Back to the biology in systems biology: What can we learn from biomolecular networks?

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Abstract

Genome-scale molecular networks, including protein interaction and gene regulatory networks, have taken centre stage in the investigation of the burgeoning disciplines of systems biology and biocomplexity. What do networks tell us? Some see in networks simply the comprehensive, detailed description of all cellular pathways, others seek in networks simple, higher-order qualities that emerge from the collective action of the individual pathways. This paper discusses networks from an encompassing category of thinking that will hopefully help readers to bridge the gap between these polarised viewpoints. Systems biology so far has emphasised the characterisation of large pathway maps. Now one has to ask: where is the actual biology in ‘systems biology’? As structures midway between genome and phenotype, and by serving as an ‘extended genotype’ or an ‘elementary phenotype’, molecular networks open a new window to the study of evolution and gene function in complex living systems. For the study of evolution, features in network topology offer a novel starting point for addressing the old debate on the relative contributions of natural selection versus intrinsic constraints to a particular trait. To study the function of genes, it is necessary not only to see them in the context of gene networks, but also to reach beyond describing network topology and to embrace the global dynamics of networks that will reveal higher-order, collective behaviour of the interacting genes. This will pave the way to understanding how the complexity of genome-wide molecular networks collapses to produce a robust whole-cell behaviour that manifests as tightly-regulated switching between distinct cell fates — the basis for multicellular life.

INTRODUCTION: MIND THE GAP OF MINDS

Ever since molecular biology came into existence as a discipline devoted to studying the function and functioning of genes some 40 years ago, scientists have entertained the idea of gene regulatory circuits, and even of genome-scale genetic networks, for understanding how the activity of genes — by influencing each other — ultimately translates into the phenotypic behaviour of an organism. In the absence of gene-specific data, the concepts of genetic networks were explored mostly at the level of abstract, generic models, without molecule-specific information. Despite intriguing insights into the fundamental properties of the collective behaviour of a large number of interacting components, such work remained largely unnoticed by practitioners of molecular biology, who emphasised the cloning, characterising and categorising of individual genes. Molecular biologists — both experimental and computational — used to studying specific, measurable biochemical details, were not ready for the abstraction required to handle networks as an object of study. It was only the recent availability of gene-specific data for genome-scale networks of molecular interactions, made possible by high-throughput genomic technologies, that revived the interest in the idea of large gene networks.

The arrival of genome-scale information on networks, ranging from networks of metabolic reactions to gene regulatory and protein interaction...
networks,\textsuperscript{8–10} has led to a recent spate of publications in which the topology ('wiring diagram') of molecular networks was analysed.\textsuperscript{11–18} When reading the literature, however, it is important to be aware of two camps of researchers with philosophically opposite mindsets and, hence, disparate motivation. These two camps can be labelled the ‘globalists’ (or, ‘generalists’) and the ‘localists’ (or, ‘particularists’) (Table 1). Historically, a similar polarisation existed in brain research, with globalists emphasising the idea that the entire network of neurons in the brain collectively determine behaviour by distributed information processing, while the localists believed more in localising a brain function to a particular anatomical region.\textsuperscript{19} It is now known that both were right.

The first wave of work on genome-wide networks was conducted mostly by physicists in the globalist’s perspective, so that networks were analysed in a more general sense, ie as an entity in their own right.\textsuperscript{11,12,14,15,20} In this globalist’s abstraction the identification of individual genes and their relationships and functions were not the primary objectives of analysis. Thus, this early work on networks failed to attract the attention of scientists in the localists’ camp, which consists largely of mainstream molecular biologists habituated to characterising specific genetic pathways one at a time.

There is a sharp, natural, but unarticulated intellectual disconnect between the two camps because of the fundamentally different motivation of the two groups of scientists. The globalists are typically interested in ‘deeper’ principles of complex systems,\textsuperscript{21–24} they are attracted to biology as a new source of complexity, which led to the notion of ‘biocomplexity’.\textsuperscript{25–28} They now seek molecule-specific data, made possible by technological advances, to validate their ideas.

