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GENE EXPRESSION

Systematic tuning of expression

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In addition to genetic mutations, alterations of gene expression levels are known to affect many traits, but quantitative explorations of precisely how gene expression levels affect fitness are scarce. A new study systematically interrogates the consequences of gene expression changes in yeast, with implications for evolutionary and biotechnological optimization.

In *Saccharomyces cerevisiae*, Keren *et al.* inserted a library of 130 barcoded synthetic promoters — spanning a 500-fold range of expression levels at narrow intervals — upstream of 81 target genes. This resulted in approximately 10,000 different strains, each with a single gene driven by a synthetic promoter.

Pooled populations of these strains were then cultured in defined media, with either glucose or galactose as the main carbon source. High-throughput sequencing across the paired promoter barcode and target gene barcode was used to quantify how the representation of each strain changed between the start of the experiment and after 11 generations. This timescale is sufficient to allow the effects on growth rates (used here as a measure of fitness) to be clearly seen, yet is too short to be confounded by the effects of acquired genetic mutations. For each targeted gene, the researchers could then plot how the high-resolution range of expression levels (which were known from separate reporter gene experiments in this study) influenced fitness.

The effect of expression levels on fitness was highly gene-specific. For some genes, fitness was insensitive to expression changes. However, for most genes, changes in expression relative to the wild-type expression had fitness effects, which were typically detrimental. For these genes, there was variability in how much deviation from wild-type levels was tolerated before fitness effects were seen.

The consequences of changes in expression levels were largely consistent with the known biological functions of the genes: fitness in galactose media was substantially affected by altered expression of core galactose metabolism genes, whereas growth in glucose media was more sensitive to expression levels of glycolysis and fermentation genes. The results also provided numerous insights into biological function; for example, fitness in galactose was positively correlated with levels of enolase 2 (ENO2) but anti-correlated with levels of ENO1, suggesting that these paralogous enzymes have evolutionarily diverged to catalyse reactions in opposite directions. Overall, across all genes tested, wild-type expression levels were closer to optimal for growth in glucose than galactose, suggesting that laboratory *S. cerevisiae* has evolved adaptations to glucose as a carbon source, while remaining considerably non-optimal for galactose use.

Finally, by cross-referencing with data on intercellular variability in expression of the targeted genes, the authors showed that genes with lower expression noise are less tolerant of changes in gene expression; hence, this lack of tolerance may have been the evolutionary force driving the low expression noise.

This approach has the potential to be applied in various additional contexts and species. One key application is for generating strains that have gene expression levels optimized for synthetic biology applications, such as for growth in unfavourable conditions or for the production or degradation of molecules of interest.

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ORIGINAL ARTICLE Keren, L. *et al.* Massively parallel interrogation of the effects of gene expression levels on fitness. *Cell* **166**, 1282–1294 (2016)