

- Seth, R.B., Sun, L., Ea, C.K., and Chen, Z.J. (2005). *Cell* 122, 669–682.
- Si, K., Giustetto, M., Etkin, A., Hsu, R., Janisiewicz, A.M., Miniaci, M.C., Kim, J.H., Zhu, H., and Kandel, E.R. (2003). *Cell* 115, 893–904.
- Si, K., Choi, Y.B., White-Grindley, E., Majumdar, A., and Kandel, E.R. (2010). *Cell* 140, 421–435.
- Skaug, B., Jiang, X., and Chen, Z.J. (2009). *Annu. Rev. Biochem.* 78, 769–796.
- Zeng, W., Xu, M., Liu, S., Sun, L., and Chen, Z.J. (2009). *Mol. Cell* 36, 315–325.
- Zeng, W., Sun, L., Jiang, X., Chen, X., Hou, F., Adhikari, A., Xu, M., and Chen, Z.J. (2010). *Cell* 141, 315–330.

# Regulatory Revolution: Evolving the “Anti-LacI” Repressor

Christopher J. Marx<sup>1,2,\*</sup>

<sup>1</sup>Department of Organismic and Evolutionary Biology

<sup>2</sup>Faculty of Arts and Sciences Center for Systems Biology

Harvard University, 16 Divinity Avenue, Cambridge, MA 02138, USA

\*Correspondence: cmarx@oeb.harvard.edu

DOI 10.1016/j.cell.2011.07.015

Much of adaptation is based upon changes in gene expression, but the emergence of new regulatory logic has not been observed directly. Now, Poelwijk et al. report evolving the *lac* repressor (LacI) to reverse its regulatory logic, resulting in an “anti-LacI” that represses transcription when bound to its “inducer.”

From the origin of new body-plans (Carroll, 2008) to beneficial changes in beak morphology (Mallarino et al., 2011), the evolution of gene regulation is critical in adaptation over many timescales. Even when populations of microbes adapt in the laboratory, promoters and regulatory proteins are disproportionately recovered as targets of beneficial mutations (Barrick et al., 2009). The selective advantage of experimentally evolved changes in expression has typically arisen from altered levels of the affected gene products, but what about the evolution of new regulatory logic itself?

Most laboratory experiments use a constant environment for selection, and thus, developing new responses to changes in the environment tend not to be particularly advantageous. Furthermore, it is unclear how many mutations, perhaps acting in concert, are needed to change the qualitative properties of a gene regulatory system. In this issue, Poelwijk et al. present a tour de force study in which they use an elegant synthetic system to apply alternating selective pressures on a genetic module of *Escherichia*

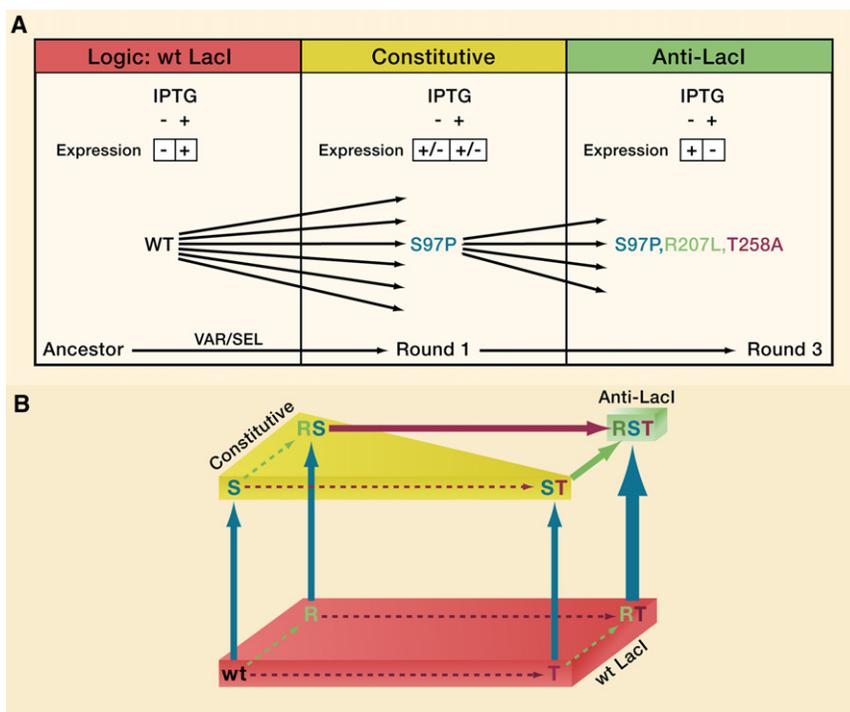
*coli* (Poelwijk et al., 2011). In the end, the authors coerce the *E. coli* lactose repressor (encoded by *lacI*) to have exactly the opposite logic as it does naturally.

