DRUG DEVELOPMENT RESEARCH 72:121–129 (2011)

DDR

Research Overview

In Silico Genome-Scale Modeling and Analysis for Identifying Anti-Tubercular Drug Targets

Dong-Yup Lee,[1–3]* Bevan Kai Sheng Chung,[2,3] Faraaz N.K. Yusufi,[3] and Suresh Selvarasu[3]

[1]*Department of Chemical and Biomolecular Engineering, National University of Singapore, Singapore*

[2]*NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore*

[3]*Bioprocessing Technology Institute, Agency for Science, Technology and Research (A*STAR), Singapore*

Strategy, Management and Health Policy				
Enabling Technology, Genomics, Proteomics	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT *Mycobacterium tuberculosis* is the deadly pathogen responsible for causing tuberculosis in humans, continuing to infect and kill millions of people globally. Despite the availability of a number of anti-tuberculosis drugs and advances in high-throughput drug discovery technology there is an urgent need for designing novel anti-tubercular treatments due to growing parasite resistance and compromised immune systems in some patients. Therefore, it is highly necessary to develop systematic approaches that can facilitate the drug discovery by identification of drug targets in effective and efficient ways. In this sense, with the availability of whole genome sequence, application of genome-scale modeling is becoming increasingly important for deriving rational drug target identification. This approach is indeed powerful in unraveling the metabolic behavior of pathogens and helps in identifying most relevant metabolites/genes as drug targets, which are experimentally testable. Herein, we present a review on the application of genome-scale modeling and analysis in the context of identification of anti-tubercular drug targets. Drug Dev Res 72:121–129, 2011.

Key words: genome-scale metabolic model; constraints-based flux analysis; *Mycobacterium tuberculosis*; anti-tubercular drug targets

INTRODUCTION

Tuberculosis (TB) remains a major threat to health, especially in developing countries, with an estimated 9 million new cases and 2 million deaths in 2009 [WHO, 2009; Lonnroth et al., 2010]. The disease is caused by the bacterium *Mycobacterium tuberculosis*, which is transmitted through aerosol droplets from infected patients. Although TB is curable, the standard treatment regimens can last 6 months or longer. This lengthy treatment period, along with other factors, makes it difficult to maintain proper drug adherence, and as a result drug-resistant strains of tuberculosis have emerged [Kaplan et al., 2003]. Multidrug-resistant (MDR) tuberculosis is tolerant to first-line drugs, isoniazid and rifampicin [Frieden et al., 1993], while extensively drug-resistant (XDR) tuberculosis is immune to first-line anti-tuberculosis drugs such as quinolone and at least one of the second-line drugs (capreomycin, kanamycin, or amikacin) [Shah et al.,

*Correspondence to: Dong-Yup Lee, Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore 117576.
E-mail: cheld@nus.edu.sg

Published online in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ddr.20408

2007]. Most recently, extremely drug-resistant (XXDR) tuberculosis cases have emerged that cannot be eradicated with the currently available drugs [Migliori et al., 2007]. Further complicating the situation is the rising number of tuberculosis patients co-infected with HIV, which increases the number of cases with active tuberculosis [Gandhi et al., 2010] and has made tuberculosis a leading cause of death in patients infected with HIV [Ghebreyesus et al., 2010]. Table 1 describes the list of currently available drugs and their effects on treating tuberculosis. However, the emergence of drug-resistant tuberculosis, HIV co-infection, and other complex challenges has made it clear that current drugs are not sufficient to tackle this epidemic. Novel treatments for tuberculosis are urgently required but these new drugs will have to address a number of issues in order to be successful [Tomioka and Namba, 2006]. Firstly, adequate new drug targets and mechanisms should be identified and employed to combat MDR and XDR strains of tuberculosis. Since treatment regimens often involve several drugs, it is also essential to know the interaction of newly developed drugs with others, as well as interactions with retrovirals for patients with HIV. Finally, these new drugs must be cost efficient and able to be administered in short courses, thus allowing for the improvement of the patient's drug adherence. Currently, ten compounds are in various stages of clinical development for tuberculosis [Ma et al., 2010]. However, much remains to be done to discover effective treatments for this disease.

Towards this end, in silico approaches are being employed to discover new drugs for tuberculosis, including high throughput screening and computational systems biology approaches. Microbial genome sequencing, in particular the completed genome of *M. tuberculosis* [Cole et al., 1998], has made it possible to reconstruct comprehensive metabolic networks that can be analyzed in silico to identify potential drug targets [Anishetty et al., 2005; Verkhedkar et al., 2007]. By analyzing the genome and identifying coding regions, a network of predicted metabolic enzymes and reactions can be constructed. The topology of this network can then be analyzed using various graph theoretic approaches to find alternative pathways, highly connected central metabolites, and other interesting global properties [Jeong et al., 2000; Ma and Zeng, 2003; Tanaka, 2005]. More recent techniques have focused on identifying essential metabolite targets in the network, so-called "load points" and "choke points" [Rahman and Schomburg, 2006]. A choke point is an enzyme/metabolite on the network that either produces a unique product or consumes a unique substrate, i.e., removing the particular metabolite enzyme will ensure that no downstream metabolites can be produced following that particular route. The load number on an enzyme is a measure of how critical that enzyme is to the proper functioning of the enzyme as it reflects how many different metabolic pathways use that particular enzyme/metabolite (how many shortest paths pass through that particular metabolite). For targeted development of a drug to affect a pathogen effectively, the drug must target a choke point in the bacterial metabolic network that has a high load point number. Doing so ensures that the

