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Research Paper

In Silico Observations and Analysis of Metabolic Pathways

Bioinformatics

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ABSTRACT

Metabolic signatures are efficient markers of prime importance in health and disease diagnosis. Metabolic pathway observation and analysis is considered to be one of the promising areas of research. Understanding of the interactions among physical entities in the cell elucidates the underlying mechanisms in normal, disordered and disease states. Pathway structure knowledge offer delicate biological insights to know higher-order functions of the biological systems. In this review attempt has been made to be familiar with important databases and relevant in silico tools and programs which are generally built to archive, organize, visualize, map and analyze metabolic pathways.

INTRODUCTION

Growth in the gene and protein sequences as a result of high-throughput experiments and next generation sequencing has enabled systematic analysis of biochemical functionality in normal and diseased states. Having geno-proteomic information in hand, it is apparent to know the involved signaling pathways. Pathways facilitate to know the intricacy of the underlying cellular set-ups. Metabolic pathway study highlights the detection, physiological endpoints and quantification of metabolites under study. It also lets us know the effect of surrounding factors influencing the system. In addition to disease diagnosis and prognosis, pathway studies are useful in toxicology, comparative and pharmacogenomics. Reaction networks and metabolism has been valuable for drug design. Microarray and mutagenesis have enabled many known genes and their products. These genes are annotated with statistically enriched pathways. It is believed that numerous pathways are still not fully understood and many connections are missing in established pathways. Programmed de novo restoration of metabolic pathways is a challenge to establish connection in unknown reactions with bridging of gaps. Synthetic reengineering of natural metabolic pathways in some organisms has allowed novel metabolite production of medical and industrial importance. Interestingly species specific natural pathways are altered dynamically to redesign them and may be implemented in a different species.

The metabolic network of any organism is complex which could be broken in to sub networks called metabolic pathways which are basic functional building blocks of the entire network. Metabolic studies are predominantly based on raw data arising from nuclear magnetic resonance, gas and liquid chromatography mass spectrometry to recognize and compute metabolites from biological samples. For any species under consideration, genes are consigned to fitting points in referenced pathways built on orthology (Galperin and Koonin, 1999). Alterations are introduced to the current state of living systems and afterward changes are observed on intermediate metabolites at precise time scales. The connections among components of a pathway are derived by metabolic profiling and comparison with altered genetic and nutritional conditions. Metabolic pathways are believed to be robust and evolve continuously in different physiological status, energy level and redox potential. Environmental dynamics might encourage the preservation or the loss of metabolic pathways, or they might simply moderate gene expression via a signaling cascade prompting a pathway turned on or off. Keeping in mind about the large data sets, it is essential to use computational power for understanding the pathways. Algorithms are required to process, visualize and analyze the data from a variety of platforms. In addition, systematic simulation studies on metabolic pathways rely on accurately predefined reaction network models. A large number of metabolic pathways remain unknown and many reaction steps are still missing even in known pathways. As the experimental confirmation of reaction networks remains difficult, in silico methods are still necessary to assume likely pathways. Computational methods are developed and many are underway for reconstructing combined metabolic pathways based on literature or reference-based frameworks and chemical transformations across species.

PATHWAY DATABASES

Biomolecular interaction data are generated, organized in the form of pathway databases. Informatics resources of reaction networks and chemical genomics are vital for understanding structure, properties and activity of biologically relevant molecules. These resources are necessary for knowing structural and physicochemical relationships among compounds. Pathway databases are also essential for a wide range of utilities like analysis of high-throughput data and computational approximation of phenotypes. Examples of some studied pathway databases are Panther (Thomas et al., 2003), TransPath (Choi et al., 2004), Reactome (Joshi-Tope et al., 2005), KEGG (Kanehisa et al., 2012), SPIKE (Elkon et al., 2008) etc. Pathway databases such WikiPathways (Pico *et al.*, 2008) and communicates cell signaling, transcriptional and metabolic pathways, as diagrams made of nodes and links. Most of the pathway databases facilitate visual exploration of molecular pathway. In Metscape, Gao et al., (2010) employed data from earlier created databases to draw the correlations between genes and metabolites. Signaling pathways, gene regulations and interaction network resulting data are interspersed in databases. These interaction databases are integrated in a flawless connection network highlighting multifunctional aspects. ConsensusPathDB (Kamburov et al., 2010), a meta-database integrates functional interactions from diverse resources. Updated pathway databases of many species have come up with wide-ranging descriptions of the metabolic network. Protein interactions and families are annotated in highly formalized pathway databases. For a global view of interactive networks, geno-chemical informations are taken in to consideration. The STRING (Franceschini et al., 2013) database provides a global perspective for many organisms. LUCApedia (Goldman et al., 2013) is used to inspect genes in the early evolution of metabolic pathways linked to ancient life by corroborating them against the rest of the database. Recently, algorithms have also been proposed to semi-automatically merge descriptions of the metabolic network of the same organism (Chindelevitch et al., 2012). Some databases archive human specific metabolic network information. For example, Stobbe et al., (2013) implemented C2CardsHuman, combining the knowledge of the other human pathway databases. The systematization of data about RNA metabolic pathways and enzymes taking part in RNA maturation and degradation is essential for the full understanding of these processes. RNApathwaysDB (Machnicka et al., 2013), an online resource about maturation and decay pathways involving RNA as the substrate.

