DNA recognition that may be amplified or modified by protein-protein interactions in vivo. Such ideas can be tested by assaying combinations of interacting proteins with bacterial one-hybrid experiments and protein-binding microarrays. Further, DNA-binding specificity is only one step in the complex process of transcription regulation. Thus, understanding how this large and important superfamily of DNA-binding homeoproteins ultimately functions in vivo is still a work in progress, but one that now benefits from the first edition of an almost unabridged dictionary of homeodomain-DNA-binding specificities.

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Forging New Ties between *E. coli* Genes

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A recent study in *Nature* (Isalan et al., 2008) has examined the effects of systematically adding new transcriptional interactions in the bacterium *Escherichia coli*. Surprisingly, the majority of the engineered connections have no effect on growth, and in some cases the new connections enhance fitness. These findings reveal insights into the robustness and evolvability of gene networks.

Systems biology measures the response of biological networks to systematic perturbations. In many cases, the perturbations involve direct targeting of genes and proteins using knockouts or knockdowns, protein overexpression constructs, or natural variation in the form of single nucleotide polymorphisms (Beyer et al., 2007). Recent work by Isalan et al. (2008) published in *Nature* takes the concept of systematic perturbation to a new level. In this study, the alterations are not to genes or proteins themselves, but to gene and protein interactions. Although the effects of adding or removing interactions have been studied before—for instance using reverse one-hybrid or reverse two-hybrid assays (Vidal et al., 1996)—Isalan et al. alter the transcriptional networks of the bacterium *Escherichia coli* on an unprecedented scale.

Their experimental design is as follows. Any transcriptional regulatory network can be viewed as the superposition of two types of interactions: the set of interactions among transcription factors in which "regulators regulate regulators" (Simon et al., 2001) and the set of interactions connecting transcription factors to downstream responder genes. It is this first network of "regulators regulating regulators" that creates interesting network structures and dynamics such as feed-forward and feed-back loops.

The goal of Isalan et al. was to perturb this network by adding each possible regulatory connection between a pair of transcription factors (Figure 1A). To add a new connection from a given factor A to factor B, the DNA promoter targeted by factor A was placed immediately upstream of the open reading frame (ORF) encoding factor B. All pairwise combinations (A,B) were considered within a set of 22 *E. coli* transcription factors, which were chosen to represent a range of general and specific regulatory functions. Each rearrangement was introduced into *E. coli* cells on plasmids and, in some cases, also by direct insertion into the genome. Plasmids also encoded a green fluorescent protein (GFP) so that the transcriptional output could be measured in addition to the overall impact on the growth rate of the organism. Note that each transcription factor gene was also left in its original genomic location, such that the net effect was merely to add interactions to the natural network, not to take any away.

What might be the possible consequences of adding a new regulatory input to a given transcription factor? As it turns out, there are at least two. Most simply, it is likely that the expression of the fac-

tor is increased to a higher level, which should depend on the strength of the new promoter element upstream. Indeed, most rewired transcription factors (>72%) were expressed at higher levels than in wild-type cells. Strikingly, however, the expression level of a transcription factor was found to depend more strongly on the identity of the transcription factor itself. Some factors, such as RpoE or FlhDC, were always highly expressed regardless of which promoter sequence was placed in front of them. Other factors, such as ApoY or FhlA, seemed never to be highly expressed regardless of their promoter sequences.

Although the authors did not attempt to explain this finding, some possible mechanisms might be differences in translational efficiency due to codon bias or unidentified regulatory elements (transcriptional or posttranscriptional) in the coding regions. Certainly, forces such as codon bias have been well documented (Ermolaeva, 2001), but the observation that these forces are stronger than the gene promoter is interesting and will likely provide fuel for many future studies. In this regard, it should be easy to test for correlations between codon usage (alternatively GC content or other sequence properties) and the expression level observed in the rewired networks.