TABLE 1. Currently Available Drugs for *M. tuberculosis*

Drugs	Group	Effect on *M. tuberculosis*
First line		
Ethambutol	Other	Affects bacterial cell envelope (arabinogalacton layer)
Isoniazid	Other	Affects bacterial cell envelope (arabinogalacton layer)
Pyrazinamide	Other	Inhibits the synthesis of fatty acids in *M. tuberculosis*
Rifampicin	Rifamycins	Inhibits nucleic acid synthesis in pathogen
Streptomycin	Aminoglycosides	Inhibits protein synthesis in the bacteria
Rifapentine	Rifamycines	Inhibits nucleic acid synthesis in pathogen
Second line		
Amikacin, kanamycin	Aminoglycosides	Inhibits protein synthesis in the bacteria
Ciprofloxacin, levofloxacin, moxifloxacin	Fluoroquinolones	Inhibits DNA replication
Ethionamide, prothionamide	Thioamides	Affects bacterial cell envelope (mycolic acid layer)
Cycloserine	Other	Affects bacterial cell envelope (peptidoglycan layer)
p-aminosalicylic acid	Other	Inhibits nucleic acid synthesis in pathogen
Third line		
Rifabutin	Rifamycins	Inhibits nucleic acid synthesis in pathogen
Clarithromycin	macrolides	Inhibits protein synthesis in the bacteria
Linezolid	Oxazolidone	Inhibits protein synthesis in the bacteria
Thioacetazone	Other	Useful in preventing resistance to more powerful drugs like isoniazid and rifampicin
Thioridazine	Phenothiazine	NA

enzyme/metabolite affected by the drug stops the production of a number of metabolites required by the pathogen for growth. Important enzymes/metabolites in the *M. tuberculosis* metabolic network thus determined through choke-point analysis can be systematically "knocked out" one by one in in-silico experiments.

In addition to the network topology based methods described above, the constraints-based flux analysis approach has also been used to gain insights into the metabolic networks of several organisms [Kim et al., 2008; Lee et al., 2005; Price et al., 2003, 2004]. This method is based on a stoichiometric model of the metabolic network comprised of mass-balance equations, and can provide detailed insight into how an organism's metabolism will react to various perturbations such as gene knockout or down-regulation. In this review, we look at genome-scale in-silico metabolic modeling and analysis, the issues involved in their construction, and various computational approaches towards identifying novel drug targets for tuberculosis.

IN SILICO IDENTIFICATION OF ANTIMICROBIAL DRUG TARGETS

The selection of a drug target can be influenced by scientific, medical and strategic considerations [Knowles and Gromo, 2003]. The ultimate goal of pathogenic studies is to devise a strategy to kill the pathogen of interest. Hence, it is desirable to identify some protein targets, which are functionally essential to the pathogen's survival, such that the disruption of the function of any of these proteins by drug action will bring about cell death. However, due to cellular robustness attributed to the existence of redundant pathways, the identification of such essential targets is not trivial. Sophisticated computational algorithms can be used to explore redundant pathways [Lee et al., 2005; Lee et al., 2000; Wilhelm et al., 2004], which can be filtered from further analysis in the process of drug target identification.

In most cases like *M. tuberculosis*, the pathogen resides within the human host, thus the drug target should ideally not be homologous to any essential protein in the host lest we run the risk of inducing adverse effects on the host. Host proteins that can bind to antibacterial drugs to produce adverse effects are referred to as "antitargets" [Recanatini et al., 2004]. Furthermore, we can also evaluate the druggability of identified targets to filter out non-druggable targets [Owens, 2007]. In order to account for all these various considerations of drug targeting, the proper use of computational capabilities to design software and databases is important for researchers to harness the large knowledge base of *M. tuberculosis.* Online databases such as DrugBank [Wishart et al., 2008] and Potential Drug Target Database (PDTD) [Gao et al., 2008] provide users with comprehensive information on established drug targets, drug interactions, and even potential binding proteins. Various computational procedures have also been developed to identify and prioritize drug targets based on network topology and protein orthology [Doyle et al., 2010; Hasan et al., 2006]. Hence, in this review we will focus on the use of a particular computational procedure that exploits genome-scale metabolic models of *M. tuberculosis* to identify drug targets.

IN SILICO MODELING AND ANALYSIS OF MICROBIAL METABOLISM

In silico analysis of the complex behavior of an organism is made possible by genome-scale metabolic modeling. The advent of high-throughput techniques enabled genome sequencing and annotation, which in turn provided the necessary information to reconstruct genome-scale metabolic models. Since the first successful application of genome-scale metabolic modeling to predict metabolic capabilities of *Escherichia coli* [Edwards et al., 2001], numerous genome-scale metabolic models have been reconstructed for describing the metabolic behavior of various organisms including pathogens such as *Staphylococcus aureus* [Becker and Palsson, 2005; Heinemann et al., 2005; Lee et al., 2009], *Pseudomonas aeruginosa* [Oberhardt et al., 2008], and *Salmonella typhimurium* [AbuOun et al., 2009; Raghunathan et al., 2009]. To date, more than 50 such reconstructions have been developed for organisms from all domains: Archaea, bacteria, and eukarya [Oberhardt et al., 2009].

