Motifs, modules and games in bacteria

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Global explorations of regulatory network dynamics, organization and evolution have become tractable thanks to high-throughput sequencing and molecular measurement of bacterial physiology. From these, a nascent conceptual framework is developing, that views the principles of regulation in term of motifs, modules and games. Motifs are small, repeated, and conserved biological units ranging from molecular domains to small reaction networks. They are arranged into functional modules, genetically dissectible cellular functions such as the cell cycle, or different stress responses. The dynamical functioning of modules defines the organism's strategy to survive in a game, pitting cell against cell, and cell against environment. Placing pathway structure and dynamics into an evolutionary context begins to allow discrimination between those physical and molecular features that particularize a species to its surroundings, and those that provide core physiological function. This approach promises to generate a higher level understanding of cellular design, pathway evolution and cellular bioengineering.

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Abbreviations

JNK	c-Jun amino-terminal kinase
MAPK	mitogen activated protein kinase
TBP	TATA-binding protein

Introduction

Whole-genome/high-throughput techniques open questions about entire organismal function and make feasible comparisons of the behavior of different organisms and their mutants. The number of computational tools used to perform and quantify these comparisons has multiplied [1–11]. This new fare is generating a more complete view of cellular function, by exposing and investigating the extensive networks of interconnections amongst cellular components and processes.

Analysis and simulation of network dynamics can verify that all the data on a particular pathway are consistent; it can test and generate hypotheses about network structure, the fundamental operating principles governing network function and the role of feedback and protein modifications. It can also predict the effects of mutation, environmental perturbation and pharmaceutical actions [12]. Topological analyses look for metrics and patterns of interconnections across and between networks [13•,14, 15,16•]. Evolutionary analysis on the level of networks and pathways is also now possible, together with more traditional physiological and molecular evolutionary investigations. Dynamics, topology and evolution are all interconnected, because evolutionary forces constrain dynamics, and the functional imperatives of dynamics canalize topology. Moreover, investigations into these topics provide clues on network decomposition (the identification of functionally significant subnetworks such as motifs and modules or other, yet to be discovered, organizational units besides operons and regulons) [17].

Network-oriented approaches have extended questions of similarity and design far beyond the level of single genes and proteins, to how networks translate perturbations into dynamical behavior of the cell, how they are the same and different across many different species, and why behavior is different in one species from that in another, despite a good deal of network homology.

In this review, we organize recent work on these network topics into a framework for thinking about how intracellular networks regulate cellular behavior and why they do it the way they do. The framework is built on the concepts of motifs, modules and games.

Motifs

Cellular regulation is achieved through the complex network of interactions among biochemicals and cellular structures. The challenge to understanding the dynamic function of these networks, composed of perhaps tens of thousands of reactions among thousands of distinct chemical species, lies in this very complexity. It is therefore important to find ways of simplifying the description of these networks to facilitate analysis. One such attempt is in the identification of motifs (small, repeated, perhaps evolutionarily conserved regulatory subnetworks, classifiable on the basis of function, architecture, dynamics, or biochemical process) [17–19]. Regulatory motifs proposed to date, with the help of mathematical systems theory and complementary experiments, include switches, amplitude filters, oscillators, frequency filters, noise filters and amplifiers, combinatorial logic, homeostats, rheostats, logic

Table 1

Proposed regulatory motifs classified on the basis of dynamic function.