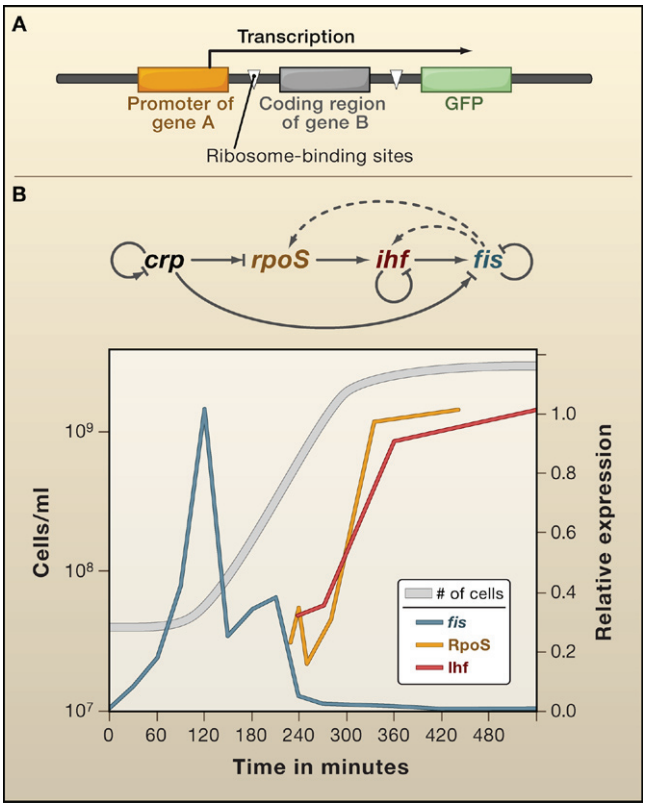

Figure 1. Transcriptional Rewiring in *E. coli*

(A) General design of the constructs used by Isalan et al. (2008) for transcriptional rewiring in *E. coli*.

(B) (Top) A static network of transcriptional interactions among four factors involved with the transition to stationary phase in *E. coli*. Interactions are activating (arrows), repressing (bars), or dual (arrows and bars). Dotted interactions are new perturbations that might be expected to dramatically change the average expression of one or more factors based on the vast differences in timing in the wild-type circuit. (Bottom) Timing of gene expression in wild-type *E. coli*. As cells enter stationary phase (>300 min), expression of *fis* decreases while expression of RpoS and Ihf increases (right vertical axis). Time courses of expression represent previously reported data: RpoS (Lange and Hengge-Aronis, 1994), Ihf (Ditto et al., 1994), and *fis* (Walker et al., 2004). Each curve is normalized so that its maximum value is equal to one. The corresponding cell growth curve is shown in gray (left vertical axis).

Beyond affecting the overall expression level, a second major consequence of adding new regulatory inputs to a transcription factor is to alter the dynamics of its expression (Figure 1B). For factors such as RpoS and Fis, which are normally growth-phase regulated (Lange and Hengge-Aronis, 1994; Walker et al., 2004), these dynamics could be attenuated by placing their ORFs downstream of a constitutive promoter. Conversely, the promoters targeted by these factors should confer growth-phase-dependent regulation to a transcription factor that is normally not dynamically regulated. To further explore such cases, one next step might be to directly measure the dynamic time-dependent expression profiles of each rewired transcriptional variant using GFP-fluorescence microscopy on individual *E. coli* cells in culture. To hone in on network dynamics, it would be especially interesting to examine the rewiring of cell-division-associated transcription factors such as RcsB and SdiA, which were not among the 22 transcription factors surveyed in the present study.

