation. Numerous studies have shown that model microorganisms can efficiently protect themselves from lethal stresses if they are exposed to mild levels of stress in advance (10, 13–17). In our experiment, *E. coli* cells growing in their optimal growth temperature were conditioned by a mild, nonlethal temperature elevation before a severe heat shock. In this setup, the control population was challenged by only the severe heat shock.

Fig. 1 *B* and *C* shows, respectively, the measured fitness advantage of preparation before addition of lactose or before exposure to a severe heat shock at various time intervals (the preparation period between the two signals). Noticeably, the dependency of the fitness advantage on the preparation period is qualitatively similar in these two diverse systems—when the delay is very short, early preparation offers little to no advantage. Similarly, when the delay is too long preparation becomes disadvantageous as well. An optimal time interval is, thus, obtained that maximizes the use of conditioning. These results can be intuitively understood after taking into account that early preparation is likely to be energetically costly to conditioned cells.

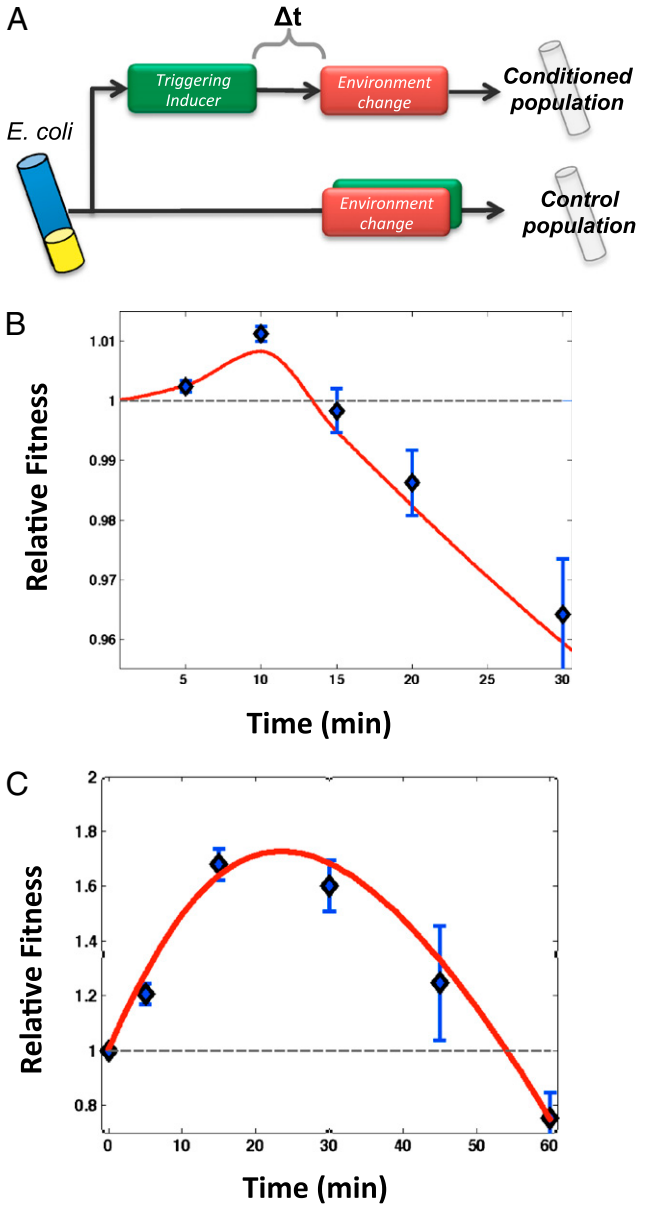

Fig. 1. Fitness advantage of early preparation. (*A*) Experimental setup exposing cells to two signals sequentially or simultaneously. Experiments measuring fitness advantage of conditioning (*B*) before lactose addition and (*C*) before severe heat shock. Blue bars denote the SEs of three repeats. The red graph marks the model predication (*SI Text*).

Phenomenological Model. The results of our experiments show a qualitatively similar dependency of the fitness advantage on the preparation period. This similarity between the metabolic and stress systems suggests that a single phenomenological model might be sufficient to capture the underlying dynamics that govern the relative fitness of conditioning under diverse environments.

