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MINIREVIEW

Shape-Dependent Control of Cell Growth, Differentiation, and Apoptosis: Switching between Attractors in Cell Regulatory Networks

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Development of characteristic tissue patterns requires that individual cells be switched locally between different phenotypes or "fates;" while one cell may proliferate, its neighbors may differentiate or die. Recent studies have revealed that local switching between these different gene programs is controlled through interplay between soluble growth factors, insoluble extracellular matrix molecules, and mechanical forces which produce cell shape distortion. Although the precise molecular basis remains unknown, shape-dependent control of cell growth and function appears to be mediated by tension-dependent changes in the actin cytoskeleton. However, the question remains: how can a generalized physical stimulus, such as cell distortion, activate the same set of genes and signaling proteins that are triggered by molecules which bind to specific cell surface receptors. In this article, we use computer simulations based on dynamic Boolean networks to show that the different cell fates that a particular cell can exhibit may represent a preprogrammed set of common end programs or "attractors" which self-organize within the cell's regulatory networks. In this type of dynamic network model of information processing, generalized stimuli (e.g., mechanical forces) and specific molecular cues elicit signals which follow different trajectories, but eventually converge onto one of a small set of common end programs (growth, quiescence, differentiation, apoptosis, etc.). In other words, if cells use this type of information processing system, then control of cell function would involve selection of preexisting (latent) behavioral modes of the cell, rather than instruction by specific binding molecules. Importantly, the results of the computer simulation closely mimic experimental data obtained with living endothelial cells. The major implication of this finding is that current methods used for analysis of cell function that rely on characterization of linear signaling pathways or clusters of genes with common activity profiles may overlook the most critical features of cellular information processing which normally determine how signal specificity is established and maintained in living cells. © 2000 Academic Press

Key Words: **cell shape; signal transduction; cytoskeleton; Boolean networks.**

INTRODUCTION

Tissue morphogenesis is driven by changes in cell shape, growth, and function that are coordinated in both time and space. Individual cells receive multiple simultaneous inputs, yet somehow they are able to rapidly integrate these signals so as to produce just one of a few possible cell fates (distinct phenotypes). During development of an epithelial gland or a branching capillary network, for example, an individual cell will proliferate, while neighboring cells only micrometers away turn on entirely different gene programs that lead to differentiation or apoptosis [1]. The conventional approach used to understand cell regulation focuses on how each of these distinct functional pathways is regulated. However, to fully understand developmental control, we need to explain how cells can be consistently switched between these different gene programs in the local tissue microenvironment when stimulated by a variety of environmental cues.

The greatest progress in our understanding of developmental control has been made in the area of cell regulation by soluble hormones and growth factors. The general paradigm is that these soluble factors bind to specific cell surface receptors and elicit a linear cascade of biochemical reactions or "signal transduction" events which lead to activation of genes that are specific for one cell fate or another (e.g., growth versus differentiation). One central "mitogenic pathway" that has emerged is the ras–raf–MAPK/ERK signaling cascade which mediates activation of growth-related genes which drive cell cycle progression and entry into S phase [2–4]. Various mitogenic signals with different chemistry (e.g., EGF, PDGF, FGF, etc.) converge on this same signaling pathway [5].

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TABLE 1

Genes and Proteins with Paradoxical Effects on Cell Fate Determination

	Growth	Differentiation/quiescence	Apoptosis	Survival
c-ras	Most cell lines (−) p16 expression	Fibroblasts (+) p16 [77]; (−) rho [93]	Fibroblasts (−) NFκB; (−) PI3K [9, 10]	Fibroblasts (+) NFκB; (+) PI3K [9, 10]
c-myc	Fibroblasts (+) serum [71]		Fibroblasts (−) serum [71]	
NF-κB	Various cell types, via cyclin D1 [78]	Immune resp. inflammation, stress [79–81]	Glutamate-activated neurons; activated T cells [82]	Many cells [82, 83]
MAPK-ERK1/2 (p44/p42)	PC12 (+) EGF (transient) [84]; fibroblasts, via cyclin D1 [2]	PC12 (+) NGF (prolonged) [84, 85]; HL60 (+) PKC [63]; smooth muscle cells (+) COX-2 [87]	Cytokine-treated β cells [88]	Most cells, PC12 [89]
MAPK-p38	Fibroblasts (+) FGF2 [100]	Fibroblasts via cyclin D1 suppression [2]	PC12 (−) ERK [89]	
SAPK/JNK			PC12 (−) ERK [89]; many cells, stress-induced [90]	Fibroblasts on fibronectin [91]
Rho	Fibroblasts (microinjection) [94]; in fibroblasts in suspension [86]		Fibroblasts (−) serum [92]	Several cell types; basic survival [101]
pRb		Most cells (−) serum [96]	Epithelial cells in suspension [95, 96]	Neurons during development [97]
E2F-1	Most cells (promotes S phase entry)		Epithelial cells in suspension [95]; fibroblasts (−) serum [96, 98]	
Bcl-2		Lymphocyte [99]		Most cells

Note. Shown is a selection of genes and signaling proteins (left column) which have been shown to promote different or opposite cell fates (top row) depending on the circumstances, analyzed as indicated in the columns at the right: cell type, treatment, and role of other signaling molecules. (+) Cell fate observed in presence of indicated factor; (−) fate observed in its absence.

