



Adaptive evolution by optimizing expression levels in different environments

M. Madan Babu and L. Aravind

National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD 20894, USA

Organisms adapt to environmental changes through the fixation of mutations that enhance reproductive success. A recent study by Dekel and Alon demonstrated that *Escherichia coli* adapts to different growth conditions by fine-tuning protein levels, as predicted by a simple cost-benefit model. A study by Fong *et al.* showed that independent evolutionary trajectories lead to similar adaptive endpoints. Initial mutations on the path to adaptation altered the mRNA levels of numerous genes. Subsequent optimization through compensatory mutations restored the expression of most genes to baseline levels, except for a small set that retained differential levels of expression. These studies clarify how adaptation could occur by the alteration of gene expression.

Environmental change and adaptation

All organisms actively maintain homeostasis despite living in changing environments. However, this equilibrium can break down if organisms encounter challenges that exceed their innate adaptations to cope with atypical conditions [1]. Nevertheless, some individuals in a population might have particular genetic mutations that enable them to survive in unfamiliar environmental conditions. Accordingly, these mutants would have a higher reproductive success (i.e. a greater fitness) than would individuals without these mutations, resulting in a higher frequency of the mutations in the surviving gene pool. Thus, the process of natural selection drives organisms to adapt better to new and changing environmental conditions.

Adaptive mutations can alter a wide range of physiological processes. For example, adaptation to a new toxic compound in the environment could arise because of mutations that cause: (i) overproduction of a protein that neutralizes the effect of the compound; (ii) an increase in the efficiency of a transporter that removes the compound from the cell; or (iii) an increase in the catalytic efficiency of the enzyme that processes the toxin. Hence, to understand how organisms adapt, one must identify the underlying mutations and determine how they might confer reproductive success.

The bacterial model of adaptive evolution

Bacterial models have been crucial for the establishment of the neo-darwinian paradigm of evolution. The advantages of easy cultivation in well-defined conditions, short generation

times and viability of frozen stocks mean that bacteria can be used to track and compare changes in fitness between ancestor and descendent strains [2]. Importantly, bacterial models can be used to investigate directly the evolution of organisms that are exposed to environmental changes. This can be done by subjecting a stock culture of bacteria to a range of new test environments to isolate survivors and identify the molecular changes that have enabled them to adapt to the new environment.

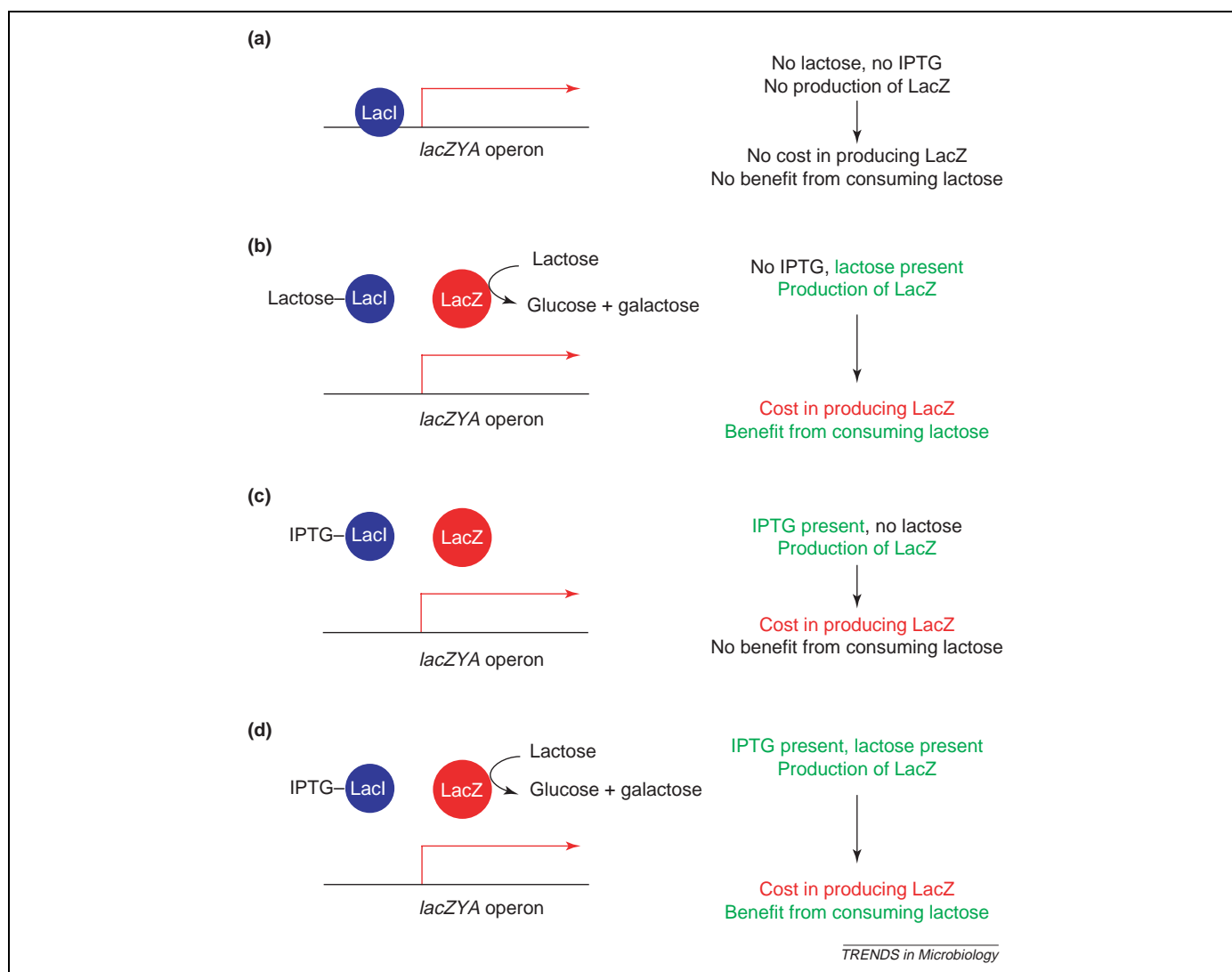
Bacteria can adapt by optimizing protein expression levels

