

# Only Two Ways to Achieve Perfection

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The functional repertoire of a network is determined by its topology. Ma et al. (2009) analyze enzyme networks with three nodes and take a reverse-engineering approach to ask how many core network topologies can establish perfect adaptation, the ability to reset after perturbation. Surprisingly, the answer is just two.

How many different ways can a biological network be wired to produce the same outcome? Many studies have focused on characterizing the behaviors of specific network topologies (Alon, 2007; Brandman and Meyer, 2008; Tyson et al., 2003). Using a complementary approach, Ma et al. (2009) now analyze the entire space of all possible topologies for enzyme networks with three nodes to identify only those that exhibit a specified behavior, namely perfect adaptation. Perfect adaptation is the unique feature of networks that have the ability to reset their output to the original levels after a transient response (Figure 1). Networks with perfect adaptation display both sensitivity (large peak output response) and precision (the output returns to prestimulation levels). Examples include many important biological processes, such as light sensing, calcium regulation, and chemotaxis.

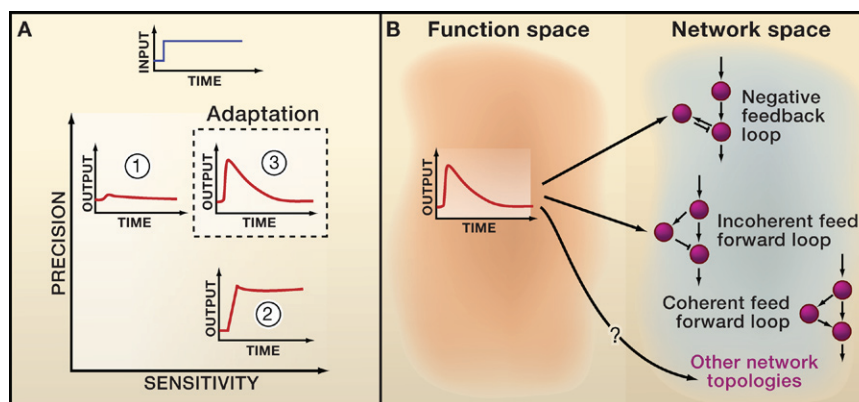
Recent work has shown that a surprisingly small number of motifs are highly enriched in diverse biological networks (Alon, 2007). Some motifs are specific to certain classes of networks. For example, two-node positive feedback loops are involved in decision making in developmental networks (Alon, 2007). Other motifs, such as feedforward loops, are very common. Feedforward loops can be classified into two groups, coherent and incoherent, based on whether the direct and indirect paths from the input to the output nodes have the same type of effect on the output node (Figure 1B). In coherent feedforward loops the direct path and indirect path have similar influences (that is, both are positive

regulators or both are negative regulators of the output node), whereas in incoherent loops the direct and indirect paths counter each other (Alon, 2007).

What are the special characteristics of these motifs that make them so prevalent in nature? Even networks with small numbers of components have the potential for generating a wide range of behaviors, including the ability to oscillate or filter noise (Brandman and Meyer, 2008; Tyson et al., 2003). Although many small networks can function as detectors of environmental change, relatively few display perfect adaptation. Despite a wide variety of biochemical implementations, systems showing perfect adaptation share a common engineering design principle: embedded integral

control, in which the time-averaged output is fed back into the system (Yi et al., 2000). Integral control can be achieved through negative feedback loops, which are commonly used in engineered devices that are designed to maintain a constant level of output, such as thermostats and cruise-control systems in automobiles. In addition to negative feedback, incoherent feedforward loops can also have properties of perfect adaptation, even though at first glance they lack apparent feedback loops (Ingalls et al., 2006).

Are there other undiscovered motifs capable of achieving perfect adaptation? In a prior effort exploring “network space,” random gene networks were “evolved” to optimize fitness functions for



**Figure 1. Network Topologies for Perfect Adaptation**

Perfect adaptation is a feature of networks that have the ability to reset their output to the original levels after a transient response.

(A) Networks that ignore an input have low sensitivity but high precision (1), and networks that have high sensitivity but low precision have outputs that do not readily return to the initial steady (2). In contrast, networks with perfect adaptation have both high sensitivity and high precision (3).