While we tend to assign specific functions to signaling molecules and pathways (e.g., mitogenic, apoptotic), the reality is that the information conveyed by the signal transduction machinery often cannot be localized to an individual cascade, rather it is distributed among numerous pathways. On one side of the spectrum, activation of a single signaling receptor results in fanning out of the biochemical signal, such that a very large array of genes becomes induced. For instance, when more than 6000 genes were monitored with DNA microarrays, activation of the FGF or PDGF receptor induced the expression of over 60 genes [6]. Even activation of a signaling molecule far down in the mitogenic pathway (the c-myc transcription factor) induced expression of 27 different genes [7]. On the other end, the same signaling molecule can produce paradoxical effects. Ras promotes growth via MAP kinases ERK1/2; however, in the absence of NF-κB or PI-3K, activation of the same pathway results in programmed cell death [8–10]. Functional characterization of key regulatory molecules by their overexpression or inhibition has revealed a number of similar examples in which a protein can be assigned paradoxical functions depending on the cell type or activity state of other regulatory proteins (Table 1). Taken together, these findings raise the question of how signal specificity is established and maintained in living cells [11, 12].

This picture is further complicated by the fact that diffusible factors alone are not sufficient to fully explain cell fate regulation. Structural cues also affect cell behavior: normal cells need to adhere to an insoluble extracellular matrix (ECM) substrate in order to survive and proliferate. Furthermore, loss of anchorage-dependence is a hallmark of transformation [13] which results in disorganization of tissue architecture [14]. As with soluble factors, ECM molecules bind to specific cell surface receptors—integrins—and thereby activate intracellular signaling pathways that govern whether a cell will proliferate, differentiate, move, or die [15–19]. Importantly, integrin-generated signals also converge with those elicited by growth factor receptors. For instance, cell binding to ECM directly activates the ras–raf–MAPK/ERK pathway and also modulates its activity in response to stimulation by

soluble growth factors [20–22]. Convergence of ECM- and growth-factor-induced transduction cascades upon a common signaling pathway provides one possible molecular mechanism for the observed synergism between growth factors and ECM in switching cells from quiescence to growth [23] as well as from growth to apoptosis [19].

Despite this complex network of signaling pathways, activation of chemical events elicited by specific molecular recognition of cell surface receptors is not sufficient to explain how cell fate is controlled within the physical context of growing tissues in which cells are both bound to ECM and surrounded by multiple growth factors. The answer to this riddle of form generation may lie in the fact that tissues are also exquisitely sensitive to mechanical forces (e.g., hemodynamic forces in blood vessels, compression in bone, tension in skin and muscle, and cell-generated forces in developing tissues) which are transmitted over ECM to individual cells [1]. Importantly, these mechanical stresses may differ locally (beneath one cell versus its neighbor) due to local changes in ECM turnover that alter scaffold compliance or through contraction of underlying cells (e.g., mesenchymal condensation). Both of these alterations are characteristically observed in regions of enhanced cell growth during tissue morphogenesis in the embryo.

In vitro studies similarly demonstrate that cells can be switched between entirely different gene programs through alterations of ECM structure or mechanics that produce changes in cell shape, independent of growth factor binding or integrin binding [24–27]. For example, when growth factor-stimulated capillary endothelial cells were cultured on microfabricated adhesive islands of varying size that were coated with a saturating density of ECM molecules, spread cells on the larger islands proliferated; retracted and rounded cells on tiny islands underwent apoptosis, and cells on the intermediate islands that promoted a moderate degree of spreading switched into a differentiation mode and formed hollow capillary tubes. These results confirm those from past studies which demonstrated tight coupling between cell shape and function in a wide variety of cell types using various experimental techniques [28–32].

Analysis of the mechanism of shape-dependent growth control in endothelial cells revealed that cell distortion does not alter the classic ras–raf–MAPK/ERK signaling pathway. Instead, cell spreading was found to act many hours later in the cell cycle and to harness the same molecular machinery that is responsible for control of the G1/S transition by growth factors [26]. Specifically, cell extension permitted signals elicited by binding of growth factor receptors and integrins to upregulate cyclin D1 and downregulate the cdk inhibitor, p27, thereby promoting Rb hyperphosphorylation and passage through this late G1 restriction point. In contrast, even though growth factors and ECM activated common early signaling events, cyclin D1 and p27 protein levels remained respectively low and high in cells that were prevented from spreading. Importantly, this gene activation pattern in round cells is nearly identical to the cell arrest induced by growth factor withdrawal and by inducing cell rounding from within by disrupting the actin cytoskeleton [26, 33, 34]. So, once again, different regulatory cues converge on a common biochemical switching mechanism, as indicated by activation of a common profile of signaling activities.

The molecular mechanism by which a cell shape change is translated into these biochemical signals is currently not known; however, several lines of evidence suggest that tension-dependent integrity of the actin cytoskeleton is essential for this form of cell cycle control [1]. For example, disrupting the actin cytoskeleton using cytochalasins and simply inhibiting cytoskeletal tension generation without altering cell shape using pharmacological modulators of actomyosin-based contractility both produce a block in G1 progression similar to that induced by cell rounding [26, 33, 35]. In fact, cytomechanical measurements have shown that isometric cytoskeletal tension is increased in spread cells [36] and that tension-driven restructuring of the cytoskeleton promotes formation of a microcompartment specialized for protein synthesis (containing poly(A) mRNA and ribosomes) directly at the site of integrin binding surrounding the focal adhesions [37]. Moreover, focal adhesion formation is itself dependent on cell spreading and associated tension generation within the cytoskeleton [25, 38]. This might represent a direct mechanism for transferring mechanical inputs into growth signals, given that cyclin D1, a rate-limiting protein in G1 progression, and other growth-related proteins are translationally regulated [39, 40]. Furthermore, many mRNAs associate directly with the cytoskeleton [41, 42]. Taken together, these findings suggest that cell shape and behavior may adapt to the mechanics of the cell's microenvironment which, in turn, may be modulated through localized changes in ECM remodeling during tissue development.

