Design principles of genetic regulatory networks

The functioning of genetic regulatory networks is a central question of modern molecular biology. That cells with identical genetic makeup are able to perform very different functions (e.g. brain vs. skin cells) is a consequence of the difference in their gene expression. Gene expression is regulated by the activity of proteins known as transcription factors (TFs), which may be influenced by molecular signals[1]. These are the basic components of a gene circuit (Fig. 1). Circuit design is assumed to be a result of natural selection, which acts to produce a system best suited to perform a certain function under given environmental conditions. Circuits with just one TF are known as elementary and have been studied extensively[2]. An example of a gene circuit, the *lac* operon, is shown in Fig. 2.

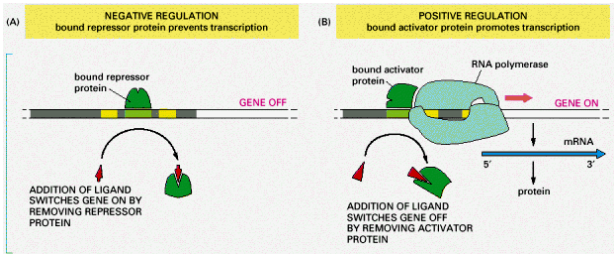

Fig 1 *Examples of signal-dependent gene regulation. Adapted from Ref. 1, Fig. 7-36.*

The aim of our research is to elucidate the design principles of both elementary and more complex genetic circuits. We do this by comparing systems of different designs but seemingly capable of performing the same basic function. We investigate subtler performance criteria such as temporal responsiveness and robustness to determine the functional implications of mechanistic differences between the circuits. Mathematical modeling is very well suited for this task, given that in experiment it is often very difficult to compare two alternative systems without the results being clouded by the effects of irrelevant differences between them.

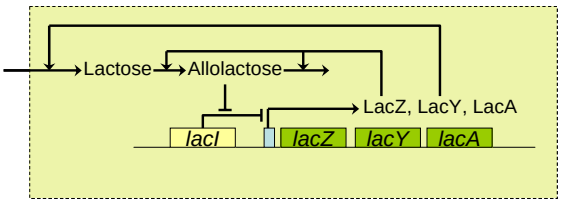

Fig. 2 *Gene regulation in the* lac *operon of* E. Coli. *The vertical arrows represent positive and blunted arrows negative control.*

We study several types of genetic circuits. The first one is an example of a *quorum sensing* (QS) system which has lately attracted significant attention in both experimental and modeling communities. Quorum sensing is a process in which bacteria communicate by secreting and detecting signaling molecules called autoinducers, whose concentration is correlated with the cell density. At high cell density, the high autoinducer concentrations trigger a signaling cascade that leads to the expression of genes not active at low cell densities. This mechanism is employed if the expression of a certain gene is advantageous when bacteria are in a group but ineffective or even harmful otherwise. For example, bacteria invading a host do not want to make their presence known until there are enough of them to launch an effective attack. Thus this cell-cell signaling provides a mechanism of social interaction in bacterial communities.

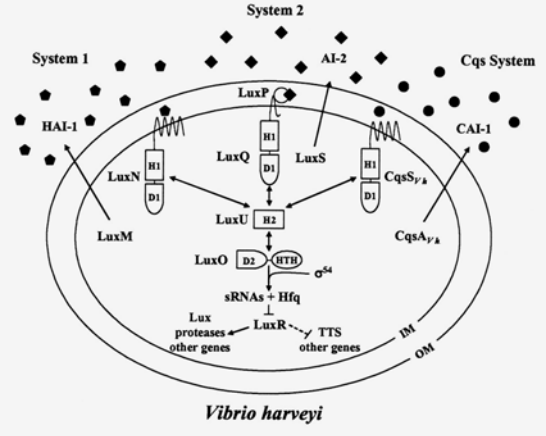

Fig. 3 *Quorum sensing in* V. harveyi. *Adapted from Ref.3.*

The particular system we are interested in is the one used by the bacterium *Vibrio harveyi* which has a unique composition, with three autoinducers (HAI-1, AI-2 and CAI-1) that act in parallel[3] (Fig. 3). It has been shown experimentally that this system has different sensitivities to all three signals, and it has been proposed that HAI-1 is used for intraspecies, AI-2 for interspecies and CAI-1 for communication between closely related species. We are developing deterministic and stochastic models of this system to explore the role of redundancy in its design. For that purpose systems with one, two and three autoinducers are being compared. A model of a multispecies bacterial colony is also being

