

Negative Feedback in Genetic Circuits Confers Evolutionary Resilience and Capacitance

David C. Marciano,^{1,*} Rhonald C. Lua,¹ Panagiotis Katsonis,¹ Shivas R. Amin,^{1,2} Christophe Herman,¹ and Olivier Lichtarge^{1,3,4,*}

¹Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA

²Biology Department, University of St. Thomas, Houston, TX 77006, USA

³Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX 77030, USA

⁴Computational and Integrative Biomedical Research Center, Baylor College of Medicine, Houston, TX 77030, USA

*Correspondence: david.marciano@bcm.edu (D.C.M.), lichtarge@bcm.edu (O.L.)

<http://dx.doi.org/10.1016/j.celrep.2014.05.018>

This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/3.0/>).

SUMMARY

Natural selection for specific functions places limits upon the amino acid substitutions a protein can accept. Mechanisms that expand the range of tolerable amino acid substitutions include chaperones that can rescue destabilized proteins and additional stability-enhancing substitutions. Here, we present an alternative mechanism that is simple and uses a frequently encountered network motif. Computational and experimental evidence shows that the self-correcting, negative-feedback gene regulation motif increases repressor expression in response to deleterious mutations and thereby precisely restores repression of a target gene. Furthermore, this ability to rescue repressor function is observable across the Eubacteria kingdom through the greater accumulation of amino acid substitutions in negative-feedback transcription factors compared to genes they control. We propose that negative feedback represents a self-contained genetic canalization mechanism that preserves phenotype while permitting access to a wider range of functional genotypes.

INTRODUCTION

While a DNA mutation represents a relatively long-lasting source of variation, a cell also faces more transient variations due to stochastic fluctuations of individual components in protein networks and transcriptional systems. One means of maintaining stability of the transcriptional system output involves a negative-feedback loop, in which a transcriptional repressor binds its own promoter to control its expression. When repressor levels fall, a concomitant increase in expression from the repressor's promoter restores repressor levels. This simple genetic circuit enables stable expression levels despite fluctuations in the repressor's gene copy number, cellular RNA polymerase activity, the repressor's mRNA levels, and the cellular concentration of the repressor protein (Raj and van Oudenaarden, 2008; Becskei

and Serrano, 2000; Thattai and van Oudenaarden, 2001). Other important characteristics of the negative-feedback motif include the potential to generate oscillations in gene expression (Elowitz and Leibler, 2000; Levine et al., 2013), the ability to both speed and linearize the response of gene expression to inducing signal (Rosenfeld et al., 2002; Nevozhay et al., 2009, 2013), and even the capacity to increase output variability under certain conditions (Singh and Hespanha, 2009; Toni and Tidor, 2013). Each of these characteristics may contribute to the prevalence of the negative-feedback architecture in over 40% of *Escherichia coli* transcription factors (Rosenfeld et al., 2002; Thieffry et al., 1998).

Initial theoretical work broadly suggested negative feedback could provide robustness to multiple sources of system variation, including nonlethal mutations (Barkai and Leibler, 1997; Hlavacek and Savageau, 1995; Savageau, 1974). However, nearly all the subsequent theoretical and experimental investigations focus upon negative feedback's dynamic properties within the cell without examining its effect upon mutational robustness. More recently, negative feedback was experimentally shown, in *Saccharomyces cerevisiae*, to reduce the variation in gene expression levels after the system-wide introduction of genomic mutations (Denby et al., 2012). However, it is unknown what role negative feedback has upon the capacity of an individual transcriptional circuit or transcription factor to tolerate variation in the form of amino acid changes. Fundamental understanding of transcription-factor robustness to mutation is of particular importance because complex transcription factor repertoires have recently been implicated in the transition from single cellular life to complex multicellular lineages that have embryonic development (de Mendoza et al., 2013).

Here, we demonstrate that a negative-feedback loop buffers against mutations that would otherwise be deleterious to the transcription factor. This simple, frequently encountered network motif can thereby expand the range of substitutions a protein can tolerate while maintaining cellular function. This may give negative-feedback transcription factors a greater capacity for the storage of silenced mutations that can be unleashed during times of stress as observed with proteins that are clients of the molecular chaperone Hsp90 (Jarosz and Lindquist, 2010).