CELL FATES AS ATTRACTORS IN CELL REGULATORY NETWORKS

Our current understanding of cell regulation involves such a complex picture of the underlying molecular machinery that it is commonly necessary to break it down into individual signaling pathways that link activated receptors to gene induction in order to gain any insight into the mechanism of control. The concept of linear signaling pathways assumes that the instruction for a cell is encoded in the molecular structure of

the ligand (whether soluble or insoluble) and its specific recognition by its cell surface receptor. The instruction is then passed down to the nucleus by a series of molecular recognition events. In contrast, work on cell shape control has revealed that a generalized mechanical stimulus—cell shape distortion—is sufficient to modulate cell sensitivity to specific regulatory cues and, thereby, to govern whether individual cells will switch between different gene programs. More importantly, this "nonspecific" stimulus can direct cells to take on the same fates (and hence, activate the same signaling intermediates and end-target gene programs) as molecular factors that bind with high specificity to their cognate cell surface receptors. But how could cells have evolved this capacity to trigger development along common pathways whether stimulated by specific chemical conformations or by external mechanical stresses devoid of any information encoded by molecular specificity? Moreover, how can a gradual change in a physical parameter over a broad range, such as cell shape (round to spread), be translated into just these same distinct cell fates? Perhaps by addressing these fundamental questions we may gain further insight into the mechanism by which living cells integrate and process regulatory information.

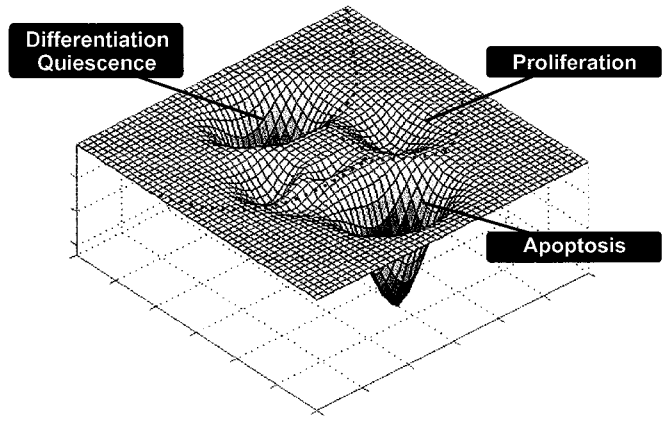


FIG. 1. Attractor landscape representation of cell fate. A hypothetical "potential landscape" representing the N-dimensional state space compressed into two dimensions (xy) for visualization purposes. Every position in the xy plane would correspond to a network state. The vertical axis (z) represents a potential function, an "energy equivalent," representing some distance measure of a network state to the attractor state. Lowest points in the valleys correspond to attractor states that represent cell fates in our model. In this example, the apoptosis state is the deepest and broadest valley, reflecting the fact that apoptosis often appears as a default program which is triggered by a large variety of stimuli.

The paradigm of pathways linking receptors with genes has led to the identification of many new signaling molecules over the past decade. In organisms whose entire genome has been sequenced, more than 10% of the genes appear to encode signal transduction proteins [43]. As we enter the postgenomic era, we must ask: can identification of all these signaling proteins and their assignment into distinct functional pathways lead to full understanding of developmental control and cell fate regulation? Importantly, the findings that cellular signaling involves "distributed information" with intense cross-talk between pathways, that a single signaling molecule produces pleiotropic effects (e.g., activates many genes), and that the same signaling protein may produce opposite effects depending on the chemical and physical context in which it acts, strongly suggest that the concept of linear signaling pathways is inappropriate [44, 45]. Thus, a new paradigm is required for further advancement in this field. Careful analysis of developmental control reveals that the characteristic phenotypes cells exhibit—growth, quiescence, differentiation, apoptosis, motility, etc.—represent *emergent* behaviors that arise through collective interactions among various different genes and signaling components. As described above, these pathways are not linear connections between receptor and gene; rather they are elements of a complex network of interacting signaling components.

How can one begin to approach the problem of biological network behavior and, specifically, address the question of how distinct cell fates emerge? One possible handle on this problem is the finding that gradual variations in a single control parameter, such as cell shape, can switch living cells between distinct gene programs, including growth, differentiation, and apoptosis. This behavior is reminiscent of phase transitions—abrupt macroscopic changes between qualitatively discrete stable states (e.g., liquid versus gas or solid)—that are observed in physical systems. Thus, the various fates that a cell may experience can be viewed as "cellular states," and the switches between these states may then be viewed as biological phase transitions.

With this formulation as a handle, it is then possible to describe the collective ordered behavior of the cell's information processing system and the relation between cell fate switching and possible underlying control elements in precise terms, without focusing on the properties of the individual molecular components. For example, the fact that different stimuli, or a single stimulus acting over a specific range, lead to the same distinct phenotype (e.g., growth), suggests that cell fates can be viewed as common end programs or "attractors" within a regulatory network [46]. To visualize attractors, imagine a potential landscape containing multiple valleys with hills in between; a droplet of rain which lands on this terrain and rolls down the energy potentials is always attracted to one of the same set of possible valleys or "basins of attraction," eventually coming to rest in a stable end-state (attractor) at the bottom of one of these valleys (Fig. 1). The position of the droplet at any time may be viewed as the internal

state of the cell which, when activated by some stimulus, rolls along the potentials always falling into one of the same set of possible valleys, or in this case, cell fates.