Reconstruction of Genome-Scale Metabolic Model

A genome-scale metabolic model is typically reconstructed using a bottom-up approach to provide a comprehensive genotype-phenotype map [Thiele and Palsson, 2010]. The starting point of model reconstruction lies in the organism's genome sequence, where its metabolic capabilities are encoded. The annotation of cryptic nucleotide sequence with meaningful biological function requires sophisticated techniques that are typically performed as part of the genome sequencing study [for a review, see Stein, 2001]. The first genome annotation of *M. tuberculosis* was published together with its sequence in 1998 by Cole et al. [1998] and subsequent re-annotation was carried out by Camus et al. [2002]. Hence, the first task in model reconstruction, which can be (semi)-automated as demonstrated elsewhere [Suthers et al., 2009; Tsoka et al., 2004], is to identify relevant metabolic reactions to be included and assign appropriate gene-protein-reaction associations to each reaction. The draft model generated by this process typically contains minor errors, due to incorrect genome annotation, inappropriate selection

of metabolic reaction, and the presence of metabolic gaps in the network. Subsequently, the next step of model refinement aims to solve these problems through manual curation and produce an in silico "viable" metabolic model that can be used for simulations and analysis. Interested readers are referred to Thiele and Palsson [2010] for a better understanding of genome-scale metabolic model reconstruction. In view of increasing interest in the field of genome-scale metabolic model reconstruction, a systematic protocol was also developed for carrying out the process of model reconstruction [Feist et al., 2009; Reed et al., 2006; Thiele and Palsson, 2010]. In the case of *M. tuberculosis*, the pathway-genome database (PGDB), which is accessible online (http://biocyc.org/MTBRV/), can be a good starting point for genome-scale metabolic model reconstruction.

Constraints-Based Flux Analysis

In general, biological systems obey the laws of physics and chemistry. Thus, it is possible to predict the physiology of an organism based on the knowledge of all the physicochemical constraints that are imposed on the system [Hartwell et al., 1999]. Constraints-based flux analysis employs this concept to elucidate cellular physiology where chemical constraints, in the form of reaction stoichiometry, and physicochemical constraints, in the form of reaction capacity and reversibility, are introduced to define the feasible solution space for metabolic flux distribution [Price et al., 2004]. This solution space captures all possible metabolic states that are achievable under unperturbed condition, which is sometimes known as "wild-type." In most cases, we are interested in a single unique solution that closely describes the in vivo cellular physiology. Thus, we resort to optimization algorithms to determine a solution that best reflects the true metabolic state [Price et al., 2004]. The implementation of optimization requires the definition of a cellular objective that is typically assumed to be biomass growth or can be systematically selected using several computational methods depending on the cellular environment [Gianchandani et al., 2008; Knorr et al., 2007; Ow et al., 2009; Schuetz et al., 2007]. Hence, after reconstructing the metabolic model of organism of interest, we can simulate its cellular phenotype by solving the following linear programming (LP) problem:

$$\max\ v_{\text{growth}}$$

$$\text{Subject to: } \sum_j S_{ij} v_j = 0 \quad \text{(Stoichiometric constraint)}$$

$$v_j^{\min} \le v_j \le v_j^{\max} \quad \text{(Capacity and reversibility constraint)}$$

where S_{ij} refers to the stoichiometric coefficient of metabolite i in reaction j and v_j, to the flux of reaction j. A detailed mathematical formulation can be found in Palsson [2006].

For identifying drug targets, we need to evaluate gene or reaction essentiality since we aim to kill the pathogen or attenuate its cellular activity by nullifying the function of certain essential gene(s). In this regard, by specifying both $v_j^{\min}$ and $v_j^{\max}$ to zero for some reaction(s), we created an in silico mutant strain that can be tested for viability by solving the above LP problem.

GENOME-SCALE METABOLIC MODELING AND ANALYSIS OF *M. tuberculosis* FOR TARGET IDENTIFICATION

M. tuberculosis has the ability to survive for a long time during the dormant stage with low or no metabolic activity and is capable of reactivating at a later time point. It has complex fatty acids characteristics involving mycolic acids, phenolic glycolipids, and mycoceric acids that may contribute to the resilience of the organism under adverse conditions. Due to this fact, *M. tuberculosis* can survive under a wide range of environments such as low oxygen [Rosenkrands et al., 2002], extreme pH levels [Wayne and Sohaskey, 2001], and so on. The hypoxic conditions from low oxygen environments led to high expression of heat shock proteins HspX and Rv2031c, thus, enabling its survival [Cunningham and Spreadbury, 1998]. In addition, the pathogen can also use CO_2 as the sole carbon source. The presence of reductive citrate cycle in *M. tuberculosis* can enable fixation of CO_2 leading to its survival. These observations indicate that the combination of different resistive characteristics makes *M. tuberculosis* develop resistance to a wide range of currently available drugs. This necessitates the need for a complete understanding of the pathogen's metabolic behavior and investigating the discovery of new drug targets using in silico approaches in a systematic manner.