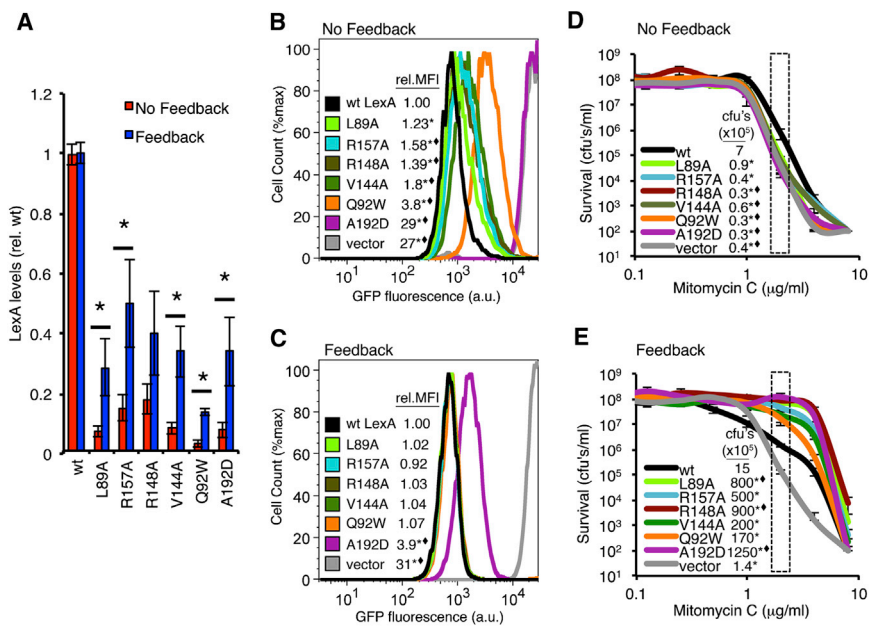


Figure 1. Negative Feedback Diminishes the Deleterious Effects of Mutations

P_{trc} (No Feedback) or the native *P_{lexA}* (Feedback) drive expression of LexA and mutants thereof.

(A) Semiquantitative western blot data of steady-state LexA expression levels. Mean values are relative to isogenic plasmid encoding wild-type LexA. Significance based on t test: *p < 0.05. Error bars represent SEM of four or more biological replicates.

(B) Flow cytometry data showing the distribution of each population's GFP fluorescence (arbitrary units [a.u.]) for the No Feedback constructs. Inset table reports median fluorescence intensity relative to isogenic plasmid encoding wild-type LexA (rel. MFI). Statistics were performed against the rel. MFI of the plasmid-encoded wild-type LexA strain (*p < 0.001 in t test; ♦, p < 0.05 in Dunn's test).

(C) Flow cytometry data as in (B) but with the Feedback constructs.

(D) Survival curves of the No Feedback constructs determined from the number of colony-forming units (cfu) able to form on LB-agar plates containing increasing concentrations of the DNA-damaging agent mitomycin C. Inset table

corresponds to mean survival (cfu/ml, × 10⁵) at 2 µg/ml (dashed box). Statistics were performed against isogenic plasmid encoding wild-type *P_{trc}-lexA* (*p < 0.05 in t test; ♦, p < 0.05 in Dunn's test). Error bars represent SEM of at least seven replicates.

(E) Mitomycin C survival curves for the Feedback constructs. Statistics were performed against isogenic plasmid encoding wild-type *P_{lexA}-lexA* (*p < 0.05 in t test; ♦, p < 0.05 in Dunn's test). Error bars represent SEM of six or more replicates.

See also Figure S1 and Table S1.

RESULTS

Assaying the Effect of Negative Feedback on Destabilized LexA Mutants

LexA represses over 40 genes whose activation constitutes the SOS response to damaged DNA (Friedberg et al., 2006). Additionally, LexA represses its own expression and therefore is an example of a direct negative-feedback loop. In order to test our hypothesis that negative feedback provides robustness to mutation, we generated LexA mutants that increase degradation rate (Figures S1A–S1C and Supplemental Results). Next, we cloned our set of LexA mutants behind either a *P_{trc}* promoter that lacks negative feedback or the native *P_{lexA}* promoter containing two LexA binding sites (Lewis et al., 1994). These constructs were then transformed into LexA-deficient *E. coli*. In agreement with our hypothesis, the negative-feedback promoter rescues protein levels of all the destabilized LexA mutants (Figures 1A and S1D). This indicates that autoregulatory negative feedback can compensate for a mutation-driven reduction in LexA levels by dynamically responding with an increase in LexA synthesis. Thus, negative feedback provides mutational robustness to substitutions that destabilize the LexA repressor.

We also examined the consequence of the repressor's mutational robustness upon a downstream gene targeted by LexA for repression. To do so, we used flow cytometry to monitor GFP expression from a chromosomally encoded *P_{sulA}-gfp* reporter that is repressed by LexA (Pennington and Rosenberg, 2007). In the absence of feedback, the median fluorescence of the destabilized LexA mutants increases significantly and to varied degrees (Figure 1B; Table S1). In stark contrast, repressor

function is restored under the control of the negative-feedback promoter and the fluorescence of the L89A, R157A, R148A, V144A, and Q92W mutants is indistinguishable from wild-type LexA (Figure 1C). Even the severely impaired A192D substitution, which was equivalent to the empty vector control in the *P_{trc}* construct, is partially restored for repressor function in the presence of a negative-feedback loop. Overall, the *gfp* reporter's experimental data represent the increased mutational robustness provided by negative feedback for mutations that are affecting LexA turnover. These results show that the architecture of the gene's transcriptional circuit can dramatically influence the phenotypic consequence of repressor mutations.

