Systems Biology and Its Application to the Understanding of Neurological Diseases

Pablo Villoslada, MD,[1] Lawrence Steinman, MD,[2] and Sergio E. Baranzini, PhD[3]

Recent advances in molecular biology, neurobiology, genetics, and imaging have demonstrated important insights about the nature of neurological diseases. However, a comprehensive understanding of their pathogenesis is still lacking. Although reductionism has been successful in enumerating and characterizing the components of most living organisms, it has failed to generate knowledge on how these components interact in complex arrangements to allow and sustain two of the most fundamental properties of the organism as a whole: its fitness, also termed its robustness, and its capacity to evolve. Systems biology complements the classic reductionist approaches in the biomedical sciences by enabling integration of available molecular, physiological, and clinical information in the context of a quantitative framework typically used by engineers. Systems biology employs tools developed in physics and mathematics such as nonlinear dynamics, control theory, and modeling of dynamic systems. The main goal of a systems approach to biology is to solve questions related to the complexity of living systems such as the brain, which cannot be reconciled solely with the currently available tools of molecular biology and genomics. As an example of the utility of this systems biological approach, network-based analyses of genes involved in hereditary ataxias have demonstrated a set of pathways related to RNA splicing, a novel pathogenic mechanism for these diseases. Network-based analysis is also challenging the current nosology of neurological diseases. This new knowledge will contribute to the development of patient-specific therapeutic approaches, bringing the paradigm of personalized medicine one step closer to reality.

Ann Neurol 2009;65:124–139

"Information is not knowledge."—*Albert Einstein*

Systems Biology: Underlying Principles, Approaches, and Applications

Although a formal definition of systems biology has not yet been widely accepted, most researchers agree in that it represents an integrative approach that attempts to understand higher-level operating principles of living organisms, including humans.[1,2] In biomedical research, not all scientific questions are directly accessible to experimentation. There is a hierarchy of scientific questions whose levels are determined by the generality of the answers sought. The lower levels in this hierarchy deal with narrowly restricted and specific phenomena. These are the kinds of questions that the reductionist experimenter feels more comfortable in addressing. On the other end of the spectrum, higher-order, abstract, and general questions are not usually directly amenable to an experimental test. Reductionist thinking mandates that these questions be broken down into more specific terms that can be translated directly from experimental results.[3] In stark contrast, systems biology strives to understand these higher-order properties whereas examining the complexity of the system under study. Systems biology heavily relies on the construction, utilization, and integration of inductive models, a creative process for which a considerable degree of abstraction is crucial. In this sense, abstraction consists of replacing the part of the system under consideration by a model of similar but simpler structure.

Traditionally, neuroscientists have coped with the enormous complexity of the brain in a purely reductionist way: by subdividing it into anatomic regions and characterizing each of their cellular compositions and basic functions in isolation. Although that approach has enjoyed remarkable success, the next challenge is to translate such valuable information into a better understanding of how several higher-order properties of the brain, such as memory, learning, and behavior, emerge from such a complex interplay (see the Table for a glossary of terms). Under the reductionist paradigm, a positive correlation between a single biological parameter and the occurrence of a disease is of-

From the [1]Department of Neuroscience, Hospital Clinic–Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain; [2]Department of Neurology and Neurological Sciences, Stanford University, Stanford; and [3]Department of Neurology, University of California, San Francisco, CA.

Address correspondence to Dr Villoslada, Department of Neurology, Hospital Clinic – IDIBAPS, Villaroel 170, 08036 Barcelona, Spain. E-mail: pvilloslada@clinic.ub.es

Potential conflict of interest: Nothing to report.

Received Dec 21, 2007, and in revised form Dec 16, 2008. Accepted for publication Dec 16, 2008.

Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ana.21634

ten considered a major success, even though the complete pathogenic mechanism may remain largely unknown. Notably, this approach has been followed by the pharmaceutical industry, despite a remarkably low rate of success (only 11% of new therapeutic targets reach the market as new drugs).[4] Systems biology focuses on understanding not only the components of a given system, thus complementing the reductionist approach, but also the effect of interactions among them and the interaction of the system with its environment. Like physiology, systems biology is deeply rooted in the principle that the whole is more than the sum of its parts.[5]

Biological systems offer multiple examples of collective properties, those in which the behavior of the whole cannot be predicted from the detailed study of individual components. In systems biology, as in the science of complexity, those intrinsic properties are referred to as *emergent*.[6] Emergent properties are present in all physiological systems and include the maintenance of blood volume, blood pressure, tissue pH, or body temperature. Emergent properties in the brain include the processes of learning, memory, and emotions that cannot be explained fully even by the detailed study of single neurons. Analyses based on single-cell neurobiology or molecular biology are useful for characterizing the individual behavior of each component. However, these properties are the result of nonrandom interactions of highly specialized cells assembled in large networks, and they can be understood only by not disrupting these structures. Based on this and other examples of systems composed of interconnected elements (eg, acquaintances among people, the Internet, airports), inductive models have been created that display surprisingly generalizable properties.[7,8]

Although systems biology strives to combine techniques obtained from seemingly disparate disciplines, it mainly represents a way of thinking.[9] Traditionally, the study of body dynamics, as functional systems, has been the focus of physiology. In this sense, systems biology can be regarded as contemporary physiology, one that uses methods from mathematics, physics, and computer sciences to integrate and analyze genetic, molecular, physicochemical, functional, and behavioral data obtained in the laboratory.[10,11] A systems approach to research already has been established in several fields of biology, such as ecology, enzymology, and computational neuroscience. Such an approach is necessary by virtue of the large amounts of data generated by high-throughput methods such as gene chips and proteomics. Moreover, we now realize that the human genome is more complex and has many more levels of regulation than previously anticipated,[12,13] and systems biology is expected to provide a comprehensive interpretation of its organization.

Robustness and Disease

Two of the most critical emergent properties of all living systems are robustness and adaptability.[8,14] *Robustness* refers to the ability of a system to maintain its basic functions even in the presence of perturbations, such gene mutations or environmental fluctuations.[15–17] However, when robustness alone is unable to cope with such challenges, organisms must be able to adapt to them and to evolve. Understanding these higher-order properties requires the formulation of a series of inductive analogues or models that capture the critical aspects of the system and neglect the details, thus shedding some of the initial complexity and making it amenable to study.

One example of such an approach is systems control theory, developed and used for decades by engineers and physicists in the construction of advanced technological equipment, ranging from thermostats to the radar and guidance systems on airplanes.[9,18] Systems control theory has been successfully adapted to the detailed study of the delicate balance between robustness and adaptability in living systems.[19] In thinking like engineers, biologists have been able to create machine-like analogues that faithfully explain the properties under study. Through the use of these metaphors, such studies have shown that robustness in life relies on several fundamental principles. First, fail-safe mechanisms such as redundancy and diversity (eg, gene duplication or overlapping pathways) enable the organism to function even if one of its molecules or pathways is affected to a reasonable degree. Second, positive and negative feedback and feed-forward mechanisms result in properties such as multistability, oscillations, and signal amplification, respectively.[16,17] These mechanisms bestow an organism with control functions such as ON/OFF switches and autoregulation (homeostasis). Third, the networked architecture of internal components allow task parallelization, thus containing local damage and preventing its spreading to the full system. Finally, functional and physical properties, although related, work independently (decoupling). In this way, changes at the physical level (ie, protein misfolding) may not translate to the functional level, resulting in pathology. Chaperones are designed to deal with misfolding and with unstable intermediate states in the final protein structure.

By integrating these pieces of our inductive model into a "general solution," diseases can be viewed as a breakdown in the robustness of normal physiological systems. Chronic diseases would represent the establishment of a new, but undesired, robust state.[20]

Advantages of a Systems Biology Approach to Neurological Diseases

Computational neuroscience, neuroinformatics, neurophysiology, and other newly developed disciplines are