(B) In a forward approach, analysis of the motif properties reveals a repertoire of functions that each motif can perform. In contrast, the goal of a reverse engineering approach is to identify a set of all possible network topologies that can perform a specific function. Ma et al. (2009) show that perfect adaptation can result from only two different network topologies: negative feedback loops and incoherent feedforward loops.

adaptation (Francois and Siggia, 2008). By continuously improving fitness, these studies found that networks could be evolved to achieve perfect adaptation. Interestingly, only two classes of network topologies emerged, both of which were governed by integral feedback control. However, the number of all possible network topologies is large, raising the possibility that other classes of network topologies capable of perfect adaptation have been overlooked.

In this issue, Ma et al. report the results of a comprehensive analysis of enzymatic networks in search of topologies capable of producing robust perfect adaptation (Ma et al., 2009). As with previous studies (Francois and Siggia, 2008), Ma et al. use two quantitative measures of a network's response: sensitivity and precision (Figure 1A). Sensitivity measures the difference between the output response and its initial steady-state value, whereas precision measures the difference between the steady-state values before and after stimulation. The authors consider a minimal framework of three-node topologies: one node for receiving inputs, one node for transmitting output, and one regulatory node (Figure 1B).

The authors model rate equations in their network using Michaelis-Menten kinetics (though they report that their conclusions also hold for additional schemes, including ones based on mass action). The authors further assume that nodes have fixed total concentrations that could be interconverted between active and inactive forms through interactions with the other active enzymes in the network. This assumption is valid on timescales that are considerably faster than protein synthesis and degradation, such as might be appropriate for studying signal transduction. When considering the possibilities of positive, negative, or no regulation between any two nodes, the number of all possible topologies with three nodes is quite large, with a total of ~16,000 different circuits containing at least one direct or indirect connection from the input to output node. Then, for each topology, Ma et al. randomly selected 10,000 sets of network parameters and characterized their responses to an input in terms of

sensitivity and precision. Finally, they estimated for each network topology the number of parameter sets whose behaviors show perfect adaptation, with values for sensitivity and precision above predetermined thresholds.

Their exhaustive search of topologies and their scan of network parameters identify 395 robust topologies (that is, topologies that show properties of perfect adaptation for at least 10 parameter sets). Reassuringly, each of these topologies contains at least one embedded negative feedback loop or incoherent feedforward loop, the two topologies already shown to achieve perfect adaptation. In both cases, the regulatory node plays an essential role. In negative feedback loops the regulatory node acts as a "buffer" (integrating the difference between network response and steady-state output), whereas in incoherent feedforward loops the regulatory node acts as a "proportioner" (negatively regulating output proportionately to input). More importantly, properties of perfect adaptation are not found for any topologies with three nodes that lack either of these two core motifs. In a complementary mathematical study of network responses to small perturbations, the authors again identify the same two classes of topologies as the only ones capable of achieving robust perfect adaptation without fine-tuning of parameters. The ability to systematically exclude all other topologies greatly limits the number of possible network architectures that have to be considered in reverse engineering perfectly adaptive systems and in the design of new synthetic networks.

Clearly, this computational approach can be used to search for other connections between circuit topology and network functions (Figure 1B). There are many possible extensions of this work. Larger and more complex networks can be examined for new motifs. Alternatively, the behaviors of small motifs, embedded in increasingly larger networks, can also be studied. The assumption that the amount of an enzyme in a network is constant may not hold in some cases, such as in transcriptional networks, and it may be intriguing to test the necessity of

this assumption. In a different direction, network behavior can be studied with varying concentrations of interacting molecules; stochastic effects may dramatically change network behavior. Spatial localization can also play an essential role in determining the behaviors of signal transduction networks.

There are, of course, limits to computational searches. Although the scale of the search presented in Ma et al. is laudable, the region of parameter space explored is still less than 4% of the total parameter space. Their mathematical analysis, based on small perturbations and linear approximations to the network dynamics, may miss behaviors possible for nonlinear systems away from steady state. Extensions of this approach to networks with higher numbers of nodes or spatially non-uniform networks may be stymied by the vastness and complexity of combinatorial searches. Finally, when studying network topologies in a stochastic or spatial context, coarse-grained parameter searches may prove inadequate as even simple circuits can produce a wide range of behaviors depending on the localizations and concentrations of interacting molecules (Altschuler et al., 2008). Nevertheless, the approach taken by Ma et al. provides inspiration for future investigations into such questions.