Although control of the *sulA* promoter is essential to *E. coli* survival (Li et al., 2010), LexA has a broader contribution in orchestrating the SOS response to DNA damage (Friedberg et al., 2006). Therefore, we examined the resistance level of our mutants to the DNA-damaging agent mitomycin C. In the absence of feedback, each of the mutants with compromised cellular expression levels display kill curves that nearly overlap the empty vector control and have a decrease in survival at 2 µg/ml mitomycin C (Figure 1D). Based upon each of the previous experiments, we anticipated that negative feedback would be able to shift survival of the mutants back to levels observed with plasmid-encoded wild-type LexA. Surprisingly, when the system includes negative feedback, several of the mutants with increased in vivo degradation actually displayed markedly higher mitomycin C resistance levels than plasmid-encoded wild-type LexA (Figure 1E). This observed phenomenon could relate to mathematical analysis indicating that faster degradation rates of a negative-feedback repressor will shorten the response

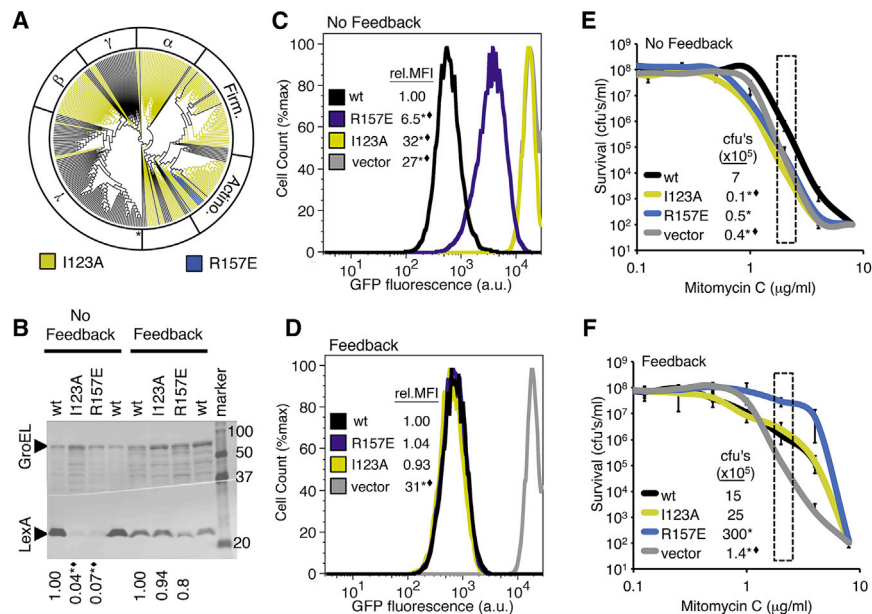


Figure 2. Transit to Distant Branches of LexA Family Sequence Space Requires Negative Feedback

(A) LexA family tree with highlighted branches containing the R157E (indigo) or I123A (gold) substitutions relative to *E. coli* LexA sequence (asterisk). Outer ring labels correspond to branches of Firmicutes (Firm.) and Actinobacteria (Actino.) phyla with the Proteobacteria phylum further divided into classes (gamma, beta, and alpha).

(B) Western blot of soluble, whole-cell lysate from the indicated LexA mutants. Blots were cut at ~37 kDa and probed separately with either anti-GroEL (top), to determine differences in loading density) or LexA antibody (bottom). Mean densitometry values relative to the corresponding wild-type LexA plasmids are reported below the blots. Statistics were performed against the isogenic plasmid encoding wild-type LexA (* $p < 0.001$ in t test; \blacklozenge , $p < 0.05$ in Dunn's test) across at least four biological replicates.

(C) Flow cytometry data showing fluorescence intensity (arbitrary units [a.u.]) of the wild-type, mutant, or isogenic empty vector populations without feedback. Inset table reports median

fluorescence intensity relative to isogenic plasmid encoding wild-type LexA (rel. MFI). Statistics were performed against the rel. MFI of the plasmid-encoded wild-type LexA strain (* $p < 0.001$ in t test; \blacklozenge , $p < 0.05$ in Dunn's test).

(D) Flow cytometry data as in (C) but with the negative-feedback constructs.

(E) Survival curves for each P_{trc} -LexA construct against the DNA-damaging agent mitomycin C with inset tables reporting the mean surviving colony-forming units (cfu/ml, $\times 10^5$) observed in the boxed region (2 μ g/ml). Statistics were performed against the isogenic plasmid encoding wild-type LexA (* $p < 0.05$ in t test; \blacklozenge , $p < 0.05$ in Dunn's test). Error bars represent SEM of six or more replicates.

(F) Survival curves as in (E) but with the negative-feedback constructs.

See also Figure S2 and Table S2.

time to an inducing signal (Rosenfeld et al., 2002). The faster turnover of these LexA mutants may tune the system to more quickly respond to DNA damage.