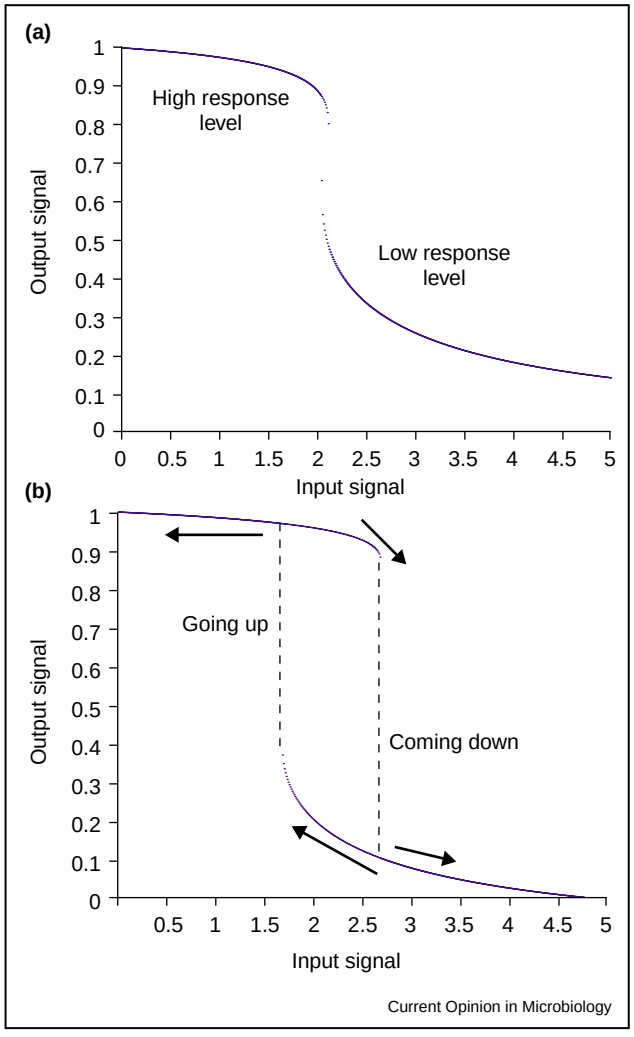
Motif	Function	Mechanisms	Examples
Switches	Digital control Computation Signal integration, amplification and noise rejection	Transcriptional control, cooperativity [23,95], Zero-order [26] cascades [24,25] Multi-input [26] Cross-repressive feedback [30,31] Positive feedback [32,33••,35] Invertible DNA and ratio-based control [29•]	*fim* in *E. coli* Phage lambda Quorum sensing MAPK and c-Jun amino terminal kinase (JNK) pathways in *Xenopus* Synthetic switches [31,33••]
Oscillators	Temporal/sequence loop Synchronize to environment Reject noise Carry signal	Relaxation, harmonic, ring oscillators Negative feedback with high gain or a delay Positive feedback Combinations of positive and negative feedback [42,45,54].	Cell cycle cAMP Circadian rhythms Glycolysis [43] Cytosolic Ca^{2+} Synthetic oscillators [46,96–98]
Biphasic amplitude filters	Tune phenotype to environmental niche Auto-regulation Computation Amplitude multiplexing	Differentially activating binding affinity clusters [29•] Scaffolds [41] Concentration-dependent pathway activation/repression [39]	*fim* temperature tuning [29•,99,100] gltBDF [37] TBP [38]
Bandpass frequency filters	Interpret dynamic signals Filter noise Demodulate Demultiplex	Third-order chemical reactions Excitable media bandpass filter [53] Integral feedback [55] Saturated kinase and phosphatase activity Receptor desensitization [50,54,101]	Interleukin-2 activation by Ca^{2+} [52] Neural growth cones cAMP frequency decoding
Memory	Event tracking Sequencing Process control Temporal integration of signals	Multi-stability DNA inversion Receptor methylation DNA methylation [102] Histone acetylation Phosphorylation timers [103] Hysteresis and delays [29•,63•]	Developmental switches Cell cycle Sic1 [103] Shufflons Type 1 piliation, Chemotaxis
Noise filters	Precise regulation from noisy components.	Negative feedback Redundancy Cascades Checkpoints Delay lines [36,58,104] Frequency filters [53]	MAPK cascades [105] Cell cycle and flagellar synthesis checkpoints; Negative feedback [33••]
Noise amplifiers	Population heterogeneity, antigenic variation.	Noise controlled bistability [30] DNA rearrangement Slipped-strand mispairing [34]	Lambda phage [30] pap *fim* *his* Shufflons [34]

gates and memory elements (Table 1; [18,20]). We describe just a few of these examples below.

