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co-exist, and maintain a dynamic micro-ecosystem in intestinal canal.

These microorganisms interact with their host through enzyme facilitated metabolism and co-metabolism, maintain a tight relationship between interaction and equilibrium. The accumulation, consequence, and toxicity of the drug in the host intestine, are influenced by these microorganisms. So, it is recognized that the activities of these microorganisms will be helpful for human health. The unbalance of these activities can direct to human diseases, and influence tissue regeneration and reparation. So, some researchers listed micro-ecological balancing in the contents of Regenerative Medicine.

Perspectives

Tissue engineering progressed rapidly in this short 20 years since its emergence on stage. Its research scope and contents extended greatly. Currently, many of regenerative tissues, such as artificial skin and soft bone that already came into market, can be embedded on big animals. It is not exaggerative to say that in near future there will be much more tissue engineering products coming into market.

However, it is very difficult to construct organs with ordinary physiological functions, especially for those important living organs. The capability to form a complicated functional organic organ is even unknown. So, the so-called "Organ Oriented Epoch" is meaningless till now. Regenerative Medicine and Substitution Surgery will still exist on the stage and complement each other for a long time.

The relationship between stem cell and cancer is as follows: cancer cell is similar to stem cell in the rapid proliferation and infection. If cancer was produced by irregular stem cell, the research in the traits of stem cell could be very helpful to us to discover and effectively cut tumor in advance.

Regenerative Medicine still faces ethic issues. The barriers where can be broken through, and where cannot are still under careful consideration and solved gradually in the future.

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The Current State and Perspectives of Systems Biology

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Abstract: Emerging as a new field in biology recently, Systems Biology provides a branch new way to study the biological activities in organisms. In order to decode the complexity of life systematically, systems biology integrates the "-omics" and uses the high throughput methods from transcriptomics, protomics and metabonomics to detect the dynamic activities in cell; and then, it incorporates bioinformatics methods to integrate and analyze those data, and simulate the biological processes based on the model built from those integrated data. In this paper, the current state, the research field and the methods for the Systems Biology are introduced briefly, and then, several ideas about future development in this field are also proposed.

Keywords: systems biology, biology system modeling, high throughput, gene regulatory network, and protein-protein interaction network

In the 20th century, the research methods used in biology were mostly reductionistic approach. However, this thinking pattern is hardly utilized to effectively and

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systematically study biological activities in organisms even it was successfully used in the discovery of many life experiences as well as in the improvement of the recognition to life activities. Accompanied with "Post-genome era", based on the gradually generated huge bio-informatics data, the improved high throughput bio-analytical equipments as mass-spectrometer, gene-chips, and methods of mass collection, integration, and mining technology for these bioinformatics data, the systematical study in life activities becomes possible. Systems Biology, derived from the cross-disciplinary area of bioinformatics and computational biology, targeting on systems and interrelationship between systems, becomes today's new spot in biological research.

The concept of Systems Biology provides a new direction for the research in cell and physiological processes. Different from traditional methods focusing on a single gene and its encoded protein, Systems Biology views biology through integrative way. The major point of Systems Biology is that the subsystems in an organism are inner-related. This further leads to the exploration of new methods to describe the interactions between these sub-systems. In general, Systems Biology emphasizes on systematical research, integration and interaction. Systems Biology analyzes complicated biosignal interactions between multiple layers from genome DNA, mRNA, proteins, metabolism and signal pathways, as well as gene regulatory networks and protein-protein interaction networks. Based on this, we can understand the cooperated activities within organism, and clarify reasons to cause diseases and functions of medicines. Therefore, if the achievement in biology in last century is described as "reductionistic approach", then, we can say that today's Systems Biology is an "integrative approach", which turns narrative biology into measurable and predictable biology.

Major objectives of Systems Biology

Systems Biology studies the following topics in different levels [1]:

1. System structure: gene regulatory and biochemical networks, as well as physical structures.

2. System behavior: qualitatively and quantitatively study the system dynamics; establish theoretical models to predict system behavior.

3. System regulation: study the mechanisms that how a system can regulate cell states.

4. System design: design, improve and reconstruct biosystem based on clarified theories.

Contents of Systems Biology

The research exploration of Systems Biology is on the frontier of contemporary life science. Currently, It includes the following topics: signal transduction modeling, genetic regulatory network, metabolic pathway, protein-protein interaction, biomolecular marker discovery, and drug screening as well as drug efficacy modeling.