\begin{table}[h]
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\begin{tabular}{|c|c|c|}
\hline
\textbf{Level of original focus} & \textbf{The ‘localist’ (‘particularist’) view} & \textbf{The ‘globalist’ (‘generalist’) view} \\
(Those who see the trees first) & & (Those who see the forest first) \\
\hline
\textbf{New field created} & Gene- and pathway-centric & Network-centric \\
\textbf{Use of hypothesis} & ‘Systems biology’ & ‘Biocomplexity’ \\
\textbf{Hypothesis at level of individual pathways. No systems-level hypothesis: research becomes ‘discovery-driven’.} & ‘Gene A inhibits Gene B, is required for function X, etc.’ & ‘Example hypotheses: ‘Hub proteins are important’ \\
\textbf{Philosophy} & System is complicated & ‘Power-law architecture favours ordered dynamics’ \\
& Properties of systems lie in the property of the components & System is complex \\
& Comprehensiveness & Higher-order system properties emerge from collective behaviour of components \\
\textbf{Practical aims of study} & The whole equals the sum of the parts & Holism \\
& To characterise exhaustively the biochemistry of (all) individual pathways and their ‘functions’ & The whole is different from sum of parts \\
& To describe idiosyncrasy & To understand generic aspects of genome-scale networks as an entity with its higher-order properties \\
\textbf{Gene identity in models} & Of primary interest. Specific models with nominal genes and their idiosyncratic properties & Of secondary interest. Models with anonymous genes as generic entities may sometime suffice \\
\textbf{Network topology} & Precise biochemical characterisation and categorising of physical and regulatory interactions in specific pathways & Analysis of large scale features, based on global statistics of local network features (degree distribution, modularity, clustering) \\
\textbf{Network dynamics} & Detailed modelling of individual small circuits (modules) in separation as a low-dimensional dynamic system & Global dynamics of network maps into whole cell behaviour (cell fates) \\
\textbf{Function} & Focus on local cellular functions (eg protein synthesis, vesicle transport, filopodia extension, DNA repair, etc) associated with a specific pathway & Emphasise emergent whole-cell behaviour, such as switch between discrete cell phenotypes (cell fates) \\
\textbf{Typical non-biologist partners} & Computer scientists, engineers & Physicists \\
\hline
\end{tabular}
\caption{Polarity of two points of view in network biology. The table represents a caricature of extreme positions for illustration purposes.}
\end{table}
By contrast, the localists’ view is rooted in classical molecular biology, hence is shaped by decades of devotion to the study of individual cellular pathways that represent to them linear causal relationships. But the prevalence of pleiotropy and convergence in cell signalling, and of crosstalk between pathways, has led to the increasing awareness that understanding gene function requires that one reaches beyond the narrow focus on individual pathways. The advent of massively-parallel analysis and high-throughput genomics and proteomics technologies finally led to the acceptance of the idea that molecular pathways are just parts embedded in one genome-scale system, leading to the explicit inception of ‘systems biology’. Nevertheless, despite considering networks and quantitative modelling, the pathway-centric ideology shines through in this new initiative towards ‘integration’. To the localists, integration essentially means the combination of different kinds of large datasets in order to find correlations — to or at least to facilitate the generation of hypotheses on relationships between individual genes, proteins and biological functions. Thus, the systems biology approach seeks comprehensive, qualitative and quantitative descriptions of all constituent parts, rather than an understanding of a higher-order quality that may arise from the collective action of the individual parts. The implicit notion is that extension of the existing ways of analysing individual pathways to the exhaustive, simultaneous characterisation of all the molecular interactions in the genome will suffice for understanding how living organisms work. Concretely, the emphasis on the analysis of vast amounts of biochemical detail has led to the involvement of computer scientists for developing computational tools that have become indispensable for representing, mining and modelling the bewildering amount and heterogeneity of collected information.

Another natural extension of pathway-centricism to the entire system, which has become a major research focus of systems biology, is to reconstruct the topology of a network by systematic experimental identification of interactions or to reverse engineer it by inference from measurement of network dynamics.

In summary, whereas globalists are attracted by the complex, seeking to understand general principles giving rise to the whole, and are ready to abstract away specific details, localists-turned-systems biologists are more inclined to attack the complicated, and seek comprehensiveness of detailed description. Because of the unique blend in biology of universality and idiosyncrasy, both approaches are complementary to each other and will be necessary in spite of a persisting, unarticulated mental divide (Table 1).

This paper discusses molecular networks in a broadly encompassing category of view, going beyond the characterisation of network maps, and addresses the fundamental questions that investigators of both ideologies studying living systems will ultimately have to address: what do networks tell us about the biology of an organism?

FROM NETWORKS IN BIOLOGY TO THE BIOLOGY OF NETWORKS

As with the information in DNA and amino acid sequences, what networks can tell us about the biology of an organism can be divided into two broad domains: evolution and function (Table 2).

While comparative sequence analysis proved to be a powerful tool for determining taxonomic relationships and can shed light on the history and mechanism of genome evolution, it remains of limited use for predicting gene function. Although conservation can point to functional importance, and sequence homology (e.g. catalytic motifs) allows the prediction of the biochemical function of a protein, the road from sequence to gene function and,
subsequently, to drug development turned out to be bumpier than was anticipated in the dawn of the genomic revolution. It is often overseen that the regulation and the context of expression of a gene is part of what defines its biological function. For example, based on sequence analysis, one readily finds that the protein cyclo-oxygenase-2 (COX-2) has the same enzymatic activity as COX-1, namely, a cyclo-oxygenase in the synthesis of prostaglandins. This information in itself was of little use until it was established that COX-2 is regulated differently than COX-1, and thus has a different biological function. Being induced in inflammatory tissues, as opposed to the constitutively active COX-1, COX-2 obviously serves a different function and thus provides a more specific drug target than its non-regulated paralogue. Thus, what determines the distinctive biological functions of COX-1 and COX-2 is encoded in their regulation, not in their enzymatic activity. Furthermore, a regulatory protein, such as Ras, myc or NFkB, can have multiple, disparate, if not opposite, functions (eg either cell proliferation or differentiation/ quiescence or apoptosis), depending on the cellular context, ie the presence of other proteins, and the cell state and type.

Hence, despite great efforts spent in functional annotation, it is only in rather exceptional cases that a specific physiological function can unconditionally and unambiguously be assigned to a given protein as a separate entity, defined just by the coding region of its gene. It is likely that most proteins, notably regulatory proteins, exert their biological function as a member of a molecular network.