Switches

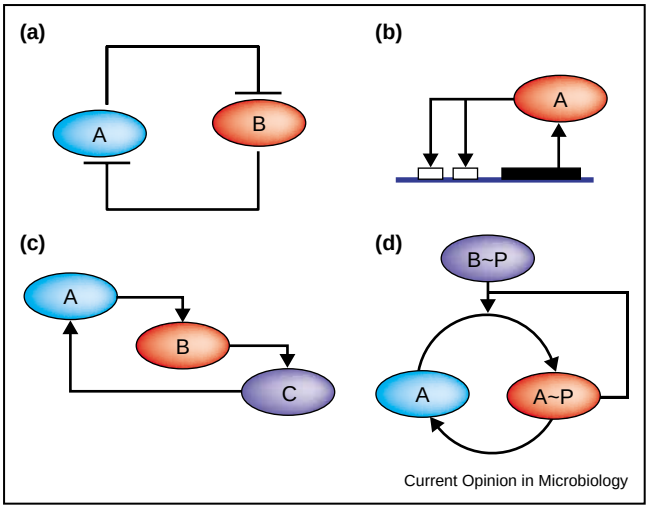
Regulatory switches enable cells to respond to environmental or intercellular signals with an all-or-nothing response. Switches control eukaryotic development (e.g. vulvar development in *Caenorhabditis elegans*) and many bacterial stress responses (e.g. alternative metabolic pathways, pili expression, sporulation and competence). Switches can be memory-less, like a doorbell (Figure 1a), or multistable, like a light switch. They are randomly triggered or tightly controlled, and manifested by single cells or populations, as in quorum sensing [21,22]. Elementary memory-less switching mechanisms include the cooperative activation or repression of gene expression [23]; cascade ultrasensitivity, arising in mitogen-activated protein kinase (MAPK) cascades [24,25]; multi-input cascades, as found in glycolysis [26]; zero-order ultrasensitivity, postulated for futile cycles operating near saturation [27] and observed in the formate/lactic dehydrogenase cycle [28]; and ratio-controlled

Figure 1



Memory-less **(a)** and bistable **(b)** switches. (a) A memory-less switch can be 'on' or 'off' depending on the level of the input signal, but cannot be 'set' by the transient application of a stimulus. (b) Bistable switches are hysteretic, meaning that different stimulus-response curves are generated depending on whether the system begins in the 'on' or 'off' state. These systems have memory, as a transient input stimulus can potentially 'set' a bistable switch to an 'on' or 'off' state.

Figure 2



Bistable switching mechanisms. Bistable switching mechanisms include **(a)** cross-repressive feedback with cooperativity, in which A inhibits B cooperatively and B inhibits A [30,31]; **(b)** cooperative auto-activation of gene expression, for example if gene product A activates its own expression in a cooperative manner; **(c)** ultrasensitive cascades with feedback as postulated for a MAPK cascade switch in *Xenopus* oocytes [35]; and **(d)** zero-order sensitivity with feedback, for example in auto-catalyzed phosphorylation/dephosphorylation reaction cycles operating near saturation.

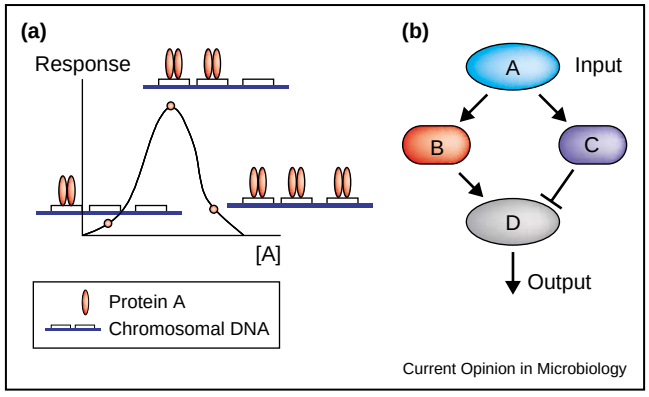
activation, characterized by differential activation of a process by two competing regulatory proteins, as found in the network controlling the probability of type 1 pili expression in *Escherichia coli* [29•].