Table. **Concepts and Examples in Systems Biology**

Concept	Description and Example
System[2,9]	A set of connected entities forming an integrated whole. e.g., The cell is a system composed by molecules (entities) with well-defined properties (division, metabolism, among others); the body is another system composed by cells
Systems thinking[9]	Systems thinking is a framework that is based on the belief that the component parts of a system can best be understood in the context of relations with each other and with other systems, rather than in isolation. e.g., Explaining the physiology using feedback exemplified the attempt to understand the influences of some biological process with each other
Complexity theory[2,9,57]	A discipline of physics studying complex systems by focusing on the generation of emergent properties in systems; *complexity* is not equivalent to *complicated*. e.g., Self-organization refers to the generation of complex structures with well-defined properties without a master program (ie, generation of liposomes when lipids are mixed with water) (see also Emergent properties and Robustness)
Nonlinear dynamics[10]	A discipline of physics that studies how systems evolve with time, involving a lack of linearity; a nonlinear system is any problem where the variable(s) to be solved for cannot be written as a linear sum of independent components. e.g., The majority of systems in nature are nonlinear (linear analysis is a simplification); electroencephalographic signals are typically nonlinear because they are the result of complex interactions between neurons and not just the sum of each neuron's activity
Control theory[12]	A discipline of engineering that deals with the behavior of dynamical systems. e.g., The analysis of molecular networks as a set of positive and negative feedbacks, mimicking the analysis of an electric circuit
Feedback control[1,2,19,43]	A process whereby some proportion of the output signal of a system is passed (fed back) to the input. e.g., Cortisol regulation by the hypothalamus-pituitary-adrenal axis
Robustness[15–17]	Maintenance of the properties and function of a system in the presence of perturbations. e.g., Homeostasis is a classic physiological example: the body maintains pH, glucose, temperature, and so forth in the presence of changes in the environment (feeding, weather, among others)
Adaptability[14,15]	A positive characteristic of an organism that has been favored by natural selection; sometimes also called *fragility* in comparison with robustness. e.g., Genetic mutations are rare but present in human genome allowing adaptation to the environment (ie, thalassemia mutation protects against malaria)
Emergent properties[2,10]	Physical properties of a system arising as an effect of complex causes and not analyzable simply as the sum of their effects. e.g., Glucose levels stability is the result of many interacting subsystems in the body (food intake, insulin, glucagons, cortisol, and so on); cognition is an emergent property of the brain
Network[7,8]	Any interconnected group or system that shares information (ie, Internet, social networks). e.g., Gene networks are the set of genes that, through their proteins, interact with each other; neuronal networks are the set of neurons connected through synapses participating in common tasks (ie, learning)
Network analysis[8]	I. Network architecture (topology):
	1. Microscale. e.g., Motif: recurring circuits of interactions between nodes (ie, gene interaction loop)
	2. Mesoscale. e.g., Module: group of physically or functionally linked molecules (nodes) that work together to achieve a distinct function (ie, cell-cycle genes)
	3. Macroscale. e.g., Scale free: only a few nodes are highly connected (hubs), and the majority of the nodes are poorly connected (eg, Internet)
	II. Network dynamics:
	1. Mathematical modeling with differential equations
	2. Synchronization analysis
	3. Feedback analysis

Table. **(Continued)**

Concept	Description and Example
Network topology[8]	The architecture of nodes and links in a network that defines how information flows through the network:
	Nodes: elements of a network. Node: a gene, protein, or cell
	Links: interactions between nodes. Links: regulation of gene expression by transcription factors
	Hub: a highly connected node. Hub: p53 protein in cell cycle
	Degree or connectivity: number of links per node
Network dynamics[8,43,83]	Changes in network properties over time. Deletion of a gene, emergence of new motifs or modules by mutations
Gene regulatory networks[1,42,43,83]	Collection of DNA segments in a cell that interact with each other through their RNA and protein expression products, thereby governing the rates at which genes in the network are transcribed into messenger RNA. Interferon regulatory network
Pathway[77,83]	Metabolic pathway: a sequence of chemical reactions in a cell undergone by a group of molecules leading to a predicted functional outcome. e.g., Amyloid pathway in Alzheimer's disease pathogenesis
	Neural pathway: a neural tract connecting one part of the nervous system with another. e.g., Dopaminergic pathways: neural pathways in the brain that transmit the neurotransmitter dopamine
Pathway modularity[77,83]	A module is a self-contained component of a system, which has a well-defined interface to the other components. e.g., Metabolic modules in the cells: cell cycle, glycolysis, energetic function (mitochondria), receptor-signaling system
Pathway analysis[77,83]	Study of a biochemical pathway instead of single genes or molecules. e.g., Axon guidance pathway, amyloid pathway, oxidative stress pathway
Dynamic disease[10,54,55]	A disease whose pathogenesis is mainly caused by the appearance of new dynamics of the organism behavior, independently of the underlying pathogenesis. e.g., Several brain injuries (tumor, trauma, and so forth) may affect neural networks generating seizures; Parkinson's disease symptoms are the results of disturbance of cortical-subcortical neural networks; epilepsy and movement disorders are dynamic diseases
Neuromodulatory system[72,73]	The neural network that modulates the overall activity of the excitatory (glutamatergic) and inhibitory (GABAergic) networks. e.g., Dopaminergic, cholinergic, neural peptides neural networks
Systems biomarker discovery[92]	Identification of new biomarkers based in systems properties of the molecules involved. e.g., Ongoing
Systems drug discovery[83,84,91]	Development of new therapies using systems biology analysis and/or aimed to modifying the system properties of the tissue involved (in comparison with target-centric drug discovery). e.g., Ongoing
Bottom-up approach[1,3]	Piecing together systems to create grander systems to gain insight into its emergent properties. e.g., Identifying molecules and pathways implicated in the pathogenesis of neurological diseases, providing an integrated model of the pathogenesis
Top-down approach[3,55]	Breaking down a system to gain insight into its compositional subsystems. e.g., Starting from the observable phenomena (cognition), identifying the domains and neuronal networks involved through electroencephalogram, functional magnetic resonance imaging, or cognitive studies and developing models of it

devoted to the study of emergent properties of the brain.[21,22] Although approaches aimed at deciphering how the brain operates are gradually migrating from deterministic to systems based, the two approaches currently coexist somewhat separately.[23] Although efforts for identifying gene variants associated with intelligence, memory, or social skills are currently under way, neuroscientists acknowledge that the information-processing capabilities and ultimate brain behavior result from the dynamic interplay of complex networks of synapses.

The identification of a gene defect associated with a given disease is often followed by attempts to develop therapies aimed at correcting its function, with the hope that this may restore homeostasis. For example, our current understanding of familial Alzheimer's dis-

ease (AD) has been quite productively focused on the identification of genetic and molecular perturbations in the amyloid-β and -τ pathways.[24] However, the presence of genetic heterogeneity and lack of knowledge about the relative contribution of each defect to the overall phenotype has delayed progress in the development of therapeutic approaches.[25] Recent studies demonstrated that the pathways affected in individuals with familial and sporadic AD are not the same. Furthermore, they also highlighted the contribution of additional processes such as axonal pathology,[26,27] myelination,[28] neuroinflammation,[29] ischemic insult,[30] and aging[31] to the overall pathogenesis. Intriguingly, it is not uncommon to observe the opposite phenomenon, in which alterations in the same pathway (amyloid) lead to different disease phenotypes such as AD versus inclusion body myositis. It has recently been shown that computational models can be used to analyze perturbations in multiple pathways over long periods that may be responsible for the observed neuronal damage in AD.[31] Indeed, the strong correlation between the development of neurodegenerative diseases and aging suggests that even minor imbalances in these pathways sustained for several years can finally overpower innate repair mechanisms and result in the neurodegenerative trait known as AD.[32]

Many of the genes and pathways altered in one neurological disease are also commonly dysregulated in others, even if they do not share the same pathophysiology, age of onset, or outcome. Some disease-activated pathways may represent an attempt by the brain to restore homeostasis. Examples of this behavior are the induction of small heat shock proteins with a guardian-like protective role in the brain such as αβ-crystallin, the production of restorative secreted factors such as neurotrophic factors, and the activation of microglia.[33–35] Physiological systems, including the central nervous system, may possess a limited ability to deal with genetic or environmental perturbations, so that different insults (eg, trauma, ischemia, metabolic imbalances) trigger the same potentially restorative pathways. Depending on genetic predisposition and environmental exposures, a given individual will go on to experience development of one of a diverse array of conditions, such as Parkinson's disease (PD), dementia, leukodystrophies, and multiple sclerosis (MS), all of which share common pathological processes.

Indeed, a recent large-scale analysis of brain transcripts from neuropathological specimens demonstrated unexpected similarities between MS and the metabolic diseases hexosaminidases. These include the shared expression of a constellation of inflammatory markers such as cytokines, heat shock proteins, and other stress proteins. Some of the most abundant transcripts found in the affected brains from MS, Tay–Sachs, and Sandhoff's disease cases, but not in normal control cases, include osteopontin, αβ-crystallin, major histocompatibility class II, prostaglandin D_2, calcyclin, apolipoprotein E, metallothionein, dnaJ, monoamine oxidase B, S-100, and calponin.[36–38] Notably, these results were not seen in sphingolipidoses. These data suggest that there is an unexpected inflammatory component in biochemical disorders of the brain. Interestingly, these findings may have therapeutic implications. When mice with deletions in the immunoglobulin Fc receptor are bred with mice carrying the Tay–Sachs and Sandhoff's mutations, there is amelioration of disease.[39] Fc receptors are present in MS lesions, and deletion of Fc receptors also ameliorates experimental autoimmune encephalomyelitis, an animal model of MS.[36] These data suggest that two inherited biochemical disorders of neural development can be modulated by regulating pathways usually considered to be solely inflammatory. This again illustrates that common mechanism or response to injury can be activated in response to different insults or pathogenic mechanisms. Moreover, such commonalities can be better understood from an integrative view of the many brain pathways at work during central nervous system injury.

Neurological Diseases Can Be Studied in the Context of Complex Networks

Among other approaches, systems biology uses network-based analyses as a strategy for integration of data from genetic, gene expression, proteomic, and neurobiological experiments with the ultimate goal of identifying pathways involved in the pathogenesis of neurological diseases. The representation of real-world systems by the network analogy represents another example of inductive modeling that helps scientists approach the complexity of the problem in question. Networks are systems of interconnected entities. When networks are studied in aggregate, certain properties emerge from them that cannot be derived from the individual analysis of each of their components. In graph theory, networks are defined by nodes (representing genes, proteins, or cells) and links, representing the interaction among nodes. In homeostasis, biological networks are constantly challenged and subjected to internal or external perturbations (ie, via gene mutations or environmental changes) that are normally counterbalanced by the network's robustness and adaptability (ie, networks maintain their properties and continue to perform their functions). However, if perturbations affect key components of a network or are sustained for a long period, a qualitative change occurs such that the whole system is pushed toward a different (in this case, pathological) steady state or "disease network."