But are cell fates structured as attractors within the cell's regulatory networks? In support of this hypothesis, regulation of cell function appears to involve selection of preexisting (latent) behavioral modes of the cell, rather than instruction by specific binding molecules [47, 48]. Moreover, nonspecific pharmacological stimuli that activate multiple proteins across several signaling pathways often trigger expression of the same set of cellular phenotypes. General inhibition of serine/threonine kinases with staurosporine or elevation of protein tyrosine phosphorylation using orthovanadate induces apoptosis in many cell types [49, 50]. Differentiation of many cell types also can be turned on by nonspecific agents, including DMSO or ethanol [51–55]. In these cases, it appears that simultaneous perturbation of multiple targets in different pathways results in the channeling of the biochemical effects into common end-programs and hence the same set of distinct cell fates.

The dynamic nature of this switching between different phenotypes, combined with the finding that diverse stimuli produce the same set of end responses, suggests that cell fates are indeed organized as attractors. Thus, the different fates a cell may experience—differentiation, growth, quiescence, motility, apoptosis, etc.—indeed do appear to be preexisting behavioral modes that are wired into cells' regulatory networks. Furthermore, there may be more than one low energy well at the bottom of an attractor (Fig. 1), such that the same cell can experience, for example, more than one state of "quiescence." The transition between different cellular states in this type of dynamic regulatory system would result from a selection process that can be as equally triggered by a nonspecific control parameter (e.g., general kinase inhibitor, cell spreading) as by a soluble growth factor that binds to specific transmembrane receptors. In other words, the functional state of the cell "self-organizes" itself: no external instruction is required, only a stimulus that the cell can perceive.

GENETIC NETWORKS AND SIGNAL TRANSDUCTION NETWORKS AS BOOLEAN NETWORKS

If living cells use this type of dynamic regulatory network, then how do these attractor states (latent behavioral modes or cell fates) self-organize themselves through collective interactions among different molecular components? One mathematical approach that may be used to understand information processing and generation of a macroscopic collective behavior within this type of complex regulatory network is the use of Boolean networks composed of binary genes or signaling molecules interconnected by regulatory interactions (Fig. 2a) [46, 56, 57]. This idealized model is sufficient, for example, to reveal how ordered collective gene activity patterns spontaneously emerge within certain classes of networks while circumventing the need to know quantitative details about all of the internal biochemical interactions [57, 58]. A Boolean network is a mathematical formulation which consists of interconnected binary elements, such as genes or proteins that can be either ON = 1 (e.g., representing a kinase that is expressed and in the phosphorylated, catalytically active state) or OFF = 0 (representing the silenced gene, or the expressed, but inactive kinase). The digitization allows the use of the Boolean formalism to characterize the effects of the collective actions of the inputs on any individual network element (e.g., a regulatory protein). For instance, in the simple case of two inputs per element, an AND function implies that a target protein is turned ON only if both inputs ("upstream regulators") are ON; a NOT IF function requires that one of the inputs be ON and the other OFF to turn the target ON (Fig. 2a, see Fig. 3 for details) [59]. The activity statuses of all the N genes or proteins within the network form an activity pattern; each pattern at a given time t constitutes a *network state,* $S(t)$ (Fig. 2b). Together, all the possible activity patterns (network states) define the complete *state space* (Fig. 2c) in which every point represents a cellular state (i.e., the pattern of gene or protein activities within the network at any given time).

Because of the constraints imposed by the interconnections between different signaling elements within the network, most of the activity patterns that are theoretically possible are unstable, that is, they violate the logical rules of the Boolean function. For example, genes A and B cannot be simultaneously ON, if A inhibits B, and A and B cannot both be OFF, if A activates B. Unstable states migrate along trajectories in the state space until they reach stable ones as the Boolean functions are executed and the network dynamically changes its activity profile over time. Trajectories can converge, like rivers in a landscape, and flow into one of a few possible attractors resulting in establishment of a stable, discrete pattern of gene or signaling protein activities. Attractors may represent either a single stable state that is self-reinforcing once formed (i.e., when the activity profiles are "updated" over time, they regenerate the same activity profile) or a stable series of states that together form a small loop ("limit cycle"), as shown on the bottom right of Fig. 2c. Such cycling attractors would correspond, for example, to repeating gene activation patterns that recur during repetition of each cell cycle [46].

The set of activity profiles in the state space that lead to the same attractor form the *basin of attraction* of that attractor. Basin boundaries divide the state space