Often, genome-scale metabolic models have been used to achieve the following objectives: (1) derive biological knowledge from high-throughput data, (2) guide strain improvement studies, (3) generate novel testable hypotheses, (4) examine multi-species relationships, and (5) uncover network properties [Oberhardt et al., 2009]. Since *M. tuberculosis* is a pathogen, it is imperative to look for anti-tubercular drug-targeting. Hence, all of the objectives, except (2), are relevant for understanding the physiological behavior of *M. tuberculosis* and exploiting the potential points of fragility in its cellular system in order to nullify its pathogenicity. These objectives can be achieved via various mathematical modeling and analysis approaches: (in the order of increasing complexity) interaction-based,

constraints-based, and mechanism-based [Stelling, 2004]. On one end, large-scale interaction-based models can be easily constructed to study network properties but they cannot indicate the strength of interaction, which is the reaction flux. On the other end, the mechanism-based approach can elucidate dynamic variation of metabolic fluxes but a genome-scale model will require the input of a large number of kinetic parameters, which cannot be easily determined due to lack of experimental data [Llaneras and Pico, 2008; Singh and Ghosh, 2006]. Hence, the constraints-based approach is usually preferred for genome-scale modeling to evaluate the steady-state metabolic flux distribution under stipulated environmental and genetic conditions. Thus, constraints-based flux analysis can be a powerful tool to identify drug-targets for TB.

The application of constraints-based flux analysis to identify potential drug targets in *M. tuberculosis* has been demonstrated in various studies. The earliest study by Raman et al. [2005] applied constraints-based flux analysis on a small-scale metabolic model, consisting only of the mycolic acid pathway, to identify essential genes. These genes were verified with a previous experimental study using tranposon site hybridization [Sassetti et al., 2003] and their feasibility as drug targets was also evaluated through sequence analysis of the gene products with human proteome. Subsequent studies [Jamshidi and Palsson, 2007] adopted a similar approach except for the reconstruction of the genome-scale metabolic model of *M. tuberculosis*. In addition, Beste et al. [2007] created a web-based interface for the implementation of constraints-based flux analysis that can be accessed via the URL (http://sysbio.sbs.surrey.ac.uk/tb/) while Jamshidi and Palsson [2007] introduced the concept of hard-coupled reaction sets to group potential drug targets that are metabolically equivalent. In another study by Raman et al. [2008], constraints-based flux analysis was used to carry out a double-gene deletion study and this procedure was included as part of a comprehensive target identification pipeline that also considers other aspects of target feasibility. The purpose of double-gene deletion analysis is to identify synthetic lethal target combinations, which can be of great interest in drug development since a recent discovery of drug targets in *M. tuberculosis* describes the synthetic lethal combination of maltosyltransferase (GlgE) and glucosyltransferase Rv3032 whose inhibition can induce DNA damage [Kalscheuer et al., 2010]. Recently, approaches involving protein-protein dependencies at genome-scale were also examined to identify efficient metabolic disruption strategies in *M. tuberculosis* [Kushwaha and Shakya, 2010; Raman and Chandra, 2008; Raman et al., 2009].

Constraints-based flux analysis can predict overall cell growth but is inadequate for the evaluation of dynamic cellular response to drug influence. Thus, a mathematical framework, which integrates enzyme kinetics and constraints-based flux analysis, was proposed to evaluate cell growth dynamics and elucidate cellular physiology in *M. tuberculosis* with the aim of drug targeting [Fang et al., 2009]. Although such an integrated framework gives good prediction of cell growth dynamics, it requires a priori information of the kinetic parameters. Unless the enzyme kinetics are well characterized or sufficient experimental data has been generated, this methodology is unlikely to produce results of the same quality reported by Fang et al. [2009]. Another integrative approach that combines gene expression data with modeling was developed by Colijn et al. [2009]. The basic idea of this approach, known as "E-flux," is to constrain the solution space using additional information provided by gene expression data, thus providing a more relevant description of the metabolic states of *M. tuberculosis*. Since gene expression forms the basis of metabolic flux determination, the capability to process and analyze gene expression data is critical to the quality of the results produced by E-flux. Figure 1 depicts the reconstruction of a genome-scale metabolic model and subsequent in silico approaches that can be applied for the *M. tuberculosis* network in order to design novel drug targets.

In addition, there have been other improvisations of constraints-based flux analysis to identify drug targets. An approach adopted by Kim et al. [2010] analyzes metabolite essentiality instead of the gene or reaction essentiality. In this study, Kim et al. [2010] suggested a metabolite-oriented approach, which combined metabolite essentiality with chokepoint analysis. In fact, metabolite essentiality is based on the concept of metabolite turnover rate or "flux-sum" that was developed earlier [Kim et al., 2007] while the chokepoint analysis has been developed by Yeh et al. [2004] to identify drug targets for *Plasmodium falciparium*. This approach has been successfully employed to find potential drug targets for *Helicobacter pylori, Staphylococcus aureus*, and *M. tuberculosis* that can inflict system-wide damage on the pathogen when they are inhibited. Such a metabolite-oriented approach has also been further developed to characterize how perturbation of individual metabolites can affect cellular metabolism [Chung and Lee, 2009]; this method can be extended for drug target identification and prioritization in the future.