The *E. coli* lactose repressor, encoded by the *lacI* gene, is one of the best understood transcriptional regulators. It binds the *lac* operator and represses transcriptional initiation in the absence of its inducer such as allolactose or its analog isopropyl  $\beta$ -D-1-thiogalactopyranoside (IPTG), but once LacI binds IPTG, the repressor releases from the operator (Figure 1A).

In order to select for the opposite response, that is repression in the presence of IPTG and lack thereof in its absence, Poelwijk et al. construct an operon with the *lac* operator controlling two genes that permit expression to be rewarded (by resistance to the antibiotic chloramphenicol) or punished (by sensitivity to sucrose) depending upon which chemical is added to the medium. Poelwijk et al. also include in the operon the reporter gene *lacZ*, which encodes  $\beta$ -galactosidase, to quantify total expression of the operon.

From here, the strategy is simple. First, iteratively introduce a hearty dose of variation specifically in *lacI* by PCR mutagenesis. Next, select upon this variation by alternating periods of growth in medium supplemented with sucrose and IPTG with growth in medium with chloramphenicol and not IPTG (i.e., to select for the opposite logic). Then, repeat.

What are the chances that LacI would readily reverse its response to inducer and become the “anti-LacI”? After the first round of variation and selection, the results did not seem promising. In fact, the evolution appears to reach a roadblock. LacI variants that abandon the wild-type logic (which led to maximal punishment emerged) exhibit no logic at all; they simply allow constitutive expression of the downstream genes, completely independent of IPTG (Figure 1A). After the next round, however, Poelwijk and colleagues find variants that perform the desired inverse logic, and these variants dominate after the final, third round. The authors then carefully analyze the genetic basis of this adaptation. Reversing the logic of the Lac repressor is indeed



**Figure 1. Constraints and Epistatic Interactions Involved in Evolving the Anti-LacI**

(A) After introducing variation into the *lac* repressor (LacI) and alternately selecting upon two markers expressed from the *lac* operator in a manner opposite to wild-type (WT) function, an “anti-LacI” protein emerged after the third round that is capable of this inverted regulatory logic. After the first round, variants simply had constitutive expression; however, apparent constraints limited later clones to descend primarily from one particular variant (serine 97 to proline), which enabled later changes to the anti-LacI phenotype.

(B) Epistatic interactions between the three mutations that are collectively sufficient for anti-LacI function. Beneficial mutations are indicated by solid arrows (dashed arrows are neutral mutations) transitioning to a different neutral network of genotypes with equivalent phenotype. Only “S” (serine 97 to proline) is beneficial on all backgrounds, whereas “R” (arginine 207 to leucine) and “T” (threonine 258 to alanine) are beneficial only in concert and even then only when S is also present.

an incredible result, and it will be exciting to see which aspects of this study will be repeatable.

Why did the progress toward the inverted logic get “stuck,” albeit temporarily, at the beginning of the evolution? When Poelwijk and colleagues sequence *lacI* variants from the final population of cells, they find that most of them share one particular mutation, a serine 97 converted to a proline, suggesting that the cells share a common ancestry. Interestingly, this mutation alone simply confers constitutive expression of the downstream genes. For the emergence of the anti-LacI activity, it turns out that interactions between beneficial mutations are essential.

The role of epistasis (i.e., how the phenotypic consequence of a mutation depends upon the presence of other

mutations) in adaptation has been addressed in numerous systems recently, ranging in scope from individual genes to entire physiological networks, and these different systems reveal quite different patterns. The now classic example in an individual protein is the analysis of antibiotic resistance (i.e., cefotaxime resistance) in *E. coli*. Five mutations in the  $\beta$ -lactamase gene increased bacterial resistance by  $\sim 100,000$ -fold (Weinreich et al., 2006), but each mutation’s contribution was rather violently affected by which other mutations were present. This resulted in a rugged adaptive landscape that blocks most possible paths to the fittest allele.

In contrast, two recent studies (Chou et al., 2011; Khan et al., 2011) found that epistatic interactions between beneficial mutations in multiple genes were dramati-

cally more gentle than those within genes. Instead of intense, erratic interactions, there was a general trend of diminishing returns; the same mutation was less beneficial as the overall fitness increased. On this smooth adaptive landscape, every possible trajectory to maximal fitness would be selectively accessible. However, epistasis contributed substantially to the deceleration of adaptation.