What is less obvious is what networks can tell us about biological evolution (Table 2). Representing an intermediate level of information, between the level of DNA sequence and that of organismal phenotype, network structures are more complex than DNA sequences, yet simple enough for a mathematical representation using the formalism from graph theory. Thus, by serving, to all intents and purposes, as an 'extended genotype', networks open an entirely new window on the workings of evolution. Before discussing what networks can teach about the evolution of the organism, the author will firstly summarise a few recent, salient findings on network topology obtained from the ‘globalist’ point of view.

GLOBAL TOPOLOGY OF NETWORKS

The ‘wiring diagram’ of the entire network can readily be drawn from data on relationships between biomolecules, including metabolic reactions, physical protein interactions (heterodimeric complexes) and transcriptional gene regulation. Network topology information is available for metabolic networks in various microorganisms and, more recently, for a genome-wide protein–protein interaction network of the yeast Saccharomyces cerevisiae.
Genome-wide gene regulatory network data are currently available for *Escherichia coli*. In the terminology of graph theory, a network consists of nodes (vertices) that can be connected by links (edges) (Figure 1). Genome-wide networks consist of thousands of nodes, and thus are referred to as ‘complex networks’ by contrast with the networks with low node numbers studied in traditional graph theory. Moreover, an important aspect of biological networks is that they grow over time, by contrast with the networks with constant node numbers seen in traditional graph theory. Thus, biomolecular networks have a history of experiencing an increase in the number of nodes. What catches the investigator’s interest is every topological feature that deviates from that of a random network, in which *N* nodes are randomly connected by *L* links. The non-random structures found in complex networks, then, provide the starting point for further research into their origin and functional significance.

To illustrate this, focus on the results obtained for the most complete eukaryotic protein interaction network available, the protein interaction network of *S. cerevisiae*, which is based on genome-wide data of pair-wise protein–protein interactions (Figure 1). It is important to note that the incompleteness and inaccuracies in the yeast protein interaction dataset, mostly due to potentially false-positive links, do not essentially affect the global statistics, as comparisons of various datasets by the authors in the cited works have shown. Many questions posed by the globalist can be addressed with incomplete and not perfectly accurate datasets, while the localist agenda requires precise and detailed information. One elementary global feature that was striking for biologists — especially those who think in categories of isolated pathway modules named after their putative cellular functions — is that, despite the overall sparseness of the network connectivity...

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**Figure 1**: Global picture of the topology of large (complex) networks. Upper panels: graph of the network, displayed using Pajek software (http://vlado.fmf.uni-lj.si/pub/networks/pajek/). A. Computer generated, not growing random network, *N* = 500 nodes, mean connectivity degree *<k> ~ 4*. Note the dominating giant component (containing 487 connected nodes). B. Computer generated, growing network using the generative algorithm with preferential attachment of new nodes, *N* = 483, *<k> ~ 4*. (The algorithm necessarily leads to one single component). C. Yeast protein interaction network consisting of *N* = 2,165 proteins, with *<k> ~ 4*, based on the CORE data set. Lower panels: probability density distribution *P(k)* in log–log plots. The density distribution exhibits a straight line for B, C indicating a power–law (scale-free) distribution.
(three to four connections per protein, on average), the vast majority (>75 per cent) of the proteins in yeast participate in a single, large sub-network of connected nodes, a so-called ‘giant component’\(^\text{12}\) (Figure 1). Although counterintuitive at first sight, this finding is not unexpected from a graph theory point of view. Given some minimal connectivity (for a growing, random graph, at least \(k = 1/8\) links per node on average\(^\text{66}\)), such a giant component is practically unavoidable in protein interaction networks (where average \(k/C^2\))\(^\text{25}\)), and is thus robust in a fundamental way. Hence, this feature does not even qualify as a non-random feature that would point to a particular biological purposefulness.

By contrast, Barabasi and coworkers describe an interesting global topology feature that indeed deviates from that of a random graph.\(^\text{12,20}\) If all the nodes \(i\) of the network are treated as one population, and \(k_i\) of each node \(i\) (ie the number \(k\) of interaction partners that node \(i\) has) is measured and displayed as a distribution, then the occurrence probability \(P(k)\) of each value of \(k\) exhibits, approximately, a power-law distribution, \(P(k) \sim k^{-g}\), rather than — as would be the case in random networks — a Poisson distribution. The exponent \(g\) is a characteristic constant (Figure 1). A power-law distribution implies that the more nodes that are sampled, the higher is the mean value of \(k\) for the sampled population. Thus, there is no stable mean that is characteristic for an ensemble of proteins (nodes), and such protein interaction networks are called ‘scale-free’. Although it remains debated as to whether the degree of distribution precisely follows a power-law, its ‘heavy-tailed’ nature implies that there are more proteins with a high-degree \(k\) than one would expect in a random network: the protein interaction network is biased to contain more ‘hubs’ than one would expect.

