

Conference report

ASM Conference “Integrating Metabolism and Genomics”,
Montreal, Quebec, Canada, 30 April–3 May, 2004

The Conference “**Integrating Metabolism and Genomics**” (**IMAGE**) was organized by the American Society for Microbiology (ASM) and took place from April 30 to May 3, 2004, in a hotel in Montreal, Quebec, Canada. The Conference IMAGE provided a context for understanding the interface between genomics and metabolism in a large variety of microorganisms, illustrating nicely the potential, the challenges and the limitations of the post-genomics approaches. Because no parallel sessions were organized, the conference provided a real opportunity to learn about the recent genomics-guided developments obtained in a variety of microbiological systems beyond the own field of interest. The Conference brought together 175 scientists; one half from outside de USA (20% were Canadian) and 16% from corporate/industry. The format of ASM Conferences, focused on topics of scientific relevance, encourages interactive exchange and the participation of graduate students and post-docs. For IMAGE, ASM had awarded travel grants to 19 graduate students and new post-docs; nevertheless, this was an expensive meeting.

The scientific program was organized in seven plenary sessions, with five presentations each (two of them selected among the posters) and two poster sessions. Session II was fully dedicated to the yeast *Saccharomyces cerevisiae* and all the sessions were of unquestionable interest to the community involved in yeast research or dedicated to any other microbial system in this new field of genomics-guided research.

1. Session I : setting the stage

Robert La Rossa (DuPont de Nemours & Company, Wilmington, DE), who belongs to the ASM Conferences Committee and was one of the Conference organizers, opened this first session, illustrating the promises and limitations derived from the gene expression profile work carried out. Among the examples provided were

the changes occurring in *S. cerevisiae* genome-wide expression caused by treatment with the herbicide sulfometuron methyl (SM), an inhibitor of amino acid biosynthesis. A potential link between SM sensitivity and ergosterol metabolism was uncovered in this study and confirmed by genetic analysis. **Eberhard Voit** (Medical University of South Carolina, Charleston, SC) in his presentation “Of math and microbes: Improbable partners for a new era of metabolic-systems analysis” put in evidence the importance and the challenges of the mathematical analysis of metabolic systems, now that techniques permit to produce dense time series of in vivo measurements of the expression of genes at a genomic scale and of many simultaneous concentrations of metabolites. **Hirotsada Mori** described the impressive activity in the area of genomics carried out at the Nara Institute of Science and Technology, in Japan. **Valerie de Crecy-Lagard** (The Scripps Research Institute, La Jolla, CA) exemplified how comparative genomics can be used as a tool to identify novel enzymes and pathways. By combining occurrence profiling, chromosome clustering and homology searches, she showed how candidate genes for missing functions in the RNA modification field were predicted and experimentally tested. She also discussed how these methods can be used to allow a more precise characterization of protein information that is wrongly annotated in the databases. This introductory session was completed with **Peter Karp**'s (SRI International, Menlo Park, CA) presentation on “Bioinformatics integration of genomic and metabolic data”. He focused on the SRI's Pathway Tools software, which is freely available to academics and provides a rich computational environment for integrating, visualizing, and analyzing metabolic and genomic data. Given an annotated genome as its input (such as a Genbank file), the software predicts the metabolic pathways of the organism. The pathways and genome are stored within a Pathway/Genome Database (PGDB) that serves as a platform for further refinement and analysis of the gen-

ome and metabolic network. The pathway prediction algorithm relies on SRI's MetaCyc database, which describes 497 experimentally elucidated metabolic pathways and 1665 enzymes from 207 organisms. Users can query PGDB data using new Perl and Java application program interfaces.

2. Session II: lessons from the yeast community

This session, fully dedicated to the yeast *S. cerevisiae* with the genome sequenced since 1996, was opened by **Mark Johnston** (Washington University, St. Louis, MO). He focused on the numerous and sophisticated mechanisms for sensing glucose in yeast and responding to it appropriately. The profile of the yeast transcriptome revealed that the two main glucose-sensing and -signaling pathways are highly interconnected in a regulatory circuit. One of these pathways represses expression of many genes using the Mig1 transcriptional repressor, which is regulated by the glucose-sensitive Snf1 protein kinase. The other pathway involves Snf3 and Rgt2, glucose sensors in the plasma membrane, which generate a signal that inhibits the Rgt1 transcription repressor, resulting in glucose induction of expression of *HXT* genes encoding glucose transporters. The importance of the emerging regulatory circuits to ensure a rapid and sensitive response of the cell to changing levels of glucose was discussed. **Steve Oliver** (University of Manchester, Manchester, UK) highlighted the importance of data integration and the key requirements for the move from functional genomics to systems biology. He presented a method denominated “metabolic footprinting” that may be used to distinguish between different physiological states of wild-type yeast and between yeast single-gene deletion mutants, by focusing not on the measurement of intracellular metabolites but on the direct, mass-spectrometric monitoring of extracellular metabolites present in the spent culture medium. He also compared the different perspectives provided by transcriptome analysis and by metabolomic analysis, using data relating to both the nutritional and growth-rate controls in *S. cerevisiae*. The integration of metabolic models and “ome” data, was left to **Jens Nielsen** (Technical University of Denmark, Lyngby). He emphasized the role of bioinformatics in the development of mathematical models that can integrate information from different levels, e.g. from measurement of different “omes”. **Eduardo Agosin** (Pontificia Universidad Católica de Chile, Santiago, Chile) showed results on gene expression profiles and metabolic fluxes during the dynamic and complex process of wine fermentation in which yeast cells are subjected to multiple-stress conditions. Remarkably, metabolic fluxes throughout glycolysis were found to diminish, despite the increase of expression of glycolytic genes, and the fluxes within