Unlike memory-less switches, bistable switches are hysteretic and so can be 'set' (possibly irreversibly) to an 'on' or 'off' state by the transient application of a stimulus (Figure 1b). Bistable switching motifs, particularly important to developmental and transformational processes, include cross-repressive feedback loops with cooperativity, as found in the lambda phage [30] and synthetically constructed in *E. coli* [31]; positive feedback with cooperativity, as seen in c-Jun amino-terminal kinase (JNK) circuits [32] and tested in the synthetic yeast switch [33••]; site specific DNA inversion, found in networks controlling surface structures like pili and flagella [29•,34]; and many memory-less switching architectures wrapped in feedback (for example, the autocatalytic ultrasensitive MAPK cascade in *Xenopus* oocytes [35]) (Figure 2). Bistable switches are thought to control developmental and transformative processes because of their ability to 'remember' a stimulus and maintain a state indefinitely. Memory-less switches, however, are likely to serve as signal-thresholding components in larger systems, or control processes requiring reversible on/off control. Even in reversible control, however, a small amount of hysteresis can prevent 'switching chatter', rapid, unproductive cycling between 'on' and 'off' states triggered by intracellular noise [29•,36].

Biphasic amplitude filters

A biphasic amplitude filter is a device that amplifies an input signal only if it is within a specific range, thereby allowing a process to be triggered by a particular environmental or intracellular condition. In theory, the serial connection of two oppositely oriented switches can implement a biphasic response; however, recent analyses have uncovered alternative mechanisms. One proposed mechanism for biphasic control involves multiple DNA-binding sites with differential affinities and regulatory effects (Figure 3a). This motif was thought to tune type 1

Figure 3



Biphasic amplitude filtering mechanisms. **(a)** A DNA-binding affinity clustering mechanism. Protein A binds to chromosomal DNA, and contributes to the regulation of a process, for example gene expression or DNA inversion. Chromosomal DNA has several binding sites for A, with varying binding affinities. When protein A occupies only the strong binding sites (left, centre), the response is activated, whereas when protein A occupies all the binding sites (strong plus weak, right), the response is inhibited. **(b)** A pathway-level mechanism. Activating (B) and inhibitory (C) signaling pathways are stimulated by the same signal (A) and lead to the same response element (D). A biphasic response will result if B is activated (ultrasensitively) at low levels of A, and if C is a strong inhibitor and is activated only at high levels of A.

pili expression to mammalian body temperature [29•], and appears to contribute to β-galactosidase operon control [37] and the control of a TATA-binding protein (TBP) gene by TBP-promoter-binding factor (TBFP) [38]. A biphasic response can also be achieved through the interaction of two signaling pathways stimulated by the same input signal (Figure 3b; [39]). This mechanism was used to explain why Xbra is induced in a narrow window of activin concentration during mesoderm induction in *Xenopus laevis* [39,40]. Protein scaffolds, like those associated with MAPK cascades, have been hypothesized to serve the same purpose. For any generic scaffold there exists a concentration value optimal for signal propagation [41].

Clocks and oscillators

Cells are thought to have evolved clocks and oscillators to control growth rate, to adapt to periodically varying environmental conditions (e.g. circadian rhythms), to control information flow, for example in neurons, and perhaps to facilitate crosstalk and carry multiple signals in the same medium (similar to frequency modulated [FM] channels on a radio) [20] or to bypass the desensitization brought about by constant stimuli [42]. Neural and cardiac rhythms are associated with ion channels; metabolic oscillations in glycolytic flux in yeast depend upon enzyme activity [43]; calcium oscillations and pulsatile intercellular signals involve receptor activity or transport process modulation; and circadian rhythms are dominated by gene expression control. Although their means of control differ, there are common regulatory themes in the generation of oscillations: negative-feedback loops with high gain and/or delays, destabilizing positive feedback, or combinations of the two [44].