Previous work has shown that adaptive mutations typically alter the physicochemical properties of proteins [3]. Several studies have also shown that mutations affecting the amount of protein expressed are an alternative adaptive mechanism [2,4,5]. However, the biological consequences of this mechanism were not well understood until Dekel and Alon [6] recently provided a quantitative model of the mechanism. The authors demonstrated the role of alterations in protein production by measuring the costs and benefits to bacteria of expressing proteins at varying levels in different environments. This work focused on the use of lactose as an energy source by *Escherichia coli* by monitoring the expression levels of the β -galactosidase enzyme (LacZ), which hydrolyzes lactose into glucose and galactose (Figure 1). The study proceeded in three stages (Figure 2).

Stage 1: measuring the cost and benefit of expressing proteins

Dekel and Alon estimated the cost to *E. coli* of producing LacZ when no lactose was present in the medium. This cost, arising from the production of a superfluous protein, draws cellular resources away from essential functions, thereby reducing growth rates. Cost was measured as the change in growth rate of cells concomitant with the induction of varying levels of LacZ, caused by systematically changing the concentration of isopropyl- β -D-thiogalactopyranoside (IPTG) – a constitutive LacZ inducer. By contrast, the production of sufficient amounts of protein to assimilate a substrate, when available, drives growth and provides a benefit to the organism. Thus, the benefit to *E. coli* of expressing LacZ was measured as the increase in growth rate in response to systematic changes in lactose concentration in the medium. High levels of LacZ expression were ensured by using saturating levels of the LacZ inducer IPTG (Figure 2a).

Corresponding author: Babu, M.M. (madanm@ncbi.nlm.nih.gov).



TRENDS in Microbiology

Figure 1. Regulation of the *lacZYA* operon. **(a)** In the absence of lactose or IPTG, the LacI repressor protein (blue) binds to the promoter and represses expression of the *lacZYA* operon (red arrow). **(b)** When lactose is present in the medium, small amounts of the carbohydrate bind to the LacI repressor protein and release it from the promoter. This enables expression of the LacZ enzyme (red circle), which converts lactose to glucose and galactose to derive energy, reflected in the increase in growth rate of cells. **(c)** In the presence of IPTG, a constitutive inducer that cannot be metabolized by LacZ, the LacI repressor binds to IPTG and is released from the promoter. Energy is spent producing LacZ, with no benefit because lactose is absent from the environment. This is reflected in the decrease in cell growth rate. **(d)** In the presence of IPTG and lactose, energy is spent producing LacZ and, simultaneously, energy is gained from using lactose in proportion to its availability in the environment. In such instances, producing a sufficient amount of enzyme derives maximum energy and drives growth. No change, no product and no benefit denoted by black text; change, presence of product and benefit incurred denoted by green text; cost incurred denoted by red text.