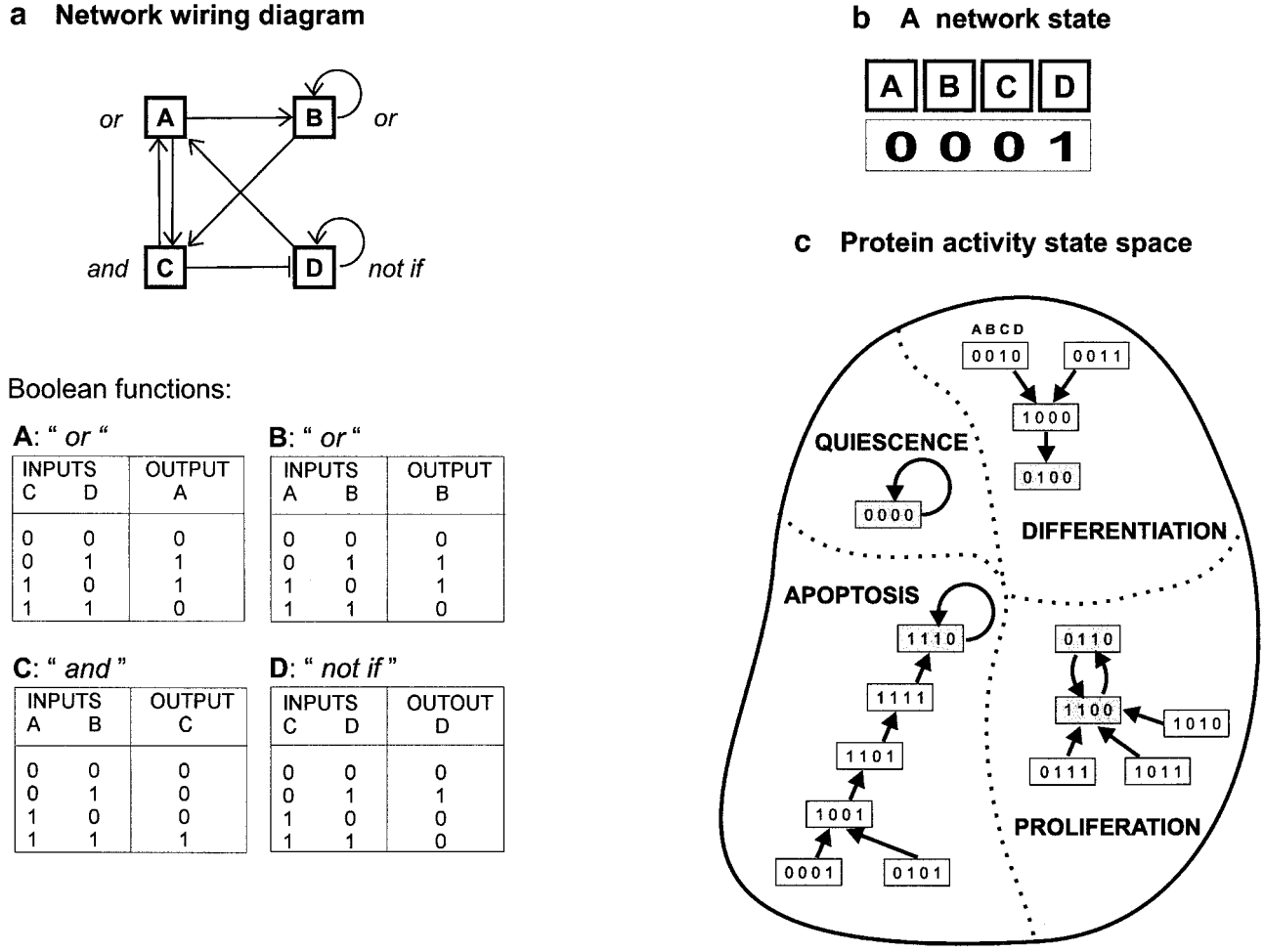


FIG. 2. Basic principles of Boolean network models and cellular states. A four-element network is used as an example to illustrate how attractors arise from the interactions between different network elements. (a) The wiring diagram of the four elements, A, B, C, and D, which could represent genes or proteins of a regulatory network. In this idealized case, all elements have two inputs that can include input from the element itself, representing a feedback loop. The thin arrows indicate specific regulatory interactions. Each element is assigned a Boolean function, as shown in the corresponding boxes below (for details, see Fig. 3), which dictates how the input is processed into an output (i.e., the activity state of that element). (b) Network state $S(t)$. *Each element can be ON* (*represented by* 1) *or OFF* (0). *The set of all the ON/OFF configurations for all elements defines the network state at a given time point t.* (c) The state space and attractors. All possible network states (in this case $2^4 = 16$) together form the state space, in which every box is a network state. Following the rules defined by the wiring diagram and the Boolean functions, the network states transition into each other, as indicated by the thick arrows which represent the trajectories. Attractor states are depicted in gray. Note that trajectories can converge, but not diverge, due to the rules of the network, resulting in the formation of basins that drain into the attractors. The entire state space is divided into basins of attraction whose boundaries are shown with a dotted line. Each attractor and its basin can be equated to a cell fate, such as differentiation, quiescence, apoptosis, or proliferation. The proliferation attractor is a limit cycle (in this case, containing two states that transit in each other) which corresponds to the oscillatory gene expression patterns characteristic of repeated passage through the cell cycle. Note that thin arrows in (a) represent physical interactions constituting the wiring diagram while the thick arrows in (c), the abstract state space, are dynamic processes (trajectories).

which results in compartmentalization of the total possible state space (Figs. 1 and 2c). Importantly, the state space of this regulatory network therefore has an internal structure that imposes certain dynamics onto the global activity pattern of the interacting components—genes and signaling proteins in the case of living cells. The dynamics of regulatory switching within this type of regulatory network can be pictured as a marble on a landscape with broad valleys, narrow troughs, and pits; the marble may explore various initial paths; however, it is eventually forced to follow a certain trajectory that leads to one of the few final attractor states (Figs. 1 and 2c). The position of the marble at any given time in the state system of the cell's regulatory network represents a transient pattern of gene activation or protein signaling protein activity. The choice of the final attractor state depends on the attractor landscape, the starting point, and any