Our model focused on two alternative response strategies, the conditioned response (CR) and the naïve direct response (DR), in an environment that consists of two stimuli, S_1 and S_2. We present the equations of the mathematical model in a few stages. First, we derive the organism's response function—a function describing the time-dependent changes in the R_2 response level under a given response strategy. The gain and cost effects are manifested as dynamic changes in the basal growth rate and are derived from the response function. By integrating over changes in the growth rate, we calculate the fitness of each response strategy in the given environment. Finally, the cumulative effects are used to yield an experimentally measurable fitness parameter.

The basal growth rate of an organism changes on encounter with a stimulus, and it is affected by two opposing influences: the cost of mounting the response (denoted c) and the benefit gained from responding (denoted b). Thus, in a steady state, the change in the growth rate (denoted δ) is the product of the gain and cost coefficients: $\delta = b \cdot c \cdot \delta_{basal}$. Using this representation, the relative fitness of an organism (F) in a changing environment can be represented as the sum of all changes relative to the basal growth rate (Eq. **1**):

$$F = \int_0^\infty b(t) \cdot c(t) dt. \qquad [1]$$

To model the time-dependent dynamics of the R_2 response, we relied on a function describing an exponential approach to a steady-state protein level (Eq. **2**),

$$Y(t) = Y_{st}(1 - e^{-\alpha t}), \qquad [2]$$

where t is the time from induction, Y_{st} is the steady-state level of the protein, and α is the dilution/degradation rate. For simplicity, active degradation of the protein is neglected, and hence, α equals the growth rate δ, which is often assumed in bacteria (18). This basic function is used to develop the two alternative response functions $r_{DR}(t)$ and $r_{CR}(t)$, which denote the relative

response level (normalized to Y_{st}) under the DR and CR, respectively (Fig. 2, response panel) (Eqs. **3** and **4**):

$$r_{DR}(t) = \begin{cases} 0 & t \le \Delta t \\ 1 - e^{-\alpha(t-\Delta t)} & t > \Delta t \end{cases} \text{ and} \quad [3]$$

$$r_{CR}(t) = 1 - e^{-\alpha t}. \quad [4]$$

The benefit gained under each regulation strategy is taken as the temporary increase in the basal growth rate. Here, we assume that the gain is linearly proportional to the response level at a given time point and is gained only when S_2 is present (Fig. 2, gain panel). Because the benefit coefficient (b) depends on the biological system, a scaling parameter is added (κ), and the coefficient is set to one if no benefit exists (Eqs. **5** and **6**):

$$b_{DR}(t) = 1 + \kappa r_{DR}(t) \text{ and} \quad [5]$$

$$b_{CR}(t) = \begin{cases} 1 & t \le \Delta t \\ 1 + \kappa r_{CR}(t) & t > \Delta t \end{cases}. \quad [6]$$

The cost under each regulation strategy is modeled through a temporary decrease in the basal grow rate. We assume that the cost originates from processes such as transcription and translation occurring at a constant rate (19, 20). Thus, unlike the gain, the cost is constant per time unit and proportional only to the production rate (β). Additional and more elaborate cost functions featuring time dependency of the cost on the induction period are presented in *SI Text* (Eqs. **S10**–**S13**). Because the R_2-normalized production rate, β, is a binary parameter ($\beta = 0$ or $\beta = 1$ at the presence and absence of the stimulus, respectively) in both regulation strategies, the cost coefficient is modeled using only a system scaling factor (η) and is set to one if no cost exists (Eqs. **7** and **8**):

$$c_{DR}(t) = \begin{cases} 1 & t \le \Delta t \\ 1 - \eta & t > \Delta t \end{cases} \text{ and} \quad [7]$$

