Mathematical Biosciences 231 (2011) 69–75

Contents lists available at ScienceDirect

Mathematical Biosciences

journal homepage: www.elsevier.com/locate/mbs

To signal a conjunction of many inputs negative regulation is likely

Jay E. Mittenthal *, Lihua Zou [1]

Department of Cell and Developmental Biology, University of Illinois at Urbana-Champaign, B107 Chemical and Life Sciences Laboratory, 601 South Goodwin Avenue, Urbana, IL 61801, USA

ARTICLE INFO

Article history:
Available online 15 February 2011

Keywords:
Network motif
Negative regulation
Conjunction
Multi-input network

ABSTRACT

Cells make many transitions from an old to a new phase of activity – between inactive and active states of an enzyme, or between phases of the cell cycle. If a cell is to survive, molecular prerequisites for functioning in the new phase should be available before a transition occurs. The cell's survival is more likely if a regulatory network gates the transition, preventing its occurrence until the prerequisites are available. Suppose a specific conjunction of inputs is required for a network, from which a single output governs the transition. Then we suggest that cells are likely to use negative regulation – a gating network based on a logical disjunction of signals for the absence of prerequisites – rather than positive regulation – a logical conjunction of signals for their presence. That is, if a logical conjunction of *n* prerequisites *A1 AND A2 AND ... AND An* is needed in the new phase, a negative regulatory network is likely to enforce the corresponding logical disjunction, *NOT* (*NOT A1 OR NOT A2 OR ... OR NOT An*). Five examples illustrate this conclusion. Arguments based on performance criteria support the hypothesis: negative regulation is more economical than positive regulation, because networks for computing *OR* can use fewer and simpler parts than those for computing *AND*. Negative regulation can increase reliability, because a mechanism that uses fewer, simpler parts is less likely to fail. And, a negative regulatory network can be more robust – less susceptible to errors resulting from noisy input.

1. Introduction

Biological design principles are useful when designing molecular networks. These principles help to choose the most effective network topologies and dynamics that implement a given biological function. For example, either positive or negative regulation can switch a regulated molecule from inactive to active. In positive regulation, or activation, the presence of a prerequisite activates a regulated molecule. In negative regulation, or disinhibition, the regulated molecule is inhibited in the absence of a prerequisite. When the prerequisite is available, the regulated molecule is disinhibited.

Previous studies have provided design principles for the positive *vs.* negative regulation of transcription. Savageau [1] proposed that the transcriptional regulation of a bacterial operon depends on the demand for the activity of the pathway that the operon encodes. The demand is high for a pathway if its gene products are used often, as in a catabolic pathway that degrades an abundant sugar, or an anabolic pathway that synthesizes an amino acid not abundant in the environment. Demand theory is a use-it-or-lose-it principle for the evolution of regulation. It says that a high-demand pathway is likely to have positive regulation, in which an activating transcription factor binds to a regulatory site most of the time. A low-demand pathway is likely to have negative regulation, in which a repressor binds most of the time. Only these pairings are selectable, responding strongly to selection pressure: with negative regulation, if the regulator protein does not bind to its DNA site, the operon is constitutively active. Its high output has little selective consequence if demand is high, but high output is likely to be disadvantageous if demand is low. Hence there is only selection to maintain negative regulation if demand is low. By an analogous argument, a pathway should have positive regulation in a high-demand environment. Extensive evidence supports this conclusion.

Shinar et al. [2] argued that demand theory neglects differences in fitness between the modes of regulation. However, the modes do differ in fitness, because binding of a regulator protein to its DNA site protects the site from non-specific binding that can cause errors in transcription. Most of the time positive regulation protects an often-used operon from such errors, and negative regulation protects a rarely-used operon. These are the correlations that demand theory predicts. In a population genetic model for the evolution of regulation, Gerland and Hwa [3] examined how the fitness

* Corresponding author. Address: 605 W. Oregon St., Urbana, USA. Tel.: +1 217 384 6468.

E-mail addresses: mitten@illinois.edu, mitten@life.uiuc.edu (J.E. Mittenthal), lihuazou@gmail.com (L. Zou).

[1] Present address: Department of Cancer Biology, Dana-Farber Cancer Institute, Smith Building 1068, 44 Binney Street, Boston, MA 02115, USA.

0025-5564/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.mbs.2011.02.001

cost of mutations that prevent transcription factor binding depends on parameters of the model. A use-it-or-lose-it principle applies for a small population subject to environmental variations with long time scales. However, a wear-and-tear principle that favors minimal use of regulators applies for a large population having environmental variations on a short time scale.

Thus the pattern of regulation can depend on diverse factors, including fitness, selectability, population size, and time scale of environmental variations. To seek other principles that favor positive *vs*. negative regulation, we looked for other factors that may affect a network's evolution or performance [4–6]. Network size is such a factor. Several criteria favor the evolution of the smallest network that can perform specified functions. A smaller network is more economical. A cell must perform many processes with limited resources – limited space, time, coding capacity and error correction. The cell can perform more processes faster with smaller networks that use fewer kinds of molecules in higher concentrations. Also, a smaller network is more reliable, in that it has fewer proteins that can mutate. And, a smaller network may be more likely to evolve, because fewer mutations are needed to produce it. (The validity of this argument depends on the available parts and on the fitness of evolutionary intermediates. A relatively large network might evolve through cooption of preexisting network fragments with several parts, or through an evolutionary path with higher-fitness intermediates than those producing a smaller network. However, regardless of the evolutionary path that generates a network, economy favors restructuring of a larger network into the smallest one that can perform specified functions while meeting specified performance criteria.)