Other non-random topological features that deviate from those of a random graph have been identified in the yeast interaction network. For example, hub proteins tend not to interact with each other.\(^\text{14}\) Moreover, the proteins or metabolites in real networks have a higher tendency than the nodes in random networks to be organised into clusters (‘cliques’) in which the partners of a given protein are also likely to interact with each other.\(^\text{13,17}\) Together with the scale-free property, these features give rise to hierarchical modularity, as has been described for metabolic networks.\(^\text{15}\) The yeast protein network also contains a significant number of proteins that have low connectivity degrees, yet are ‘central’ in that they lie on many of the ‘shortest paths’ that connect any pair of proteins.\(^\text{57}\) Of interest also is the recent study of ‘network motifs’ across the network. Network motifs are subgraphs, ie locally defined patterns of interaction between a small number of nodes, such as triangles, branching structures, etc.\(^\text{5,16,18,68}\) In gene regulatory (transcription) networks, links represent transactivation and are, therefore, arrows rather than undirected connections; thus, transcription networks form directed graphs. Such digraphs exhibit a higher diversity of network motifs than the networks from protein interaction databases, which yield undirected connections. Specifically, Shen and coworkers found that in bacterial gene regulatory networks, certain motifs of directed links were significantly over-represented, such as the feed-forward loop (Figure 2).\(^\text{18}\)

In view of all of these non-random features of network topology, two interdependent questions immediately come to mind: how do such structures originate, and how do they affect biological function?

**NETWORKS AND EVOLUTION: ADAPTATION VERSUS INTRINSIC CONSTRAINTS**

A fundamental question in biology is why organisms are the way they are: why does a given trait exist, how does it arise? It is obvious that the non-random network features discussed above can now be
treated as elementary phenotypic traits. In an encompassing view of evolution, one can distinguish between two fundamentally distinct, but not mutually exclusive, groups of mechanisms that can contribute to the very existence and nature of a given trait in living systems. Because authors use a large variety of terms with essentially identical or overlapping core meaning, here they will simply be called ‘Type I’ and ‘Type II’ mechanisms, to avoid semantic ambiguity or bias (Table 3).

The Type I mechanism concerns the (neo-)Darwinian principles of selection and adaptation. Traits can (smoothly) vary in a random fashion due to mutations; and selection (in a given environment) of the fittest (of the inheritable) variants drives the features towards a(n) (local) optimum in terms of functionality. Because of the underlying connotation of directedness or finalism (even if the mutations themselves are not), the adaptation mechanism is in essence equivalent to the engineer’s notion of optimisation of a task by deliberate design, or the ‘tinkerer’s’ trial and error approach. The vast majority of biologists think in categories of this mechanism to explain almost every feature. The Type II mechanism comprises a variety of alternative, ie non-adaptationist mechanisms, summarised here under the general term ‘intrinsic constraints’. The mechanisms have in common the fact that they embody a set of rules that impose constraints so as to make particular features unavoidable, ie intrinsically robust, independent of a notion of usefulness or self-maintenance in the face of external perturbation.69,70 This implies that no external force, like adaptation or any other notion of functionality or purposeful design, is required. ‘Constraint’ is a creative rather than inhibitory force, for it is that which prevents (‘constrains’) a system from settling down in a random, undifferentiated, pattern less state. The intrinsic constraints can be imposed by elementary physical laws (eg spherical shape), allometric relationships,

Figure 2: Hypothetical mechanism for the generation of a feed-forward loop in the absence of selection for functionality. 1. Primordial, auto-regulative gene X encodes a transcription factor that binds to its own cis-regulatory element, ‘X-responsive element’ (XRE). 2. A second auto-regulative gene A, together with its cis-regulatory region, ARE, (eg, a regulon) is inserted into the 5’ region of X (eg mediated by transposable elements) in the reverse direction. If XRE is a bidirectional promoter element, XRE can contribute to transactivation of A. (This is the case in the arabinose operon, which is part of a feedforward-loop.18) 3. Duplication of the DNA segment ‘Gene A-ARE-XRE’ creates a new gene that is responsive to both A and X. Due to subsequent diversifying mutations, Gene A becomes Gene B; ARE is partially deleted because it is redundant. Now factor X regulates itself and Gene A via XRE, while both products, A and R, regulate gene B. On top of this structural and procedural scheme, which facilitates the spontaneous creation of a feed-forward loop, natural selection may then do the fine tuning, eg change strength and modality of regulation by point mutations in the respective cis or trans regulatory regions.
architectural and mechanical principles (eg force–balance rules in tensegrity) and, importantly, self-organisation processes, such as symmetry-breaking patterns (see below). Other non-adaptive mechanisms include historical (phylogenetic inertia, genetic drift, frozen accidents, etc) and developmental constraints (restrictions in the ‘manufacturing path’). A distinct school of thinking explicitly opposite to adaptionism is ‘structuralism’, which stresses the study of structures independent of selection for functionality.

Self-organisation is part of ‘complexity theory’, which emerged as a field of study in its own right in the 1980s but which still lacks a rigorous definition, its novelty and necessity is still being questioned by some scientists. (This paper will not refer to theories that address the question of macroscopic evolution and the origin of the complexity of systems itself, which is a different matter.) Nevertheless, by unifying various theoretical concepts aimed at understanding the origin of ordered, non-random, patterns through self-organisation, the approach of the complex system theories has added substantial momentum to the community of researchers that emphasise Type II explanations for particular features. By providing astonishingly simple explanations for the inherent robustness of many counterintuitive patterns, such as stripes, spirals, fractals, power-law relationships, etc, which are found in living as well as non-living systems, complexity theory may provide some of the ‘deeper principles’ of organisation which embody the design constraints and which structuralists were seeking.