$$c_{CR}(t) = 1 - \eta. \quad [8]$$

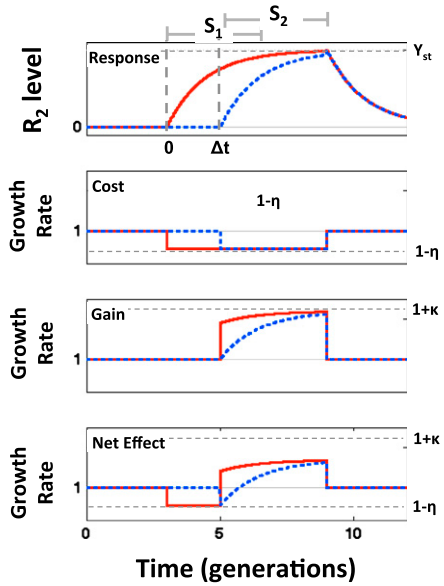

Fig. 2. Calculation of the relative fitness in an example habitat. The blue and red graphs mark DR and CR, respectively. The stimuli are marked on top.

Using the gain and cost coefficients, we can integrate over all temporal changes in the growth rate (Fig. 2, net effect panel) to calculate the fitness of the two alternative response strategies in a stimuli-coupled environment (S_1 was followed by S_2) (Eqs. **9** and **10**):

$$F_{DR}^{Coupled} = \Delta t + \int_{\Delta t}^{\infty} (1-\eta)\left(1 + \kappa\left(1 - e^{-\alpha(t-\Delta t)}\right)\right) dt \text{ and} \quad [9]$$

$$F_{CR}^{Coupled} = (1-\eta)\Delta t + \int_{\Delta t}^{\infty} (1-\eta)(1 + \kappa(1 - e^{-\alpha t}))dt. \quad [10]$$

The relative fitness after solving the integrals is (Eq. **11**)

$$\Delta F_{CR-DR}^{Coupled} = (1-\eta)\kappa\frac{1 - e^{-\alpha\Delta t}}{\alpha} - \eta\Delta t, \quad [11]$$

whereas in an uncoupled environment (only S_1 appears), the relative fitness of each response strategy is reduced to the integral over the cost coefficients (Eq. **12**):

$$\Delta F_{CR-DR}^{Uncoupled} = -\eta T_{S1}. \quad [12]$$

We can now incorporate Eqs. **11** and **12** into a single fitness equation to calculate the fitness in a composite environment that includes both coupled and uncoupled appearances of the two stimuli (Eq. **13**):

$$\Delta F_{CR-DR} = p\left((1-\eta)\kappa\frac{1 - e^{-\alpha\Delta t}}{\alpha} - \eta\Delta t\right) - (1-p)(-\eta T_{S1}), \quad [13]$$

where p denotes the conditional probability that S_2 will occur given that S_1 occurred. Note that the expression is calculated for an average encounter with S_2 (weighed by p).

The new fitness expression (Eq. **13**) is useful, because it can be applied to calculate an experimentally measurable fitness parameter, the population size ratio (Eqs. **14**):

$$N_{CR}/N_{DR} = \exp(\delta_{basal} \cdot \Delta F_{CR-DR}). \quad [14]$$

A population ratio above one indicates that, in the tested environment, early preparation is advantageous, whereas a ratio below one indicates a parameter combination that selects for the direct response strategy.

Conditioned Stress Response. Under some environments, the fitness advantage of early preparation can be manifested as a single time-independent event. Such a benefit function is useful when considering stressful environments in which the increased survival of a cell is proportional to the R_2 level at the time point of stress exposure. These environments can be modeled by discarding the accumulative gain ($\kappa = 0$) and adding a new non-integrative benefit parameter B. After this manipulation, the fitness equation (Eq. **13**) becomes (Eq. **15**):

$$\Delta F_{CR-DR}^{Stress} = p(B - \eta\Delta t) - (1-p)(-\eta T_{S1}). \quad [15]$$

Because B is expected to depend on the preparation level at the time of S_2 exposure, we modeled B as linearly dependent on the R_2 level at the time of encounter and used a new scaling parameter κ^* (Eq. **16**):

$$B = r_{CR}(\Delta t)\kappa^*. \quad [16]$$

Note that κ^* is proportional to the maximal fold protection that can be entailed by early preparation (Eq. **17**):

$$\kappa^* = \ln(\textit{fold protection})/\delta_{basal}. \quad [17]$$

Exploring the Parameter Space. The relative fitness equation allows us to quantitatively study the relationship between key biological and environmental parameters. One interesting aspect that can be explored is which environments are expected to select for early preparation. We used the model to address two prototypic predictable ecologies: an environment in which growth conditions improve and stressful environments.