1. Signal transduction

The process of signal transduction controls the survival and apoptosis of cell. The expectation of Systems Biology in this area is that, through the establishment of the model for the process of signal transduction, we can find the protein-protein interaction network for the process, and explain its effect on gene regulation and disease occurrence (i.e., the tumor). And then, it is further expected to provide valuable information for the medical formula of curable medicines. Recently, the quantitative analysis on signal transduction pathway warmed up gradually. To setup a mathematical model to implement this quantitative analysis becomes the hot spot. Currently, researchers mostly used continuous system dynamics to study the problems related to this field. Up to now, the mechanisms of several signal transduction pathways were studied: phosphoinositide-calcium signaling pathway [2], mitogen activated protein kinase system (MAPK) [3], JAK-STAT system [4], as well as ion channel and receptor systems [5].

2. Gene regulation

The research for gene regulatory networks is to construct gene regulatory network prototype in the whole genome scope with methods of bioinformatics and computational biology by integrating mass gene expression profile data generated by high throughput technologies, e.g. microarray, interaction information between protein and

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DNA, and results from other experiments.

The interactions between transcription factors and target genes can be expressed by a directed graph, on which the nodes represent transcription factors as well as target genes, the edges represent their regulatory relationships. The final regulatory network should be a very complicated multiple layered system. Currently, the methods for the research in gene regulatory network are generally categorized in: clustering methods, expression profile and ChIP-chip, expression profile with transcription start site analysis, as well as machine learning.

Clustering analysis [6] is based on a hypothesis that genes with similar expression profile can be regulated by the same transcription regulatory mechanism (can be controlled by the same transcription factors with same binding site), and possibly have same biological functions. The limitation of these traditional clustering methods is that they only consider gene expression similarity under all the conditions, in fact most of the genes can only express in certain specific conditions. So, in order to break through this confine, a series of new computation methods were introduced in clustering analysis [7]. These clustering methods treated both columns and rows in the gene expression matrix, hence are called "bi-clustering".

ChIP-chip method [8] is to combine the co-immunoprecipitation of an antibody for a specific transcription factor and microarray experiment to identify the DNA fragments bound by the transcription factor. Even though ChIP-chip data can provide direct evidence of interactions between transcription factor and the promoter region of its target genes, they cannot prove the binding effect of the transcription factor to its targets, nor tell whether the regulation of the transcription factor to its target gene is positive, negative or neutral. The analysis method based on expression profile and promoter sequence [9] is to recognize functional motifs through a statistical definition on the cooperation of motifs: if the expression coherence score of genes containing both motifs in their promoters is significantly greater than that of genes containing either motif alone, then the pair of motifs is considered 'synergistic'. The methods of machine learning include Linear model [10], Bayesian model [11], Boolean network [12], SVM (support vector machine) [13] etc. Linear model supposes that the expression level on the node of network depends on the linear combination of the expression levels of nearby nodes. Bayesian model is based on Bayesian probability theory, and describes related genes by an acyclic direct graph. So, it is well utilized to study the local gene regulatory network. Boolean network describes the two states of gene nodes in the expression network as "on" and "off". Either signal transduction pathway or gene regulatory network can select Boolean network; and in the network, each signal protein or each gene holds the state of either active or inactive. Probabilistic Boolean network is an improved version of Boolean network. It not only maintains the benefits of Boolean network, but also effectively handles indefinite situations. Recently, SVM was introduced into the research of gene regulation network, through the training of the network, SVM provide a statistical possibility to every transcription factor and its coupled target gene, and then the regulatory network was established based on these statistical possibilities.