Stage 2: optimizing the cost–benefit function to predict optimum expression levels

Having calculated the cost and benefit to *E. coli* of expressing LacZ in varying concentrations of lactose, the authors calculated the optimum expression level of LacZ that would maximize the growth function for environments containing low, medium or high lactose concentrations (Figure 2b). The growth function is the difference between the cost and the benefit of expressing LacZ in a given lactose environment.

Stage 3: bacteria evolve and adapt by changing protein expression levels

Finally, Dekel and Alon carried out adaptive evolution experiments in which *E. coli* cells were cultured in different conditions (low, medium or high concentrations of lactose) over many generations. The authors found that, after ~500 generations, cells evolved to reach a

state in which they expressed LacZ at levels that were predicted by the cost–benefit optimization model. Cells grown in low lactose concentrations ceased *lacZ* expression by introducing a deletion near the promoter. However, cells grown in medium lactose concentrations expressed moderate amounts of the enzyme, and cells grown in high lactose concentrations evolved to express high levels of LacZ (Figure 1c). These mutations occurred within the coding region and resulted in silent or conservative changes to LacZ, implying that the mutations altered mRNA or protein stability or modified the rate of translation.

For understanding the diversification of protein expression levels, this study established the use of simple econometric principles, which have proved useful elsewhere in evolutionary biology. Thus, bacterial cells adapt by rapidly (within ~500 generations in 30–35 min) optimizing protein expression levels to maximize growth.

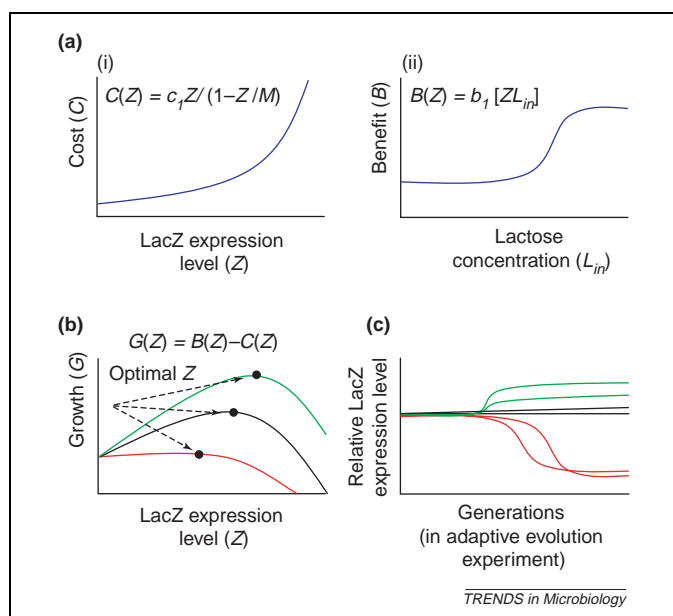


Figure 2. Use of the cost–benefit function to predict optimal LacZ expression level. (a) Cost, $C(Z)$, and benefit, $B(Z)$, functions of LacZ expression (Z) in *Escherichia coli* cells. (i) Cost of LacZ expression is measured as the reduction in relative growth rate with respect to wild-type cells when varying amounts of LacZ (variable Z) are expressed in the absence of lactose and with variable amounts of IPTG. (ii) Benefit of LacZ expression is measured as the increase in the relative growth rate of *E. coli* cells that express maximal levels of LacZ (constant Z) when grown in varying amounts of lactose and with saturating amounts of IPTG. Forms of the best-fit equations of the observed experimental data for the cost and benefit functions are shown. M , c_1 and b_1 are constants estimated from experimental observations. (b) Prediction of optimal LacZ level with the predicted relative growth rate difference, $G(Z)$, as a function of LacZ expression. Optimization of the cost–benefit function predicts that, for each environment with varying amounts of lactose, there is an optimum level of LacZ expression that maximizes growth. Low lactose concentration shown in red, medium lactose concentration shown in black and high lactose concentration shown in green. (c) Observed levels of LacZ expression in adaptive evolution experiments over 500 generations. *E. coli* cells were cultured to enable adaptation to different environments of lactose concentration. Cells accumulated mutations that abolished or decreased LacZ expression in an environment with a low lactose concentration (red), showed similar levels of LacZ expression to wild-type cells in an environment with a medium lactose concentration (black), or increased LacZ expression in an environment with a high lactose concentration (green), as predicted by the cost–benefit optimization model of Dekel and Alon [6].