A mechanism for cAMP oscillations in *Dictyostelium* has been proposed to involve both positive-feedback and negative-feedback loops [42,45]. Transport of extracellular cAMP into the extracellular medium creates a positive-feedback loop that drives cAMP synthesis, creating a sharp increase in production, upon which a negative-feedback loop — created by cAMP-induced receptor desensitization — allows cAMP to drop to minimal levels, thus setting the stage for the beginning of the next cycle.

Circadian rhythms in *Drosophila* and cyanobacteria partly originate from the negative feedback exerted by a protein on the expression of its gene [46–49]. A positive-feedback loop involving calcium-induced calcium release (CICR) was used to explain cytosolic Ca^{2+} oscillations [50]. The eukaryotic cell-cycle is also an oscillator, albeit an unconventional, quasi-digital one because of the existence of checkpoints and composite switching modules [51].

Bandpass frequency filters

To interpret dynamic signals and function in the presence of noise, cells must be able to filter, and perhaps demodulate and de-multiplex frequency-domain signals. Although it is clear that cells perform this type of processing, as demonstrated by the sensitivity of interleukin-2 expression to the frequency of cytosolic calcium oscillations [52], or the frequency-selective decoding of cAMP pulses into slime-mold development, the mechanisms responsible are largely unknown. However, modeling studies have produced possible mechanisms, including excitable biochemical enzyme networks and certain bimolecular reactions [53], phospho-transfer cycles operating near saturation [50,54], and integral feedback, like that found in chemotaxis [55]. All of these architectures behave like bandpass filters, amplifying a signal only if it oscillates at a particular frequency. If multiple pathways act as bandpass filters at different frequencies with respect to the same signaling molecule, then the molecule could potentially act like a FM channel, efficiently carrying multiple signals and controlling different cellular processes.

Noise-related motifs

Whether considering regulatory dynamics dominated by switching, oscillations, frequency or amplitude filtering, or simple homeostasis, a central mystery in biology concerns the dichotomy between noisy intracellular components and precisely regulated cellular processes. Results from computational studies [56] and laboratory experiments [57] have suggested that intracellular noise is sometimes a product of random bursts of protein production, primarily arising during translation. Negative feedback, redundancy, cascades, checkpoints, delay elements

and frequency filters are motifs that achieve reliability in the face of this uncertainty [36,58].

There are also motifs that exploit noise. Antigenic diversity and population heterogeneity that 'spreads risk' over multiple phenotypes is produced by mechanisms that couple intracellular noise to an ordered process, for example noise-triggered bistability [30], DNA rearrangement and shuffling, and slipped-strand mispairing mechanisms [34]. Cells also appear to use noise to enhance a signal, as in the phenomenon of stochastic resonance [59].

Interacting motifs control complex processes

Motifs do not function in isolation. Complex processes such as growth, the cell cycle, maintenance, developmental programs, motility and pathogenic processes are controlled by motifs connected in elaborate hierarchical and feedback structures. For example, Tyson and colleagues [51,60,61] proposed a mechanism for cell–cycle regulation predicated on the serial, irreversible invocation of three devices: a G1 → S phase bistable switching network composed of two cross-repressive feedback loops (between Ste9 and Cdc2–Cdc13, and between Rum1 and Cdc2–Cdc13); a G2 → M phase bistable switch (implemented by cross-repressive feedback between Wee1 and Cdc2–Cdc13, and cross-activating feedback between Cdc2–Cdc13 and Cdc25); and a mitotic oscillatory module generated by a negative-feedback loop (Cdc2–Cdc13 activates Sp1, which destroys Cdc2–Cdc13). Each of these switching and oscillatory loops is a regulatory motif. Together, these motifs function as a 'fuzzy' digital oscillator with intracellular-signal checkpoints and a system 're-set' induced by cell division.

Motifs can be nested and overlapping, as demonstrated by the network-controlling type 1 piliation in *E. coli*, which creates piliated populations in the bladder through the combined action of four motifs: an invertible DNA element; a ratio-controlled switch; an amplitude tuner capable of reading the temperature and increasing piliation at mammalian body temperature; and a delay line using feedback as memory to prevent rapid cycling between on and off switching states [29•]. This system provides an example of how integrated regulatory motifs in a network can function to both shape and filter intracellular noise, thereby creating environmentally tuned heterogeneity in a cell population.