developed to assess the functional consequences of interspecies communication. In addition to gene regulation, this system requires the modeling of signal transduction.

The second system is the well-studied *lac* operon in *Escherichia coli* (Fig. 2). In this circuit external lactose is imported into the cell by lactose permease (LacY) and degraded to allolactose by β−galactosidase (LacZ); allolactose then binds and blocks the activity of LacI, an inhibitor of the expression of the *lacZYA* transcriptional unit. Thus, starting from a state of low *lacZYA* expression the system is switched to a high expression state due to two (indirect) positive feedback loops involving lactose, allolactose, *lacZ*, *lacY* and their products. This system has been found to accommodate bistability (two stable steady states) associated with hysteresis for physiological parameter values in a series of recent theoretical papers[4]. However, bistability has never been observed experimentally, except in the case when an artificial inducer (IPTG), which is not degradable by LacZ, is used instead of allolactose. We are trying to understand this discrepancy between theory and experiment. Also, the *lac* circuit has the potential for responding adaptively to stimuli due to the presence of both a fast and a slow feedback loop (not shown). We are investigating whether this interesting behavior, commonly observed in neural circuits, is biologically relevant and, if it is, what are its consequences in this particular system.

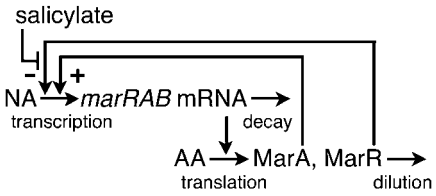

Fig. 4 marRAB *regulation circuit.*

Finally, we are studying the behavior of the *mar* circuit in *E. coli*. This circuit, a simplified version of which is shown in Fig. 4, is a *binary* circuit, having two transcription factors, MarA and MarR. MarA activates expression of many target genes involved in *multiple antibiotic resistance.* In our model, *marRAB* is expressed as the external concentration of salicylate is increased and the salicylate/MarR complex is formed, inhibiting the repression by MarR. A model with realistic parameter values gives good agreement with experiment (Fig. 5). An intriguing aspect of this circuit is that a similar steady state behavior can be expected without the positive regulation of *marRAB* by MarA. Preliminary studies of otherwise equivalent systems with and without positive autoregulation indicate that a step change in the salicylate concentration can cause a significantly faster response in the system without the positive feedback. These studies suggest that the reason for additional regulation might lie somewhere other than in the acceleration of the temporal response. Also of interest is that at least two other proteins, SoxS and Rob, bind to DNA and positively regulate expression of *marRAB*. The competition of these proteins in binding to DNA is

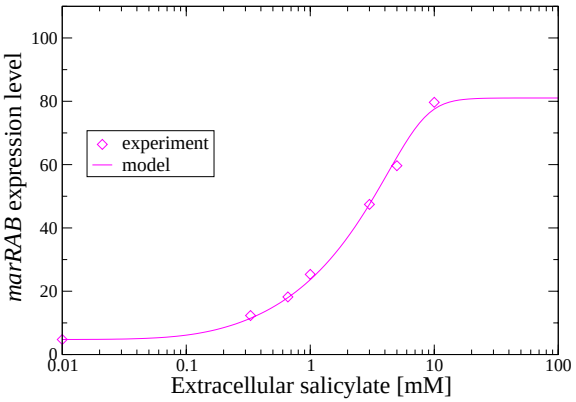

Fig.5 *Experimental data*[5] *and a theoretical curve for the steady state expression of* marRAB.

References

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