Role of Negative Feedback in Facilitating Sequence Divergence of the LexA Family

The LexA substitutions discussed so far are either absent or very rarely occur in its homologous sequences. In order to determine if negative feedback could act as a bridge to distant sequences in the LexA family, we made additional mutants that substitute the native residue with one occurring in another branch of the LexA family tree. The R157E substitution is present in a small branch of Gram-positive Actinobacteria, while the I123A substitution occurs frequently outside the γ -proteobacteria branches (Figure 2A). These positions were shown to be under selection and predicted to have epistatic interactions with other residues in LexA (Wilkins et al., 2013). R157 was also suggested to contribute to RecA-mediated LexA autoproteolysis (Kovačić et al., 2013; Wilkins et al., 2013). In *E. coli*, both substitutions increase degradation as shown by reduced steady-state expression levels (Figure 2B); however, negative feedback restores expression to levels indistinguishable from the plasmid-encoded wild-type control. Likewise, interrogating the *suIA-gfp* reporter with flow cytometry shows negative feedback fully restores repressor function of these mutants (Figures 2C and 2D; Table S2). Finally, in the absence of negative feedback, both the I123A and R157E mutants have reduced survival to DNA dam-

age induced by mitomycin C treatment (Figure 2E). However, in the P_{lexA} negative-feedback construct, survival is either rescued to wild-type levels (I123A) or enhanced (R157E) as seen with the previous LexA mutants (Figure 2F). Although a mathematical model described below predicts negative feedback will partially rescue mutants affecting turnover, the full restoration to wild-type expression levels and repressor function is remarkable (Figure S2). These results suggest the I123A and R157E substitutions may also affect other features of the system such as LexA dimerization, binding to target gene promoters, or, as previously proposed, interaction with RecA (Kovačić et al., 2013; Wilkins et al., 2013).

Modeling the Negative Feedback/Mutational Robustness Relationship

To further support the hypothesis that negative feedback enhances mutational robustness of a repressor, we modeled the transcriptional circuit controlled by LexA in *E. coli*. In this elementary transcriptional circuit (Figure 3A), a destabilizing mutation that increases the rate of LexA turnover reduces the LexA pool. LexA represses its own promoter, and thereby the immediate consequence is increased protein synthesis from the *lexA* gene. The ability of the output LexA protein to govern its own expression is a closed-loop control system that is expected to compensate for mutations that alter parameters of the system. Indeed, negative feedback reduces the sensitivity of LexA levels to changes in either protein removal or synthesis rate when

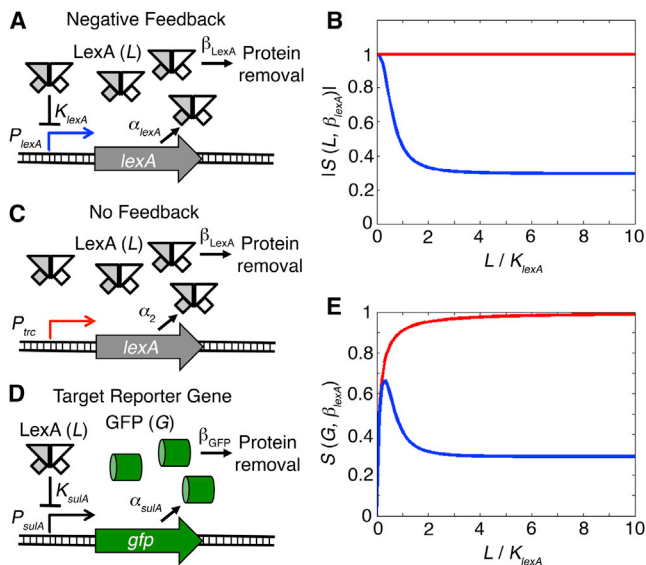


Figure 3. Modeling the Effect of Negative Feedback on Sensitivity to Changing System Parameters

(A) A schematic model for the LexA transcriptional circuit includes LexA levels (L), production rate (α_{lexA}), degradation/dilution rate (β_{LexA}), and repression level (K_{lexA}).

(B) Analytical results for the parameter sensitivity of L with respect to β_{LexA} in the presence (blue line) or absence (red line) of negative feedback showing reduced sensitivity of the negative-feedback system to changes in relative LexA concentrations (L/K_{lexA}).

(C) Schematic model of a LexA circuit without feedback.

(D) Transcriptional circuit of a reporter gene (*gfp*) fused to the *sulA* promoter that is repressed by LexA.

(E) Analytical results for the parameter sensitivity of GFP levels (G) with respect to β_{LexA} in the presence (blue line) or absence (red line) of negative feedback. See also Figure S3 and Table S3.

compared to the system without feedback (Figures 3B and S3A). This is because, in the absence of negative feedback, steady-state levels are directly affected by changes in either protein removal or synthesis rate with no mechanism to modulate the synthesis rate (Figure 3C). Using biologically plausible parameters for L and K_{lexA} (2.2 μ M and 1.1 μ M, respectively; Table S3), we find that the *E. coli* LexA system operates within the regime of robustness to variation around $L/K_{lexA} = 2.0$ (Figure 3B). The sensitivity of GFP expression to changes in LexA degradation rate, synthesis rate, or promoter binding is similarly reduced by negative feedback. (Figures 3D, 3E, and S3B). The model indicates that negative feedback can counteract parameter-changing mutations through reduced sensitivity in LexA protein levels and, consequently, target gene expression levels.