the TCA cycle were also found to decrease while genes of the TCA cycle were slightly overexpressed. These observations clearly indicate that only by the integration of metabolic fluxes and gene expression profiles data it is possible to obtain a clear picture of the cell response to alterations occurring in its environment. This session dedicated to yeast was completed with a presentation by **Isabel Sá-Correia** (Instituto Superior Técnico, Lisboa, Portugal). She gave a picture of the yeast responses, suggested by quantitative proteomics, following a period of adaptation preceding the resumption of cell division under stress induced by the widely used herbicide 2,4-D. The presence of higher amounts of a number of proteins involved in the stress response, in protein and mRNA degradation and in carbohydrate metabolism, was registered in stressed cells. Remarkably, the up-regulation of amino acid biosynthetic enzymes in 2,4-D-stressed cells correlated with the reduction of the amino acid pools in the cell, possibly due to the disturbance of vacuolar and plasma membrane functions.

3. Session III: impact of genomics on metabolic engineering

Greg Stephanopoulos (Massachusetts Institute of Technology, Cambridge, MA), one of the Conference organizers, discussed the topic of evolutionary pathway optimization in *Escherichia coli*, starting by recognizing that the identification of genes that should be targeted in order to bring about a desired phenotype change is still an open question. As an aid in discovering putative genes impacting network properties and cellular phenotype, his group undertook a global genome-wide stoichiometric analysis. The metabolic fluxes such as to optimize growth were determined and the genome was scanned for single and multiple gene knockouts leading to improved product yield while maintaining acceptable growth rate. As a result of this approach, that can be extended to identify genes whose overexpression would be beneficial for a particular pathway, novel gene knockout targets were identified that improved lycopene accumulation in *E. coli*. The use of a library of promoters applied to facilitate the overexpression at different levels of target genes for pathway improvement was also reported. **Peter Yorgey** (Microbia Inc., Cambridge, MA) proposed a comprehensive approach for developing enhanced microbial biomanufacturing processes by the coordinate regulation of many cellular processes. Computational methods that allow integration of transcriptional and metabolic profiling data to decipher interrelationships between gene expression patterns and metabolite production were described. In combination with bioengineering tools, these methods permit rapid enhancement of metabolite production in commercial strains, as exemplified for the lovastatin-produc-

ing fungus *Aspergillus terreus*. This approach can be applied in interactive cycles to enhance existing biosynthesis as well as to establish entirely new biomanufacturing processes. **Jim Liao** (University of California, Los Angeles, CA) focused on the network component analysis of transcription regulatory networks in bacteria. He described a novel method, denominated Network Component Analysis (NCA), to determine the dynamics of the activities of various transcription factors during a physiological process. This approach utilizes both DNA microarray data and partial information regarding the membership of regulons as defined by each transcription factor in question. Remarkably, as compared with other approaches, it utilizes biological information regarding regulatory network topology, even when it is incompletely defined, and allows deconvolution of multiple regulatory pathways. **Wei Lian** (University of Minnesota, Minneapolis, MN) dedicated his presentation to the regulation of secondary metabolism in *Streptomyces coelicolor*. Whole-genome DNA microarrays were used to examine the temporal transcription of mutants where 20 known or putative regulatory genes, including many two-component systems, were knocked out individually. The session was completed with the contribution of **Armin Ehrenreich** (Institute of Microbiology, University of Göttingen, Germany), focused on the analysis of carbon metabolism in *Bacillus licheniformis* by genome sequencing and DNA microarrays.

4. Session IV: complex regulatory systems

This session started with a presentation by **Barry Wanner** (Purdue University, West Lafayette, IN) on the stochastic changes in expression for an *E. coli* gene controlled by the PhoB/PhoR two-component regulatory system. It is believed that cells exploit stochasticity to achieve genetic and nongenetic diversity for generating populations capable of surviving in different environments. Therefore, a better understanding of the stochastic behavior of native promoters in the context of well-defined signal transduction regulatory networks will have broad implications in cell biology. The approach exploited in the work described in this presentation consisted in the measurement of gene expression in single cells within isogenic populations carrying a promoter fusion to the fluorescent report protein GFP in a FACS flow cytometer. **Virgil Rhodius** (University of California at San Francisco, San Francisco, CA) discussed the exploitation of genome-wide expression studies, bioinformatics and comparative genomics to identify, in *E. coli*, the target genes regulated by the sigmaE factor which mediates the regulation of the extracytoplasmatic stress response. **Susan Gottesman** (National Institutes of Health, Bethesda, MD) reviewed the current status of computational and experimental

methods of detecting small RNAs in microbes. Although overlooked in genome annotation, it is becoming increasingly clear that these small RNAs are critical cell regulators. **Alyssa Redding** (University of California, Berkeley, CA) used proteomics to study the changes occurring in gene expression levels in response to oxygen stress in the sulfate-reducing anaerobic bacterium *Desulfovibrio vulgaris*. **Salvador Flores** (Instituto de Biotecnología-UNAM, Cuernavaca, Mexico) described results of the characterization of the adaptive response to the use of glucose as carbon source in a PTS-deletion derivative of *E. coli*, based on gene expression profiling, RT-PCR and ¹³C-tracer experiments-metabolic modeling.