There are two main approaches for studying networks: (1) structural, also referred to as *topological analysis,* which examines the architecture of the system (network connectivity patterns); and (2) dynamic anal-

ysis, which examines how networks evolve over time by changes in the number of nodes and in their connections. Topological analysis is based on the statistical properties of the network, such as the degree of connectivity, mean path length, or clustering coefficient (see Barabasi and Oltvai's[8] review). Many real networks display the "small-world" property, in which several nodes are locally connected among each other but loosely connected to others, creating a modular structure. Recent studies have demonstrated that, in small-world networks, it is possible to navigate from one particular region to any other in just a few steps (eg, the well-known phenomenon of "six degrees of separation" among all people in the world[7]). In scale-free networks, the majority of nodes are poorly connected with other nodes, but a few nodes (hubs) are highly connected (ie, Google is an example of a hub in the Internet because millions of other pages are just one click away). One of the most salient properties of scale-free networks is that they are robust against random attacks and sensitive to selective targeting (ie, a single directed attack at Yahoo will cause more damage to the Internet than will several thousand random attacks at home PCs). These properties are good examples of the "general solutions" that can be achieved by deductive analysis of data from several unrelated systems. Although these network properties are characteristic of hard-wired networks, biological networks are less fixed. Thus, considerable efforts are now under way to characterize the dynamics of biological networks.

Networks and Systems Control

When networks are studied at the local level, other interesting topological elements, such as modules and motifs, emerge. A module is a set of nodes participating in similar functions that cluster together (ie, there are more connections among themselves than there are with any of the other molecules in the network).[40,41] Examples of modules are abundant in analyzing key biological functions. Many apoptotic events are centered on caspases, whereas the cell cycle is coordinated via cyclins. At the center of many key processes associated with inflammation, the nuclear factor-κB pathway commands a huge position of importance. Caspases in apoptosis, cyclins in the cell cycle, and nuclear factor-κB represent some examples of these so-called local structures.

Network motifs are responsible for many basic control mechanisms such as negative or positive feedback and feed-forward signaling (Fig 1). The study of local motifs integrates the topological and dynamic features of complex networks.[42,43] Feedback loops are an efficient mechanism applied by cells to ensure precise control over gene and protein expression, even in the presence of noise from other cellular components. A positive feedback enables the generation of bi(multi)stable responses depending on the duration and intensity of the initial stimulus. Bistability is the proposed mechanism behind the storage of information and cellular decisions (ie, cell phenotype).[42,44] Bistability is the existence of two stable states, and it is generated by an abrupt transition in the dynamics of a system that generates the new state (ie, activation of a gene expression pattern leading to a permanent cell phenotype). A negative feedback loop is an efficient mechanism for maintaining product levels in a tightly regulated range, and it is one of the most basic cellular mechanisms for controlling homeostasis.[45] Oscillatory dynamics is common in signaling pathways, such as nuclear factor-κB pathway. More complicated dynamics can appear with combinations of complex network motifs that include positive and negative feedback loops. Finally, feed-forward motifs provide a redundant mechanism for the transmission of molecular information by extending the duration of the signal and ensuring its arrival at the intended site. Recent studies have shown that these and other basic control motifs are present in large regulatory networks in yeast, bacteria, and humans, and form the basis for information processing through cells.[42,43] The abstract representation of complex biological relationships using engineering tools provides another example of the widespread use of inductive models in systems biology. By mainly focusing on certain aspects of the real world, these models can provide important clues about how these systems work, although it will take time before we know the degree to which that conceptual representation provides insight into the actual mechanisms.

Like engineering-based systems-control analysis, the study of complex sets of biological entities in the context of networks may demonstrate common properties relevant for disease pathogenesis that cannot be predicted by analyzing each entity in isolation. Several studies have questioned whether proteins associated with diseases share any particular network property, such as higher connectivity, preferential modular architecture, or involvement in more than one biological pathway.[46–49] Although still in their infancy, these approaches may provide important insights toward understanding the pathogenesis of neurological diseases and designing new therapeutic strategies. For example, if disease-related proteins interact with several other proteins (are hubs) within their networks (eg, p53 in cancer), any pharmacologically induced modulation will affect the overall proteome network and may induce significant side effects. Moreover, by relating mechanism of action and side effects through network analysis, new applications of existing drugs may be inferred.[50]

A recent study analyzing the protein network involved in inherited ataxias exemplifies the power of this approach.[49] In this study, Lim and colleagues[49] exam-

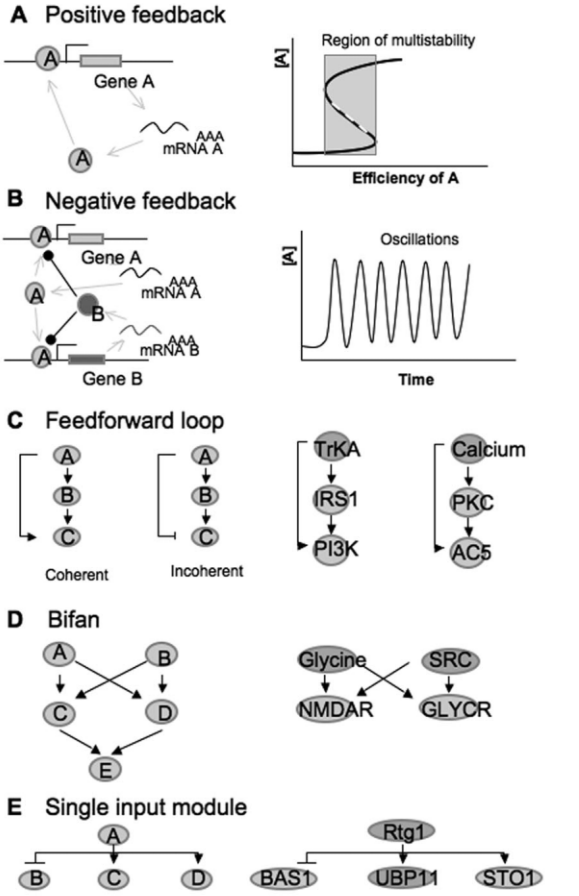

Fig 1. Genetic circuits. (A) In positive feedback loop (left), the expression of a gene product is stimulated by its own expression, thus enabling the generation of bistable responses (right) depending on the duration and intensity of the initial stimulus. (B) Another basic type of control is negative feedback loops (left). When levels of gene A increase, expression of gene B is induced, which represses expression of gene A (and its own expression). When levels of A fall below a threshold, its production is stimulated because of the absence of the repressor B. This control mechanism yields oscillations around a mean value (right), which, in turn, depends on other parameters of the system such as synthesis and degradation rates. (C) Feedforward loops (FFL) consist of three genes: A, B, and C. Gene A is a regulator of B and C (left). FFL is coherent if the sign (activation or suppression) of the path A-B-C is the same as the sign of the path A-C. If the signs do not match, the FFL is incoherent. FFL can reject transient inputs and activate only after persistent stimulation (ie, TrkA and calcium signaling, right). (D) The bifan motif (left), composed of two source nodes directly cross-regulating two target nodes, is able to act as signal sorter, a synchronizer, or a filter (ie, glycine pathway, right). It also provides temporal regulation of signal propagation. (E) In the single-input-module motif (SIM), modulator A controls the expression of a group of species (left). This motif can generate an ordered expression program for each of the components under regulation of A (ie, rtg1-mitochondrial pathway in yeast, right). mRNA = messenger RNA; NMDAR = N-methyl-D-aspartate receptor; PI3K = phosphatidylinositol 3 kinase; PKC = protein kinase C.

ined the biological relations between the mutated proteins in hereditary ataxias. They hypothesized that if mutations in different genes lead to death of Purkinje neurons in all ataxias, they should be part of common biological pathways critical for the survival of these cerebellar neurons. To answer that question, they performed a topological network analysis of the protein–protein interaction network. Starting from the 23 proteins previously known to be involved in hereditary ataxias because of the presence of known mutations in their genes, they identified the contextual network of 3,607 proteins in which the ataxia network was included. The gene ontology analysis showed that proteins affected in ataxias are functionally clustered in related pathways (Fig 2A), with overrepresentation of pathways related to RNA splicing, ubiquitination, and the cell cycle. This finding suggests that RNA splicing plays a critical role in Purkinje cell degeneration. By analyzing the ataxia network, Lim and colleagues[49] were able to confirm involvement of the previously described pathways and the recently discovered *Puratrophin-1* as an ataxia gene[51] having strong interactions with *Ataxin-1* (see Fig 2B), even though *Puratrophin-1* was not included in the original list of ataxia genes. Overall, the protein network analysis of human ataxias provided an integrated view of these diseases as RNA splicing diseases promoting the death of Purkinje neurons. This aspect of systems biology will open new avenues for the development of new diagnostic tests and therapies.

