

Building Networks From the Ground Up: Robustness and Predictability of Regulatory Modules

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Genetic networks are composed of many small groups of interacting genes, called modules, that regulate each others' expression over time. In an attempt to understand the dynamical behavior of gene expression, synthetic and systems biologists have focused on building the simplest one-, two-, and three-gene modules. One goal is to assemble such modules into larger networks whose behavior is predictable from the dynamics of the component parts. Another goal is to understand the intrinsic properties of transcriptional networks, such as the remarkable robustness to parameter variation that they display in vivo.

To investigate the predictive abilities of the reductionist approach, and to identify the sources of dynamical robustness in regulatory networks, we modeled two classes of network building blocks: “activator” and “repressor” switches (AS and RS). Each switch consists of 2-4 nodes, representing mRNAs and proteins, that regulate each other’s transcription or translation (Fig. 1A,B). We represented each module as a set of ordinary differential equations, and studied their behavior when we combined them into two-module networks with regulatory interactions between the switches (Fig. 1C). By simulating the networks computationally, we were able to explore thousands of variations that would have been impossible in vivo, giving us a comprehensive picture of each network’s dynamical repertoire.

We simulated each two-module network with a range of parameter sets chosen to encompass the range of dynamical behavior for that network. For each set, we varied all individual parameters and measured how much the overall dynamics shifted in response. Networks in which the steady states, and the set of initial conditions leading to each steady state, remained consistent despite parameter variation were considered robust. We found that networks containing RS modules consistently displayed a more robust response (Fig. 2A).

The regulatory interactions between modules suggested different behavior for each two-module network. For example, we predicted that the positive regulation between modules in the AS1→AS1 network shown in Fig. 1C would prevent the steady state in which AS1 = (1,1) and AS2 = (0,0). We found that networks with RS modules conformed much more reliably to the predicted outcomes than networks with AS modules (Fig. 2B).

Why are predictability and robustness correlated (Fig. 2C)? Regulation from other genes restricts the dynamical range of a module. Modules that readily respond to such restriction improve their network's robustness by allowing parameter variation to move them only among the reduced group of allowed states. Less predictable networks, by contrast, sometimes occupy states discouraged by the regulation, and thus preserve a wider space of possible outcomes. As

their parameters vary, they shift among these destinations, displaying reduced robustness. The predictable RS switches, therefore, also produce highly robust two-module networks.

Especially during development, when switch-like decisions are common, organisms require genetic networks that respond reliably and robustly to upstream regulation. Our data suggest that the predictable, robust class of “repressor-switch” (RS) modules will be overrepresented in gene regulatory networks when these are well-characterized on a genomic scale.

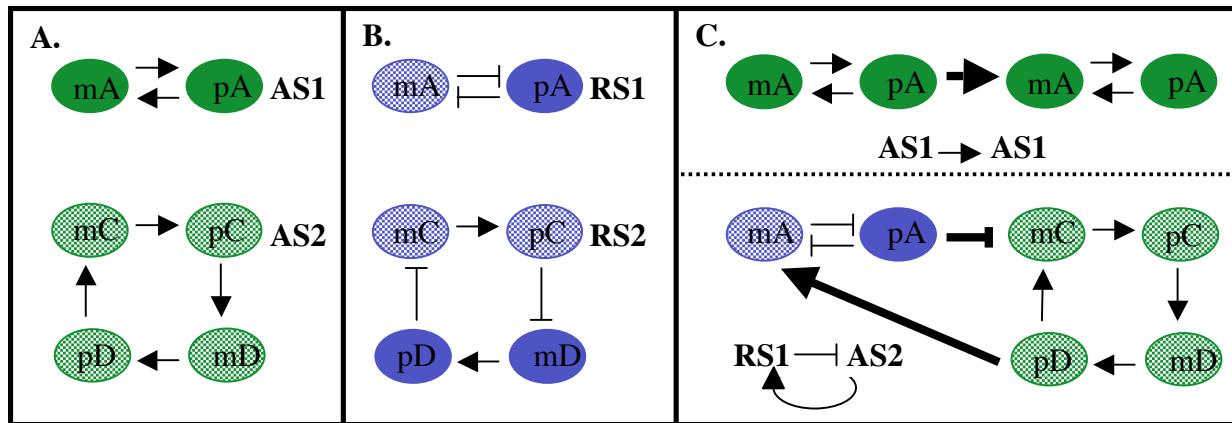


Figure 1:

A: “Activator” switches (AS) are positive feedback loops, where each mRNA or protein node encourages the production of the next node’s mRNA/protein by translation or transcriptional regulation. Stable steady states for these switches are all nodes at 0 concentration ((0,0) or (0,0,0,0)) or all nodes at maximum concentration ((1,1) or (1,1,1,1)). **B:** “Repressor” switches (RS) contain two negative regulatory interactions, resulting in switches for which the stable states are (1,0)/(1,1,0,0) or (0,1)/(0,0,1,1). **C:** Examples of two-module networks with positive and/or negative regulation between the modules. We studied all 62 possible two-module networks.