Another example of how interconnected motifs generate complex behavior can be found in the segment polarity network in *Drosophila*, a collection of bistable switches and a homeostat arranged to produce robust spatial patterning [62•].

Motif searching via pattern recognition

Although we have called the architectures in Table 1 'motifs', it remains to be seen how pervasive or evolutionarily conserved they really are. There is a need for objective measures to identify regulatory motifs based on over-representation and phylogeny, in addition to dynamics. High-throughput technologies present this opportunity. Alon and colleagues [15,63•] recently searched an *E. coli* network connectivity database for over-represented patterns and revealed just a few themes: feedforward loops, single input modules and dense overlapping regulons, with feedback notably absent. These studies were based, however, on the transcriptional network alone. Many prokaryotic feedback structures contain at least one protein–protein link, and thus would not show up in a purely transcriptional network.

A similar approach with a strong experimental component, applied by Young and colleagues [16•] to *Saccharomyces cerevisiae*, identified six regulatory motifs: autoregulation, multicomponent loops and regulator chains in addition to the three patterns found in the *E. coli* transcriptional network. Possible dynamics for these patterns include reduced response time (positive feedback) or increased stability (negative feedback) of gene expression for the autoregulatory loops, multistability or oscillations for the multicomponent loops, transient-rejecting switching for the feedforward architecture, signal integration and process control for the multi-input motifs, and simple temporal logic for the regulatory chains. It is difficult, however, to draw conclusions without accounting for post-translational regulation and the specific kinetics of DNA–protein interactions.

Diversity of dynamics and designs

Most of the architectures in Table 1 behave as advertized in some parameter regimes, but not in others. For example, cross-repression in itself is not adequate for bistability; among other restrictions cooperativity is also required [35]. Moreover, depending on the gain and the delay, negative feedback can stabilize a process or generate oscillations. If we are to fruitfully use motifs to analyze large networks at a higher 'device' level of abstraction, there is work to be done deriving necessary and sufficient conditions on functional parameter regimes for each motif, and in experimentally determining if a proposed function of a motif is central to the biology, or merely incidental. For example, is the biphasic response of scaffolds [41] vital to their function in larger networks, or secondary to their role in bringing molecules into close proximity? Further experiments are needed to determine this.

Although we have described and organized these networks in engineering terms, the use of the metaphor is unproven, and the number of motifs that defy an engineering lexicon is unknown. Examples like these spark a question as to the purpose and genesis of such extensive diversity. Are different designs implementing seemingly identical functions selected on the basis of demand [64], robustness to fluctuations in system parameters [65,66],

evolvability [65], signal integration, optimization [67,68•], or primarily by accident? Putting the diversity under a single functional umbrella is a starting point for this sort of inquiry.

Modules

Although most biologists believe life to be modular on nearly every level, few agree on what constitutes a module. Network-level modules are defined variously as chemically isolated, operating on different time or spatial scales, functionally buffered, robust, independently controlled, plastic in composition and interconnection, evolutionarily conserved, clustered in the graph-theory sense, phenomenological, and any or all combinations of the above. This definition is very similar to that of a motif, and, according to some definitions, the two are indistinguishable. For the moment, we distinguish the two by emphasizing small size and recurrence for motifs, endowing modules with larger size, and perhaps a composition dominated by interconnected motifs. Below, we present some examples of modules, as defined by different criteria.

Depending on one's definition, modules can be identified and tested by *in vitro* or *in silico* reconstructions, perturbation studies, applications of graph theory to network diagrams, or phylogenetic analyses. Developmental networks, such as the segment polarity network in *Drosophila* and Notch–Delta signaling, are considered modular because *in silico* experiments reveal them to be robustly capable of generating their purported functions [62•,69]. The implicit argument is that if such a network of 'known' components and interconnections were not a module, it would be unlikely to robustly exhibit the correct behavior. It remains to be seen, however, if robustness implies modularity and whether robustness is a result of stabilizing selection or merely a biproduct of complexity and the need for developmental stability [66,70].