In another study, Miller and coauthors[31] applied a network analysis to DNA array studies from the CA1 region of the hippocampus of patients with AD and normal aging individuals. Instead of reporting a list of differentially expressed genes between both conditions, an approach with low probability of replication, they used weighted gene coexpression network analysis to group results into a nine functionally relevant pathways. They found that synaptic transmission, extracellular transport, immune response, mitochondrial and metabolic processes, and myelination were associated with disease progression. Some of these modules correlate with the degree of cognitive impairment measured with the Mini-Mental test or with the pathological burden measured using neurofibrillary tangle quantification. Moreover, a commonality between AD and aging was found in the involvement of mitochondrial processes and synaptic plasticity. In addition, Miller and coauthors[31] found new evidence supporting the role of demyelination and oligodendrocyte dysfunction in AD progression, which was related to the preselinin-1 pathway. Overall, by providing a pathway view of AD pathogenesis, these authors provided a new set of mechanistic hypotheses about AD that can be tested in future studies. The benefits of these kinds of approaches would be exemplified in that they help in

the design of new therapies targeting the pathways involved.

In addition to studying ensembles of genes or proteins, network analysis offers a potentially useful way to improve the classification of neurological diseases by closing the gap between causative factor, pathogenesis, and disease phenotypes.[52] Recently, Goh and researchers[47] analyzed data from the Online Mendelian Inheritance in Man (OMIM) database and characterized the full set of disease–gene associations in what they called the "human diseasome" (Fig 3). In this analysis, two genes are linked together if they are responsible for causing the same disease, and two diseases are linked together if they share at least one defective gene. The authors found that the products of genes associated with phenotypically similar disorders tend to cluster together. Indeed, experiments demonstrated that proteins affected by the same disease interact in such networks more frequently with each other than with those not associated with the disease. These results support the concept of disease-specific functional modules. Analysis of the components of networks may show certain common responses to brain pathology and offers a new formalized conceptual approach to assess disease susceptibility. Thus, network analysis and other systems approaches can be helpful for neurologists in the process of classifying diseases.

We must clarify that the methods identified earlier are used to find patterns in data. Depending on the nature of the data and the method used, the pattern found is mapped formally in a geometric point-to-point manner to an abstract, idealized pathway or network. As with any other model, the pathways and networks described here are inductive concepts, and no quantitative mapping is possible between the conceptual system and the real system. Nevertheless, biomedical research relies heavily on conceptual models, and the formal introduction of these should come as no surprise to the reader. An important contribution of these models is that they provide the guidelines needed to construct analogue mechanisms (a task that remains to be done) using the irreplaceable methods of experimental biology. The map between those mechanisms and biology can be iterated in a concrete manner.[53]

Dynamic Analysis of Neurological Disease

Chronic diseases can be distinguished by their onset (acute, subacute, or progressive) and their subsequent

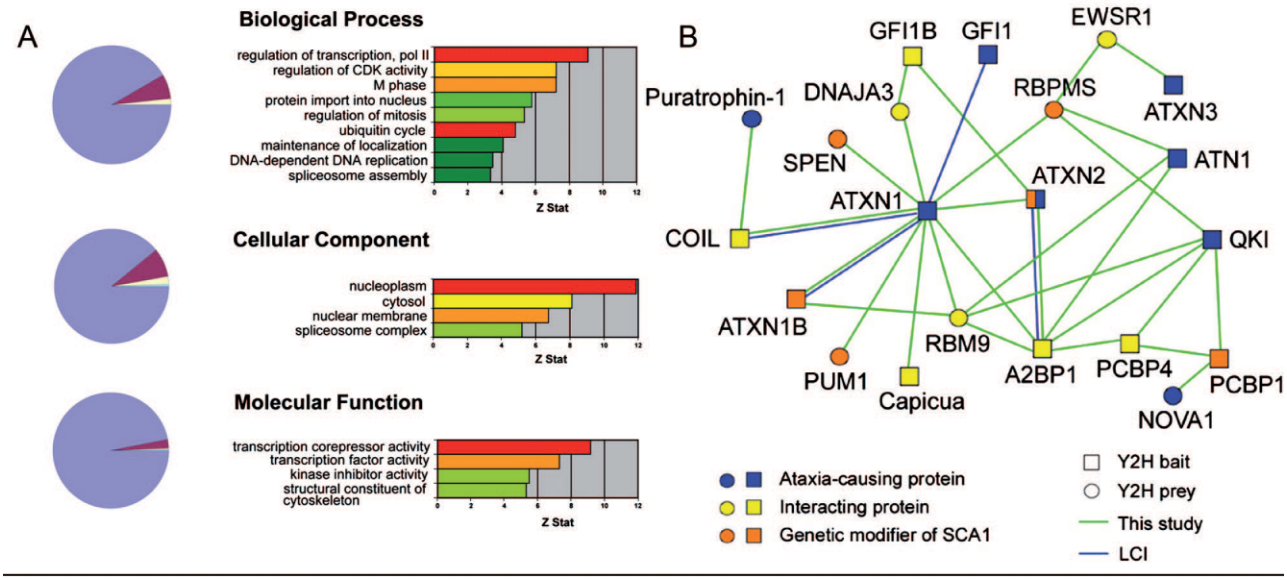

Fig 2. Ataxia network. By performing protein interaction studies, bioinformatics search, and network analysis, Lim and colleagues[49] hypothesized the existence of a network, pathway modules, and functions that may relate the genes associated with human hereditary ataxias. (A) Enriched gene ontology (GO) categories of the ataxia network. Pie charts depict the relative number of GO terms enriched in each of the three branches of the GO structure when using the whole genome and/or the hORFeome as reference distributions. Enriched categories are identified as those significant ($p < 0.05$) after adjusting for multiple testing. Bar charts depict the enriched GO terms from the common set (whole genome and hORFeome). The number of bars shown is less than the total number of common enriched categories because bars represent only the terminal GO terms. The length of the bar is the standardized enrichment score. (B) Graphic representation of an ataxia subnetwork. Several interactors of ATXN1 appeared to be genetic modifiers as well. Nodes in yellow represent interacting proteins, hypothesized ataxia-related proteins are in blue, and putative genetic modifiers of SCA1 pathology are in orange. Squares *and* circles *indicate two-hybrid baits or prey, respectively. Edge colors represent the different hypothetical interactions: green (from this study) and blue literature curated interactions (LCI). (Reprinted from Lim and colleagues,[49] by permission.)*

