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Current Opinion in Biotechnology

Diseases as network perturbations

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The tremendous amount of the data obtained from the study of complex biological systems changes our view on the pathogenesis of human diseases. Instead of looking at individual components of biological processes, we focus our attention more on the interaction and dynamics of biological systems. A network representation and analysis of the physiology and pathophysiology of biological systems is an effective way to study their complex behavior. Specific perturbations can trigger cascades of failures, which lead to the malfunctioning of cellular networks and as a result to the development of specific diseases. In this review we discuss recent developments in the field of disease network analysis and highlight some of the topics and views that we think are important for understanding network-based disease mechanisms.

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Current Opinion in Biotechnology 2010, **21**:566–571

This review comes from a themed issue on
Systems biology
Edited by Vitor Martins dos Santos and Jiri Damborsky

Available online 13th August 2010

0958-1669/$ – see front matter

DOI 10.1016/j.copbio.2010.07.010

Introduction—the systems approach to human disease in the context of P4-medicine

Systems biology is changing the future of medicine, which will become preventive, predictive, personalized and participative, a paradigm called P4-medicine [1]. One of the major drivers of this transformation will be the availability of low cost high throughput sequencing in combination with the development of high throughput multiple parameter molecular measurements — of RNA, proteins and metabolites — high resolution imaging and data processing and data storage beyond the petabyte range. Equally important however will be the realization that we need a new conceptual framework to describe and model diseases in their complexity. Such a framework needs to take into account the highly complex multifactorial nature of the causes of disease pathogenesis. These factors include genetic variation, epigenetic modifications and many genome–environment interactions. Looking at diseases as 'perturbations of networks' can provide such a framework and help to translate insights from systems biology into the practicalities of personalized and preventive medicine.

Genotype–environment interactions are highly nonlinear. Intuition as a tool for predicting the behavior of a system upon external perturbation fails in most cases and we contend that we need to apply the more formalized language of graph theory, vector algebra and nonlinear dynamics to properly make predictions. A graphical representation and mathematical description of diseases in their perturbed networks builds upon the advances made in other disciplines, such as engineering, physics and mathematics. Looking at diseases as a perturbation of networks can also serve as a conceptual basis to identify new drug targets and new innovative therapeutic strategies. Here we briefly review recent developments in the field of disease network analysis and highlight some of the topics that we feel will stay at center stage in the coming years.

Diseases can be viewed as specific types of network perturbation

Cells employ regulatory and signaling pathways that connect a large number of constituent parts of the system, like proteins, DNA, RNA, and metabolites, to coordinate multiple functions. One of the roles of this complexity is to permit cells to adapt to changing conditions. In order to understand the mechanisms underlying biological processes, we need to know not only the identity of the components that constitute the biological system, but also the ways they interact with each other. A network representation of these systems has been proven to be a powerful framework for their study. The analysis of the topology and dynamics of these biological network representations is thus essential to understand their complex and adaptive behavior.

Perturbation of cellular systems

Cells are constantly exposed to multiple simultaneous input cues from the environment, such as temperature and pH changes, external agents inducing DNA damage, and chemicals, which can modify proteins in posttranslational modifications. Cells often have to cope with rapid changes in the concentration of specific proteins and RNAs, the rate of enzyme catalysis, chromatin conformation, and also the allosteric regulation and interactions between proteins, transcription factors or the DNA-binding of transcription factors [2,3]. In addition, noise in the transcription and translation mechanisms lead to internal fluctuations in gene expression, protein and

metabolite concentrations [4•,5,6]. Consequently, the required levels of mRNA, proteins and metabolites for maintaining the cellular function need to be re-established with time or alternatively, changes in cellular behavior, such as proliferation, apoptosis, differentiation into mature cell types, and activation of differentiated cells, need to reflect changes in these informational molecules.

This fact is nicely illustrated by Kauffman's model of the attractor landscape of a gene regulatory network [7,8]. In this model, a gene expression profile reflects a state of a gene regulatory network, which evolves depending on the network's wiring towards an equilibrium state that is determined by the regulatory interactions. There are an enormous number of possible configurations in the gene expression state space. Nevertheless, cell fates (distinct functional phenotypic states of cells) correspond to these stable equilibrium states (attractor states) of the dynamic system on the basis of an underlying gene regulatory network. The process of changing functional phenotypic states then corresponds to trajectories in the gene expression state space.