Examining Sequence Divergence Rates of Negative- and Positive-Feedback Transcription Factors

Negative feedback clearly contributes to LexA's tolerance to mutations in our genetic system. However, if this is a general principle, negative feedback transcription factors should be diverging more rapidly than other proteins with a shared evolutionary history. Each negative-feedback transcription factor we analyzed (ChbR, LexA, LysR, Mall, Mlc, and NagC) is more diver-

gent than proteins within their respective gene circuits (Figures 4A–4C and S4A–S4C). We also analyzed the AraC transcription factor that can display either negative feedback or positive feedback for transcription of the *araBAD* operon depending upon the presence of its ligand (Englesberg et al., 1969) but has only negative feedback for its own promoter (Lee et al., 1981; Reeder and Schleif, 1993). AraC is also more divergent than the target genes (Figure 4D). In contrast, the positive-feedback transcription factors CdaR, PhoB, and FucR diverged at an equivalent or lower rate (Figures 4E, 4F, and S4D). We note that the PhoB system may also display negative feedback at high induction levels through phosphatase activity of PhoB's cognate histidine kinase, PhoR, as has been shown with the PhoQ/PhoP two-component system (Ray and Igoshin, 2010; Yeo et al., 2012). Overall, these results suggest a broader, fundamental role of transcriptional circuit control mechanisms in influencing the evolutionary rate of transcription factors.

DISCUSSION

The tolerance of a protein's structure to amino acid substitutions enhances the ability to evolve new functions by allowing it to explore a larger fraction of sequence space that can form a folded protein (Bershtein et al., 2006, 2008; Bloom et al., 2005, 2006; Tokuriki and Tawfik, 2009). Variants that trade stability for new functions are often met with subsequent second-site suppressor mutations that increase protein stability and expression levels while maintaining activity against the introduced substrate (Huang and Palzkill, 1997; Soskine and Tawfik, 2010). However, in contrast to the emergence of mutations that can suppress stability defects, the consequence of negative feedback is both immediate and dynamic. Not only are destabilized mutants instantly compensated for by increased production, but also the production rate is set to specifically address the severity of the mutation. Moderately destabilized mutants are met with a mild increase in production, while highly destabilized mutants are balanced with even higher rates of production. Furthermore, the increased mitomycin C resistance of our destabilized LexA mutants under negative-feedback control supports the assertion that phenotypic robustness can facilitate adaptation to a new condition by allowing a genotype to explore a wider sequence space (Wagner, 2012). Our results also align with the concept of the molecular chaperone, Hsp90, acting as an evolutionary capacitor that permits the accumulation of phenotypically silent mutations that are then unmasked during times of stress (Jarosz and Lindquist, 2010). For LexA, negative feedback reduces the impact of mutations and therefore may allow amino acid changes with cryptic phenotypes to persist until a new selection regime is encountered (in this case, elevated DNA damage levels). Overall, we provide a conceptual model in which network architecture modifies a transcription factor's resiliency toward detrimental mutations and may even functionalize mutations that destabilize the protein.

Initially, one may expect the mutational robustness provided by negative feedback to create a Muller's ratchet effect wherein deleterious mutations accumulate until the buffering capacity is exhausted and the system fails or the repressor becomes highly unstable. However, the immediate rescue of a partially functional