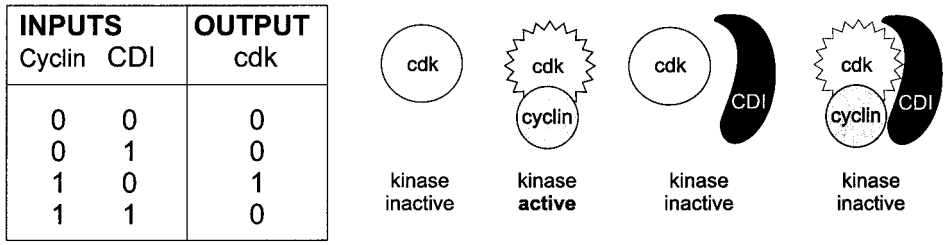


FIG. 3. Example of how to encode a regulatory protein interaction in a Boolean function. cdk represents a cyclin-dependent kinase (e.g., cdk4) that is a signaling protein which receives two inputs from its upstream regulators, one a cyclin (e.g., cyclin D1) and the other a cdk inhibitor (CDI; e.g., p27). The cdk requires association with a cyclin to be activated; the CDI binds to the cyclin–cdk complex [102] and inhibits cdk activity. This regulation can be encoded with a NOT IF Boolean function shown in the bottom of Fig. 2a: The cdk is ON (= 1) (active kinase) only IF CDI is NOT present (= 0) and the cyclin is present (1).

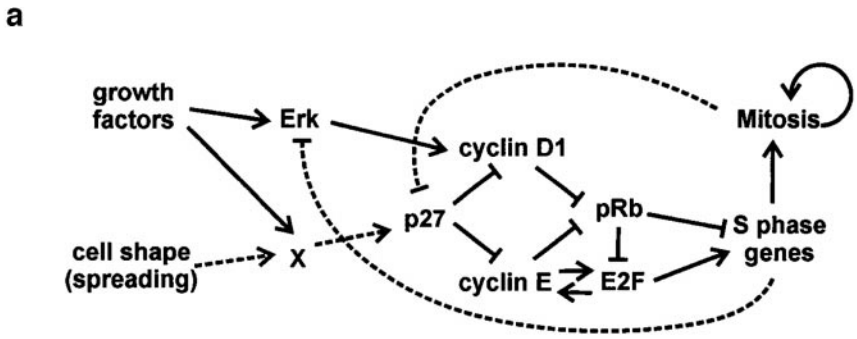
influences that may perturb the dynamics of the protein or gene activity pattern.

In summary, the generic dynamic properties of Boolean networks predict that if the cell organizes its regulatory network in this manner, then multiple attractor states (distinct stable gene activity patterns or signal transduction profiles) will spontaneously emerge as a result of internal network interactions. This model is therefore consistent with the proposal that the different cellular states characteristic for any particular cell type (e.g., growth, differentiation, apoptosis, motility, etc.) may be viewed as attractor states within the cell's regulatory network [46]. The depth of the valleys in the attractor landscape indicate that attractors are mutually exclusive states that are inherently stable to perturbations, such as the random "flipping" of the activity status of any individual network element. This is also a property exhibited by living cells. For example, differentiation and proliferation are well known to be mutually exclusive and robust [60]; moreover, in many cell systems just quitting the proliferation state by overexpressing the cell cycle inhibitor p21 forces the cell to automatically enter the differentiation program [61–64]. But how can the intrinsic stability of an attractor state be overcome? Network simulations reveal that it is necessary to change the activities of *multiple* network elements in order to cause this type of dynamic regulatory network to switch between different self-stabilizing attractors [65]. Thus, each transition requires the modification of the activity status of a precise set of regulatory molecules. Importantly, these transitions correspond to the switch of cell fate that is induced by external stimuli which trigger pleiotropic effects, such as when cells are stimulated by soluble mitogens, adhere to ECM, or are exposed to mechanical stresses [1]. Herein might lie a basic design principle behind the wiring architecture of regulatory networks, such as those observed in living cells, that are dominated by convergence and pleiotropy. In this context, "master switch" genes, such as MyoD [66] or PPARγ [67], that transform cells between entirely different lineages (e.g., from fibroblasts or fat cells to muscle cells), may be viewed as elements which precisely activate the correct set of genes and proteins which leads to major changes in their activity patterns, thereby triggering the transition of the network state to a new attractor representing an entirely new differentiation state. (Such transitions would correspond to transdifferentiation and require that the new attractor, the differentiation attractor of another cell type, is accessible from the original state. This in turn depends on the local structure of the state space as defined in the genome-wide wiring diagram [65].)

BOOLEAN NETWORK SIMULATIONS MIMIC BEHAVIORS OF LIVING CELLS

If the cell's regulatory networks self-organize into multiple alternative cellular states in this manner, then a nonspecific control parameter that elicits pleiotropic changes in intracellular signaling, such as a physical force or cell distortion, would be able to evoke similar signaling trajectories into a common set of attractors and, hence, the same cellular responses that are induced by specific biochemical signals which contain regulatory information in their three-dimensional structure. Given that we observed precisely this response in our past studies on shape-dependent control of cell function, it may therefore be useful to explore whether experimental results obtained during analysis of the switching between different phenotypes with living cells are consistent with the existence of this type of information processing system which is based on self-organizing dynamic networks.