The stability of these high-dimensional attractors guarantees that changes in the levels of expression and activation of interacting proteins will not modify the cell phenotype, but be constrained around the attractor. On the other hand, regulatory signals or stochastic fluctuations inducing changes in expression of multiple genes in a combinatorial manner would be able to produce an attractor switch. The existence of attractor states, determined by thousands of genes and their connection to cell fates has been experimentally verified [6,9,10]. The convergence of different trajectories in the state space to attractor states, which correspond to differentiated cell states, as well as the reversion back to attractor states after local perturbations, have been observed by monitoring gene expression changes in different multi-potent cell lines.

Mathematical models have been proposed for the analysis of cell signaling networks determining cell fate decisions [11,12]. These models, which rely on a logical formalization of known molecular interactions, are able to describe cross talk between these signaling pathways and to allow monitoring of dynamical effects resulting from perturbations of the system.

Network perturbations leading to human diseases

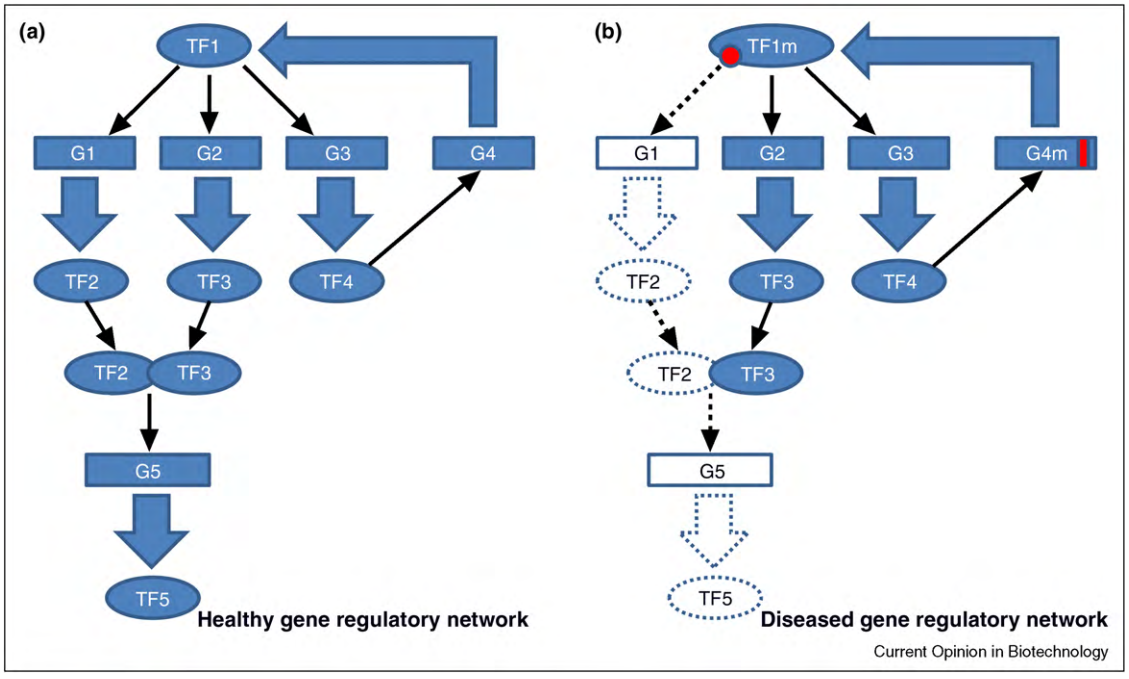
Despite the robustness of cellular networks in maintaining their performance against a wide range of perturbations and noise sources and levels, these networks can exhibit an extreme fragility towards certain (even seemingly much smaller) specific perturbations [13]. This tradeoff between robustness against a large number of perturbations and fragility against some other perturbations can explain the formation of diseases [14,15]. For example, although many mutations have no effect on a specific phenotype, mutations of certain genes or a particular combination of them can trigger cascades of failures, which lead to the gene regulatory network malfunctioning and therefore to the appearance of disease phenotypes [13,16] (Figure 1). Indeed, several human diseases, such as Huntington's disease, Cystic Fibrosis, and Sickle cell anemia are monogenic, resulting from the mutation of one gene; whereas in many cases diseases such as cancer, diabetes and Alzheimer's disease are multi-genic, caused by mutations in multiple genes. It is also clear that the single causal gene diseases are modified in their phenotypes by additional genes, sometimes called modifier genes, whose identities can shed light on the disease networks. In this sense there are very few, if any, truly single-gene diseases. In addition, environmental effects (some of them causing epigenetic modifications) can also impact interactions among genes, as toxins present in the environment may further degrade already weakened pathways, or stimulate them to transition into pathological states.