Another popular definition of network modularity (probably because it allows an analyst to use the most widely available data — DNA sequence and microarray) involves gene co-expression, with or without promoter region motif correlations and environmental context dependence [71•,72••,73]. The module-identification algorithm developed by Barkai and colleagues [71•] implicitly assumes such a definition (context-dependent co-expression). Applied to yeast microarray data gathered in multiple environmental conditions, their fixed-point algorithm revealed a modular structure of context-dependent and potentially overlapping transcription 'modules', a view complemented by the nifty 'combinograms' of Church and colleagues [72••], which combine gene expression and promoter-sequence analyses. Interestingly, an analysis of yeast knockout microarray data, analyzed by Vilo and colleagues [74] with the construction and topological analysis of a 'disruption network' graph, finds the network to be dominated by a single connected component, and thus not modular in topology. These seemingly contradictory results hint at the plasticity of regulatory structures and a murky relationship between network topology and dynamics. Barabasi and colleagues [13•] tried to resolve this inconsistency by suggesting that metabolic networks are modular in the graph theoretic sense only if one allows for hierarchical modularity. Many graph theoretic analyses are handicapped, however, by neglect of the stoichiometry inherent to biochemical reactions, and by the exclusion of enzymes and their different post-translational and complexation states.

Other approaches to module identification focus on evolutionary conservation. Evolutionary arguments, in conjunction with dynamical explication, contribute to the identification of partner switching modules in the general stress-response network in *B. subtilis* [75], and gated pore modules in bacteria [76], although the former might be better classified as a motif than a module. Applying evolutionary analysis and gene-classification information, Huynen and colleagues [77] found significant correlations between modularity and patterns of gene gain and loss in three different strains of *Pyrococci*, thus introducing a new means for cross-validating modules on the basis of sequence comparisons and providing an entrée for module co-evolution and member 'centrality' studies. More recently, Huyen and colleagues used conservation of gene order in operons across unrelated genomes to identify 800 putative transcriptional modules [78•].

Why should networks be modular? One theory is that modularity is necessary for robustness and evolvability, reducing the potential lethality of mutations [79] and facilitating the generation of variation [65,80]. This theory predicts that lineages with relatively greater degrees of modularity in given traits should exhibit higher rates of diversification, a prediction borne out in studies of holometabolous and hemimetabolous insects [81]. Modularity could arise spontaneously in evolutionary systems in response to environmental variation, as suggested by Lipson and colleagues [82], or, as Fontana suggests [83], from the properties of a space he calls a 'pretopology', a non-metric biophysical map between genotype and phenotype. Other possibilities are that modular behavior need not imply modular organization at the network level at all [84], or, at the other end of the spectrum, that modular structure is a pervasive vestige of early evolution as a communal project [85].

Games

Motifs and modules recur across many different organisms and scales of networks. There is a high degree of — but not perfect — conservation of the components of the underlying networks. But which network components and architectural features exist to ensure survival in a particular environment? Which provide fundamental

function? Which aid competition and commensalism? And which are evolutionary spandrels? If, however, differences in network design are primarily for survival, how does one understand the relationship between design, phenotype and environmental niche?

Evolutionary game theory [86], a merger between game theory and population biology, provides a language for this sort of inquiry and is becoming an increasingly popular tool to explain phenotype-expression patterns and the compositional dynamics of viral and bacterial populations. In evolutionary games, microbes compete for a larger share of descendants and thus long-term survival by evolving strategies (inheritable traits) whose payoff (Darwinian fitness — average reproductive success) depends on the strategies of other microbes. This framework can be used to explain and predict phenotype-expression patterns as evolutionary stable strategies in a game pitting microbe against microbe, and microbe against nature.