FUTURE DIRECTIONS AND CHALLENGES

Genome-scale metabolic network models have been extensively used in elucidating the systemic

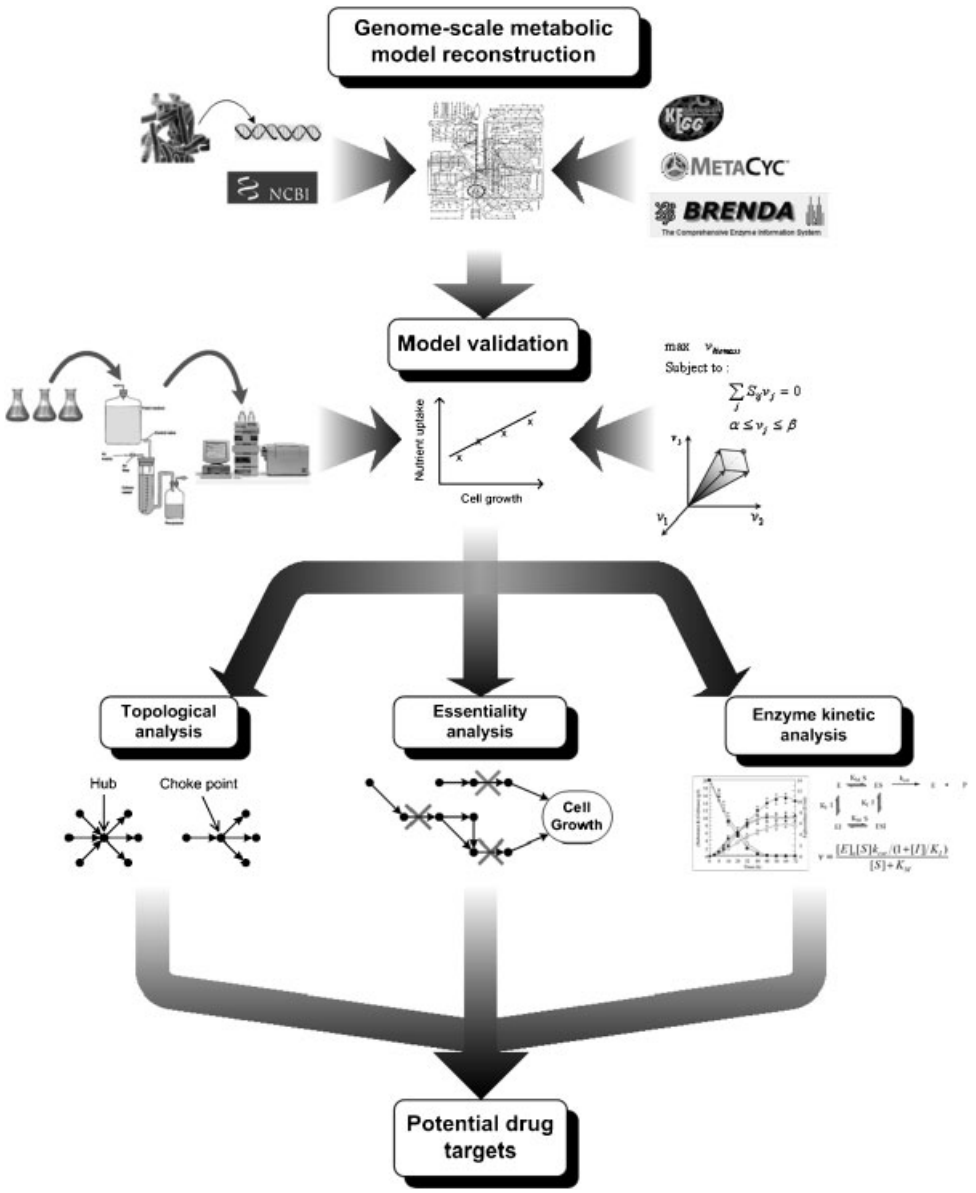

Fig. 1. Reconstruction of genome-scale model of *M. tuberculosis* and investigation of various computational approaches for identifying drug. The diagram illustrates how the genome-scale model is reconstructed with publicly available resources. Experimental data are then used to validate the model. Subsequently, various computational approaches such as choke point, synthetic lethality, essentiality, and enzyme kinetic analyses are employed for drug targeting. Cross indicates gene deletion.

properties of organisms as well as to an extent in determining the drug targets for harmful pathogens. Nevertheless, major efforts are needed in integrating the drugs and disease information along with the systems-level modeling. The advancements in computational and experimental chemistry have led to synthesis of several novel drug compounds. Thus, developing a database with the information of disease and currently available drugs can be beneficial for the process of further enhancements in drug discovery. When such information is made accessible, combining it with in silico modeling can help us unravel the complex behavior of pathogens and suggest new drug targets and drug compounds, followed by experimental validation and database updates. This implies that the process of new drug development for TB should be iterative with significant efforts from both computational and experimental experts. Initial efforts on automating similar drug-targeting approaches have been successfully made and validated experimentally, extracting the relationship exhibited by different variants of drug-resistant *M. tuberculosis* strains and

their evolution [Singh et al., 2006]. The whole approach can be further improved by adding another layer of information in the form of signal transduction and regulatory networks [Cui et al., 2009] as well as high-throughput "-omics" data although heterogeneous data integration remains a major challenge. Recently, a structure-based approach has been combined with *M. tuberculosis* proteome data for drug discovery [Holton et al., 2007; Ioerger and Sacchettini, 2009] while information on carbohydrate-binding proteins was used to find new drug targets mitigating the effect of tuberculosis [Ernst and Magnani, 2009]. In future, such integrated systems biology approaches can act as a valuable tool to understand cellular functioning and identify potential drug targets for inhibiting or preventing the growth of bacterial pathogens.