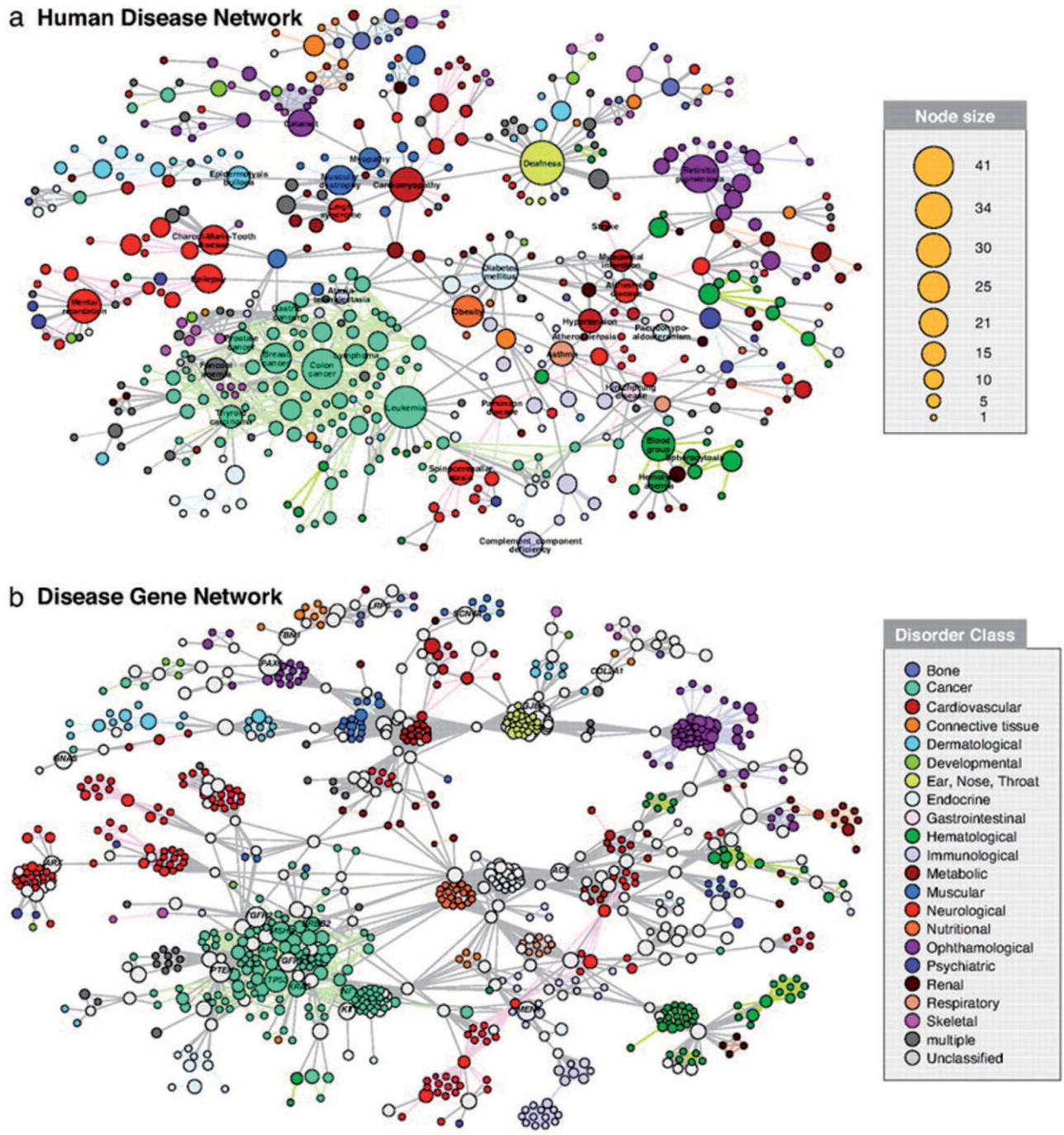

Fig 3. Human disease network. By relating diseases with gene mutations available at OMIM, Goh and researchers[47] built a network in which diseases were grouped based on their genetic commonalities. (A) The human disease network: Each node corresponds to a specific disorder colored by class (22 classes). The size of each node is proportional to the number of genes thought to contribute to the disorder. Edges between disorders in the same disorder class are colored with the same (lighter) color, and edges connecting different disorder classes are colored gray, with the thickness of the edge proportional to the number of genes shared by the disorders connected by it. (B) The disease gene network: Each node is a single gene, and any two genes are connected if implicated in the same disorder. In this network map, the size of each node is proportional to the number of specific disorders in which the gene has been implicated. The resulting network challenges current disease classifications based on phenotypes (syndromes), with implications for diagnosis and therapeutics. (Reprinted from Goh and researchers,[47] by permission.)

clinical course (self-limiting, relapsing remitting, cyclic, or chronic progressive). Recognition of the critical value of time and temporal rhythms, combined with the knowledge that particular temporal rhythms respond best to certain treatment strategies, often provides a basis for therapy. Chronic diseases develop over variable but extended periods until a phenotype manifests. This time dependency suggests that the normal physiological processes are robust but subject to failure, possibly because of a number of mechanisms (eg, redundancy, network topology). Only the prolonged exposure to pathological insults is capable of shifting the system to another alternative robust, but unfortunately pathological state. Under this scenario, complex diseases arise because of abnormalities in the underlying physiological control mechanisms, more so than in any perturbation of one or a limited number of specific molecular events.[10,54–57] Thus, development of therapeutic strategies aimed at reestablishing the altered dynamics may be another rational approach toward cures of diseases.[58]

The concept of dynamic diseases is deeply rooted in neurology.[58,59] The first descriptions of dynamic diseases came from the phenomenology of seizures, paroxysmal movement disorders, and psychiatric diseases such as bipolar disorders.[60–63] A classic example is the development of seizures, which can be identified by changes in the normal dynamics of the electroencephalogram (EEG). Epileptic foci, which can be generated by many different kinds of neuronal insults, generate a new rhythm that spreads through brain networks, creating a pathological brain dynamic.[60] Movement disorders, such as tremor or gait abnormalities in PD or Huntington disease, are also examples of alterations in the dynamics of physiological systems.[61] The motor symptoms of PD have been associated with increased activity of the subthalamic nucleus, resulting in excessive inhibitory outflow from the basal ganglia to the thalamus and brainstem.[64] High-frequency deep brain stimulation modulates the activity of the subthalamic nucleus and restores normal dynamics of the motor circuit.[65] Dopaminergic therapy also suppresses the activity of the subthalamic nucleus, modulating the dynamics of the corticosubcortical system.[66]

The longitudinal analysis of the cellular network in the basal ganglia in patients with PD using positron emission tomography and analyzing the spatial covariance in the data has identified a motor pattern characterized by increased pallidothalamic and pontine metabolic activity. This increased activity is associated with reduction of premotor and posterior parietal cortical regions (Fig 4).[67] Disease progression was associated with increasing metabolism in the subthalamic nucleus, internal globus pallidus, dorsal pons, and primary motor cortex.[68] This analysis demonstrated that cognitive impairment in PD patients was associated with a different cellular network, with reduced metabolic activity in the prefrontal and parietal cortices, and relative increases in the dentate nuclei and cerebellar hemispheres.[69] Assessment of the effect of deep brain stimulation and dopamine therapy in the dynamics of the basal ganglia network[70] demonstrated that both therapies produced metabolic reductions in the precise areas that are chronically stimulated in PD by the subthalamic nucleus. These target areas for the subthalamic nucleus included the putamen-globus pallidus, cerebellar vermis, and parietal cortex. Although both approaches showed different effectiveness in individual regions, the overall activity of the network was similar, and both approaches correlated well with clinical improvement. Thus, such studies highlight the critical role of brain dynamics in the development of neurological diseases, which would not be unraveled through dissection of the different components, and how such dynamics may be rationally targeted through therapy.

Several methods allow for the monitoring of brain dynamics, including EEG, positron emission tomography, and functional magnetic resonance imaging. These are examples of descriptive models whereby particular aspects of brain function can be measured. Pattern recognition methods are then applied to reconcile the obtained data, and the inductive model investigators then have insight about how that part of the brain works. Methods such as nonlinear dynamic analysis, computational models, or network analysis can be useful for achieving such reconciliation between data and model. For example, although slow periodic EEG discharges are common in encephalopathies, encephalitis, and tumors, their underlying pathogenesis remains unknown, thus preventing the development of new therapies. Frohlich and colleagues[71] performed a dynamic analysis of a computational model of the human cortex to better understand the generation of slow periodic EEG discharges. In particular, they examined the deafferentation caused by neuronal loss. To simulate the deafferentation process, they used data from Creutzfeldt–Jacob disease and tested different degrees of deafferentation for modeling different degrees of neuronal loss and disease severity. They found that different degrees of deafferentation influence the neural network dynamics, with a critical degree (80%) of deafferentation after which a new periodic dynamic of slow waves emerge (Fig 5). This resulted in poor information transmission through the neuronal network. These unexpected results are a paradoxical effect of brain plasticity. Thus, this modeling approach benefits the understanding of encephalopathies because it provides information about how much neural networks can cope with brain damage and why this new slow dynamics emerge. Moreover, such findings may lead to the development of new therapies for preventing sec-