Many paths lead to effectively similar adaptive endpoints

A recent study by Fong *et al.* [7] helped to clarify the sequence of changes that leads to environmental adaptation. Upon transferring fresh *E. coli* cells to multiple parallel replicates of lactate or glycerol media, the authors used microarrays to monitor mRNA expression profiles to track the changes in *E. coli* cells during the course of adaptation to their new environment. The organism was considered to have adapted to the new medium if its growth rate was stable in successive generations. It was observed that: (i) initially, numerous genes were differentially expressed, suggesting that the first fixed mutations affected global regulatory processes; (ii) several subsequent compensatory mutations restored the expression of most genes to baseline level, leaving only a few genes with altered expression that were essential for adaptation; and (iii) endpoints that were similarly adapted could result from the alteration of expression of different gene sets. This study illustrates that mutations with generic effects on gene expression are more likely to occur than are specific mutations in a single pathway that lead to a tailor-made

adaptation. Whereas pleiotropic mutations cause widespread alterations of gene expression, it is possible that the inherent robustness of regulatory networks provides a buffer for survival, thereby enabling a rapid coarse adaptation followed by fine-tuning through compensatory mutations.

Practical implications and future directions

In another study, Fong *et al.* [8] showed that strains with different genetic backgrounds can evolve to optimize growth and maximize lactic acid production. The authors used the metabolic network of *E. coli* to predict and create knockout strains that resulted in high levels of lactate production. These mutants were cultured in a constant environment that enabled them to adapt to optimize growth, coupled with maximum levels of lactate production. In this context, the study by Dekel and Alon [6] might enable an objective calculation of growth functions (e.g. for engineered mutants) to optimize growth for maximal production of a given biomolecule. Alternatively, the application of such studies to understand changes in protein expression during the adaptation of bacterial pathogens to host niches could aid the design of therapeutics.

The monitoring of adaptive changes in gene expression using microarrays [7–9] could also be extended to more-complex organisms such as humans to investigate geographically distinct populations that have adapted to local environmental and pathogenic challenges over varying time periods. For example, such studies could elucidate the role of subtle evolutionary optimizations of gene expression that might underlie conditions such as diabetes and obesity. Thus, genome-scale analyses of gene expression help the understanding of evolution in real time.

Acknowledgements

We gratefully acknowledge the intramural research program of the National Institutes of Health for funding our research. We thank S. Balaji for useful comments about the manuscript.

References

- Smith, J.M. (2000) *The Theory of Evolution*, 3rd edn, Cambridge University Press
- Elena, S.F. and Lenski, R.E. (2003) Evolution experiments with microorganisms: the dynamics and genetic bases of adaptation. *Nat. Rev. Genet.* 4, 457–469
- Hall, B.G. (1983) Evolution of new metabolic functions in laboratory organisms. In *Evolution of Genes and Proteins* (Nei, M. and Koehn, R.K., eds), pp. 234–257, Sinauer Associates
- Ibarra, R.U. *et al.* (2002) *Escherichia coli* K-12 undergoes adaptive evolution to achieve *in silico* predicted optimal growth. *Nature* 420, 186–189
- Orr, H.A. (2005) The genetic theory of adaptation: a brief history. *Nat. Rev. Genet.* 6, 119–127
- Dekel, E. and Alon, U. (2005) Optimality and evolutionary tuning of the expression level of a protein. *Nature* 436, 588–592
- Fong, S.S. *et al.* (2005) Parallel adaptive evolution cultures of *Escherichia coli* lead to convergent growth phenotypes with different gene expression states. *Genome Res.* 15, 1365–1372
- Fong, S.S. *et al.* (2005) *In silico* design and adaptive evolution of *Escherichia coli* for production of lactic acid. *Biotechnol. Bioeng.* 91, 643–648
- Ferea, T.L. *et al.* (1999) Systematic changes in gene expression patterns following adaptive evolution in yeast. *Proc. Natl. Acad. Sci. U. S. A.* 96, 9721–9726