As an example of improvement in growth conditions, we consider a metabolic upgrade. For this, we used the gain and production cost previously measured for the *E. coli* in the lactose-metabolizing system (19). Because such pathways typically include a limited number of metabolic genes, they are expected to bear only a low cost. Additionally, because the benefit is gained only from a relatively short period of the superior nutrient breakdown, we expect only a moderate gain from early preparation. Sugar metabolism by *E. coli* passing through the mammalian digestive tract is one such ecology that fits this environmental prototype (10).

Focusing on the environmental parameters (p and Δt) reveals the environmental setups that can support a conditioned response strategy in such a limited biological system (Fig. 3*A*). As the figure shows, CR is expected to be beneficial in at least moderately predictable environments ($p > 0.5$) and for a limited range of delay periods. However, the potential fitness advantage under such circumstances is relatively low, saturating at only a few percent in terms of population size ratio. The dependencies between additional model parameters are further explored in Fig. S1.

Now let us consider stress preparation (10–17). Such environments are characterized by the potential high benefit of preparation (e.g., a 50-fold protection was measured recently in cell survival) (10). Remarkably, this increased survival sometimes relies only on a handful of genes (e.g., one gene underlying a 20-fold protection against heat in *E. coli*) (16).

Fig. 3*B* shows the model's predications for a modest stress system characterized by a low cost and only a modest protection potential. The increased survival of heat-conditioned *E. coli* and *S. cerevisiae* against severe diverse stresses that are facilitated by a single chaperone is an example of such a cellular system (14, 16). Although the results in this prototypic type of environment recapitulate the basic dynamics revealed in the growth improvement example (Fig. 3*A*), the parameter subspace that selects for CR is considerably larger, and the fitness advantage is one order of magnitude larger. Noticeably, in such a preparation for stressful changes, even poorly predictable environments ($p < 0.2$) can select for CR at an optimal delay time. Likewise, a wide range of delay periods selects for CR (e.g., up to 10 generations in a moderately predictable environment) ($p = 0.5$).

A complementary stress system can also be addressed. Fig. 3*C* focuses on predications for a system characterized by high cost and high-protection capacity. This setup reflects a genome-wide predicative response that underlies a considerable protective capacity. Two examples that fit this prototypic system can be found in the increased survival of conditioned *S. cerevisiae* to oxidative stress (10, 21) and increased survival of glucose-conditioned *C. albicans* to oxidative stress (12), which both involve the expression of hundreds of genes. The analysis reveals that, although CR can yield a remarkable fitness advantage for some value combinations, it is much less robust to long delay periods compared with the modest stress system. This restricted capacity under long delay periods arises from the high cost of a genome-wide response.

To conclude, the three systems explored above share qualitatively similar fitness dynamics that reflect the advantage of conditioning in highly predictable ecologies characterized by a high gain to cost ratio. Among the important differences are

