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NEWS AND VIEWS

Modularizing gene regulation

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As our ability to tinker with cellular components increases, the possibility of designing and controlling the functioning of living systems seems closer than ever.

In the pioneering monograph 'What is life?', Erwin Schrödinger pointed out the now obvious fact that living organisms obey the well-established laws of physics (Schrödinger, 1945). Yet, there is a long way from the atomic interactions at the molecular level to the behavior of even the simplest bacterium. So far, the type of predictive behavior that has been so successful in physics, chemistry, and engineering is largely missing in biology. Biological systems are not static; they evolve and adapt, grow and die. They are also intrinsically stochastic and live in a fluctuating environment. All these contingencies make quite a challenging task to apply the well-established principles of engineering to biology.

In a recent issue of *Nature*, Guido *et al* (2006) take an important step in this direction: building up networks of genes from well-characterized modules and obtaining the expected behavior in all its details. This type of challenge boils down to the core of systems biology, which aims at predicting the systemic properties in terms of the properties of the components and their interactions (cellular processes in terms of molecular interactions, organismal behavior in terms of populations of cells, and so on). If this program can be carried out at all levels of organization, building up successively on the previous one, it would be possible to design and control the functioning of living systems.

Guido *et al* engineered a promoter controlled by lambda cI and the *lac* repressor to allow simultaneous repression and activation of a gene in the bacterium *Escherichia coli*. The authors studied its behavior in synthetic gene networks under increasingly more involved conditions, ending with the promoter being controlled by the product of the gene it controls. The novelty with respect to previous work is the construction of a particularly complex promoter and, most remarkably, the verification of the extremely detailed predictions that were made from the behavior of the simpler components. The predicted behavior included not only the average values of protein content but also a detailed quantification of cell-to-cell variability. Such predictive power is needed to bring synthetic biology to engineering grounds.

How scalable is this type of approach? It is illustrative to take a look first at the other extreme of synthetic biology, in which many networks are randomly constructed with the hope of obtaining the wanted behavior among the resulting networks. For instance, Guet *et al* (2002) used a combinatorial approach to construct networks of three genes controlling their promoters in a highly interconnected way. The resulting networks displayed a myriad of different types of behavior but, remarkably, the functioning of many of these networks could not be explained in terms of the known properties of their components.

The components used by Guido *et al* originate from the two systems that led to the discovery of gene regulation, and we know a great deal about them. One might ask, would the approach of Guido *et al* work in a set up like that of Guet *et al*, that is, when multiple components are assembled adjacent to each other on the same DNA molecule? The answer does not follow straightforwardly. It just happens that both the *lac* repressor and lambda cI can loop DNA (Müller-Hill, 1996; Ptashne, 2004). Thus, placing two promoters on the same DNA strand relatively close to each other would induce the formation of DNA loops (Figure 1). DNA looping has been shown to have strong effects in gene regulation. It enhances

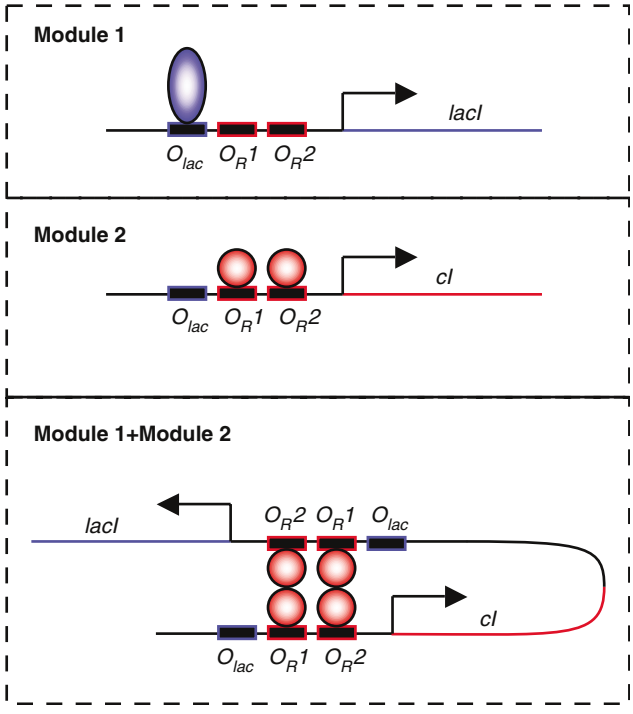

Figure 1 Modular crosstalking in synthetic gene networks. A promoter with binding sites for lambda cI dimers (O_R1 and O_R2) and for the *lac* repressor (O_{lac}) controls the production of the lac repressor (encoded by *lacI*) in module 1 and lambda cI (encoded by *cI*) in module 2. The *lac* repressor and lambda cI dimers are represented by blue ellipsoids and red circles, respectively. When the two modules are put together, two pairs of lambda cI dimers bound at different operators can loop DNA and octamerize, forming a tetramer of dimers.

the repression level about hundred times in the *lac* operon (Müller-Hill, 1996) and it couples two operators that are over 2 kb apart in phage lambda (Ptashne, 2004). Therefore, the formation of DNA loops would affect the behavior of the system in ways that are not present in the original components (Vilar and Saiz, 2005).

This potentially emergent behavior illustrates a general theme that is present in biology at all levels: increasingly complex systems can pick up details that were hidden in simple setups. To carry on with the modular approach, crosstalking would need to be prevented. In the case of Guido *et al*, it is possible to devise mutant *lac* repressors and lambda cI proteins that do not induce DNA looping (Müller-Hill, 1996; Ptashne, 2004). Thus, in principle, it would be easy to tweak the modules to prevent them to be tangled in DNA loops.

As our ability to put together different components increases, the main challenges for a large-scale bottom-up approach to gene regulation become double-edged: how to avoid the unwanted emergent behavior in an engineered design and how to use the emergent behavior to tackle more sophisticated tasks. Naturally occurring systems seem to be placed in a middle ground where some degree of modularity is present with extensive crosstalking between different modules (Hartwell *et al*, 1999; Martinez Arias and Stewart, 2002). In this regard, nature seems to have followed a quasimodular approach. Perhaps, a more practical avenue to go up in complexity will be to build the core structure of the system and artificially evolve the details (Yokobayashi *et al*, 2002).

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