VISUALIZATION, MAPPING AND NETWORKING OF METABOLIC PATHWAYS

Pathways are compilations of functional units resulted from the interplay of interacting genes, RNAs, proteins and small molecules. Mapping genes or proteins into the context of pathways let us enable knowing their functions and interactions in and among organisms. Graphical depiction is a successful way to understand biological pathways. Concept of graphical display of molecular interactions in the form of maps is perhaps

one of the best approaches to maneuver metabolic research. In last years, graphical illustrations of biodiagrams are started by many developers. SBML (Hucka et al., 2003) and BioPAX standards are used for biological pathway databases contents (Stromback et al., 2005). Other advanced standard programs are CellDesigner (Kitano et al., 2005), SBGN (Le Novere et al., 2009). Ordered modeling of cellular behavior is a major challenge as sometimes complexes share some of their proteins. Attempts for calculable illustrations comprise the development of markup languages. A number of software tools have also been designed to construct pathway models which are typically built on graphical models wherein nodes represent genes, proteins or chemical compounds, and edges represent various types of relations or families. Pathway visualization programs such as MapMan (Thimm *et al.*, 2004) and KaPPa-View (Tokimatsu *et al.*, 2005) display metabolite and transcript levels on predefined pathways. While some pathway viewers creates static network images with hyperlinks implemented without the use of Java, BioPP (Viswanathan, 2007) display static images with Perl and CGI with annotations mapped to the nodes as hyperlinks. AVIS (Berger *et al.*, 2007), a network drawing tool uses asynchronous JavaScript and XML (AJAX) and underlying Perl libraries to draw static networks. With the integration of the KEGG, Predictome databases and search engines for co-expressed genes/proteins, VisANT 3.0 (Hu et al., 2007) supports exploratory pathway analysis, with multi-scale editing and harmonious visualization of expression data. Advances in nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) have allowed for quantifying hundreds of metabolites in body fluids. Various commercial and free stand-alone analysis tools dedicated to metabolomic data support in aligning and binning peaks in MS and NMR spectra provide functionality for annotating peaks with metabolites. The MassTRIX software (Suhre and Schmitt-Kopplin, 2008) translates NMR spectra into metabolic compounds and maps them into KEGG pathways together with genome information. Web-based metabolomics data analysis tool MetaboAnalyst (Xia et al., 2009) provides biomarker-phenotypic data analysis. Another development is ProMeTra (Neuweger *et al.*, 2009) allowing pre-computed and customized maps in scalable vector graphics (SVG). Oberhardt et al. (2009) contextualized high-throughput and hypothesis-driven interrogation of multi species network. Visual analysis has been shown to be effective in exploratory analysis with bioinformatics tools.