To illustrate the idea of Boolean networks and shape-dependent switching between attractors, we simulated the dynamics of the signaling system within capillary endothelial cells as a simple Boolean network which consists of cell cycle genes and proteins and whose wiring diagram and mathematical processing functions are based on the known signaling interactions (biochemical or functional) between these differ-



network element	Erk	D1	p27	(X)	E	E2F	pRb	(S)	(M)
input 1	GF	Erk	(M)	GF	E2F	E	D1	pRb	(M)
input 2	(S)	p27	(X)	spread	p27	pRb	E	E2F	(S)
boolean function	not if	not if	implicat	and	implicat	not if	nand	not if	not if

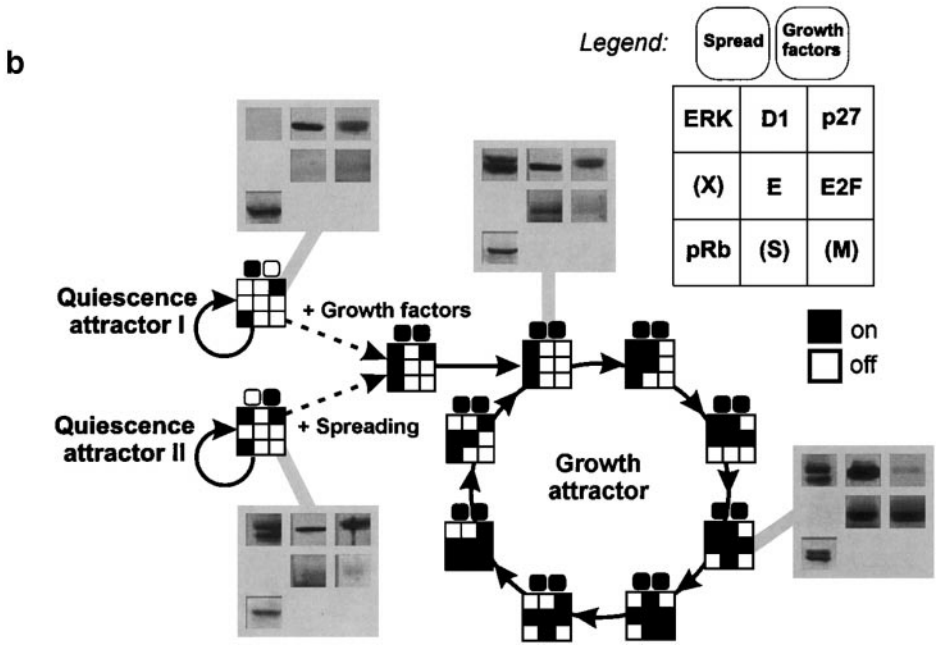


FIG. 4. Computer simulation of gene and protein signaling activity patterns within a small model Boolean network compared with experimental results relating to the switch between growth and quiescence in living cells. (a) The wiring diagram used in the dynamic network model consists of nine growth-related genes or proteins; logical placeholders are used for S-phase genes (S) and the process of mitosis (M) and unknown processes involved in transduction of the shape signal into a biochemical signal response (X). The system is under the control of two external inputs: growth factors (GF) and cell spreading (shape). Simple arrows indicate positive stimulation; barbed ends denote inhibition; dashed lines represent indirect effects. (M) was used in the simulation to close the circle and represents collectively the events downstream of (S). The self-limiting property of mitosis was implemented by a negative feedback loop. For simplicity, every gene was allowed to have only two inputs corresponding to the best characterized upstream regulators. The table summarizes the Boolean functions of the network elements with the names of the Boolean function in the bottom row. Implicat, "implication," a Boolean function in which the output is always ON except in the situation in which one of the inputs is ON and the other is OFF. Nand, "not and," a Boolean function in which output is always ON except if both inputs are ON (for details see [46, 59]). (b) State space structure of the network described in (a) obtained from the computer simulation and compared to experimental results obtained in studies with capillary endothelial cells cultured on ECM-coated adhesive islands of varying size that were created with a micropatterning technique [26]. The small 3×3 checkerboard grids represent the network states with the activities of each of the nine genes or signaling proteins highlighted in the nine corresponding boxes of the checkerboard (see legend). Black squares indicate the ON = 1 status of the gene or protein; the two larger squares with rounded corners at the top of each checkerboard denote the status of the external inputs to the network: presence or absence of GFs and of cell spreading. Solid arrows signify transitions between network states upon execution of the Boolean functions; dashed arrows indicate transition between attractors resulting from changes in the two external inputs. Results of experimental monitoring of gene protein signaling protein activities in endothelial cells are shown in the gray inset boxes for four of the network states. For cyclin E and E2F, mRNA

ent regulatory components (Fig. 4a) [26]. For example, as shown in the table in Fig. 4a, if growth factors and spreading were present (both ON = 1), then due to the Boolean AND function, the output of X would also be ON (= 1), whereas due to the NOT IF function, cyclin D1 would be induced (ON = 1) only if p27 was OFF = 0 ("not on") and ERK was ON(= 1). The entire signaling network was idealized in this manner to allow exactly two inputs per element; it also contains some logical placeholders instead of specific genes, such as (S), representing S phase, and (M), representing mitosis. We then compared the gene and signaling protein activity status of the attractor states (3 × 3 checkerboard in Fig. 4b) which spontaneously arise within this computer-simulated network with the observed activity profiles obtained in our past experimental studies with living endothelial cells (insets with images displaying results from Western blots or PCR analysis are shown in Fig. 4b) [26]. This analysis revealed that in the presence of soluble growth factors and cell spreading the theoretical network exhibited a single, limit-cycle attractor corresponding to the proliferation state (*growth attractor* in Fig. 4b). In this attractor, the cell visits a series of network states repeatedly; this movement corresponds to the recurring gene and protein activity patterns characteristic of passing through the cell cycle progression multiple times. The gene and protein activity patterns of the individual states which form the state cycle as cells undergo cell cycle progression are very similar to the biochemical activity profiles of genes and proteins measured within cultured endothelial cells (insets, Fig. 4b). Upon removal of growth factors or induction of cell rounding (removal of spreading), the network transitions into single-state attractors (*quiescence attractors I* and *II* in Fig. 4b) which are unstable in the presence of both stimuli (i.e., growth factor and spreading). These two single-state attractors correspond to quiescent states which differed slightly in their gene and signaling protein activity profiles, much like what is observed in living endothelial cells which stop growing and enter slightly different G0/1 quiescence states when they are prevented from spreading or cultured in the absence of soluble mitogens [26]. Fibroblasts similarly experience different forms of quiescence when growth is blocked by serum removal or detachment from ECM [68, 69].