Given that network rewiring can alter both the level and the time-dependent behavior of transcription, it is surprising that very few (<5%) had a deleterious affect on growth. Exactly why cells are so tolerant to the perturbations is unclear, but a key piece to the puzzle is likely to come through a better understanding of the expression dynamics. For instance, one hypothesis is that the transcriptional network variants that most affect the growth rate are those that most dramatically alter the dynamics of gene expression. This could be easily tested by including cell-cycle-dependent factors and/or by generating time-dependent GFP expression profiles. In addition, much of the dynamic network behavior may not yet have been surveyed, given that the bulk of the data reported in Isalan et al. were collected in rich media. Indeed, they do show that one rewiring mutant in particular (rpoS→ompR) confers a strong selective advantage during stress due to prolonged stationary phase or heat shock.

Initially, the authors anticipated that gene expression levels should be most strongly affected when new transcriptional interactions create feed-forward or feed-back loops in the regulatory network. They also expected that the most deleterious interactions should be those involving the most highly connected factors in the network. Neither of these expectations was borne out by the data. Isalan et al. present these negative results as the next chapter in the ongoing debate over the importance of network connectivity and network motifs to the function of biological systems (Barabasi and Oltvai, 2004). Although feed-forward loops and other small regulatory motifs can work well when engineered de novo, this new work illustrates that it may be dangerous to conclude that these topologies are functionally relevant when extracted from a much larger in vivo network. Alternatively, the lack of correlation with network structure may be due to the fact that the promoter constructs altered transcriptional output less than expected.

In summary, the systematic rewiring of gene networks in *E. coli* by Isalan et al. provides a powerful new resource for studying gene transcription and network evolution. The perturbed networks show that it is very easy for organisms to optimize their growth by creating new network connections, a strategy that could be useful for creating new phenotypes with applications in biotechnology. The work leads to more questions than answers, in particular concerning why promoter elements do not affect gene expression as strongly as expected. Perhaps most importantly, the rewired networks make it clear that, despite the extensive information about *E. coli* pathways compiled in public databases, we may know less about its gene regulatory network than we previously thought.

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LUSH Shapes Up for a Starring Role in Olfaction

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In the fruit fly *Drosophila*, odorant-binding proteins are secreted into the fluid that bathes olfactory neurons. Laughlin et al. (2008) now challenge the assumption that the odorant-binding protein LUSH passively transports its pheromone to a specific olfactory receptor. Instead, LUSH undergoes a conformational change upon pheromone binding that is sufficient for neuronal activation.

The signal transduction events that underlie the detection of odors are now commonplace in the textbooks of Biology 101. Individual neurons each express one of a large, diverse family of G protein-coupled odorant receptors (GPCRs). The molecular features of a volatile chemical activate a specific subset of receptors that stimulates cAMP-mediated signaling, resulting in the opening of ion channels and depolarization of the corresponding neurons. Breathe in, it works great! However, recent studies investigating insect olfaction reveal that this system is not the only mechanism to initiate the perception of smell. The first surprise came from reports that the insect "GPCR" is topologically reversed in the membrane and appears to function directly as a ligand-gated ion channel (Sato et al., 2008; Wicher et al., 2008). As a second surprise, elegant work from Laughlin and coworkers in this issue of *Cell* now shows that odorant neurons in *Drosophila* that detect pheromones are not directly activated by the volatile ligand, but instead are activated by endogenous odorant-binding proteins (OBPs).

In the fly, odorant neurons are anatomically segregated in a protective lymph-filled cuticle in which odorants diffuse. OBPs are a large, diverse family of proteins that are differentially secreted into the lymph surrounding specific subsets of neurons. For several decades, OBPs have been known to bind and release odorants and pheromones. However, their role in the mechanism of chemosensory detection has remained enigmatic. It has been proposed that they are either odorant scavengers that downregulate the signal or transport proteins that stabilize the small molecule in the lymph and deliver it to the receptor (Pelosi, 2001). Several years ago, Dean Smith's group characterized an OBP gene, OBP76a, that encodes the LUSH protein, so named for its role in mediating alcohol avoidance (Kim et al. 1998). They found that LUSH bathes a subset of neurons including those that are activated by 11-*cis* vaccenyl acetate (cVA), a mating pheromone that mediates social