Do *in vivo* networks behave as predicted *in silico*? Leibler and others have designed, built, and tested biological networks, not all of which worked as intended (Guet et al., 2002). Further, for complex networks, interconnections among motifs may mask behaviors of individual motifs (see for example Knabe et al., 2008). Thus, the final *in vivo* story may be more complicated than reductionist approaches allow. Nevertheless, the work by Ma et al. raises the possibility that network topologies that can be used to robustly produce specific functions may one day be identified and enumerated. Such a function-topology map will be invaluable for predicting behaviors of natural networks, identifying links missing in current wiring diagrams, and engineering synthetic networks.

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# Clamping Down on Transposon Targeting

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**The sliding  $\beta$  clamp subunit of the DNA replication machinery in the bacterium *Escherichia coli* coordinates multiple functions in the cell beyond genome duplication. In this issue, Parks et al. (2009) find that the  $\beta$  clamp interacts with the transposition protein TnsE to target the Tn7 transposon to discontinuously replicating DNA at the replication fork.**

The bacterial replisome, a molecular machine originally thought to be solely dedicated to the efficient duplication of the prokaryotic genome, is now known to harbor many protein subunits that are intimately involved in the maintenance of genome organization and integrity. Indeed, the replisome is proving to be a scaffold that integrates DNA replication with processes such as DNA recombination, DNA repair, segregation of newly replicated DNA into dividing daughter cells, and cell-cycle progression. A protein that plays a pivotal role in integrating these processes within the replisome is the sliding  $\beta$  clamp, a replication processivity factor. The  $\beta$  clamp acts as a binding platform for the replicative polymerase Pol III and a diverse range of other proteins, including those involved in DNA repair and replication initiation (see Kongsuwan et al., 2006). In this issue of *Cell*, Parks et al. (2009) show that the  $\beta$  clamp has yet another function in DNA metabolism—it can be co-opted to target the bacterial Tn7 transposon, a mobile DNA element, to replicating DNA. The authors demonstrate that a direct interaction between the  $\beta$  clamp and a transposition protein called TnsE allows Tn7 to insert into DNA at replication forks.

Transposition of Tn7 (Craig, 2002) is regulated by a complex series of protein-protein and protein-DNA interactions. The transposon encodes five proteins—TnsA, B, C, D, and E—required for transposition. Unlike most other transposons, the Tn7 transposase is composed of two proteins (TnsA and TnsB) that each catalyze the cleavage of one DNA strand at both ends of the transposon in the donor DNA molecule to liberate the mobile DNA element (Figure 1). TnsC, a DNA-dependent ATPase, helps to recruit the target DNA and forms a crucial bridge between the donor and target sites of transposition. Remarkably, Tn7 has multiple transposition targeting pathways whose choice is determined by the specificity factors TnsD and TnsE (Figure 1). In the TnsABC+D pathway, TnsD recognizes a sequence in the *glmS* gene (highly conserved in many bacteria) and directs Tn7 insertion into an upstream extragenic site, thereby preserving the integrity of the *glmS* coding sequence and providing a safe haven for the transposon. In the alternative TnsABC+E pathway, Tn7 insertion occurs preferentially (and largely in one orientation) into plasmids during their replicative transfer between

bacteria (conjugation), thus promoting transposon proliferation between different bacterial species. This targeting preference is a consequence of a specialized mechanism of plasmid DNA replication (rolling circle replication) that occurs during conjugation. TnsE recognizes characteristics of the rolling circle replication that occurs on the discontinuous or lagging DNA strand (a particularly prevalent feature of conjugative replication) and preferentially binds to DNA structures with recessed 3' ends (Peters and Craig, 2001a). The TnsABC+E alternative transposition pathway also directs Tn7 insertion into replication termination regions and DNA breaks (Craig, 2002). Interestingly, a TnsC mutant protein called C\* eliminates the need for specificity factors and results in random Tn7 insertion in vivo and in vitro.

To investigate the mechanism that allows Tn7 to target replicating DNA for insertion, Parks et al. compared TnsE proteins from a number of Tn7 family transposons and identified a conserved motif resembling consensus sequences that are implicated in mediating  $\beta$  clamp binding (Wijffels et al., 2004). Assays that detect protein-protein interactions, including yeast two-hybrid