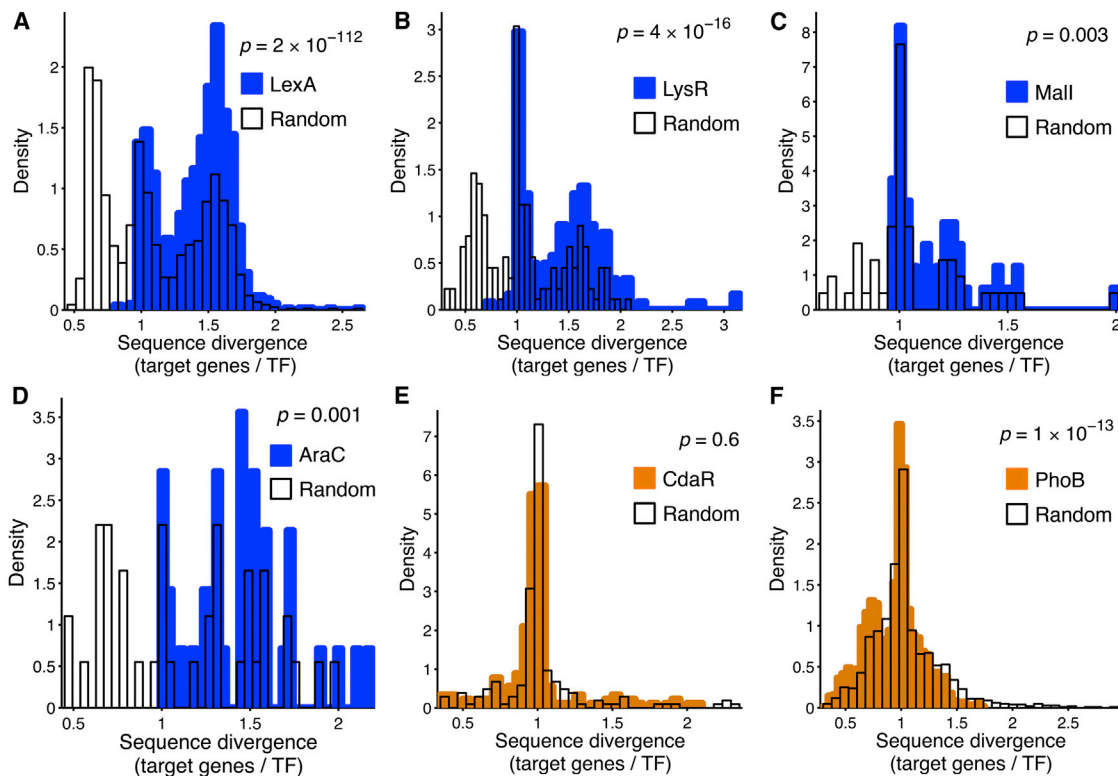


Figure 4. Feedback Influences Transcription-Factor Sequence Variation

Distributions of relative amino acid sequence divergence for prokaryotic negative-feedback (blue) and positive-feedback (orange) transcription factors are shown. Bins >1 contain bacterial transcription-factor (TF) homologs more divergent than their target genes. Hollow histograms correspond to a randomization that redistributes each data set equally to either side of 1. *p* values correspond to a Mann-Whitney *U* test between the sequence divergence data set and the randomized data set. Relative sequence divergences are shown for (A) LexA versus RecA, (B) LysR versus LysA, (C) Mall versus MalXY, (D) AraC versus AraE/AraFGH, (E) CdaR versus GudD/GarLRK/GarD, and (F) PhoB versus PstSCABPhoU. See also [Figure S4](#) and [Table S4](#).

mutant requires elevated protein expression levels of the repressor, which may come with its own fitness cost to the cell. In fact, several gene circuits with artificially elevated protein expression quickly adapt in continuous culture to reinstate an optimum expression that matches the original wild-type expression levels ([Dekel and Alon, 2005](#); [Gao and Stock, 2013](#)). In the case of our destabilized LexA mutants, overexpression may be particularly costly if the mutations potentiate protein aggregation within the cell. Negative feedback may act as a transitory bridge that preserves circuit function until one or more compensatory mutations arise that restore original circuit dynamics. In this respect, negative feedback could serve as a cyclic evolutionary capacitor for gene networks.

Canalization is a term coined by [Waddington \(1942\)](#) to describe the robustness of phenotype to perturbations in environment or genotype. Our results suggest negative feedback serves as a molecular mechanism of genetic canalization. This complements the finding in *Xenopus* embryos that negative feedback can promote developmental canalization by reducing molecular variability and thereby ensuring stable morphology between individuals ([Paulsen et al., 2011](#)). Compared to the canalization mediated by chaperones as observed in multiple eukaryotic species ([Jarosz and Lindquist, 2010](#); [Lempe et al., 2013](#)), variation suppression by negative feedback is self-con-

tained within the gene itself and can be found in replicating systems as simple as bacterial plasmids ([Paulsson and Ehrenberg, 2001](#)).

EXPERIMENTAL PROCEDURES

Sequence Analysis

In addition to a previously reported Q92W mutant that increases LexA turnover ([Smith et al., 1991](#)), we used the evolutionary trace method ([Lichtarge et al., 1996](#); [Madabushi et al., 2002](#); [Mihalek et al., 2004](#)) to identify other LexA mutants that increase in vivo degradation and reduce thermostability in vitro ([Figures S1A–S1C](#) and [Supplemental Results](#)). Sequences used in determining relative divergence distributions were obtained from UniProt ([UniProt Consortium, 2013](#)). We calculate relative divergence as the percent amino acid identity of an organism's target gene divided by the percent amino acid identity of the transcription factor that controls the target gene in the same organism. As a stringent control data set, we randomly inverted this calculation for each pair to redistribute the data to either side of 1. The *E. coli* homolog is the reference sequence for determining percent identity in each case.

Strains

Strains used in this study and the primers used to generate them are detailed in [Supplemental Experimental Procedures](#). With the exception of the BL21(DE3) strain used for LexA purification, all strains have a frameshift mutation in the chromosomal copy of *lexA*.

Mitomycin C Kill Curves

Dilutions of log-phase cultures were spotted onto lysogeny broth (LB)-agar plates containing 0- or 2-fold dilutions of mitomycin C from 8 to 0.03125 $\mu\text{g}/\text{ml}$. Surviving colony-forming units per ml were estimated after incubation of the plates at 37°C overnight.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Results, Supplemental Discussion, Supplemental Experimental Procedures, four figures, and four tables and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2014.05.018>.

ACKNOWLEDGMENTS

This project was funded by the NSF (CCF-0905536 and DBI 1062455 to O.L.; MCB1022327 to C.H.) and the NIH (GM079656 and GM066099 to O.L.; 1R01GM088653 to C.H.). D.C.M. was supported by a training fellowship from the Keck Center of the Gulf Coast Consortia, on the Training Program in Biomedical Informatics, National Library of Medicine (NLM) T15LM007093 (principal investigator: G. Anthony Gorry). S.R.A. was supported by an IRACDA training grant (K12 GM084897). This project was also supported by the Cytometry and Cell Sorting Core at Baylor College of Medicine with funding from the NIH (NIAID P30AI036211, NCI P30CA125123, and NCRR S10RR024574) and the expert assistance of Joel M. Sederstrom. We thank Benu Atri, Herman Dierick, Ido Golding, Teng-Kuei Hsu, Christian Ray, and Angela Wilkins for helpful comments and discussions.