Effects of disease-related perturbations on the gene regulatory network landscape

Transcription factors exist as a dynamic population of proteins in different structural and dynamics states, exhibiting different DNA-binding affinities for DNA response elements [17,18]. Consequently, it is reasonable to assume that different gene regulatory network states, characterized by different sets of transcription factor conformations, coexist even in an apparently uniform cell population. Since many transcription factors and proteins that bind in a functionally modifying fashion to transcription factors are allosteric proteins, mutations modifications and environmental changes may act as allosteric effectors, leading to a redistribution of the transcription factor population, and therefore inducing a redistribution of gene regulatory network states in the cell population (Figure 1). For this reason single cell analyses will be important in research of the transitions between cell states.

This particular view leads us to an important hypothesis: some of these gene regulatory network states could correspond to disease stable states, pre-existing in the gene regulatory network landscape of healthy cell populations. This idea has been proposed in an updated view of the original Kauffman's model, where the concept of cancer attractors was introduced [19•]. Cancer attractors define stable gene expression programs associated to specific gene regulatory network states that implement the tumor cell phenotype and are already present in the healthy genome. However, these cancer attractors are normally not accessible—rarely attained by normal cells. Specific genetic mutations, environmental insults and/or

Figure 1



Mutation leading to the gene regulatory network malfunctioning. In this representation of a gene regulatory network proteins are depicted as ovals and genes as rectangles, correspondingly. Thick arrows indicate genes encoding for proteins, whereas thin arrows show transcription factors acting on genes. Dashed arrows show affected steps in the gene regulatory network. **(a)** Schematic illustration of a healthy-cell gene regulatory network. **(b)** A mutation in gene 4 triggers a conformational change in transcription factor 1, which impairs its binding to gene 1, and consequently leads to a cascade of failures yielding to a malfunctioning of the gene regulatory network associated to a disease.

epigenetic perturbations might modify the gene expression state landscape allowing cells to enter these unoccupied attractors. In addition, new cancer attractors that are normally unstable states may also appear.

Although, in most cases a complete understanding of how failure of individual network components leads to network malfunctioning and consequently to disease remains a significant challenge, a systems approach to study a number of diseases has given insights into the disease mechanisms. This challenge probably constitutes the frontier of systems biomedicine.

Examples of systems level approaches to study diseases

A number of experimental and theoretical systems level approaches have been carried out in the last few years aiming at dissecting anomalies in cellular networks associated with different diseases [20–23,24•,25–29]. These studies were based on the analysis of the topology and dynamics of disease-perturbed networks. Recently, the dynamic structure of the human protein interaction network was examined to predict breast cancer outcomes [20]. The computational removal of nodes from the protein interaction network identified protein interaction hubs critical for the network connectivity. Two types of hubs were classified—inter-modular and intra-modular hubs, which display low or high correlation of co-expression with their interaction partners, respectively. Data show that inter-modular hubs, which tend to be more critical for network's connectivity, were associated with cancer phenotypes more frequently than intra-modular hubs. Indeed, gene expression levels of these hubs were strongly correlated with the expression of their interacting partners in tumors from surviving patients, but not well correlated with their expression in tumors from poor-outcome patients. Another study based on a comparison of microRNA regulatory networks (inferred networks, based on data and predicted interactions) in normal tissues and different cancer tissues (51 solid tumors and leukemias) revealed their differences in hubs (the most connected miRNAs) [21]. Furthermore, complete miRNA networks characterized normal tissues, whereas cancer tissues were represented by disconnected, disjointed sub-networks, which allowed the identification of important miRNA cliques in cancer.

