

# Structural and Functional Characterization of Combined Regulatory Network Motifs

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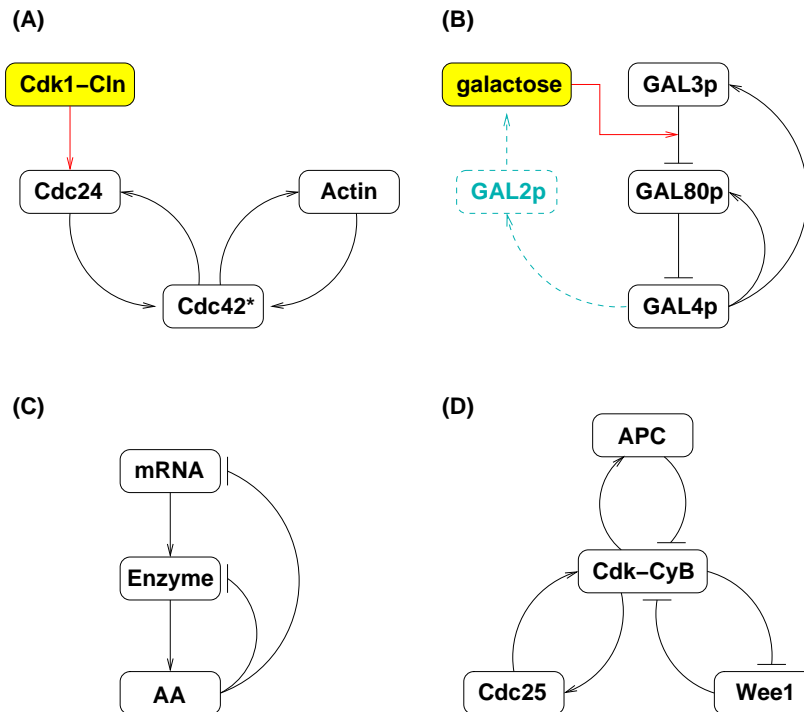
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Understanding a cell's ability to robustly respond to noisy stimuli is of central importance to harness its evolved metabolic capabilities. Efforts in genome-wide characterizations of metabolic pathways and regulatory circuits have provided unprecedented engineering strategies as well as novel insights into the robustness of complex biological systems. Although much effort has been focused on delineating structural determinants of complex cellular networks in a functional context, recent attempts to synthesize *in vivo* regulatory circuits have not yet achieved the robustness and predictability of their electronics counterparts. For instance, while the genetic oscillator circuit designed to express three genes in periodic manner [1] constituted a major break-through in synthetic biology, only a minority of the modified cells displayed oscillations and these cells had significant individual variations in the oscillation period. Also, in an effort to build integrated transcriptional logic gates [2], a pair of circuits with the *same network topology* but different effector molecules have produced inconsistent circuit function.

One explanation for this is that *in vivo* biochemical circuits are exposed to significant levels of *crosstalk* with other pathways, abrupt environmental changes, as well as stochastic fluctuations inherent in molecular systems. Even for the ideal case of determinism in the network topology and environment, uncertainty in kinetic details may change the system properties qualitatively. While a single set of chosen model parameters only represents a point in the parameter space, there is evidence to suggest that such parameter values display variation within one microbial species. Consequently, we can hypothesize that robust regulatory circuits that have survived evolutionary selection can tolerate some level of parameter variations and noisy conditions without losing their functional integrity.

To systematically evaluate information-processing properties of a selected set of biologically important circuits (Fig. 1), we have taken the *ensemble approach* where the robustness of a given circuit topology is quantitatively evaluated for an ensemble of model systems with fixed topology. To this end, we generated random ensembles of each circuit topology with different rate laws (mass action versus Michaelis-Menten-Hill) and different set of kinetic parameters. In particular, we classified purely post-translational control from transcriptional/translational control by changing the kinetic order of chemical reactions involved in activation/repression. We developed a scoring scheme that characterizes the dynamic response of each circuit, such as fold change, sensitivity, and discrepancy between input and output signals. We have first analyzed the dynamic response properties of regulatory network motifs (subnetworks of recursive minimal regulatory patterns) without any reference to the functional context in which those motifs are placed. Further, we extended this analysis to combined network motifs with distinct functional objectives.

We have investigated functionally conserved subnetworks, such as switches, oscillators, and homeostatic devices, and explore their information-processing capabilities. We discuss possible fitness benefits and implications for the evolutionary selection of these circuit topologies, proposing a rational design for *de novo* regulatory circuits.



**Figure 1:** Combined regulatory network motifs. (A) Yeast Cell polarization circuit made up of two parallel positive feedback loops. Circuit is triggered by cyclin and cyclin-dependent kinase. (B) Yeast galactose utilization circuit activated by intracellular galactose. Both positive and negative feedback loops are coupled in a nested way. (C) Tight control of amino acid biosynthesis characterized by nested negative feedback loops. (D) Conserved components of cell cycle circuit. Two positive feedback loops create bistability while the negative feedback by way of APC (Anaphase Promoting Complex) is responsible for restoring the system toward equilibrium.

## Acknowledgments

This work was performed under the auspices of the U. S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48. This project (06-ERD-061) was funded by the Laboratory Directed Research and Development Program at LLNL.

## References

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