It is important to note that the Type I and Type II mechanisms are not mutually exclusive, but are consistent with each other and can cooperate in some rather convoluted way. For example, a particular intrinsically robust structure, such as the fractal branching patterns of pulmonary bronchi or a network motif in molecular networks (see below), may simply have been exploited — because they happen to be advantageous — rather than created by natural selection. Variability and selection pressure may not have been strong enough to mould such structures from scratch by stepwise exploration of many alternative, similar but less functional forms in the ‘phenotype space’. In other words, functional adaptation may be a by-product of an inherently robust process.

Table 3: Major points of the two mechanisms for explaining the existence of distinct features in biology. Although the two mechanisms are not mutually exclusive, but complementary — and perhaps synergistic, they have become polarised means of explanation among biologists, with the majority today leaning towards Type I.

<table>
<thead>
<tr>
<th>Type I mechanism: Adaptation (natural selection)</th>
<th>Type II mechanism: Intrinsic constraints</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Neo)-Darwinian evolution: random mutation generates phenotypic variation, selection of a distinct feature based on ‘fitness’ in a given environment</td>
<td>Intrinsic inevitability (robustness) of a feature due to constraints in the mechanism of its genesis or design:</td>
</tr>
<tr>
<td>Biology as a historical science</td>
<td>– architecture constraints</td>
</tr>
<tr>
<td>(Local) optimisation of a trait for functionality</td>
<td>– physical (mechanical, energetic, allometric) constraints</td>
</tr>
<tr>
<td>Notion of purposefulness is central</td>
<td>– ‘self-organisation’, pattern formation</td>
</tr>
<tr>
<td>Gradual, cumulative evolution used to explain ‘unlikely’ complex trait</td>
<td>– developmental constraints</td>
</tr>
<tr>
<td>Adaptation is a powerful moulding force that generates traits from scratch</td>
<td>– historical constraints (phylogenetic inertia, genetic drift, etc)</td>
</tr>
<tr>
<td>Natural selection is creative</td>
<td>Biology can be historical or ahistorical</td>
</tr>
<tr>
<td>Adaptation is cause for a trait</td>
<td>No optimisation necessary, but can secondarily shape trait</td>
</tr>
<tr>
<td></td>
<td>No notion of purposefulness</td>
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<tr>
<td></td>
<td>Self-organisation used to explain ‘unlikely’ trait</td>
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<td></td>
<td>Adaptation just maintains favourable traits that are inevitable due to constraints</td>
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<tr>
<td></td>
<td>Natural selection is conservative</td>
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<tr>
<td></td>
<td>Adaptation is consequence of a trait</td>
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that creates structure based on internal design principles. Selection then does the fine-tuning.

Unfortunately, the above dichotomy — a matter of two non-competing and overlapping explanations — becomes a matter of competing, polarised points of view. The majority of modern molecular biologists gravitate towards the adaptionist perspective and put all faith in the extraordinary power of natural selection in the creation and moulding of traits. This unquestioned belief in natural selection as the core, irreducible principle of explanation in biology hinders the pluralistic endeavour to disentangle and assess the relative contribution to a trait of either mechanism. Even Darwin himself cautioned us in the introduction to *The Origin of Species*: ‘I am convinced that Natural Selection has been the main, but not the exclusive, means of modification’.82

**EVOLUTION OF NETWORK STRUCTURES: INTRINSIC CONSTRAINTS**

The dualistic scheme of explanation can now be applied to the non-random architectural features of networks. In the spirit of the prevailing bias towards adaptionism, the identification of particular network structures was almost always automatically followed by the interpretation that they must reflect some optimisation of functionality by evolution.18,83,84 Such statements, although particularly attractive to the engineer’s mind accustomed to purposeful, optimal design, are dangerous. When invoking the ‘survival of the fittest’ in the absence of rigorous analysis of the actual mechanism of the fitness associated with a trait and of its history, one can easily succumb to the inherently self-referential logic of this statement (‘if what is fit survives, then what survives must be fit’). Although pure Darwinian adaptionism applies in many specific scenarios, eg in one of its purest forms — the generation of antibiotic resistance, in most cases both mechanisms appear to contribute, in a synergistic manner, to the prevalence of a given trait.

Topological features of networks are ideal objects of study for determining the relative contribution of either type of mechanism to a distinct trait. The deviation from randomness of a network structure can be precisely determined and a mechanism for the structural and historical constraint formalised. A network feature represents an elementary phenotype, but unlike classical phenotypic traits it does not undergo ontogenesis, which complicates debates on evolution. Thus, developmental constraints do not have to be considered.

From the discussion presented above, it becomes obvious that before attributing a feature to adaptation, one needs to exclude any constraint accounting for the inherent inevitability of that structure. The demonstration that a network feature is significantly more abundant than one would expect if the network were to have been generated randomly, by comparing it with a random graph, does not suffice to support natural selection. Furthermore, the demonstration of convergence, ie similarity of a trait in the absence of genetic evidence for common ancestry, is often used as an argument in favour of adaptation.85 Other than dispelling the idea of a common ancestry, however, convergence does not exclude the role of various kinds of intrinsic constraints in enforcing a particular feature, but may instead point precisely to some universality of the constraining design rules.