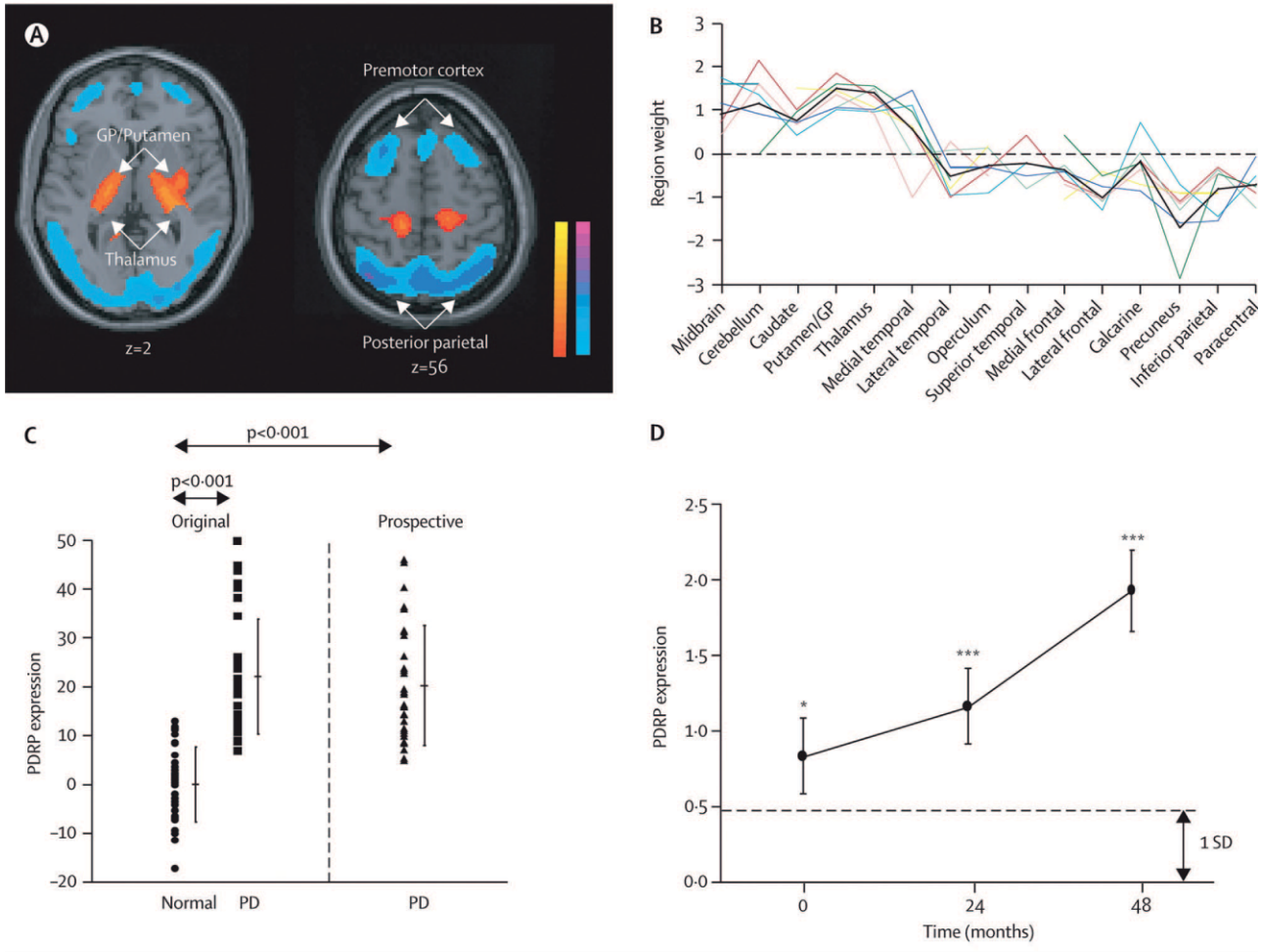

Fig 4. Parkinson's disease (PD)–related spatial covariance pattern. (A) Parkinson's disease–related spatial covariance pattern (PDRP) identified by network analysis of 18F-fluorodeoxyglucose (FDG) positron emission tomographic scans. This spatial covariance pattern was characterized by relative metabolic increases in the putamen, globus pallidus (GP), thalamus, pons, and cerebellum, and was associated with decreases in the premotor and posterior parietal areas. The display shows voxels that contribute significantly to the network (voxels with positive region weights [metabolic increases] are color coded from red to yellow; voxels with negative region weights [metabolic decreases] are color coded from blue to purple). (B) Region weights on PDRPs seen in the rest state. The patterns were characterized by substantial contributions from the putamen or GP, thalamus, and cerebellum (metabolic increases), and from the lateral premotor and parietal association regions (metabolic decreases). Black line *indicates population-averaged regional loadings across centers. (C) Network activity was increased in PD. (D) Mean PDRP activity in patients with early-stage PD followed up longitudinally at baseline, 24 months, and 48 months. Network activity increased over time. PDRP expression in the patient group was significantly increased at all three time points relative to values for healthy control subjects.* Dashed line *is one standard deviation (SD) above the normal mean.* Bars *show the standard error for the PD patient group at each time point.* Asterisks *show the significance of comparisons with control values at each time point. (Reprinted from Eckert and colleagues,[67] by permission.)*

ondary damage caused by brain plasticity in neurological diseases.

It is well known that the brain is organized in dynamic neuronal networks of excitatory (glutamatergic) and inhibitory (GABAergic) connections. The precise activity of neuronal networks is modulated by several regulatory pathways, such as dopaminergic, cholinergic, serotoninergic, or histaminergic circuits, collectively known as the neuromodulatory system.[72–74] Unlike epilepsy, in which excitatory-inhibitory networks are the main pathways affected, most neurodegenerative diseases show disruption of the neuromodulatory system (dopaminergic system in PD, cholinergic system in AD). This provides another example of emergent network properties. In this case, although the loss of a significant but random number of neurons throughout the brain may not have a tangible clinical impact, when an insult targets a critical part of the network such as the dopaminergic system in PD, it starts a cascade of failures that ultimately culminates in progressive neurological symp-

toms. Damage of this neuromodulatory system also illustrates the concept of dynamic diseases, because dopaminergic neuronal loss alters the dynamics of basal ganglia cortical circuits as described earlier, ultimately leading to clinical symptoms.

Pathway-Centered Analysis of Neurological Diseases

Pathway analysis is becoming a valuable tool for improving our understanding of molecular data generated from human studies. The studies of chemical pathways can reconcile many studies, providing valuable data but not confirming one to the other because pathway analysis is focused in the outcome of the system. By this way, the study of pathways buffers the heterogeneity because individual patients bear mutations or damage in different molecules of the same pathway leading to the same outcome (ie, protein misfolding in neurodegenerative diseases). For example, studies of hereditary PD have identified six genes involved in its pathogenesis: *SNCA* (α-synuclein), *PARK2* (parkin), *DJ-1, UCHL1, PINK1,* and *LARK2* (dardarin).[75,76] The clinical and pathological heterogeneity of individuals with such mutations has prompted researchers to redefine PD and its nosology. The functions of genes identified in hereditary PD involve lipid and vesicle dynamics (α-synuclein), the ubiquitin-proteasome system (parkin and UCHL1), mitogen-activated protein kinase kinase kinase (MAPKKK) signaling leucine-rich repeat kinase 2 (LRRK2), oxidative stress and mitochondrial function (DJ-1, PINK1, parkin), and microtubule stability. However, the lack of a comprehensive view of how these pathways interact to cause brain damage has hampered our understanding of the pathogenesis of PD.[76]

One strategy involves the use of high-throughput approaches such as large-scale DNA microarrays and proteomics in pathological specimens. The confluence of such studies demonstrates the remarkable interplay of genes regulating common pathways in a disease, a concept now termed *genomic convergence.*[77,78] Using this approach, Hauser and investigators[77] were able to map genes differentially expressed in the substantia nigra of control subjects and patients suffering from PD to five genomic loci, and they identified the mitochondrial pathways as the main candidates for the susceptibility for PD. Another example of pathway analysis and genomic convergence in PD is the integration of genetic and gene expression studies to support the involvement of the axon-guidance pathways (ephrins, netrins, semaphorins, slits and their receptors, and intermediate proteins).[79,80] The study of PD and other degenerative diseases using pathway analysis can help to integrate the biological data in functional models (pathways), coping with disease heterogeneity and providing therapeutic targets for treating such disease. In the aforementioned example of axon-guidance pathways, their involvement contributes to improving our understanding of the pathogenesis of PD with another biological function (in addition of dopamine dysfunction and oxidative stress) not previously recognized and because it provides new targets for treating PD.

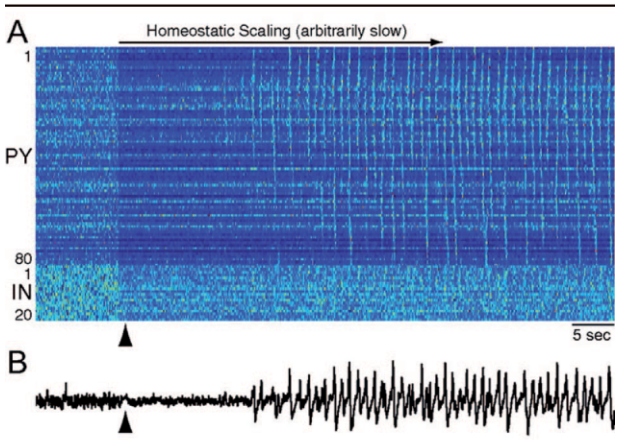

Fig 5. Computational analysis of periodic slow-wave patterns in neurological diseases. (A) Activity map of pyramidal neurons (PYs; top) and interneurons (INs; bottom). Cool and hot colors indicate hyperpolarization and depolarization, respectively. Severe deafferentation (90%) induced consecutive decline in network activity (arrowhead). *Recovery of target firing rate by homeostatic scaling resulted in prominent periodic network activation. (B) Simulated local field potentials (lfp): Before deafferentation, lfp have high-frequency activity with low amplitude. After deafferentation* (arrowhead), *the recovery of activity level is characterized by slow high-amplitude lfp oscillations. (Reprinted from Frohlich and colleagues,[71] by permission.)*

Guiding Drug Discovery and Personalized Therapy

Neurology is now entering a new therapeutic age. Instead of symptomatic therapies, neurologists are developing therapies that modify disease pathogenesis. This will impose several challenges because of the critical importance of neural dynamics in the generation of neurological symptoms. For example, full blockade of lymphocyte homing to the central nervous system in MS led to the undesired development of progressive multifocal encephalopathy in patients treated with natalizumab.[81] Similarly, vaccination of AD patients with β amyloid led to the development of autoimmune encephalitis in 10% of the patients in a clinical trial.[82] Targeting molecules without a thorough understanding of the integrated mechanisms in which they participate often leads to unexpected and sometimes catastrophic adverse effects.[83]

The approach taken by systems biology involves a different paradigm based on improved knowledge of dynamic mechanisms.[20,84,85] For example, the in vivo

transcriptional response to interferon-β was recently studied using a network-based conceptual analysis.[86] This study demonstrated that, in addition to reducing T-cell adhesion, interferon-β represses RNA transcription and protein synthesis, and induces apoptosis in these cells. These findings suggest that the most widely used therapy to control exacerbations in MS may achieve its benefits by simultaneously targeting components of interacting and intertwined mechanisms.

By targeting key steps in the dynamics of a disease at different time points and targeting different regions or modules of the molecular or cellular mechanisms governing the pathogenesis of the disease, better regulation of the system under study can be achieved. For example, a recent analysis of the gene expression network that controls T-cell activation in patients with MS allowed identification of Jagged-1 as a new therapeutic target.[85] The reductionist approach would have prevented such discovery, because levels of Jagged-1 did not differ between patients and control subjects, but network analysis identified the critical role of this gene in the set of interactions regulating T-cell differentiation in MS patients. Moreover, in vitro assays and network analysis identified a synergistic effect between Jagged-1 and interferon-β, indicating that systems drug discovery may improve our ability to identify new therapies for neurological diseases.