REFERENCES

AbuOun M, Suthers PF, Jones GI, Carter BR, Saunders MP, Maranas CD, Woodward MJ, Anjum MF. 2009. Genome scale reconstruction of a *Salmonella* metabolic model: comparison of similarity and differences with a commensal *Escherichia coli* strain. J Biol Chem 284:29480–29488.

Anishetty S, Pulimi M, Pennathur G. 2005. Potential drug targets in *Mycobacterium tuberculosis* through metabolic pathway analysis. Comput Biol Chem 29:368–378.

Becker SA, Palsson BO. 2005. Genome-scale reconstruction of the metabolic network in *Staphylococcus aureus* N315: an initial draft to the two-dimensional annotation. BMC Microbiol 5:8.

Beste DJ, Hooper T, Stewart G, Bonde B, Avignone-Rossa C, Bushell ME, Wheeler P, Klamt S, Kierzek AM, McFadden J. 2007. GSMN-TB: a web-based genome-scale network model of *Mycobacterium tuberculosis* metabolism. Genome Biol 8:R89.

Camus JC, Pryor MJ, Medigue C, Cole ST. 2002. Re-annotation of the genome sequence of *Mycobacterium tuberculosis* H37Rv. Microbiology 148:2967–2973.

Chung BK, Lee DY. 2009. Flux-sum analysis: a metabolite-centric approach for understanding the metabolic network. BMC Syst Biol 3:117.

Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon SV, Eiglmeier K, Gas S, Barry CE 3rd, Tekaia F, Badcock K, Basham D, Brown D, Chillingworth T, Connor R, Davies R, Devlin K, Feltwell T, Gentles S, Hamlin N, Holroyd S, Hornsby T, Jagels K, Krogh A, McLean J, Moule S, Murphy L, Oliver K, Osborne J, Quail MA, Rajandream MA, Rogers J, Rutter S, Seeger K, Skelton J, Squares R, Squares S, Sulston JE, Taylor K, Whitehead S, Barrell BG. 1998. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature 393:537–544.

Colijn C, Brandes A, Zucker J, Lun DS, Weiner B, Farhat MR, Cheng TY, Moody DB, Murray M, Galagan JE. 2009. Interpreting expression data with metabolic flux models: predicting *Mycobacterium tuberculosis* mycolic acid production. PLoS Comput Biol 5:e1000489.

Cui T, Zhang L, Wang X, He ZG. 2009. Uncovering new signaling proteins and potential drug targets through the interactome analysis of *Mycobacterium tuberculosis*. BMC Genomics 10:118.

Cunningham AF, Spreadbury CL. 1998. Mycobacterial stationary phase induced by low oxygen tension: cell wall thickening and localization of the 16-kilodalton alpha-crystallin homolog. J Bacteriol 180:801–808.

Doyle MA, Gasser RB, Woodcroft BJ, Hall RS, Ralph SA. 2010. Drug target prediction and prioritization: using orthology to predict essentiality in parasite genomes. BMC Genomics 11:222.

Edwards JS, Ibarra RU, Palsson BO. 2001. In silico predictions of *Escherichia coli* metabolic capabilities are consistent with experimental data. Nat Biotechnol 19:125–130.

Ernst B, Magnani JL. 2009. From carbohydrate leads to glycomimetic drugs. Nat Rev Drug Discov 8:661–677.

Fang X, Wallqvist A, Reifman J. 2009. A systems biology framework for modeling metabolic enzyme inhibition of *Mycobacterium tuberculosis*. BMC Syst Biol 3:92.

Feist AM, Herrgard MJ, Thiele I, Reed JL, Palsson BO. 2009. Reconstruction of biochemical networks in microorganisms. Nat Rev Microbiol 7:129–143.

Frieden TR, Sterling T, Pablos-Mendez A, Kilburn JO, Cauthen GM, Dooley SW. 1993. The emergence of drug-resistant tuberculosis in New York City. N Engl J Med 328:521–526.

Gandhi NR, Nunn P, Dheda K, Schaaf HS, Zignol M, van Soolingen D, Jensen P, Bayona J. 2010. Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis. Lancet 375:1830–1843.

Gao Z, Li H, Zhang H, Liu X, Kang L, Luo X, Zhu W, Chen K, Wang X, Jiang H. 2008. PDTD: a web-accessible protein database for drug target identification. BMC Bioinform 9:104.

Ghebreyesus TA, Kazatchkine M, Sidibe M, Nakatani H. 2010. Tuberculosis and HIV: time for an intensified response. Lancet 375:1757–1758.

Gianchandani EP, Oberhardt MA, Burgard AP, Maranas CD, Papin JA. 2008. Predicting biological system objectives de novo from internal state measurements. BMC Bioinformatics 9:43.

Hartwell LH, Hopfield JJ, Leibler S, Murray AW. 1999. From molecular to modular cell biology. Nature 402:C47–52.

Hasan S, Daugelat S, Rao PS, Schreiber M. 2006. Prioritizing genomic drug targets in pathogens: application to *Mycobacterium tuberculosis*. PLoS Comput Biol 2:e61.

Heinemann M, Kummel A, Ruinatscha R, Panke S. 2005. In silico genome-scale reconstruction and validation of the *Staphylococcus aureus* metabolic network. Biotechnol Bioeng 92:850–864.