A large-scale study relying on the construction of a functional protein interaction network by using different data sources has been recently performed to analyze two data sets from genome-wide glioblastoma multiforme (GBM) [22]. After mapping cancer candidate genes obtained

from different GBM samples onto the network, it was found that these genes cluster together in modules enriched in known oncogenes, tumor suppressors and genes involved in signal transduction, suggesting that there is a core network in GBM tumorigenesis.

A combined study of the human metabolic network topology and the skeletal muscle gene expression datasets has been used to identify signatures of type 2 diabetes [23]. Transcription factors and metabolites that represent potential drug targets and clinical diagnostics for type 2 diabetes were determined. In addition to metabolites from the TCA cycle, oxidative phosphorylation and lipid pathways, a perturbation analysis of the cellular metabolic network identified highly connected metabolites ATP and NAD+ as contributors for the widespread gene expression changes observed in type 2 diabetes.

A comprehensive study of the initiation and progression of murine prion disease, a degenerative neurological disorder, has revealed several striking observations [24•]. This study generated dynamical subtractive brain transcriptome data (disease minus normal) to identify the differentially expressed genes across 10 time points during disease progression in eight different inbred strain/prion strain combinations. These data were integrated into four protein interaction networks that had been delineated by histopathological data—prion replication, glial activation, degeneration of axons and dendrites and neural apoptosis. In addition several other types of data gathered at each of these time points were integrated with the transcriptome and protein network interaction data in one inbred strain/prion strain combination—sagittal brain sections stained for infectious prion protein, histopathological analyses and clinical signs. The multiple inbred strain/prion strain combinations allow us to reduce the signal to noise in the transcriptome data by subtractive analyses of various biological features from 7400 to 333 genes that appeared to encode the essence of the prion disease process. Several other observations were made: first, two thirds of the 333 gene fell into the four networks described above, whereas the remaining one-third encoded six additional networks heretofore not known to be a part of the disease process and second, the four major networks were disease-perturbed in sequential order — prion replication, glial activation, degeneration of axons and dendrites and neural apoptosis — and this has important implications both for diagnostic and therapeutic strategies.

Interrelation between different diseases

Most of the current systems level approaches to study diseases focus on a single disease, relying on network-based methods to gain insights on the molecules and pathways relevant for the specific disease. A conceptually different approach has been recently proposed to study the pleiotropic relationships between different human diseases, leading, for example, to comorbidity, instead of focusing the attention on one disease [30]. The study was based on the generation of two complementary networks: The first network is a human disease network (HDN) with nodes representing disorders that are linked through edges representing common implicated genes. The second network is a disease gene network (DGN) with nodes corresponding to disease genes and the edges formed by the diseases that result from a perturbation of the linked node genes.

This provided a theoretic framework to connect all known phenotypes and disease gene associations. This approach can lead to a revelation of some of the common genetic origins of diseases. As a result of this study the authors observed a gene expression correlation between genes associated with similar disorders, indicating the existence of disease-specific functional modules. It was interesting that they also found that the majority of human disease-related genes are nonessential and do not encode hub proteins. A selection-based model was proposed to explain differences between essential and disease genes. In a different study by combining information on cellular interactions, disease gene relationships, and population-level Medicare data, correlations between disease comorbidity and the structure of cellular networks were found [31].

Despite these efforts to understand the interrelations between different diseases based on cellular network characteristics, and in order to understand the genotype–phenotype relationships in diseases, the modeling of disease-causing mutation effects on molecular networks is required. Different mutations in the same gene can produce distinct functional mutants, which may differently affect cellular networks. This point has been addressed in a systematic analysis of how perturbations of interactome networks may differ between complete loss of gene product (node removal) or loss of specific molecular interactions (edge removal) on a large dataset of known mutations in Mendelian disorders [32•]. Mapping disease-causing mutations on available three-dimensional structures of disease proteins provided complementary information on perturbations of physical protein–protein interactions leading to changes in the interactome network.