Computational modeling can be helpful in the design of the most adequate strategies in this multitarget and multistep process, as well as in the prediction of some unexpected side effects.[87–89] For example, mathematical modeling of the multifactorial events in brain damage may allow the identification of optimal therapeutic strategies, as recently demonstrated in spinal cord injury.[90] Indeed, the integrative approach of systems biology may speed up and reduce the costs of the current drug discovery process by testing combination therapy, and identifying the best targets and dynamics to be modified using computational models and validated cell assays.[84,91] A crucial concept is that the suitability of a given protein as therapeutic target is determined by the nature of its contribution to the network's control mechanisms (pathway drug target) rather than by its aberrant activity or expression (protein drug target).[83]

These approaches can also be helpful for the identification of new biomarkers,[92] a critical step in the process of developing stratified and personalized medicine.[93] For example, network analysis of prostatic cancer identified the androgen receptor as a novel pathway and a genetic mediator of metastasis, suggesting that targeting of this pathway may rescue patients escaping androgen dependence.[94] Also, the analysis of serum proteome from patients with AD demonstrated the presence of abnormalities in the blood, indicating a deregulation of hematopoiesis, immune responses, apoptosis, and neuronal support.[95] Proteomic studies of MS lesions have demonstrated numerous unexpected pathways in various pathological stages of disease.[34] For example, elements of the coagulation cascade, including tissue factor and the inhibitor of protein C, were shown to be critical in active and chronic active MS lesions. In models of MS, paralysis could be reversed with activated protein C, an approved drug for treatment of septic shock, and with thrombin inhibitors used as approved anticoagulants.[34] Finally, a recent report suggested that transcriptional profiling of CD4 T cells in clinically isolated syndrome patients accurately predicts their conversion to clinically definite MS.[96] Together, these findings suggest that genetic and biochemical abnormalities involved in the pathogenesis of neurological diseases may affect other systems without inducing pathological effects.

Concluding Remarks

It is imperative that any scientific research, especially if it requires an interdisciplinary approach, be grounded in the philosophy of science and furnished with the logical tools that permit the translation of empirical data into useful knowledge and worthwhile means of advancement.[97] The knowledge generated in the past few decades on the pathogenesis of neurological diseases, as well as the development of high-throughput methods of analysis, biotechnology, and computational biology, has provided unprecedented opportunities for developing new disease-modifying therapies. We argue that the integration of this knowledge into the theoretical framework and the tools provided by systems biology will be an invaluable help in this process. However, therapeutic decisions made by neurologists as we treat patients will always be based on the expertise of the physician and on evidence-based medical practice. The genomic portrait of an individual may allow a predictive and personalized approach to therapy. In the near future, this process will be aided by powerful computers, with information technologies that will manage the available information from the patient's tests, the patient's medical history and genetic history, and ever-evolving scientific databases.[21] Although systems biology offers a new framework for the study of human disease, better characterization of biological processes, quantitative data, and dynamic information are still required to successfully translate this paradigm from the bench/chip to the bedside. However, we find ourselves at an exciting turning point in the history of biomedical research, where integrated methods for the analysis of complex diseases allowing the conversion of information into effective knowledge are finally within our grasp.

Note Added in Proof

Another recent review regarding neurodegenerative diseases was recently released: Deciphering complex mechanisms in neurodegenerative diseases: the advent of systems biology. Noorbakhsh F, Overall CM, Power C. Trends Neurosci 2009 Jan 8.

This work was supported by the Pathfinder Initiative on Complexity for Living Science of the European Union (ComplexDis [NEST-043241], P.V.).

Acknowledgment

We also thank C. Anthony Hunt for critical reading of this manuscript. Pathway examples for each motif were obtained from Ravy Lyengar Lab Web site at Mount Sinai (http://www.mssm.edu/labs/iyengar/resources) and from Richard Young Lab at Massachusetts Institute of Technology (http://jura.wi.mit.edu/cgi-bin/young_public/

References

1. Ideker T, Galitski T, Hood L. A new approach to decoding life: systems biology. Annu Rev Genomics Hum Genet 2001;2: 343–372.
2. Kitano H. Systems biology: a brief overview. Science 2002;295: 1662–1664.
3. Rosenblueth A, Wiener N. The role of models in science. Philos Sci 1945;12:316–321.
4. Kola I, Landis J. Can the pharmaceutical industry reduce attrition rates? Nat Rev Drug Discov 2004;3:711–715.
5. Baranzini SE. Systems-based medicine approaches to understand and treat complex diseases: the example of multiple sclerosis. Autoimmunity 2006;39:651–662.
6. Germain RN. The art of the probable: system control in the adaptive immune system. Science 2001;293:240–245.
7. Watts DJ, Strogatz SH. Collective dynamics of 'small-world' networks. Nature 1998;393:440–442.
8. Barabasi AL, Oltvai ZN. Network biology: understanding the cell's functional organization. Nat Rev Genet 2004;5:101–113.
9. Bertalanffy LV. General system theory: foundations, development, applications. New York: George Braziller, 1976.
10. Glass L. Synchronization and rhythmic processes in physiology. Nature 2001;410:277–284.
11. Kitano H. Computational systems biology. Nature 2002;420: 206–210.
12. Joyce AR, Palsson BO. The model organism as a system: integrating 'omics' data sets. Nat Rev Mol Cell Biol 2006;7: 198–210.
13. Sauer U, Heinemann M, Zamboni N. Genetics. Getting closer to the whole picture. Science 2007;316:550–551.
14. Wagner A, Fell DA. The small world inside large metabolic networks. Proc Biol Sci 2001;268:1803–1810.
15. Wagner A. Robustness and evolvability in living systems. Princeton, NJ: Princeton University Press, 2005.
16. Kitano H. Biological robustness. Nat Rev Genet 2004;5: 826–837.
17. Stelling J, Sauer U, Szallasi Z, et al. Robustness of cellular functions. Cell 2004;118:675–685.
18. Wiener N. Cybernetics. New York: Wiley, 1948.
19. Cannon W. Organization for physiological homeostasis. Physiol Rev 1929;9:397.
20. Kitano H. A robustness-based approach to systems-oriented drug design. Nat Rev Drug Discov 2007;6:202–210.
21. Villoslada P, Oksenberg J. Neuroinformatics in clinical practice: are computers going to help neurological patients and their physicians? Future Neurol 2006;1:1–12.
22. Glimcher P. Decisions, uncertainty, and the brain: the science of neuroeconomics. Cambridge, MA: MIT Press, 2004.
23. De Schutter E. Why are computational neuroscience and systems biology so separate? PLoS Comput Biol 2008;4:e1000078.
24. Mattson MP. Pathways towards and away from Alzheimer's disease. Nature 2004;430:631–639.
25. Dragunow M. The adult human brain in preclinical drug development. Nat Rev Drug Discov 2008;7:659–666.
26. Stokin GB, Lillo C, Falzone TL, et al. Axonopathy and transport deficits early in the pathogenesis of Alzheimer's disease. Science 2005;307:1282–1288.
27. Adalbert R, Gilley J, Coleman MP. Abeta, tau and ApoE4 in Alzheimer's disease: the axonal connection. Trends Mol Med 2007;13:135–142.
28. Bartzokis G, Sultzer D, Lu PH, et al. Heterogeneous age-related breakdown of white matter structural integrity: implications for cortical "disconnection" in aging and Alzheimer's disease. Neurobiol Aging 2004;25:843–851.
29. Wyss-Coray T. Inflammation in Alzheimer disease: driving force, bystander or beneficial response? Nat Med 2006;12: 1005–1015.
30. Zlokovic BV. Neurovascular mechanisms of Alzheimer's neurodegeneration. Trends Neurosci 2005;28:202–208.
31. Miller JA, Oldham MC, Geschwind DH. A systems level analysis of transcriptional changes in Alzheimer's disease and normal aging. J Neurosci 2008;28:1410–1420.
32. Palop JJ, Chin J, Mucke L. A network dysfunction perspective on neurodegenerative diseases. Nature 2006;443:768–773.
33. Hauser SL, Oksenberg JR. The neurobiology of multiple sclerosis: genes, inflammation, and neurodegeneration. Neuron 2006;52:61–76.
34. Han MH, Hwang S, Roy DB, et al. Proteomic analysis of active multiple sclerosis lesions reveals therapeutic targets in the coagulation cascade. Nature 2007;451:1076–1081.
35. Villoslada P, Genain CP. Role of nerve growth factor and other trophic factors in brain inflammation. Prog Brain Res 2004; 146:403–414.
36. Lock C, Hermans G, Pedotti R, et al. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. Nat Med 2002;8:500–508.
37. Myerowitz R, Lawson D, Mizukami H, et al. Molecular pathophysiology in Tay-Sachs and Sandhoff diseases as revealed by gene expression profiling. Hum Mol Genet 2002;11: 1343–1350.
38. Myerowitz R, Mizukami H, Richardson KL, et al. Global gene expression in a type 2 Gaucher disease brain. Mol Genet Metab 2004;83:288–296.
39. Yamaguchi A, Katsuyama K, Nagahama K, et al. Possible role of autoantibodies in the pathophysiology of GM2 gangliosidoses. J Clin Invest 2004;113:200–208.
40. Qi Y, Ge H. Modularity and dynamics of cellular networks. PLoS Comput Biol 2006;2:1502–1510.
41. Sauro HM. Modularity defined. Mol Syst Biol 2008;4: 166–167.
42. Ma'ayan A, Jenkins SL, Neves S, et al. Formation of regulatory patterns during signal propagation in a mammalian cellular network. Science 2005;309:1078–1083.