Received: January 17, 2014

Revised: April 4, 2014

Accepted: May 9, 2014

Published: June 5, 2014

REFERENCES

- Barkai, N., and Leibler, S. (1997). Robustness in simple biochemical networks. *Nature* 387, 913–917.
- Becskei, A., and Serrano, L. (2000). Engineering stability in gene networks by autoregulation. *Nature* 405, 590–593.
- Bershtein, S., Segal, M., Bekerman, R., Tokuriki, N., and Tawfik, D.S. (2006). Robustness-epistasis link shapes the fitness landscape of a randomly drifting protein. *Nature* 444, 929–932.
- Bershtein, S., Goldin, K., and Tawfik, D.S. (2008). Intense neutral drifts yield robust and evolvable consensus proteins. *J. Mol. Biol.* 379, 1029–1044.
- Bloom, J.D., Silberg, J.J., Wilke, C.O., Drummond, D.A., Adami, C., and Arnold, F.H. (2005). Thermodynamic prediction of protein neutrality. *Proc. Natl. Acad. Sci. USA* 102, 606–611.
- Bloom, J.D., Labthavikul, S.T., Otey, C.R., and Arnold, F.H. (2006). Protein stability promotes evolvability. *Proc. Natl. Acad. Sci. USA* 103, 5869–5874.
- de Mendoza, A., Seb e-Pedr s, A.,  estak, M.S., Matej ci , M., Torruella, G., Domazet-Loso, T., and Ruiz-Trillo, I. (2013). Transcription factor evolution in eukaryotes and the assembly of the regulatory toolkit in multicellular lineages. *Proc. Natl. Acad. Sci. USA* 110, E4858–E4866.
- Dekel, E., and Alon, U. (2005). Optimality and evolutionary tuning of the expression level of a protein. *Nature* 436, 588–592.
- Denby, C.M., Im, J.H., Yu, R.C., Pesce, C.G., and Brem, R.B. (2012). Negative feedback confers mutational robustness in yeast transcription factor regulation. *Proc. Natl. Acad. Sci. USA* 109, 3874–3878.
- Elowitz, M.B., and Leibler, S. (2000). A synthetic oscillatory network of transcriptional regulators. *Nature* 403, 335–338.
- Englesberg, E., Squires, C., and Meronk, F., Jr. (1969). The L-arabinose operon in *Escherichia coli* B-r: a genetic demonstration of two functional states of the product of a regulator gene. *Proc. Natl. Acad. Sci. USA* 62, 1100–1107.
- Friedberg, E.C., Walker, G.C., Siede, W., and Schultz, R. (2006). *DNA Repair and Mutagenesis* (Washington: ASM Press).
- Gao, R., and Stock, A.M. (2013). Evolutionary tuning of protein expression levels of a positively autoregulated two-component system. *PLoS Genet.* 9, e1003927.
- Hlavacek, W.S., and Savageau, M.A. (1995). Subunit structure of regulator proteins influences the design of gene circuitry: analysis of perfectly coupled and completely uncoupled circuits. *J. Mol. Biol.* 248, 739–755.
- Huang, W., and Palzkill, T. (1997). A natural polymorphism in beta-lactamase is a global suppressor. *Proc. Natl. Acad. Sci. USA* 94, 8801–8806.
- Jarosz, D.F., and Lindquist, S. (2010). Hsp90 and environmental stress transform the adaptive value of natural genetic variation. *Science* 330, 1820–1824.
- Kova ci , L., Pauli , N., Leonardi, A., Hodnik, V., Anderluh, G., Podlesek, Z.,  gur-Bertok, D., Kri aj, I., and Butala, M. (2013). Structural insight into LexA-RecA* interaction. *Nucleic Acids Res.* 41, 9901–9910.
- Lee, N.L., Gielow, W.O., and Wallace, R.G. (1981). Mechanism of araC autoregulation and the domains of two overlapping promoters, Pc and PBAD, in the L-arabinose regulatory region of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 78, 752–756.
- Lempe, J., Lachowicz, J., Sullivan, A.M., and Queitsch, C. (2013). Molecular mechanisms of robustness in plants. *Curr. Opin. Plant Biol.* 16, 62–69.
- Levine, J.H., Lin, Y., and Elowitz, M.B. (2013). Functional roles of pulsing in genetic circuits. *Science* 342, 1193–1200.
- Lewis, L.K., Harlow, G.R., Gregg-Jolly, L.A., and Mount, D.W. (1994). Identification of high affinity binding sites for LexA which define new DNA damage-inducible genes in *Escherichia coli*. *J. Mol. Biol.* 241, 507–523.
- Li, B., Smith, P., Horvath, D.J., Jr., Romesberg, F.E., and Justice, S.S. (2010). SOS regulatory elements are essential for UPEC pathogenesis. *Microbes Infect.* 12, 662–668.
- Lichtarge, O., Bourne, H.R., and Cohen, F.E. (1996). An evolutionary trace method defines binding surfaces common to protein families. *J. Mol. Biol.* 257, 342–358.
- Madabushi, S., Yao, H., Marsh, M., Kristensen, D.M., Philippi, A., Sowa, M.E., and Lichtarge, O. (2002). Structural clusters of evolutionary trace residues are statistically significant and common in proteins. *J. Mol. Biol.* 316, 139–154.
- Mihalek, I., Res, I., and Lichtarge, O. (2004). A family of evolution-entropy hybrid methods for ranking protein residues by importance. *J. Mol. Biol.* 336, 1265–1282.
- Nevozhay, D., Adams, R.M., Murphy, K.F., Josic, K., and Bal zsi, G. (2009). Negative autoregulation linearizes the dose-response and suppresses the heterogeneity of gene expression. *Proc. Natl. Acad. Sci. USA* 106, 5123–5128.
- Nevozhay, D., Zal, T., and Bal zsi, G. (2013). Transferring a synthetic gene circuit from yeast to mammalian cells. *Nat. Commun.* 4, 1451.
- Paulsen, M., Legewie, S., Eils, R., Karaulanov, E., and Niehrs, C. (2011). Negative feedback in the bone morphogenetic protein 4 (BMP4) synexpression group governs its dynamic signaling range and canalizes development. *Proc. Natl. Acad. Sci. USA* 108, 10202–10207.
- Paulsson, J., and Ehrenberg, M. (2001). Noise in a minimal regulatory network: plasmid copy number control. *Q. Rev. Biophys.* 34, 1–59.
- Pennington, J.M., and Rosenberg, S.M. (2007). Spontaneous DNA breakage in single living *Escherichia coli* cells. *Nat. Genet.* 39, 797–802.
- Raj, A., and van Oudenaarden, A. (2008). Nature, nurture, or chance: stochastic gene expression and its consequences. *Cell* 135, 216–226.
- Ray, J.C.J., and Igosin, O.A. (2010). Adaptable functionality of transcriptional feedback in bacterial two-component systems. *PLoS Comput. Biol.* 6, e1000676.
- Reeder, T., and Schleif, R. (1993). AraC protein can activate transcription from only one position and when pointed in only one direction. *J. Mol. Biol.* 231, 205–218.
- Rosenfeld, N., Elowitz, M.B., and Alon, U. (2002). Negative autoregulation speeds the response times of transcription networks. *J. Mol. Biol.* 323, 785–793.