Several kinds of networks tend to have the minimum size. Some metabolic networks are among the smallest that can perform their functions – glycolysis [7], the pentose phosphate pathway [8,9], and the tricarboxylic acid cycle [10,11]. Networks for signaling and for regulation of transcription exhibit network motifs – circuits with a few components connected in a pattern that occurs many times in a cell's networks [12,13]. These circuits need not be evolutionarily homologous. As Alon [14] has shown, non-homologous networks with the same structure can have the same dynamical behavior and play related functional roles. Network motifs are used as building blocks to construct larger networks that perform more complex functions. Network motifs seem to be the smallest and most robust of the many circuits that could carry out their functions.

The preceding arguments suggest a hypothesis of minimum size: when one considers all the functions a signaling network performs and the constraints under which it operates, the biological realizations of the network tend to be the smallest that could perform those functions under those constraints. Some recent studies support this hypothesis. The chemotaxis network of *Escherichia coli* is the smallest that provides adequate chemotactic response, given the variability in levels of its constituent proteins associated with gene expression noise [15]. Kashtan and Alon [16] simulated the evolution of model networks in response to varying combinations of goals. They found that network modules corresponding to the goals evolve. In electronic combinatorial logic circuits, modules evolved that implement *EXCLUSIVE OR*, *XOR*. (*A XOR B* means either *A* or *B*, but not both, is true.) The *XOR* modules consisted of four *NOT AND* (*NAND*) gates, the minimum *XOR* implementation in electronic engineering. (*A NAND B* means not both *A* and *B* are true.)

The most challenging problems in network design may involve networks with multiple inputs [17,18]. In eukaryotic cells, signaling networks that respond to several extracellular messengers modulate the activity of gene regulatory networks [19,20]. Several networks, including those that replicate DNA and that transcribe and translate RNA, involve assembly of multi-molecular complexes, through association of macromolecules, polymerization of monomers, or both [21,22]. Among the networks with multiple inputs are networks where a cell makes a transition from an old to a new phase of activity, for which prerequisites are required. A cell's survival is more likely if a regulatory network prevents a transition until the prerequisites are available. The network may also promote the correction of deficiencies in prerequisites. Such networks mediate stress responses, for lack of amino acids or DNA damage as discussed below, and for heat shock [23].

Here we argue that if a multi-input, single-output network signals a logical conjunction of prerequisites, negative regulation can represent the conjunction with greater robustness and with fewer parts than positive regulation. Five examples illustrate this conclusion. We then discuss some possible objections to our hypothesis and the arguments supporting it.

2. To signal a conjunction of inputs in a single-output network, negative regulation is more likely than positive regulation

2.1 In a multi-input/single output network signaling conjunction, negative regulation is more robust than positive regulation

Negative regulation provides a more robust way than positive regulation to assure that all inputs of a set are present before a transition occurs [24]. For example, in eukaryotic cells the metaphase/anaphase transition does not occur until all chromosomes are attached to microtubules at the metaphase plate. If each attachment helped to activate the transition using a shared activating signal, attachment of the last of many chromosomes would be signaled by a small fractional change in the intensity of the signal. Noise in the signal from attached chromosomes would obscure the last attachment. More formally, suppose the number of signals from each attached chromosome per unit time has a Poisson distribution with mean and variance μ. The sum of signals from n attached chromosomes has a Poisson distribution with mean and variance $n\mu$; the standard error of this mean is $\mu^{1/2}$ [25]. If n is sizeable (say, 10) and μ is small (say, 1), the mean ± standard error of the mean, $n\mu \pm \mu^{1/2}$, changes little when one more chromosome attaches.

Furthermore, since the number of chromosomes may change during evolution, with a shared activating signal the threshold for activating the transition would have to change correspondingly. By contrast, if each unattached chromosome produces the same inhibitory signal, attachment of the last chromosome removes all inhibition. Such inhibition is easier to detect reliably and is insensitive to the number of chromosomes. This rationale can be extended to other situations in which a conjunction of inputs must occur.

2.2 In a multi-input/single output network signaling conjunction, negative regulation uses fewer parts than positive regulation

Let us define the size of a molecular network as the number of parts it contains. Parts include small molecules (*e.g.* metabolites) and domains in proteins. (We do not consider networks signaling through changes in membrane potential.) Consider networks that can signal the conjunction *A1 AND A2 AND . . . AND An* of n prerequisites *Ak*, $k = 1, \ldots, n$, with a single output. Fig. 1(A) shows such a network. In it, the kth prerequisite produces a distinctive signal *Sk*. These signals bind to a receptor *R*, which must respond only when it has bound signals from all n prerequisites. Such a receptor typically includes several protein domains – that is, several parts. For example, each signal might bind to a different domain of the receptor, and allosteric interaction of these domains might determine when the receptor responds. The complex of all signals and the receptor activates a target molecule *T* that promotes the transition