43. Alon U. Network motifs: theory and experimental approaches. Nat Rev Genet 2007;8:450–461.
44. Losick R, Desplan C. Stochasticity and cell fate science. 2008; 320:65–68.
45. Smolen P, Baxter DA, Byrne JH. Mathematical modeling of gene networks. Neuron 2000;26:567–580.
46. Goni J, Esteban FJ, Velez N, et al. A computational analysis of protein-protein interaction networks in neurodegenerative diseases. BMC Syst Biol 2008;2:52.
47. Goh KI, Cusick ME, Valle D, et al. The human disease network. Proc Natl Acad Sci USA 2007;104:8685–8690.
48. Lu X, Jain VV, Finn PW, Perkins DL. Hubs in biological interaction networks exhibit low changes in expression in experimental asthma. Mol Syst Biol 2007;3:98.
49. Lim J, Hao T, Shaw C, et al. A protein-protein interaction network for human inherited ataxias and disorders of Purkinje cell degeneration. Cell 2006;125:801–814.
50. Campillos M, Kuhn M, Gavin AC, et al. Drug target identification using side-effect similarity. Science 2008;321:263–266.
51. Ishikawa K, Toru S, Tsunemi T, et al. An autosomal dominant cerebellar ataxia linked to chromosome 16q22.1 is associated with a single-nucleotide substitution in the 5′ untranslated region of the gene encoding a protein with spectrin repeat and Rho guanine-nucleotide exchange-factor domains. Am J Hum Genet 2005;77:280–296.
52. Loscalzo J, Kohane I, Barabasi AL. Human disease classification in the postgenomic era: a complex systems approach to human pathobiology. Mol Syst Biol 2007;3:124.
53. Hunt CA, Baranzini SE, Matthay MA, Park S. A framework and mechanistically focused, in silico method for enabling rational translational research. American Medical Informatics Association (AMIA) Summit on Translational Bioinformatics; 2008; San Francisco, CA.
54. Mackey MC, Glass L. Oscillation and chaos in physiological control systems. Science 1977;197:287–289.
55. Varela F, Lachaux JP, Rodriguez E, Martinerie J. The brainweb: phase synchronization and large-scale integration. Nat Rev Neurosci 2001;2:229–239.
56. McCormick DA. Are thalamocortical rhythms the Rosetta Stone of a subset of neurological disorders? Nat Med 1999;5: 1349–1351.
57. Bar-Yam Y. Dynamics of complex systems. Reading, MA: Addison-Wesley, 1997.
58. Milton J, Black D. Dynamic diseases in neurology and psychiatry. Chaos 1995;5:8–13.
59. Milton JG, Longtin A, Beuter A, et al. Complex dynamics and bifurcations in neurology. J Theor Biol 1989;138:129–147.
60. Lopes da Silva F, Blanes W, Kalitzin SN, et al. Epilepsies as dynamical diseases of brain systems: basic models of the transition between normal and epileptic activity. Epilepsia 2003; 44(suppl 12):72–83.
61. Hausdorff JM, Cudkowicz ME, Firtion R, et al. Gait variability and basal ganglia disorders: stride-to-stride variations of gait cycle timing in Parkinson's disease and Huntington's disease. Mov Disord 1998;13:428–437.
62. Llinas RR, Ribary U, Jeanmonod D, et al. Thalamocortical dysrhythmia: a neurological and neuropsychiatric syndrome characterized by magnetoencephalography. Proc Natl Acad Sci USA 1999;96:15222–15227.
63. Pezard L, Nandrino JL, Renault B, et al. Depression as a dynamical disease. Biol Psychiatry 1996;39:991–999.
64. Vitek JL, Giroux M. Physiology of hypokinetic and hyperkinetic movement disorders: model for dyskinesia. Ann Neurol 2000;47:S131–S140.
65. Olanow CW, Brin MF, Obeso JA. The role of deep brain stimulation as a surgical treatment for Parkinson's disease. Neurology 2000;55:S60–S66.
66. Schapira AH, Bezard E, Brotchie J, et al. Novel pharmacological targets for the treatment of Parkinson's disease. Nat Rev Drug Discov 2006;5:845–854.
67. Eckert T, Tang C, Eidelberg D. Assessment of the progression of Parkinson's disease: a metabolic network approach. Lancet Neurol 2007;6:926–932.
68. Huang C, Tang C, Feigin A, et al. Changes in network activity with the progression of Parkinson's disease. Brain 2007;130: 1834–1846.
69. Huang C, Mattis P, Tang C, et al. Metabolic brain networks associated with cognitive function in Parkinson's disease. Neuroimage 2007;34:714–723.
70. Asanuma K, Tang C, Ma Y, et al. Network modulation in the treatment of Parkinson's disease. Brain 2006;129:2667–2678.
71. Frohlich F, Bazhenov M, Sejnowski TJ. Pathological effect of homeostatic synaptic scaling on network dynamics in diseases of the cortex. J Neurosci 2008;28:1709–1720.
72. Yu AJ, Dayan P. Uncertainty, neuromodulation, and attention. Neuron 2005;46:681–692.
73. Hasselmo ME. Neuromodulation: acetylcholine and memory consolidation. Trends Cogn Sci 1999;3:351–359.
74. Hasselmo ME, Giocomo LM. Cholinergic modulation of cortical function. J Mol Neurosci 2006;30:133–135.
75. Hardy J, Cai H, Cookson MR, et al. Genetics of Parkinson's disease and parkinsonism. Ann Neurol 2006;60:389–398.
76. Farrer MJ. Genetics of Parkinson disease: paradigm shifts and future prospects. Nat Rev Genet 2006;7:306–318.
77. Hauser MA, Li YJ, Takeuchi S, et al. Genomic convergence: identifying candidate genes for Parkinson's disease by combining serial analysis of gene expression and genetic linkage. Hum Mol Genet 2003;12:671–677.
78. Noureddine MA, Li YJ, van der Walt JM, et al. Genomic convergence to identify candidate genes for Parkinson disease: SAGE analysis of the substantia nigra. Mov Disord 2005;20:1299–1309.
79. Lesnick TG, Papapetropoulos S, Mash DC, et al. A genomic pathway approach to a complex disease: axon guidance and Parkinson disease. PLoS Genet 2007;3:e98.
80. Lesnick TG, Sorenson EJ, Ahlskog JE, et al. Beyond Parkinson disease: amyotrophic lateral sclerosis and the axon guidance pathway. PLoS ONE 2008;3:e1449.
81. Khalili K, White MK, Lublin F, et al. Reactivation of JC virus and development of PML in patients with multiple sclerosis. Neurology 2007;68:985–990.
82. Gelinas DS, DaSilva K, Fenili D, et al. Immunotherapy for Alzheimer's disease. Proc Natl Acad Sci USA 2004;101(suppl 2):14657–14662.
83. Araujo RP, Liotta LA, Petricoin EF. Proteins, drug targets and the mechanisms they control: the simple truth about complex networks. Nat Rev Drug Discov 2007;6:871–880.
84. Butcher EC. Can cell systems biology rescue drug discovery? Nat Rev Drug Discov 2005;4:461–467.
85. Palacios R, Goni J, Martinez-Forero I, et al. A network analysis of the human T-cell activation gene network identifies JAGGED1 as a therapeutic target for autoimmune diseases. PLoS ONE 2007;2:e1222.
86. Fernald GH, Knott S, Pachner A, et al. Genome-wide network analysis reveals the global properties of IFN-beta immediate transcriptional effects in humans. J Immunol 2007;178:5076–5085.
87. Materi W, Wishart DS. Computational systems biology in drug discovery and development: methods and applications. Drug Discov Today 2007;12:295–303.
88. Aradi I, Erdi P. Computational neuropharmacology: dynamical approaches in drug discovery. Trends Pharmacol Sci 2006;27: 240–243.
89. Baranzini SE, Bernard CC, Oksenberg JR. Modular transcriptional activity characterizes the initiation and progression of autoimmune encephalomyelitis. J Immunol 2005;174:7412–7422.

90. Woolfe F, Waxman SG, Hains BC. In silico modeling of axonal reconnection within a discrete fiber tract after spinal cord injury. J Neurotrauma 2007;24:421–432.
91. Yildirim MA, Goh KI, Cusick ME, et al. Drug-target network. Nat Biotechnol 2007;25:1119–1126.
92. Danna EA, Nolan GP. Transcending the biomarker mindset: deciphering disease mechanisms at the single cell level. Curr Opin Chem Biol 2006;10:20–27.
93. Nicholson JK. Global systems biology, personalized medicine and molecular epidemiology. Mol Syst Biol 2006;2:52.
94. Ergun A, Lawrence CA, Kohanski MA, et al. A network biology approach to prostate cancer. Mol Syst Biol 2007;3:82.
95. Ray S, Britschgi M, Herbert C, et al. Classification and prediction of clinical Alzheimer's diagnosis based on plasma signaling proteins. Nat Med 2007;13:1359–1362.
96. Corvol JC, Pelletier D, Henry RG, et al. Abrogation of T cell quiescence characterizes patients at high risk for multiple sclerosis after the initial neurological event. Proc Natl Acad Sci USA 2008;105:11839–11844.
97. Fogel LJ. Biotechnology: concepts and applications. Englewood Cliffs, NJ: Prentice-Hall, 1963.