3. Metabolism pathway

Metabolism network has been analyzed by multiple methods quantitatively and qualitatively. These methods can be generally categorized into three forms: steady state analysis, kinetic analysis, as well as sensitivity analysis. According to these research aspects, there emerged many modeling methods, which were utilized in different specific metabolism networks, and gained effective results consistent with experiments. Flow balance analysis [14] is the typical method of steady state analysis and the fundamental of this method is linear programming, which defines objective function according to the instances of the organism, treats the range of reactive rates as constraints, and then solves the function to obtain reaction rates in a steady-state. Metabolic control analysis [15] is used to analyze the sensitivity of parameter, which is defined by partial differential coefficient. However, this method could be only utilized in a place with parameter changing in slight variations. Elementary flux modes [16] are minimal sets of enzymes that each can generate valid steady states, and every steady-state flux pattern can be expressed as a

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non-negative linear combination of these modes, so it can be regarded as a basis of steady-state flux space. If we have an elementary flux, then we can describe any trait of a steady state flux. All of the above methods we mentioned do not concern about kinetic properties in a metabolic network. This virtue makes the computation simpler and feasible. However, the analytical results are limited because we don't consider kinetic data. The kinetic analysis, namely, the changing of variables depending on time, can only be realized by kinetic modeling. As traditional kinetic tool, partial differential equation [17] acts as important role in the kinetic analysis in metabolic network. The difficulty in this area is to setup reaction rate table. And at the same time, if we need to establish a practical continuous model, we must also accurately evaluate the parameters for this model. This is hardly realized because it is scarce of data. In general, different modeling methods reflect the characteristics of metabolic network in different aspects.

4. Protein-protein interaction

Traditional methods of protein-protein interaction include yeast two-hybrid, affinity purification with Mass Spectrometry etc. However, to utilize these methods, we require a laboratory with better conditions, a longer experimental cycle, a huge expense, and but lower accuracy (for example, some experimental results show 50% false negative in a yeast two-hybrid experiment). In this situation, researchers in this area developed many bioinformatics methods [18]. On the one hand, the false results can be eliminated through the analysis of current experimental data, and then, the real protein interaction information is left; on the other hand, we can analyze and predict protein-protein interaction by the utilization of computation methods, then direct experiment, and further construct protein-protein interaction network in biological activities. Based on above, we can study proteins with unknown functions and explore new functions of well-known proteins. Therefore, we can provide evidences to the discoveries of new drug targets.

5. Bio-molecular marking

Molecular marking in diseased organism is always an important issue in biomedical research. It became the hot spot of biomedical research recently and had tight connections to clinical trial and the drug discovery. The differential expression of proteins can be used as marker or index to the state and phenotype change of an organic body. So, protein marking can be used to detect the disease change, trace seriousness of the disease and monitor the reaction to a specific drug used in an organic body. The application of protein marking will be helpful to the discovery of new drug, clinical toxicology, fundamental and clinical research, as well as disease diagnosis. Disease is a course instead of a state. So, single-molecule marking cannot detect the whole process of the generation of the disease and development of the disease. Current researchers used multiple molecule marking methods to effectively diagnose diseases. Thus multiple molecular markers discovery is the major direction in this research area.

6. Drug discovery

Drug discovery is one of the hottest application areas today. ADMET (absorption, distribution, metabolism, excretion, toxicity) is the major problem obsessing international pharmacy. Drug failure induced by ADMET could cause a 2-5 hundred million USD lost averagely. The basis in this is: we could not explain the interactions between precursor molecule and its target site within an organic body. In the research stages before clinical use, because of the high expense in the drug development, the current research is focusing on the optimization of the functions of macromolecules and their ligands; and this optimization is independent of the interactions between gene networks and proteins, so, the synergism of the interactions between these molecules is not considered. The current ADMET predicting methods are based on traditional Quantitative Structure-Activity Relationships (QSAR) modeling, but not display the molecular biological mechanisms in ADMET, so, the effectiveness is not as high as expected. The research in the molecular biological mechanisms in ADMET is significant for the drug development. And the methods used in Systems Engineering inaugurated new research channels for predicting ADMET and the simulations for the

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pharmacological mechanisms.

Methods used in Systems Biology

Current research in biology can be generally categorized in two classes according to its research channels. The first is Top-down: step-by-step, it puts forward proposals, establishes related model, simulates the mechanism, and compares the results; the other is Bottom-up: through mass experimentation, it collects related results, further analyzes the results, then sets up biological modeling, and make simulations, finally compares the results with experimental results. These two methods have the same functions with different directions.