In the context of these recent reports, the study by Poelwijk and colleagues represents an interesting intermediate case in which the mutations occur within a single protein, LacI, but selection acts upon the “functional” proteins that it regulates (Figure 1B). As with  $\beta$ -lactamase, the observed interactions are quite strong. However, unlike with  $\beta$ -lactamase, all trajectories to the most-fit genotype of LacI require at least one mutational step that is neutral (i.e., that doesn’t change the regulatory logic) but that is ultimately necessary for a synergy with mutations later in the evolutionary process.

This contingency of a complex phenotype upon earlier, enabling mutations draws parallels to another study examining the evolution of *E. coli* populations to metabolize citrate, which was added as an “inert” chelator in glucose minimal medium (Blount et al., 2008). Ultimately, through replay experiments, the authors found that the much-delayed evolution of the phenotype was contingent on the particular history of that population.

As discussed recently by Draghi et al. (2010) and Hayden et al. (2011), this study and the one now presented by Poelwijk and colleagues provide examples in which variation within a neutral network of genotypes, all with equivalent phenotypes, is critical to finding a region in that network that could access more fit phenotypes. The observation of a barrier to adaptation, or a “constraint,” thus appears to have followed directly from the fact that only one (or a few) mutations could enable the needed switch in logic and that to reach this logic requires a pair of subsequent mutations that are individually neutral. This challenge for adaptation is perhaps best thought of as a “statistical constraint,” rather than some immutable consequence of physical laws. The desired change in correlation between phenotypes—that is, LacI simultaneously binding to IPTG and to

the *lac* operator—is possible but simply rare in genotype space and requires multiple mutations.

Finally, the study by Poelwijk and colleagues demonstrates a remarkable case in which it is possible to predict where adaptation was going, but not whether it could get there. By parameterizing chloramphenicol and sucrose action with initial growth experiments, the authors develop models that calculate the fitness of different expression phenotypes in the face of each of these stresses. The resulting adaptive landscape across phenotypic coordinates correctly predicts the optimal phenotype that is present after the final round of selection. Indeed, this ability to translate from protein phenotypes to fitness is a fantastic success. Unfortunately though, it also reveals the remaining and rather daunting challenge of mapping mutations to the protein phenotypes themselves. Until

this critical connection is illuminated, biologists will struggle to predict constraints (absolute or statistical) in the evolution of proteins and will question whether protein phenotypes can change via large jumps even when a continuous (but nonlinear) fitness landscape acts upon their traits. Thus, we are still constrained to find empirical examples of epistatic interactions within proteins rather than broad principles.

#### ACKNOWLEDGMENTS

My apologies to the many authors I was unable to reference due to length restrictions. C.J.M. is supported by the NIH (R01 GM078209) and NSF (DEB-0845893, EAR-1024723).

#### REFERENCES

- Barrick, J.E., Yu, D.S., Yoon, S.H., Jeong, H., Oh, T.K., Schneider, D., Lenski, R.E., and Kim, J.F. (2009). *Nature* 461, 1243–1247.
- Blount, Z.D., Borland, C.Z., and Lenski, R.E. (2008). *Proc. Natl. Acad. Sci. USA* 105, 7899–7906.
- Carroll, S.B. (2008). *Cell* 134, 25–36.
- Chou, H.H., Chiu, H.C., Delaney, N.F., Segrè, D., and Marx, C.J. (2011). *Science* 332, 1190–1192.
- Draghi, J.A., Parsons, T.L., Wagner, G.P., and Plotkin, J.B. (2010). *Nature* 463, 353–355.
- Hayden, E.J., Ferrada, E., and Wagner, A. (2011). *Nature* 474, 92–95.
- Khan, A.I., Dinh, D.M., Schneider, D., Lenski, R.E., and Cooper, T.F. (2011). *Science* 332, 1193–1196.
- Mallarino, R., Grant, P.R., Grant, B.R., Herrel, A., Kuo, W.P., and Abzhanov, A. (2011). *Proc. Natl. Acad. Sci. USA* 108, 4057–4062.
- Poelwijk, F.J., de Vos, M.G.J., and Tans, S.J. (2011). *Cell* 146, this issue, 462–470.
- Weinreich, D.M., Delaney, N.F., Depristo, M.A., and Hartl, D.L. (2006). *Science* 312, 111–114.