The origin of a network motif needs to be examined in more detail. The feed-forward loop has been shown, based on a comparison with the random graphs, to be a prominent, hence ‘adaptive’ feature in the *E. coli* gene regulatory network (Figure 2).18 This conclusion is problematic, since real gene regulatory networks cannot be randomly redrawn like the nodes and links in a graph. On the contrary, evolution of the genome and, hence, of the network that it encodes, is subject to a series of...
mechanistic constraints that may significantly reduce and bias the space of the possible random network architectures represented by the immaterial, random graphs. Genes are embodied by a linear physical structure, the DNA, which rearranges in particular ways. Genome evolution occurs via a finite set of physicochemical mechanisms, including chromosome and tandem gene duplication, followed by diversification of the duplicated gene pair due to mutations of the redundant genes. Moreover, rearrangements of DNA are caused by DNA segment inversion or deletion, or insertion of DNA fragments via transposable elements, crossovers, etc. These processes, as well as promoter-generating mutations, drive the rewiring of gene regulatory networks by shuffling cis- and trans-regulatory regions. For example, the duplication of a gene, with subsequent mutations and acquisition of new properties in the coding region but not in the promoter region, would create a ‘branching’ network motif (one gene activates two genes). These mechanisms could facilitate the genesis of particular network motifs over others, independent of functionality. Figure 2 shows a hypothetical mechanism that could explain the genesis of a feed-forward loop via only a gene insertion and a gene duplication event, plus a few minor mutations.

Mechanistic and historical considerations help to place the potential effect of adaptation by selection in the appropriate light. Unlike the ad hoc assumption of an effective selective advantage, the assumption of mechanistic and historical constraints can be rigorously tested. Analysis of the DNA sequence to trace back the history of the cis- and trans-regulatory elements within a specific network motif, combined with the study of generative algorithms constrained by mechanistic considerations of DNA rearrangement, could reveal the contribution by Type II mechanisms to a network structure.

In the case of global structures, like the power-law degree distribution of $k$ discussed above, Barabasi and coworkers proposed a generative algorithm for network growth (increase in node number) that inevitably results in scale-free networks. An essential ingredient of their algorithm is that genes newly added to the existing, growing network preferentially establish a connection to an already highly connected gene. Although the model of genesis is cast in generic terms, one can now examine whether simulations of the actual physical mechanism (increase in genome size by gene duplication, rewiring by mutations, particular exon shuffling, etc) are compatible with the generic model. In fact, protein sequence comparisons that allowed the determination of the evolutionary age of a protein appear to suggest that the hubs are older and that preferential attachment might have taken place during evolution. A power-law architecture has also been found in a large number of non-biological networks, such as social and computer networks, suggesting that adaptive evolution is not likely to be the creator of this architecture.

This section on the evolution of network features concludes by emphasising again that each of the types of explanation for a particular structure — adaptation and intrinsic constraints — does not exclude the other. Evolution will secondarily, by chance, ‘coopt’ an intrinsically robust feature that happens to have desirable properties and maintain it. For example, a giant component of the network which is an unavoidable structure may coincide with functional advantages: in the case of the metabolic network, it might facilitate global coordination of energy utilisation upon change of nutritional source; and in the case of the regulatory network, global connectivity allows regulation of whole-cell behaviour, such as cell fate switches, as discussed below. Similarly, structural modularity of networks, for which several advantages have been attributed in terms of functionality and evolvability, may...
simply have been a fortuitous accident of horizontal (vector-mediated) transfer of entire clusters of genes. Such groups of genes could collectively confer a biochemical function to the host, allowing it to invade a new metabolic niche, and hence be maintained as a module by selection. Since the expression of these genes is coordinated inter se such as to perform a metabolic task, they necessarily form a network module. Finally, as proposed below, the power-law feature of regulatory networks may have the functional benefit of generating ordered global network dynamics. Thus, adaptation is part of the game, but it may be conservative, or just doing the fine tuning, rather than creative.

**NETWORK FUNCTION: FROM TOPOLOGY TO DYNAMICS**

Most functional interpretation of networks has been based on their topology alone. For example, it has been suggested that highly connected hubs in networks are more likely to be essential genes because they interact with many proteins. In fact, for the yeast proteome there is a weak association between degree \( k \) of a protein and the probability that it is essential. The power-law network has been shown to be generically more tolerant to random failure of individual nodes, but vulnerable to targeted attacks on the hubs. This would be in line with the suggestion that hub proteins have a slower rate of evolution.

Much of the topology-based reasoning about function rests on the unarticulated premise that the molecular network acts like a communication network in which some information 'flows' in the links from node to node. Although this may be appropriate for metabolic reaction networks, it certainly does not apply to networks of regulation, like the protein or transcription networks, where a link represents an influence rather than a flow. To understand functionality, it is, therefore, necessary to move from the domain of topology to that of dynamics. Network dynamics come into play when considering two essential properties of molecular regulation networks:

(i) A node \( i \) in the network (gene or protein) takes a value, \( x_i(t) \), which represents its expression or activation level, which changes with time in response to incoming influences from other nodes \( j, k, l, \ldots \), represented by the links.