Systems Biology majorly accepted the first research mode, in which the research subjects are mostly network structures within an organic body instead of the composites of a single molecule. A system could be a gene regulation network, a metabolism pathway, or a cell, a tissue, and even a whole complicated organic body. Because Systems Biology needs to study interactions between different constituents at same time, so, the utilization of high throughput and quantitative technologies is very important. Different computation methods to analyze mass data in a complicated biological system are also needed.

In the experiment design of Systems Biology, although, the research subject and the spatio-temporal measurement units for the living activities do not have big differences from before, through the utilization of high throughput analysis technology and text mining technology in current published papers, to find the relativities between different genes and proteins, and the variations of gene expressions as well as protein activities in different experimental conditions becomes more expectable (PubMed is the main source of text mining). Furthermore, sometimes, there exists big differences between traditional biology and data analysis methods currently used, and many researchers even are unfamiliar with the new analysis methods, including those different complicated methods in statistics, probability, and computation algorithm etc., and the modeling based on analysis data. So, this type of mode, which collects data in the beginning and analyzes them in the following, effectively displayed different activities within an organic body in different aspects at the same time. And the most importance is that we can observe the interactions between many living process pathways at the same time. This cannot be realized by the research methods used in traditional biology, and provide chances to study living activities in different aspects and integratively.

Research tendency

The current research in Systems Biology is focusing on the human disease research and new drug development, and has gained great achievements. Systems Biology in microbiology is deployed gradually. This deployment is concentrated on the research of metabolism pathways in the micro-organic body. Through analysises and comparisons of metabolism pathways, the optimized metabolism regulation modes, key factors, and the optimum state of the reaction are expectable, and the production rate of effective constituent is increased. Systems Biology in the applications of botany seams lagged behind those in zoology and microbiology. We believe that the research for chloroplast in plant could be one of the best research topics in Systems Biology of botany, and hold economic significance and research value. China has achieved glorious progress in photosynthesis research, and has solid foundation in the traditional biology. So, we should apply those methods used in Systems Biology to the research for chloroplast, integratively study the interactions between different factors in the process of photosynthesis, and select important regulation factors related to photosynthesis to optimize photosynthesis pathways. This will significantly influence agricultural breeding and its production rate. The secondary botany metabolism pathway is also a research subject in Systems Biology because many effective constituents in traditional Chinese medicine are the products from the secondary botany metabolism pathway. At the same time, the introduction of the research methods in Systems Biology is also required by the research in the mechanism of traditional Chinese medicine because the effective constituents in traditional Chinese medicine are polymolecules, and their corresponding target sites in human body are various, however, synergistic effects exist in these polymolecules and their corresponding target sites.

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So, to explain the effective mechanism in traditional Chinese medicine, we also need to consider Systems Biology.

Conclusion

Nowadays, Systems Biology attracted more and more considerations from biological research. In 2002, *Science* and *Nature* all published special editions and topics to discuss the progresses and perspectives in Systems Biology. The international conferences on Systems Biology also attracted more and more scientists to participate in. Research papers on Systems Biology abruptly increased in these years, the number published in 2003 doubled that in 2000. This increment accredits mostly the intensified foundation support from NIH and others. Currently in Britain, it is hardly granted by science foundation that if the proposal applying for biological related fund does not include the concept of Systems Biology. Being aware that the increment of papers on Systems Biology and its great development potential, *Nature* also issued a related special edition-"Molecular Systems Biology" in March 2005.

Recently, Systems Biology progressed greatly in USA and Europe. However, it is still in its infant stage in china and has a big gap to catch that overseas. Accompanied with the development in Systems Biology, the number of talents needed in this area will be highly increased. Being aware of this, in 2004, the Medical school in Harvard University established the first Systems Biology department to accelerate the speed of the talent fostering process. In China, we also paid high attention on the establishment of research centers for Systems Biology, for example, Shanghai Institutes for Biological Sciences (SIBS) of CAS and Shanghai Jiaotong University co-established the Center for Systems Biology; University of Science and Technology of China (USTC) and SIBS co-established China's first talent fostering base of System Biology-the department of System Biology of USTC. CAS and the Ministry of Science and Technology are preparing the establishment of the State Key lab of System Biology relying on SIBS. We believe that these establishments of research and talents fostering systems related to Systems Biology will construct a stable basis for China's Systems Biology research, and promote its development.

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