Holton SJ, Weiss MS, Tucker PA, Wilmanns M. 2007. Structure-based approaches to drug discovery against tuberculosis. Curr Protein Pept Sci 8:365–375.

Ioerger TR, Sacchettini JC. 2009. Structural genomics approach to drug discovery for *Mycobacterium tuberculosis*. Curr Opin Microbiol 12:318–325.

Jamshidi N, Palsson BO. 2007. Investigating the metabolic capabilities of *Mycobacterium tuberculosis* H37Rv using the in silico strain iNJ661 and proposing alternative drug targets. BMC Syst Biol 1:26.

Jeong H, Tombor B, Albert R, Oltvai ZN, Barabasi AL. 2000. The large-scale organization of metabolic networks. Nature 407: 651–654.

Jones D. 2005. Antibacterial drugs: tackling tuberculosis. Nat Rev Drug Discov 4:103.

Kalscheuer R, Syson K, Veeraraghavan U, Weinrick B, Biermann KE, Liu Z, Sacchettini JC, Besra G, Bornemann S, JacobsJr WR. 2010. Self-poisoning of *Mycobacterium tuberculosis* by targeting GlgE in an alpha-glucan pathway. Nat Chem Biol 6: 376–384.

Kaplan G, Post FA, Moreira AL, Wainwright H, Kreiswirth BN, Tanverdi M, Mathema B, Ramaswamy SV, Walther G, Steyn LM, Barry CE 3rd, Bekker LG. 2003. *Mycobacterium tuberculosis* growth at the cavity surface: a microenvironment with failed immunity. Infect Immun 71:7099–7108.

Kim HU, Kim TY, Lee SY. 2008. Metabolic flux analysis and metabolic engineering of microorganisms. Mol Biosyst 4: 113–120.

Kim PJ, Lee DY, Kim TY, Lee KH, Jeong H, Lee SY, Park S. 2007. Metabolite essentiality elucidates robustness of *Escherichia coli* metabolism. Proc Natl Acad Sci USA 104:13638–13642.

Kim TY, Kim HU, Lee SY. 2010. Metabolite-centric approaches for the discovery of antibacterials using genome-scale metabolic networks. Metab Eng 12:105–111.

Knorr AL, Jain R, Srivastava R. 2007. Bayesian-based selection of metabolic objective functions. Bioinformatics 23:351–357.

Knowles J, Gromo G. 2003. A guide to drug discovery: target selection in drug discovery. Nat Rev Drug Discov 2:63–69.

Kushwaha SK, Shakya M. 2010. Protein interaction network analysis--approach for potential drug target identification in *Mycobacterium tuberculosis*. J Theoret Biol 262:284–294.

Lee DS, Burd H, Liu J, Almaas E, Wiest O, Barabasi AL, Oltvai ZN, Kapatral V. 2009. Comparative genome-scale metabolic reconstruction and flux balance analysis of multiple *Staphylococcus aureus* genomes identify novel antimicrobial drug targets. J Bacteriol 191:4015–4024.

Lee DY, Fan LT, Park S, Lee SY, Shafie S, Bertok B, Friedler F. 2005. Complementary identification of multiple flux distributions and multiple metabolic pathways. Metab Eng 7:182–200.

Lee S, Phalakornkulem C, Domach MM, Grossmann IE. 2000. Recursive MILP model for finding all the alternate optima in LP models for metabolic networks. Comput Chem Eng 24: 711–716.

Lee SY, Lee DY, Kim TY. 2005. Systems biotechnology for strain improvement. Trends Biotechnol 23:349–358.

Llaneras F, Pico J. 2008. Stoichiometric modelling of cell metabolism. J Biosci Bioeng 105:1–11.

Lonnroth K, Castro KG, Chakaya JM, Chauhan LS, Floyd K, Glaziou P, Raviglione MC. 2010. Tuberculosis control and elimination 2010–50: cure, care, and social development. Lancet 375:1814–1829.

Ma HW, Zeng AP. 2003. The connectivity structure, giant strong component and centrality of metabolic networks. Bioinformatics 19:1423–1430.

Ma Z, Lienhardt C, McIlleron H, Nunn AJ, Wang X. 2010. Global tuberculosis drug development pipeline: the need and the reality. Lancet 375:2100–2109.

Migliori GB, De Iaco G, Besozzi G, Centis R, Cirillo DM. 2007. First tuberculosis cases in Italy resistant to all tested drugs. Euro Surveill 12:E0705171.

Oberhardt MA, Puchalka J, Fryer KE, Martins dos Santos VA, Papin JA. 2008. Genome-scale metabolic network analysis of the opportunistic pathogen *Pseudomonas aeruginosa* PAO1. J Bacteriol 190:2790–2803.

Oberhardt MA, Palsson BO, Papin JA. 2009. Applications of genome-scale metabolic reconstructions. Mol Syst Biol 5:320.

Ow DSW, Lee DY, Yap MGS, Oh SKW. 2009. Identification of cellular objective for elucidating the physiological state of plasmid-bearing *Escherichia coli* using genome-scale in silico analysis. Biotechnol Prog 25:61–67.

Owens J. 2007. Determining druggability. Nature Rev Drug Discov 6:187.

Palsson B. 2006. Systems biology: properties of reconstructed networks. New York: Cambridge University Press.

Price ND, Papin JA, Schilling CH, Palsson BO. 2003. Genome-scale microbial in silico models: the constraints-based approach. Trends Biotechnol 21:162–169.