Turner and Chao [87] used game theory to investigate why it is that bacterial RNA phage populations have different frequencies of the two genotypes *phi6* (the cooperator is able to manufacture shared intracellular products) and *phiH2* (the defector sequesters shared intracellular products) at different multiplicities of infection [87]. They show experimentally that the fitness of the high-multiplicity phage relative to their ancestors generates a pay-off matrix conforming to the Prisoner's Dilemma strategy of game theory, in which selfish behavior leads to a sub-optimal growth. Selfish behavior arising in a Prisoner's Dilemma game was also used to explain *E. coli* mutant proliferation dynamics to a sub-optimal state [88].

Another experimentally validated game, rock–paper–scissors, where rock crushes scissors, scissors cuts paper and paper covers rock, was used to explain why non-transitive, competitive bacterial communities can coexist only if ecological processes such as dispersal, movement and interaction occur over small spatial scales [89••]. Other theoretic interpretations of game include viral latency and lambda-phage infection strategies as hedging bets [90,91] and chromosome segregation subversion in sexual species as a poison–antidote game [92].

Evolutionary game theory and optimization studies combined with dynamical analysis have the potential for linking genotype, regulatory dynamics, phenotype, cellular behavior, population-level behavior and the vagaries of environmental forces — all necessary pieces of the 'whole organism biology' puzzle.

Conclusions

In this review, we have organized recent network analysis research into a conceptual framework for regulation comprising motifs, modules and games. The framework is designed to tell the following story — motifs, small, repeated, and conserved regulatory devices — are arranged by evolutionary processes into modules, which are larger, overlapping, and functionally significant subnetworks. Dynamic themes, implemented by interconnected regulatory motifs arranged into modules, include signal-integrating switches, amplifiers, logic devices, memory devices and oscillators that act on the single cell or at the population level. Intracellular noise, produced largely at the protein translation stage of gene expression, is controlled (by motifs) precisely to regulate processes that require tight control. The cell also exploits intracellular noise (using other motifs) to produce survival-enhancing population heterogeneity and to stabilize dynamics and amplify signals. Many networks are robust, but this robustness is balanced by fragility [19], and qualitative behaviors such as adaptation and the ordering of events appear to be more robust to perturbation than are time responses [93,94].

The interconnection of functionally diverse motifs and modules enable each cell to act as a sensor, taking in environmental and intercellular signals. It also enables a cell to act as a signal processor, amplifying, noise-rejecting, and integrating these signals; as a computer, transducing processed signals into the coordination of competing cellular processes; and as a factory, implementing deployed processes. Modules map onto phenotypes, which are designed by evolution to play out evolutionarily stable strategies in a game of survival, pitting cell against cell, and cell against nature. Games microbes play — using strategies implemented by modules coordinated through shared components and global regulators – range from the Prisoner's Dilemma, to rock–paper–scissors, to hedging bets for survival in uncertain, wildly fluctuating environments. Although networks might not be optimized for fitness, network design is constrained by the functional imperatives imposed by these 'games of life' and the competing needs for robustness and flexibility in the face of uncertainty.

If this story holds true, how might we uncover these themes better in the network designs and phenotypic behaviors of particular organisms in particular niches? The answer must lie in the detailed comparison of homologous motifs, modules and network organizations among organisms that are competing within and across niches. By looking for features of cellular networks that are conserved across all niches, we might find the fundamental units of 'function' that are the basic requirements for survival. Differences in the implementation of these functions among niches, but which are conserved within a niche (or based on a niche property such as salinity, pH or environmental variability), indicate features that have been evolutionarily selected for adaptation to that environment. Differences in homologous networks among

microbes within a niche are then either there for commensalism, competition or evolutionary drift. Programs that aim to determine these differences will have to use all three of the concepts described above — motifs, modules and games — with their attendant levels of detail ranging from molecular mechanisms through control and dynamics, to population structure and evolution. Within each of these areas, there is still challenging theory and experiment to be done, such as rigorously defining motif and module, coming up with consistent theories of network evolution, experimentally measuring dynamics in single cells, tracking population heterogeneity under varying conditions, and quantifying fitness. In any case, full elucidation of these themes is likely to emerge from deep collaborations between experimentalists, computer scientists and mathematical-system theorists, or from a new breed of biologist equally comfortable at the bench and the computer.

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- • of special interest
- •• of outstanding interest

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