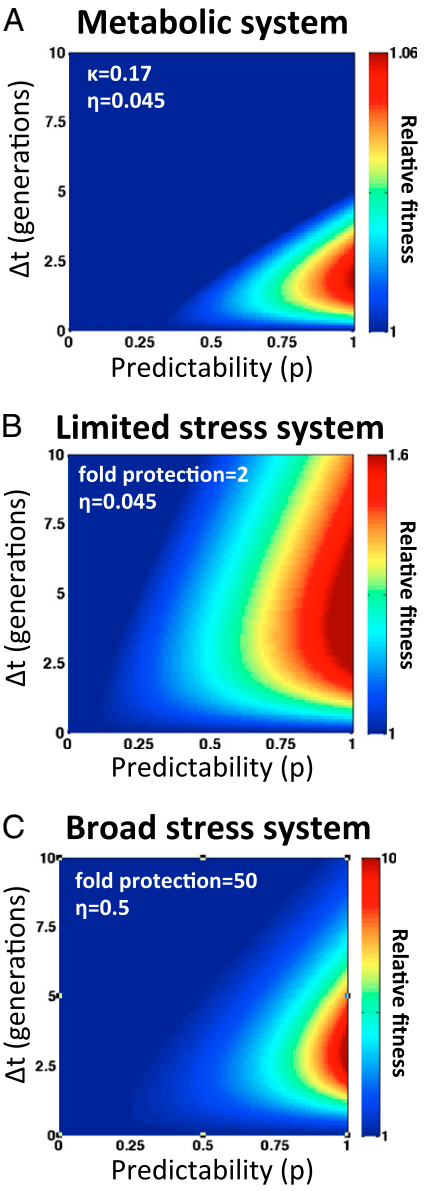

Fig. 3. Exploring the fitness landscape in different environments. Predicted relative fitness as a function of delay period and predictability in a (*A*) metabolic setup (low cost and moderate gain), (*B*) limited stress setup (low cost and moderate protection), and (*C*) broad stress system (high cost and high protection). Note the different scale of fitness in the three figures.

the scale of the potential fitness advantage, ranging over three orders of magnitude, and the ability of even poorly predictable environments to modestly select for conditioning given that other parameters are favorable. An unexpected similarity is the optimal value for the delay time between the stimuli (Δt). The optimal values are restricted to a narrow range of 1–2.5 generation times, reflecting an inherent dependency of the fitness on the response time of the biological system. The system's response time in our model is captured by the dilution/degradation parameter α. Thus, by inducing protein degradation, cells can increase α and adjust the system to shorter delay periods (Fig. S2).

Fit to Experimental Results. We used the model to readdress the experimental results measuring the fitness of early preparation (*SI Text* has a full account, and Fig. S3). In the lactose system, values of all parameters were experimentally measured: the values of the gain (κ) and the production cost (η) have been previously estimated

(19) and reproduced by us. However, an additional cost parameter is also known to exist in this system. This cost originates from excessive transport of lactose and consequent loss of membrane potential in cells already expressing the lactose permease when encountering the sugar (22). As Fig. 1*B* shows, we observed a good agreement between the model predication and experimental results without adjusting any free parameters.

Analysis of the experimental stress system also shows a good fit to the model (Fig. 1*C*). In this case, the fit was preformed after defining a feasible range for the degradation rate (α). Interestingly, an optimal fit was observed for a high degradation rate, indicating that active protein degradation and not just dilution takes place. This observation agrees with the known elevation of active protein degradation under heat stress in *E. coli* (23).

Model Expansion—Two-Phase Conditioning. The CR strategy described above represents a full commitment of an organism to the R_2 response on encounter with the preceding stimulus S_1. However, under a more adjustable strategy, S_1 might activate R_2 to a partial level only. Such partial activation reflects only a limited commitment of the cell to R_2 in response to S_1, whereas only S_2 fully triggers R_2. We term this strategy two-phase conditioned response (CR2) to indicate the two steady-state levels of R_2—first, an intermediate level when S_1 is encountered, and then, a full level when S_2 appears. A full account of this expansion is in *SI Text*, and Fig. S4.

Intuitively, CR2 can be viewed as a risk management strategy, allowing an organism to be moderately prepared for only a part of the cost. Subsequently, such a partial commitment can potentially significantly increase the environmental subspace that selects for early preparation. Fig. 4 shows our analysis of CR2 in the metabolic system previously addressed (Fig. 3*A*). As the figure indicates, conditioning can be selected even in poorly predictable and long delay periods (Fig. 4*A*). This ability is achieved through reduced commitment to conditioning (Fig. 4*B*).

Adaptive Conditioning in *E. coli* and the Intestinal Ecology. The mammalian digestive tract represents an ideal environment that can potentially select for conditioning. Microorganisms found in this ecology usually alternate between the digestive tract and the outside environment (e.g., water, sediment, and soil) (24). Moreover, focusing on the digestive tract reveals a habitat that is characterized by a sequential exposure to various stimuli. Indeed, previous studies have uncovered examples of a CR strategy both in *E. coli* (9, 10) and *V. cholerae* (11).

Here, we readdress this habitat using the developed mathematical model to test its use for natural ecologies. Specifically, during passage along the digestive tract, exposure to lactose precedes exposure to another sugar, maltose (25). We, thus, expect that this environment can potentially select for conditioning in sugar metabolism—bacteria that link between the presence of lactose and future exposure to maltose may better use maltose resources on encounter.