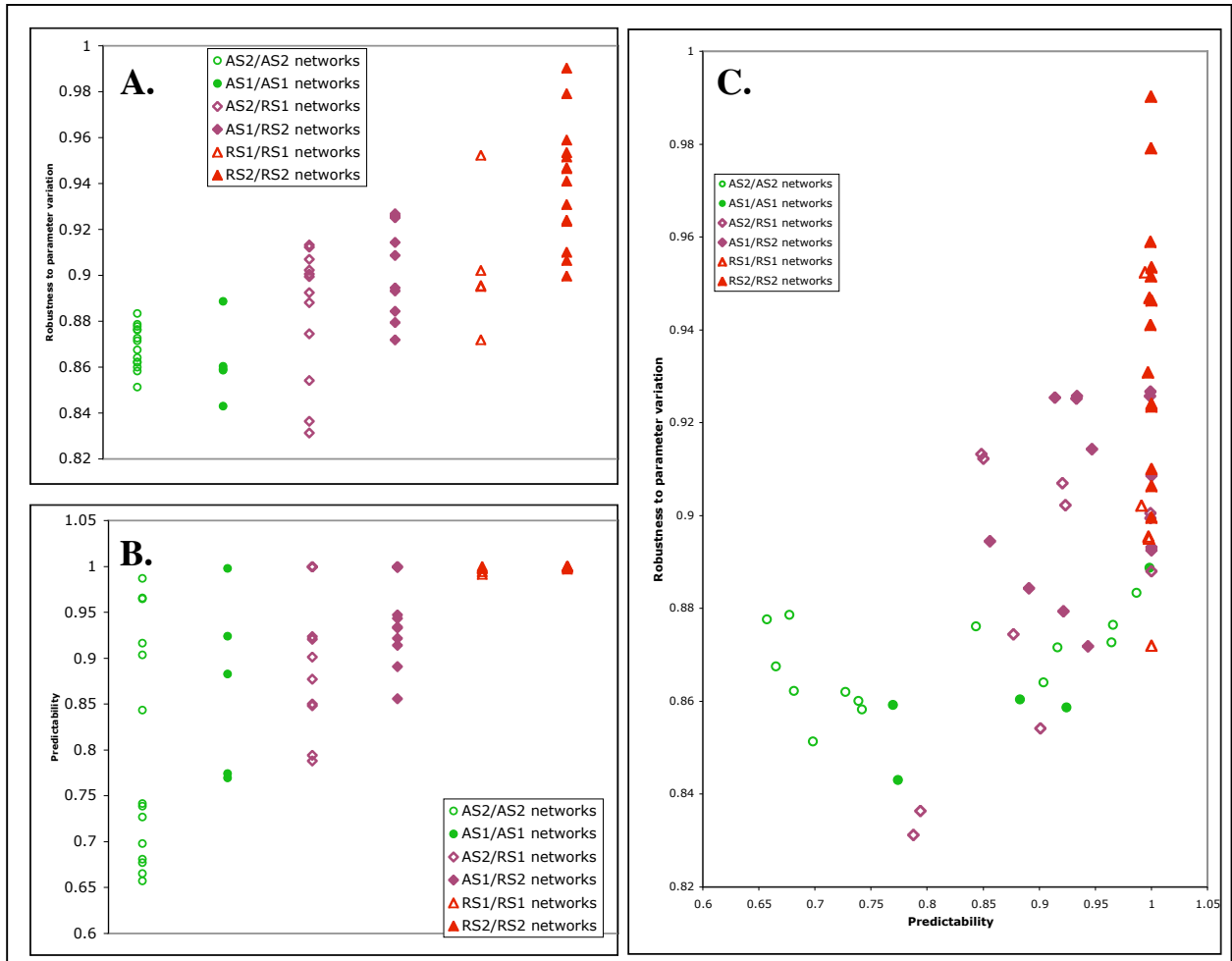


Figure 2.

A: Networks with more RS modules are more robust to parameter variation. Green points are networks with two AS modules; violet points are networks with one AS and one RS module; red points are networks with two RS modules. **B:** Networks with more RS modules are more predictable. Here, predictability is measured as the proportion of state space that is *not* in steady states that are predicted to be prevented by inter-module regulatory interactions. **C:** Robustness is correlated with predictability (correlation coefficient: 0.62).