- Savageau, M.A. (1974). Comparison of classical and autogenous systems of regulation in inducible operons. *Nature* 252, 546–549.
- Singh, A., and Hespanha, J.P. (2009). Optimal feedback strength for noise suppression in autoregulatory gene networks. *Biophys. J.* 96, 4013–4023.
- Smith, M.H., Cavenagh, M.M., and Little, J.W. (1991). Mutant LexA proteins with an increased rate of in vivo cleavage. *Proc. Natl. Acad. Sci. USA* 88, 7356–7360.
- Soskine, M., and Tawfik, D.S. (2010). Mutational effects and the evolution of new protein functions. *Nat. Rev. Genet.* 11, 572–582.
- Thattai, M., and van Oudenaarden, A. (2001). Intrinsic noise in gene regulatory networks. *Proc. Natl. Acad. Sci. USA* 98, 8614–8619.
- Thieffry, D., Huerta, A.M., Pérez-Rueda, E., and Collado-Vides, J. (1998). From specific gene regulation to genomic networks: a global analysis of transcriptional regulation in *Escherichia coli*. *BioEssays* 20, 433–440.
- Tokuriki, N., and Tawfik, D.S. (2009). Stability effects of mutations and protein evolvability. *Curr. Opin. Struct. Biol.* 19, 596–604.
- Toni, T., and Tidor, B. (2013). Combined model of intrinsic and extrinsic variability for computational network design with application to synthetic biology. *PLoS Comput. Biol.* 9, e1002960.
- UniProt Consortium (2013). Update on activities at the Universal Protein Resource (UniProt) in 2013. *Nucleic Acids Res.* 41, D43–D47.
- Waddington, C.H. (1942). Canalization of development and the inheritance of acquired characters. *Nature* 3811, 563–565.
- Wagner, A. (2012). The role of robustness in phenotypic adaptation and innovation. *Proc. Biol. Sci.* 279, 1249–1258.
- Wilkins, A.D., Venner, E., Marciano, D.C., Erdin, S., Atri, B., Lua, R.C., and Lichtarge, O. (2013). Accounting for epistatic interactions improves the functional analysis of protein structures. *Bioinformatics* 29, 2714–2721.
- Yeo, W.-S., Zwir, I., Huang, H.V., Shin, D., Kato, A., and Groisman, E.A. (2012). Intrinsic negative feedback governs activation surge in two-component regulatory systems. *Mol. Cell* 45, 409–421.