(ii) The links of the network represent influences (rather than flows) and have a modality (e.g. inhibition or stimulation). Together with interaction rules (e.g. additive effects, or some Boolean functions), they determine how the level \( x_i(t) \) of a given protein \( i \) ('output') is jointly affected by the values \( x_j, k, l \) ('inputs').

The dynamics of the system is the concerted change in the levels of \( x_i(t) \) for all of the nodes \( i \) of the network, and can be represented by the \( N \)-dimensional state vector \( S(t) = [x_1(t), x_2(t), \ldots x_N(t)] \) for a network with \( N \) nodes. While the topology is represented by a graph, the dynamics can be imagined as a more abstract \( N \)-dimensional state space in which \( S(t) \) moves its position with time \( t \) as defined by its components \( [x_1(t), x_2(t), \ldots x_N(t)] \) (see Figure 3 for details). The interactions of the network now impose restrictions on the movement of the state vector \( S(t) \): a given state can only move in certain directions in the state space because its components \( x_i(t) \) cannot change independently but influence each other. This 'frustration' of the freedom of movement is where the unfathomable complexity of large networks collapses. If the wiring diagram is 'right', this restriction will give rise to simple, robust, higher-order behaviour, as will be seen below.

Mathematically, the dynamics of the network can be described and modelled...
using a large variety of formalisms that differ in the level of 'coarse-graining'. For example, systems of ordinary differential equations, with continuous values of concentrations for $x_i(t)$ that are subjected to mass action kinetics (like chemical reactions), are often used for smaller circuits consisting of a handful of proteins or genes. This is already an abstraction, in particular when used to model gene regulatory or signal transduction, since $x_i(t)$ represents a hypothetical average of the stochastically fluctuating activity of node $i$, taken over time and over an ensemble of heterogeneous cells. At the even more
abstract end of the spectrum of models are
the discrete networks in which genes are
represented by binary, ON-OFF
switches, with Boolean functions
determining the interaction modalities.
Because of their computational simplicity,
such Boolean networks have been used as
a convenient, tractable caricature for
studying fundamental aspects of the global
behaviour of large networks using
statistical ensembles of networks of
varying topologies.4,22 In fact, Boolean
functions (e.g. AND, OR, ... ) capture the
link modalities and their cooperation
rather well/although these are, in reality,
embodied by the molecular interactions at
promoters and in protein–protein
complexes.47,99,100

Quantitative modelling of local
modules of signalling and gene regulatory
pathways that consider non-linear aspects
(such as positive and negative feedback
loops) can predict a variety of interesting
behaviours, such as robustness, multi-
stability, oscillation and switch-like
behaviour.101 These ‘emergent’ qualities
are not evident from study of the
topology, nor can they intuitively be
predicted by the linear ‘upstream–
downstream’ schemes of the
experimentalist (Figure 3). This pathway-
centred modelling, however, predicts the
dynamics only of the components of a
particular signalling module, not of
phenotypic cell behaviour.

CELL FATES AS
ATTRACTORS IN
GENOME-SCALE
NETWORKS
A globalist view of the dynamics of the
network may also, therefore, be necessary.
Two findings suggest that global network
dynamics may be relevant. First, the
existence of a giant component in the
protein interaction network12 (and
probably also in the gene regulatory
networks) raises the question of whether
there is a collective, higher-order
behaviour that arises from the large
number ($N = 1,000–10,000$) of
interdependent genes and proteins, or
whether their activity changes simply
‘average out’ and get lost over the large
population of the nodes, so that no
interesting global pattern emerges. In
other words, is the genome-wide
network (or large portions of it) wired in
such a way that it acts coherently as one
entity with a distinct ‘macroscopic
dynamics’ and, thus, contains additional
information which would not be
accessible in the study of pathway
modules in separation? Secondly, cells in
multicellular organisms exhibit a simple,
coherent whole–cell behaviour which
may precisely reflect a higher-order
dynamics of the global network: the
switching between cell fates. This strictly
regulated, rule-based systems behaviour is
robust and remarkably simple compared
with the complexity of the underlying
molecular network.100

Cells in multicellular organisms are able
to rapidly and reliably integrate a
multitude of simultaneous and often
conflicting signals from their tissue
environment, and respond by selecting
just one of a few discrete cell fates, such as
quiescence, proliferation, migration or
differentiation into distinct cell types.100

Tight regulation of the balance between
cell fates is required for the development
and homeostasis of the tissue. Cell fates
are discrete, mutually exclusive
behavioural types, as Waddington
described back in the 1940s.102 What is
particularly counterintuitive from the
localist, pathway-centred perspective, is
that the same cell fate switch can be
triggered by a broad variety of unrelated
biochemical and physical stimuli, while
the same biochemical stimulus can elicit
entirely different cell fate switches —
depending on the context.59,100 This
defies the localist notion of modular
pathways that serve a specific cellular
function. The mutual exclusivity of cell
fates is often taken for granted but is a
systems property that requires that the
pathways that have been associated with
particular cell fates be globally
coordinated. This would be consistent
with the function of a giant component in

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the regulatory networks that spans almost the entire genome (even if this structural feature is statistically almost unavoidable, and thus may not have ‘evolved’ for this function, as discussed above).