Systems level approach to drug design

Systems level approaches can yield insights into disease-related perturbations of cellular networks [33–35]. In order to attack the understanding of truly complex diseases, such as cancer, Parkinson's disease, and diabetes, we need to reconsider our strategies for drug design and selection of molecular targets for treatment. The development of a multi-target drug strategy based on a network analysis, if it can predict network responses, should give

much better results than the traditional single-target strategy [36•,37,38]. A network approach should provide the framework for designing combinatorial therapies that target less essential nodes to increase synergetic performance and decrease side effects. Furthermore, considering complex diseases as robust systems exhibiting nevertheless points of fragility may provide insights for the development of new drugs [39]. Nevertheless, for the design of appropriate drug combinations and multi-target drugs, we need to develop novel theoretical and experimental approaches to explore the dynamic complexity of cellular networks after multiple perturbations [36•,40].

The challenges

The concept of disease states as perturbations of cellular networks is gaining recognition as a fruitful conceptual framework for developing new preventive and therapeutic strategies. One of the major drivers in the past and in the foreseeable future is the enhanced ability for high throughput analysis of genomes and molecular phenotype analysis. There is a major need for more sophisticated automatation and miniaturization technologies to be used in this effort.

Equally challenging will be the dissection of the internal characteristics, such as the topology and dynamics of the cellular networks involved. The majority of biological perturbation studies thus far have employed single endpoint studies, to compare changes in physiological, molecular or cellular parameters in healthy and/or disease, including human patients, animal models and *in vitro* culture systems. It will be essential to fully dissect the state changes to have time series analyses. Otherwise we will not be able to see the complex dynamics of regulatory interactions that occur during disease pathogenesis. Automated time-lapse imaging cinematography and other imaging techniques will greatly facilitate this.

A major problem for the future will be the integration of the different networks, for example, regulatory, protein–protein interactions, proteomic and metabolic networks. The time-scales underlying these networks operate are in many cases very different and we do not have complete datasets, and good bioinformatics and computational tools available for this integration. Ideally, an integrated network approach would consider full cellular network models, which describe the dynamics of interactions among different types of molecular nodes (proteins, RNAs, genes, and other molecules), characterized by quantitative variables representing the states of these molecules (concentration, activity, expression, etc.). The challenge of characterizing and understanding 'emergent properties' of these systems will be at the heart of future biological research.

An enormous challenge for the future is dealing with the signal to noise issues that arise from all high throughput data sets. This will require the development of mathematical approaches to handle the noise—but perhaps more important will be the deployment of deep biological understanding of the relevant systems. For example, in the prion example given above about eight subtractive analyses were employed among the eight inbred strain/prion strain combinations to subtract signal for other types of biology from that of the core prion response (21a). For example, for each of the 10 time points the differentially expressed genes (diseased minus normal controls) changes seen in the congenic mice for the double knockout of the prion gene (animals that never get the disease) were subtracted from the 7400 differentially expressed initially observed. This signal to noise is a critical approach and obviously requires a very deep understanding of the biology—and it will in other examples be a critical component of identifying the core of genes responsible for specific diseases.

The analysis of disease networks requires the perturbation and monitoring not only at a cell population, but also at the single cell level. Stochastic effects, intrinsic or extrinsic noise or the switch from one attractor state to another can often be derived only at the single cell level. Microfluidics, flow sorting and other technologies will greatly facilitate such single cell studies.

We also need new mathematical tools required for a successful modeling and simulation of diseases. Maybe the desire to fully understand biological networks and predict their behavior after specific perturbations might trigger the development of new areas of mathematics, similar to what we witnessed in other disciplines. A detailed description of what we know about such needs is beyond the scope of this article, but it is clear that there are growing needs for new methods.

We need to devise efficient new tools to reliably link the tremendous genetic variation that we see among individuals with individual disease prevalence and comorbidity. The study of cellular networks underlying the link of genetic and environmental information will be at the forefront of this attempt to create the new systems biomedicine.

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