In the Boolean network simulation, the shape-dependent signal elicited a concerted response leading to the transition between quiescence and growth attractors because we explicitly fed the shape signal in as the input of a Boolean function via a putative transducer, "X" (Fig. 4a). In reality, the molecular pathways for shape-dependent control remain obscure. Still, the fact that, in living endothelial cells, shape modulation induces changes in patterns of gene activation and signal transduction that are almost identical to those produced both by specific soluble mitogens and by the computer simulation based on Boolean networks is remarkable. These findings strongly support the concept that the nonspecific "shape signal" is translated by the cell into coherent patterns of gene expression and signaling activity (cell fates) that represent attractors within the cell's dynamic regulatory network.

IMPLICATIONS FOR THE FUTURE

We have rapidly reached the limits of the current information processing paradigm in cell biology which is based on the use of linear signal transduction pathways for developmental control. This paradigm emerged from recognition of the importance of soluble hormones for cell regulation and from the fact that the functional effects of these regulators are mediated by their binding to cell surface signaling receptors. However, more recent studies have clarified that insoluble ECM molecules and mechanical forces that produce cell distortion play equally important roles in the control of the cellular phenotype. Furthermore, cell shape regulates switching between different cell fates by inducing the same gene and signal protein activity profiles that are activated by cell binding to specific growth factors and ECM molecules. This observation, combined with the increasing awareness of "distributed" information processing within cell signaling networks, emphasizes the necessity to develop a more global and integrated model of cell regulation.

In this article, we introduced the use of Boolean networks as simple idealized models of cellular information processing, particularly as it relates to switching between different cell fates. Computer simulations revealed that the different phenotypes that a particular cell may exhibit—growth, differentiation, apoptosis, quiescence, motility, etc.—may represent attractors which spontaneously arise (self-organize) within the dynamic network of molecular interactions that comprises the living cell. The existence of a basin of attraction for a cellular state massively increases the

levels were monitored by reverse PCR; cyclin D1 and p27 protein levels were analyzed using Western blots as were Erk phosphorylation and pRB hyperphosphorylation (doublet band indicates pRb inactivation). Note that for cyclin D1, basal expression was always detected; activation (ON = 1) is indicated by the increased band staining intensity. For (X), (S), and (M), the corresponding field in the experimental data inset remains empty; however, the ON or OFF status in the computer simulation is noted in the small checkerboard grid. Note that (S) turned on after about half of the cell cycle in the simulation, consistent with cell cycle progression leading to entry into S phase. (M) was aberrantly turned on transiently early in the cycle (right bottom square)—revealing an intrinsic artifact of the Boolean network that can give rise to aberrant oscillations.

odds for evolution to link the intricate biochemistry of cell fate regulation with the physical world that lacks specific molecule-encoded information. This might have facilitated the evolution of larger organisms whose development and function must satisfy the laws of physics at all size scales, yet are regulated by genes. More importantly, the concepts of Boolean networks and attractor landscapes capture many properties of the dynamics of cell regulation that otherwise would have required metaphoric descriptions to grasp, such as the recognition of a "balance of survival and apoptotic signals," "cellular decision-making," "conflicting signals," and "default programs" [70–73]. In addition, recognition of the use of this type of complex dynamic networks by cells provides a formal basis for analyzing tolerance and sensitivity of cellular systems to various types of interventions (e.g., pharmacological or infectious agents) [46, 74]. The Boolean network model described here is no doubt an oversimplification; however, it represents a first step toward development of a language for conceptualizing how information is processed within regulatory networks in living cells. Further refinements will be necessary to embrace features of real molecular interactions, including the temporal order of inputs, asynchrony of network updating, and multiple thresholds.

The implications of the use of dynamic regulatory networks by cells go much further than control of cell fate determination by cell shape. For example, the results of our Boolean network model suggest that the current approaches used in functional genomics to analyze results of DNA microarray-based gene expression profiling [75], such as clustering similar expression profiles, may overlook the most important features of cell regulation. Very simply, existing forms of gene cluster and proteomic analysis [76], at best, transform data into information. To convert information into knowledge, novel approaches are needed that address the fundamental principle of cellular regulatory systems as a whole. The concepts of dynamic Boolean networks and attractor landscapes could provide a more meaningful, albeit rudimentary, framework for such an integrative approach. Broadening our view beyond the realm of molecular recognition events to embrace physical control parameters in cell regulation, such as cell shape and mechanics, also should help to bridge the gap between test tube biochemistry and the biology that we observe within intact living cells and organisms. Continued efforts of this type which attempt to place molecular regulation in the context of cell and tissue structure will be necessary to ensure that we take the most expeditious and meaningful path forward in this newly emerging postgenomic era.

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