Do the dynamics of large networks in fact generate robust, simple, macroscopic behaviour that is manifest as regulated cell fate switching? To overcome the lack of specific information on genes and the precise network topology, yet be able to study the universal behaviour of large networks, more than 30 years ago Kauffman used random Boolean networks as models to show that, given some minimal topological constraints (such as sparse connectivity), the global dynamics of a network with \( N \) genes will be far from ‘chaotic’ (disordered), but that due to the constraints imposed by the particular gene–gene interactions, it will inevitably exhibit some ordered collective behaviour: most states \( S(t) \) are not stable, thus forcing \( S(t) \) to move in the state space until it arrives at a stable state to which unstable states will be attracted (see Figure 3 for details).3,22 These ‘attractor states’ can now be designated as the cell fates or, in Kauffman’s more general view, as the various cell types in a multicellular organism.22,100 In fact, the dynamics of a network with attractor states naturally captures the essential properties of cell fate dynamics, including mutual exclusivity, robustness and all-or-none transitions between cell fates in response not to a single ‘specific’ instructive signal but to a large variety of signals.

Although Kauffman used randomly connected networks (with restrictions only with regard to connectivity degree and type of Boolean function), he was able to show some universal dynamic properties, such as the existence of robust attractors.22 Kauffman’s pioneering work receives little attention from modern day experimental and computational molecular biologists, however,34,35 who in their localist point of view emphasise the description of specific, idiosyncratic details over abstraction and analysis of higher-order behaviour. Interestingly, it turned out that the scale-free network topology that is now found in real networks is even more likely than randomly connected networks to generate a reasonable, ‘ordered’ dynamics, in the sense of Kauffman (ie containing stable, compact attractors, consistent with cell fate behaviour).103 Thus, the self-organised scale-free topology relaxes Kauffman’s restrictions on gene network topology for producing ordered behaviour.

The idea that attractors correspond to distinct, stable phenotypic states is just one aspect of a more general view. The genome–wide molecular network and, hence, the cell do not randomly and indifferently visit all possible network states \( S(t) \). Instead, because of the interactions between the molecular constituents, and the particularity of their wiring diagram, the state space is not ‘isotropic’, but highly sub structured, or compartmentalised, into stable attractor states and their ‘basins of attraction’. Thus, the interactions between genes and proteins are wired such as to impose restrictions on the dynamics of the network \( S(t) \). The complexity of the genomic network collapses into simple, robust behaviour with relatively few stable network states — the biological cell fates. The attractors in the state space ‘landscape’ (Figure 3) may thus represent a molecular and formal explanation for Waddington’s ‘epigenetic landscape’ (Figure 4), which he proposed as an intuitive metaphor to explain the discreteness of cell fates more than 60 years ago, without the notion of genes and networks.

**CONCLUSION**

In this paper the author has discussed disparate but complementary viewpoints in biology that the burgeoning notion of system-wide networks has exposed. On the one hand, a localist view that stresses the importance of detailed pathway analysis at the level of nominal genes and proteins, and that considers a network as the sum of all the pathways; and on the
other hand, a globalist view that emphasises the additional ‘emergent’ information harboured by a network as an entity. Since living systems are both complicated and complex, both viewpoints are equally important. Network structures provide an ideal handle with which to determine the relative contribution of natural selection versus intrinsic constraints to particular traits. Global network dynamics, however, open a new perspective to cell fate decisions in multicellular organisms which will foster understanding of phenomena such as the multi-potency of stem cells.

In conclusion, however, it is important to remember that networks are just a convenient abstraction, which fail to capture some essential properties of living systems. First, they represent a level of description that is devoid of space and physicality, such as the subcellular localisation of the proteins representing the network nodes, as well as mechanical forces within cells. Secondly, in multicellular organisms, every cell contains its own individual network. One forgets that in a theoretical treatment one analyses and models a single ‘proxy network’ whose behaviour is then mentally multiplied to billions of identical replicate cells in order to represent a tissue with its billions of cells. This subconscious, amplifying projection is problematic, since there is no representative ‘average cell’ because of molecular noise and other, epigenetic variations. Instead, a population, even of clonal cells, can dramatically vary in its network state, as evidenced by the dispersion of behaviour of the same genes in individual cells within a population. Perhaps this population heterogeneity itself is a desired feature, because the dispersion of single cell behaviour establishes robust, characteristic macroscopic response dynamics for the tissue as an entity. In this case, the detailed behaviour of a single cellular network would not matter, while sloppiness within the network of individual cells may even be beneficial for the behaviour of tissues. Finally, at the tissue level, there is another network: the cell–cell communication network, mediated by secreted factors, extracellular matrix and mechanical forces. Understanding intracellular molecular network is, therefore, all but a first step towards this network of networks that makes multicellular living systems work.

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Figure 4: ‘Epigenetic landscape’ proposed by Waddington in the 1940s as a metaphor to capture the observable dynamics of cell fates. The marble represents a cell state. Since Waddington was studying development, the overall slope represents the driving force of development. As the marble rolls downwards, the cell is forced to choose between discrete fates — represented by the valleys. Reprinted from Waddington (1957), ‘The Strategy of the Genes’, Allen and Unwin, London.