The model was used to predict the regulation strategy that is most beneficial for the induction of the lactose and maltose metabolic pathways. Calculations were based on the previously estimated parameter values of the digestive tract, $\Delta t = 3$ h and $\delta = ln(2)$ (25). Unfortunately, the values of the gain and cost parameters are not known for this system, and direct measurement within the natural habitat is extremely difficult. Thus, we define a wide range of possible gain to cost ratios. Because *E. coli* responds to maltose, it is safe to assume that the gain is greater than the cost, yielding a lower boundary of unity to the ratio. As an upper boundary, we used the gain (κ) to production cost (η) ratio of the lactose system that reflects the energy yield in a carbon source superior to maltose.

Fig. 5 shows the model's predications, indicating that, under this range of possible values, a two-phase conditioning strategy is most advantageous. Thus, on encounter with lactose, cells are expected to fully induce the lactose operon and additionally, induce the maltose operon to an intermediate level. The maltose operons are expected to be fully induced only on encounter with maltose itself. Furthermore, conditioning remains most advantageous, even under alternative cost function (Fig. S5). The model predications are in agreement with the experimentally measured promoter activities for the maltose and lactose operons (10).

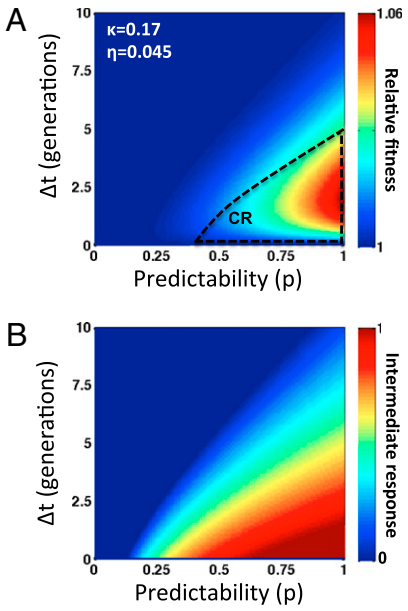

Fig. 4. Fitness landscape of two-phase conditioning in a metabolic setup (low cost and moderate gain). (*A*) The predicted relative fitness as a function of delay period and predictability. The enclosed dashed subspace denotes parameter combinations that also select for full conditioning (CR). (*B*) The optimal intermediate response level.

Discussion

Recent experimental studies suggest that diverse microorganisms adapted to reoccurring patterns of stimuli in their natural habitats by treating an early stimulus as a predictor for the likely arrival of a future environmental challenge. Here, we present a theoretical framework that helps to elucidate this adaptation and the key environmental and biological forces that affect it. The presented model was based on a minimal set of biological assumptions and was restricted to only a few key parameters. However, under a different set of assumptions, the model can be easily updated to accommodate additional, more complex biological instances.

In the scope of this paper, we addressed two prototype environments that can potentially select for early preparation: im-

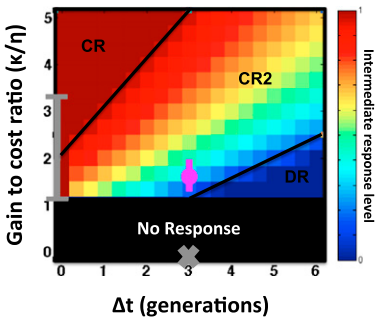

Fig. 5. Model-predicted regulation strategies in the digestive tract. The model was used to calculate the optimal intermediate level of induction after encounter with S_1 as a function of the gain to cost ratio and delay time. The color code marks the intermediate response level. The typical delay time and feasible range of gain to cost ratios are marked in gray. The magenta bar depicts the mean intermediate induction levels (0.37 ± 0.2) observed for the five maltose operons in response to lactose in the WT *E. coli* (10).

