NEGATIVE FEEDBACK CONFERS MUTATIONAL ROBUSTNESS IN YEAST TRANSCRIPTION FACTOR REGULATION

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Organismal fitness depends on the ability of gene networks to function robustly in the face of environmental and genetic perturbations. Understanding the mechanisms of this stability is a key aim of modern systems biology. Here, we hypothesized that negative regulatory feedback could stabilize gene expression against the disruptions that arise from natural genetic variation. Using the ROX1 transcription factor as a model, we showed that genetic variation results in greater steady state expression variation between genetically distinct ROX1 feedback mutant strains than among strains with the ROX1 feedback loop intact. We conclude that ROX1 feedback buffers perturbations arising from natural genetic variation and suggest that regulatory feedback may be an important element of the network architectures that confer mutational robustness across biology.

I. BACKGROUND

Robustness of organismal function in the face of perturbations is critical for fitness [1]. Much of the search for molecular mechanisms of robustness has focused on gene regulation. Characteristics of regulatory networks that confer robustness include pathway redundancy and master regulatory organization [2], phenotypic capacitors [3], paired activating and inhibiting inputs [4], and cooperative and feed-forward regulation [5]. Additionally, negative regulatory feedback, in which a biomolecule represses its own abundance, can buffer variation in gene expression [6,7]. Negative feedback may also confer network stability against the effects of mutations [1,8], but evidence for negative feedback as a driver of mutational robustness in vivo has been at a premium [9]; the relevance of this principle to natural genetic variation remains largely unknown.

In this study, we tested whether feedback on Rox1 (**R**epressor of Hyp**OX**ia) confers robustness of gene expression to natural genetic variation. This master regulator represses genes involved in both metabolite and mitochondrial biogenesis, and precise expression may be crucial for proper regulation of these cellular processes.

II. RESULTS

We evaluated feedback on Rox1 as a mechanism for robustness of gene expression to naturally occurring genetic variation. For this purpose, we developed an assay to interrogate the effects on ROX1 expression of the spectrum of variants present in a set of divergent yeast strains of environmental and laboratory origin. For each such tester strain, we crossed it to a laboratory strain bearing the wild type ROX1-GFP reporter, and we performed an analogous cross using a feedback mutant strain. Haploid recombinant progeny, each a mosaic of inheritance from the tester and laboratory parents, served as a panel of genetically distinct strains among which Rox1 expression could vary. For a given cross, we measured the median Rox1-GFP levels in a culture of each progeny strain. Eliminating feedback compromised robustness to these variants, with a wider spread of median Rox1-GFP levels across genetic backgrounds in feedback mutant strains than in wild type; the coefficient of variation across strains was two to fivefold higher in the presence of the feedback mutation. Interestingly, the extent of expression variation across recombinant progeny was a function of the tester strain parent, indicating that some testers harbored alleles with more dramatic consequences for Rox1-GFP expression than others.

III. CONCLUSION

We conclude that Rox1 negative feedback buffers the effects of natural genetic variation on ROX1 expression, establishing regulatory feedback as a determinant of mutational robustness in this system.

REFERENCES

[1] Masel J, Siegal ML (2009) Robustness: Mechanisms and consequences. *Trends Genet* **25**, 395–403.

[2] Macneil LT, Walhout AJ (2011) Gene regulatory networks and the role of robustness and stochasticity in the control of gene expression. *Genome Res* **21**, 645–657.

[3] Rutherford SL, Lindquist S (1998) Hsp90 as a capacitor for morphological evolution. *Nature* **396** 336–342.

[4] Acar M, Pando BF, Arnold FH, Elowitz MB, van Oudenaarden A (2010) A general mechanism for network-dosage compensation in gene circuits. *Science* **329**, 1656–1660.

[5] Alon U, (2007) Network motifs: Theory and experimental approaches. *Nat Rev Genet* **8**, 450–461.

[6] Nevozhay D, Adams RM, Murphy KF, Josic K, Balázsi G (2009) Negative autoregulation linearizes the dose-response and suppresses the heterogeneity of gene expression. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 5123–5128.

[7] Ramsey SA et al. (2006) Dual feedback loops in the GAL regulon suppress cellular heterogeneity in yeast. *Nat Genet* **38**, 1082–1087.

[8] Acar M, Pando BF, Arnold FH, Elowitz MB, van Oudenaarden A (2010) A general mechanism for network-dosage compensation in gene circuits. *Science* **329** 1656–1660.

[9] Kafri R, Bar-Even A, Pilpel Y (2005) Transcription control reprogramming in genetic backup circuits. *Nat Genet* **37**, 295–299.

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