In recent times, network and sub-network study help to infer data picked up from genomic experiments. Network visualization help in understanding the complexities of biological networks. Network diagrams are commonly used to visualize biochemical pathways by displaying the relationships between genes, proteins, mRNAs, microRNAs, metabolites, regulatory DNA elements, diseases, viruses and drugs. Several algorithms have been developed by integrating genomic signals with predefined networks. Examples include heaviest induced subgraph (Dittrich et al., 2008) and BioNet (Beisser et al., 2010) etc. These methods identify the sub-networks in the reference networks. The resultant sub-networks imply the functional relationships of the seed genes and additional genes. For subnetwork extraction, web-based tool NeAT combines different algorithms (Brohee et al., 2008). Genes2Networks uses a neighborhood approach to connect genes in protein interaction networks (Berger et al., 2007). Dannenfelser et al., (2011) have developed a flash-based network viewer for the visualization of small to moderately sized biological networks and pathways. Zheng and Zhao (2012) introduced GenRev, a network-based program to explore the functionally important genes. The Genome-Linked Application for Metabolic Maps (GLAMM) is an integrated interface for visualizing, reconstructing metabolic networks and construction of novel, transgenic pathways (Bates et al., 2011). Web-based visualization tool libSBGN (van Iersel et al., 2012) enables basic manipulations and biographer (Krause et al., 2013) renders and edits reaction networks integrated as library-tools dealing with network-related information.

IDENTIFICATION AND ANALYSIS OF METABOLIC PATHWAYS

Graph theory approach is applied by many researchers where the metabolic network is mapped onto a graph with the shortest computed path. The network expansion approach (Handorf and Ebenhoh, 2007) iteratively adds reactions and their products from a given network to a set of initial source compounds. Pathway Hunter Tool (Rahman et al., 2005) analyzes the shortest paths for one or more organisms and can build enzyme network of virtual organisms. Carbon metabolic fluxes can be displayed using VisANT network analysis software (Hu et al., 2007). Bayesian networks have been for data modeling and classification. BioBayesNet (Nikolajewa et al., 2007) is a web application that allows the easy modeling and classification of biological data. In contrast, other methods integrate, a priori, the knowledge of gene networks in the analysis of gene-expression data. A Potts spin algorithm was developed to cluster gene expression data by using the nearest neighbour reactions of biochemical networks (Konig et al., 2004). Rapaport and co-workers (2007) extracted gene-expression patterns of neighbouring genes in the network, involving the attenuation of high frequency signals with respect to the graph. In another approach states that elementary modes represent true functional units of metabolism and can be used to reveal transcriptional activity (Schwartz et al., 2007). However, the combinatorial explosion of computing elementary modes in large networks limits the practical use of these methods.

In the past decade, many functional enhancement analysis tools have been developed. Most of them focus on identification of enriched functional categories based on gene ontology, such as Ontologizer (Bauer et al., 2008) and FuncAssociate (Berriz et al., 2009). As functionally enriched tools are not so perceptive in the context of signaling pathways and diseases, a number of other tools have been developed for pathway-disease identification like MAPPFinder (Salomonis et al., 2007), PaLS (Alibes et al., 2008), GENECODIS (Nogales-Cadenas et al., 2009). PATIKAweb (Dogrusoz *et al.*, 2006) uses java server pages to generate pathway diagrams and arrange static images of nodes and edges. Tools such as WebInterViewer (Han *et al.*, 2004) and VisANT (Hu *et al.*, 2008) are useful for large protein–protein interaction networks utilizing JavaWebStart. Goffard et al., (2009) presented an update of PathExpress, a tool to interpret gene-expression data and explore the metabolic network without being restricted to predefined pathways. Genes are clustered based on shared features i.e. common domains, common protein complexes or signaling pathways and co-expression (Tranchevent et al., 2008). Analysis of reconstructed metabolic networks through constraint-based approaches is used computationally to study the biochemical processes. Interaction network studies have uncovered network properties resulting in the development of models describing protein interaction network evolution (Kim et al., 2008). Some approaches have elucidated important features of the metabolism of diverse organisms, identified essential genes and proposed potential metabolic drug targets. The complexity of the reconstruction process and the large number of literature sources on metabolism have often led to the generation of multiple metabolic models for the same organism. The program ClustScan (Starcevic et al., 2008) annotates DNA sequences encoding modular biosynthetic enzymes in invertebrates and metagenomic datasets. Li et al., (2009) developed R-based software (SubpathwayMiner) which facilitates subpathway identification. Integrated approaches that combine transcriptome, proteome and metabolome profiling have gained popularity and have proven to provide novel insights in the understanding of the biological systems (Cho *et al.*, 2008). MetaboAnalyst (Xia et al., 2009) is a metabolomic data processing tool which accepts a variety of input data such as NMR peak lists, binned spectra, MS peak lists, compound/concentration data etc. and offers metabolomic data processing, normalization, multivariate statistical analysis, graphing, identification and mapping. The proposed concept of molecular connectivity maps enables researchers to integrate experimental measurements of genes, proteins, metabolites, and drug compounds under similar biological conditions (Li et al., 2009). Ayydurai et al., (2010) developed new system named CytoSolve that dynamically integrates computations of smaller models that can run in parallel across different machines without the need to merge the source codes of the individual models. Tools such as CellPublisher (Florez et al., 2010) and PathVisio