provement in growth conditions and stresses. Using the model, we explored the fitness dynamics under each environment to reveal different fitness landscapes that are governed by similar dynamics. Biological systems characterized by low cost and moderate gain can support a conditioned response only in highly predictable environments with restricted delay periods (Fig. 3*A*). Additionally, the fitness advantage gained by early preparation is in the order of a few percents in population-size terms. A different picture emerges for stressful environmental setups (Fig. 3 *B* and *C*). A modest stress protective response characterized by a low cost and moderate protection capacity can be selected in a very wide range of tested delay and predictability values. This large subspace indicates that early preparation is expected to be a robust solution, even in a noisy environment (featuring varying predictability and delay periods over time). Additionally, the fitness advantage that will be gained under conditioning is larger by one order of magnitude relative to an environment that prepares the organism for an improvement in growth conditions. The complementary stress system explored, characterized by a considerable cost and high protection capacity, revealed that, although the fitness advantage gained by early preparation can reach hundreds of percents, this system is much less robust to a noisy environment because of the high cost.

Rooting our model in the realm of cellular decision making (26), we introduced the flexibility offered by the partial preparation strategy (CR2). Interestingly, we observed that this flexibility considerably increases the subspace of parameters that can select for conditioning (Fig. 4*A*). Indeed, using this expanded model, we predicted that CR2 (and not CR) would be the most advantageous response strategy for the maltose metabolic pathway (Fig. 5). This observation is in agreement with the previous experimentally measured promoter activities of maltose and lactose operons (10). In the future, additional augmentation in model complexity can be obtained. For example, a feed-forward network motif is one potential means to attain a delayed response after encounter with the first stimulus (27), thus circumventing some of the unnecessary costs caused by premature protein production. In fact, our measurements in the *E. coli* maltose system reveal such a potential example. Focusing on the temporal dynamics of maltose operons, induction in response to lactose reveals that they are not induced immediately after lactose exposure but feature a delayed response (see Fig. S1 in ref. 10).

Organisms may have evolved more clever strategies in which the gain is still maximized but the costs are minimized. One such potential solution might be represented by the poised RNA polymerase, which under stress, can be localized and stalled next to promoters of genes such as heat-shock genes (28), perhaps in preparation for stress. The rapid release of stalled polymerase was suggested to facilitate efficient responses to the dynamically changing environment, but it may occur with minimal cost production of stress response genes before stress actually occurs. Future modeling would be needed to assess the costs and benefits associated with such adaptations.

Conditioning does not need to be a deterministic response strategy. Especially in partially predictable environments, microbial population may exercise a bet-hedging strategy (6–8) in which, under an early predictive signal, only a part of the population will be conditioned and another part may remain unaffected. By expanding the model to allow heterogeneity in the population dynamics, it will be possible to clarify which subset of environmental setups selects for a combined strategy and which setups select for a purely conditioned strategy.

Materials and Methods

Strains and Media. *E. coli* MG1655 (*E. coli* Genetic Stock Center) was used for model validation. *E. coli* W2244 (29) was used as a *lacZ* mutant strain (*lacZ39Δ*) to measure additional cost.

Experiments in lactose system were done in M9 medium (M9 salts, 1 mM $MgSO_4$, 0.1 mM CaCl2, 0.05% casamino acids, 5 ng/mL thiamine) supplemented with 0.1% glycerol (Baker). Lactose (10 mM; Fluka) and IPTG (0.15 mM; Sigma) were added similarly to the method used in ref. 19. Heat-shock experiments were done in rich media (LB).

Population-Size Measurement. Population size was measured similarly to the method used in ref. 19, and experimental setup is shown in Fig. 1. In the lactose system, overnight cultures, diluted into fresh media, were grown for 1 h at 37 °C and were then transferred to a 96-well plate for IPTG and lactose treatment. Population size was monitored using a multiwell spectrophotometer at 595 nm (GENios; TECAN). The relative fitness of early preparation was taken as the OD ratio of the conditioned culture over unconditioned control culture 1.5 h after the addition of lactose. In the heat-shock system, overnight cultures, diluted into fresh media, were grown for 1 h at 37 °C. Conditioned cultures were transferred to 45 °C to invoke autoprotection. All cultures were challenged by a 52 °C water bath for 2 min and plated in 96-well plate for growth. The relative fitness of early preparation was taken as the OD ratio of the conditioned culture over the unconditioned control culture 1.5 h after the severe heat shock.

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