Price ND, Reed JL, Palsson BO. 2004. Genome-scale models of microbial cells: evaluating the consequences of constraints. Nat Rev Microbiol 2:886–897.

Raghunathan A, Reed J, Shin S, Palsson B, Daefler S. 2009. Constraint-based analysis of metabolic capacity of *Salmonella typhimurium* during host-pathogen interaction. BMC Syst Biol 3:38.

Rahman SA, Schomburg D. 2006. Observing local and global properties of metabolic pathways: 'load points' and 'choke points' in the metabolic networks. Bioinformatics 22:1767–1774.

Raman K, Chandra N. 2008. *Mycobacterium tuberculosis* interactome analysis unravels potential pathways to drug resistance. BMC Microbiol 8:234.

Raman K, Rajagopalan P, Chandra N. 2005. Flux balance analysis of mycolic acid pathway: targets for anti-tubercular drugs. PLoS Comput Biol 1:e46.

Raman K, Yeturu K, Chandra N. 2008. targetTB: a target identification pipeline for *Mycobacterium tuberculosis* through an interactome, reactome and genome-scale structural analysis. BMC Syst Biol 2:109.

Raman K, Vashisht R, Chandra N. 2009. Strategies for efficient disruption of metabolism in *Mycobacterium tuberculosis* from network analysis. Mol Biosyst 5:1740–1751.

Recanatini M, Bottegoni G, Cavalli A. 2004. In silico antitarget screening. Drug Discov Today 1:209–215.

Reed JL, Famili I, Thiele I, Palsson BO. 2006. Towards multidimensional genome annotation. Nat Rev Genet 7:130–141.

Rosenkrands I, Slayden RA, Crawford J, Aagaard C, Barry3rd CE, Andersen P. 2002. Hypoxic response of *Mycobacterium tuberculosis* studied by metabolic labeling and proteome analysis of cellular and extracellular proteins. J Bacteriol 184: 3485–3491.

Sassetti CM, Boyd DH, Rubin EJ. 2003. Genes required for mycobacterial growth defined by high density mutagenesis. Mol Microbiol 48:77–84.

Schuetz R, Kuepfer L, Sauer U. 2007. Systematic evaluation of objective functions for predicting intracellular fluxes in *Escherichia coli*. Mol Syst Biol 3:119.

Shah NS, Wright A, Bai GH, Barrera L, Boulahbal F, Martin-Casabona N, Drobniewski F, Gilpin C, Havelkova M, Lepe R, Lumb R, Metchock B, Portaels F, Rodrigues MF, Rüsch-Gerdes S, Van Deun A, Vincent V, Laserson K, Wells C, Cegielski JP. 2007. Worldwide emergence of extensively drug-resistant tuberculosis. Emerg Infect Dis 13:380–387.

Singh NK, Selvam SM, Chakravarthy P. 2006. T-iDT: tool for identification of drug target in bacteria and validation by *Mycobacterium tuberculosis*. In Silico Biol 6:485–493.

Singh VK, Ghosh I. 2006. Kinetic modeling of tricarboxylic acid cycle and glyoxylate bypass in *Mycobacterium tuberculosis*, and its application to assessment of drug targets. Theor Biol Med Model 3:27.

Stein L. 2001. Genome annotation: from sequence to biology. Nat Rev Genet 2:493–503.

Stelling J. 2004. Mathematical models in microbial systems biology. Curr Opin Microbiol 7:513–518.

Suthers PF, Dasika MS, Kumar VS, Denisov G, Glass JI, Maranas CD. 2009. A genome-scale metabolic reconstruction of *Mycoplasma genitalium*, iPS189. PLoS Comput Biol 5:e1000285.

Tanaka R. 2005. Scale-rich metabolic networks. Phys Rev Lett 94: 168101.

Thiele I, Palsson BO. 2010. A protocol for generating a high-quality genome-scale metabolic reconstruction. Nat Protoc 5:93–121.

Tomioka H, Namba K. 2006. Development of antituberculous drugs: current status and future prospects. Kekkaku 81:753–774.

Tsoka S, Simon D, Ouzounis CA. 2004. Automated metabolic reconstruction for *Methanococcus jannaschii*. Archaea 1:223–229.

Verkhedkar KD, Raman K, Chandra NR, Vishveshwara S. 2007. Metabolome based reaction graphs of *M. tuberculosis* and *M. leprae*: a comparative network analysis. PLoS One 2:e881.

Wayne LG, Sohaskey CD. 2001. Nonreplicating persistence of *Mycobacterium tuberculosis*. Annu Rev Microbiol 55:139–163.

WHO. 2009. Global tuberculosis control: a short update to the 2009 report. Geneva: WHO.

Wilhelm T, Behre J, Schuster S. 2004. Analysis of structural robustness of metabolic networks. Syst Biol (Stevenage) 1: 114–120.

Wishart DS, Knox C, Guo AC, Cheng D, Shrivastava S, Tzur D, Gautam B, Hassanali M. 2008. DrugBank: a knowledge base for drugs, drug actions and drug targets. Nucleic Acids Res 36(Database issue):D901–906.

Yeh I, Hanekamp T, Tsoka S, Karp PD, Altman RB. 2004. Computational analysis of *Plasmodium falciparum* metabolism: organizing genomic information to facilitate drug discovery. Genome Res 14:917–924.