(Luna et al., 2011) navigate along with geography. Moriya et al. (2010) presented PathPred server to predict plausible pathways from a query compound to global chemical structure alignment focusing on microbial biodegradation and biosynthesis of secondary metabolites. Similarly, PathBuilder is another pathway resource including an annotation tool (Kandasamy et al., YEAR). NaviCell exploits large maps of molecular interactions (Kuperstein et al., 2013). Further, model repositories, such as BiGG (Schellenberger et al., 2010), MetRxn (Kumar et al., 2012) were developed to integrate models and to allow comparative analyses. In addition, tools like CytoSEED (DeJongh et al., 2011), FAME (Boele et al., 2012) assist in the analysis and modeling tasks. Desktop tools that can specifically provide annotation of model components include Pathway Tools (Karp et al., 2010), the SuBliMinaL Toolbox (Swainston et al., 2011), Metannogen (Gille et al., 2011) and GEnome-scale Metabolic models Simulation, Reconstruction and Visualization (GEMSiRV) (Liao et al., 2012). Interactive tool LinkinPath (Ingsriswang et al., 2011) analyzes, map and visualize interconnected pathways and functional genomics of most organisms. Kastenmuller et al., (2011) developed *meta*P-*server* to facilitate data interpretation which provides standardized data analysis for quantitative metabolomic data acquisition and interpretation. Xie et al., (2011) reported KOBAS 2.0, which annotates an input set of genes with putative pathways and disease relationships allowing cross species sequence similarity, making it statistically meaningful. iPath2.0 (Yamada et al., 2011) is a web-based tool which summarizes the metabolism in biological systems as annotated to date. Cobalt and tungsten assimilation pathways have proposed to be established in the genomes of *Methanococcus maripaludies* and *Methanosarcina mazei*, respectively. It is suggested that in combination of systems models and evolutionary inference can only correlate metabolic fluxes and physiological changes in methanogens (Chellapandi, 2011). On the basis of docking interaction analysis, Naeem et al., (2013) proposed the schematic diagram of C3 metabolic pathway. Flux balance analysis (FBA) is used for constructing genome-scale, steady-state models of metabolic networks having applications from evaluation of potential growth media for an organism to prediction of phenotypes of knockout mutants. Latendresse et al., (2012) presented a new software technology for generating FBAmodels that accelerates model development. Cytoscape is a java-based open source software tool that is used by biomedical researchers to visualize and dynamically analyze gene/protein interaction networks (Shannon *et al.*, 2003). The protein–protein interactions (PPIs) at the system level have become increasingly important in understanding biological processes. Comparison of the interactomes of different species not only provides a better understanding of species evolution but also helps with detecting conserved functional components and in function prediction. Phan and Sternberg (2012) reported a PPIs alignment method, called PINALOG. Alignment of human and yeast PPIs reveals several conserved sub-networks. MetaboAnalyst (Xia et al., 2012) is a web-based suite for high-throughput metabolomic data analysis, to support robust statistical analysis, exploration, visualization, as well as functional interpretation. UniPathway (Morgat et al., 2012) is a manually curated resource for the pathway representation and annotation providing unambiguous representations of enzyme-catalyzed, spontaneous chemical reactions and hierarchical representation of pathways. Leader and coworkers (2011) described web-based facility 'Pathos' which can be used to analyse raw or processed mass spectrometric data and display the metabolites identified – and changes in their experimental abundance. Chindelevitch et al., (2012) described MetaMerge, an algorithm for semi-automatically reconciling a pair of existing metabolic network reconstructions into a single metabolic network model. Methods using pathways have evolved from classical test to complex systems such as impact analysis (Khatri et al., 2012). In addition, newer methods have been proposed, which target RNA-seq datasets specifically in addition to microarrays: one of these is the Gene Set Variation Analysis (GSVA) (Haenzelmann et al., 2013), which shifts the focus from gene expression to pathway expression through the generation of enrichment scores. Pathway Processor 2.0 (Beltrame et al., 2013) is an application to analyze microarray data. May et al. (2013) have developed an open-source desktop application that simplifies the process of adding database cross references and chemical structures to genome-scale metabolic models. Kotera et al. (2013) developed a supervised de novo method to reconstruct metabolic pathways from a large compound set in the reaction-filling framework.

GENOME-SCALE METABOLIC ANNOTATION AND RECONSTRCTIONS

Genome-scale metabolic network reconstruction is a labor-intensive process that relies on extensive searches in the metabolic literature to find out set of reactions to a given organism. The number of complete and draft genomes is rapidly growing in recent years, and it has become increasingly important to automate the identification of functional properties genes in these genomes. Genomic knowledge provides an insight into the metabolic capability of a particular organism based on known metabolic pathways and enzymes involved. The ability to determine a relationship between an organism's genome and its molecular physiology quantitatively, based upon current understanding of metabolism and molecular biology, continues to improve. Establishment of a quantitative model allows for the compact and integrated analysis of complex metabolic networks. Genome-scale metabolic modeling in particular has been used in pathway engineering and facilitates understanding of metabolism of microorganisms. Modeling is also a tool to elucidate missed information and recognize errors in new and existing models of metabolic networks. The comparative cross connective study of metabolism among genomes is possible. Blum et al., (2008) presented MetaRoute, a search algorithm based on atom mapping rules and path weighting schemes that returns relevant or textbook-like routes between a source and a product metabolite within seconds for genome-scale networks. Genome-scale metabolic models have proved useful in areas such as growth optimization and metabolic engineering (Thiele and Palsson, 2010). The time required to generate a reconstruction has decreased with improvements in automatic pipelines, such as the popular model-SEED (Henry et al., 2010). Despite rapid improvements in automation, extensive manual curation is still required for an accurate model. Common practice is to download the draft reconstruction and curate the model adding missing reactions from multiple databases and literature reviews. Primarily used for metabolic engineering and synthetic biology, genome-scale metabolic modeling shows tremendous potential as a tool for fundamental research and curation of metabolism. Genome-scale metabolic networks (GSMs) consist of compartmentalized reactions that consistently combine biochemical, genetic and genomic information. GSMs are often used to study genotype–phenotype relationships, to identify targets in metabolic engineering (Karr et al., 2012). Genome mining of biosynthetic gene clusters may accelerate identification and characterization of metabolites. Blin et al., (2013) developed antiSMASH, a web-based analysis platform that automates this process and detect additional classes of secondary metabolites, including oligosaccharide antibiotics, phenazines, thiopeptides, homoserine lactones, phosphonates and furans. MetaNetX.org (Ganter et al., 2013) is a website for accessing, analyzing and manipulating genome-scale metabolic networks (GSMs) as well as biochemical pathways. It consistently integrates data from various public resources and provides access to hundreds of GSMs and pathways that can be interactively compared (two or more), analyzed, manipulated and exported.

SYSTEMS BIOLOGY APPROACH

Biological processes are inherently dynamical systems. The use of systems biology approaches is becoming common in the study of metabolic and other networks to elucidate their functions and roles in human health and diseases. For many diseases, new therapies are needed to tackle problems with cost, toxicity and resistance to current drugs. Software have been developed which allow various types of modeling and analysis, such as steady state analysis, kinetic modeling, parameter estimation, sensitivity analysis, metabolic control analysis, stochastic simulation and consideration of spatial variation. Systems biology tools can be used to gain valuable insight into pathogenic processes and drug discovery. Constraint based modeling

of genome-scale metabolic networks has been widely used. Focusing on pathogen metabolic networks, Chavali et al., (2012) reviewed *in silico* strategies to identify effective drug targets, and highlighted recent successes as well as limitations associated with such computational analyses. Systems biology now extends in its scope to identify biosynthetic and metabolic lipid networks, cellular signaling networks that explicitly include lipid molecules and transcriptional and epigenetic networks where lipids play an integral role. The computational prediction of all biologically relevant or novel alternative routes in metabolic networks has numerous applications in systems biology, for example, the design of tracer or knockout experiments.

CONCLUSION

In silico study of metabolomes reveal unknown metabolic pathways and regulation. Visualizing a compound network enables tracing links among metabolites, genes, proteins and enzymes. Linking compounds and reactions to well-known metabolomic data sources supports verification revealing coordinated state of transcript and metabolite levels. As the database and tools are increasing in number there should be coordinated effort to exchange information towards establishment of common platforms for networking, graphics and analysis.

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