5. Session V: genomics, metabolism and infection

Linc Sonenshein (Tufts University School of Medicine, Boston, MA), another Conference organizer, focused on the metabolic control of virulence and other stationary-phase responses in gram-positive bacteria. The genome-wide analysis carried out revealed that in *Bacillus subtilis* the CodY protein controls hundreds of genes induced at the entry to stationary phase. Interestingly, homologues of CodY were found encoded in the genomes of most of the low-G + C gram-positive bacteria and, in the pathogenic species, some of their primary virulence factors are induced in stationary phase, apparently under CodY control. **Steve Lory** (Harvard Medical School, Boston, MA) rendered clear how genome-wide analysis may reveal the evolution of virulence traits and signaling networks in *Pseudomonas aeruginosa* while this same approach was used by **Tyrrell Conway** (University of Oklahoma, Norman, OK) to identify *E. coli* genes induced by growth on mucus, conditions designed to mimic nutrient availability in the mammalian intestine. **Danielle Légaré** (Centre de Recherche en Infectiologie du CHUL, Université Laval, Ste-Foy, PQ, Canada) described the involvement of complex metabolic pathways in drug-resistant *Leishmania*, as determined by DNA microarrays and proteomic approaches.

6. Session VI: metabolic integration

The first presentation of this session, entitled “Dissecting metabolic integration in bacteria” was given by **Diana Downs** (University of Wisconsin, Madison, WI) another Conference organizer. Focused on the thiamine biosynthetic pathway in *Salmonella enterica*, the work in her laboratory uncovered phenotypic consequences for mutations in multiple genes of unknown functions in addition to metabolic connections with known biochemical pathways. She discussed the present understanding of the integration of the thiamine biosynthetic pathway

in the cellular metabolism and the progress toward assigning function to those uncharacterized genes. The following presentation, dedicated to Phenotype Micro-Array (PM) technology, developed by Biolog Inc., Hayward, CA, for phenotypic analysis of *E. coli*, *S. cerevisiae* and other microbial species, attracted the interest of the audience. **Barry Bochner** stressed that the phenotypic assays were designed to survey in vivo the function of diverse biological pathways, including both metabolic and regulatory pathways. PM technology can be used to complement genetics and genomics approaches and allows the testing of 2000 properties (phenotypes) of a cell in about 30 min of labor and 24–48 h of incubation. He discussed examples using *E. coli* and *S. cerevisiae* as models, where knockout mutants have been phenotyped. The technology was also applied to diverse bacterial species (*Burkholderia cepacia*, *P. aeruginosa*, *Salmonella typhimurium*, *Helicobacter pylori*, *Staphylococcus aureus*, *Listeria monocytogenes*, among others) and to *Candida albicans*, *Ustilago maydis* and *Aspergillus nidulans*. In this same session, **Jim Imlay** (University of Illinois, Urbana, IL) advanced some molecular explanations for the toxicity of oxygen in *E. coli*. **Craig Stevens** (Santa Clara University, Santa Clara, CA) reported the exploitation of genome-wide transcription analysis by microarrays to get insights into the physiological strategies used by the fresh-water oligotrophic bacterium *Caulobacter crescentus* to adapt to life in low nutrient/low osmolarity environments. At last, **Robert Bender** (University of Michigan, Ann Arbor, MI) carried out a refreshing re-analysis of central metabolism in *E. coli*, considered as a misunderstood black box.

7. Session VII: genomics, metabolism and the environment

Mary Lidstrom (University of Washington, Seattle, WA), another Conference organizer, described the approach used to identify a set of metabolic modules that define methylotrophy (the ability to grow on one-carbon compounds). The study was focused on a module that interconverts formaldehyde and formate, which was identified in genomes of bacteria not known to be methylotrophs (e.g. in *Burkholderia fungorum*). Novel families of the required genes were identified by developing environmental primers and obtaining PCR products from environmental DNA, from a well-studied habitat exhibiting high methylotrophic activity, and efforts are underway to isolate and characterize the strains carrying these novel genes. Phylogenetic analysis of the novel sequences is providing new insights into the evolution of this ancient biochemical pathway, and functional analysis is providing new insights into functional transformations of C1 compounds in natural habitats. **Gary Schoolnik** (Stanford Medical School, Stanford, CA) discussed the application of genomic approaches focused

on chitin utilization by *Vibrio cholerae* in marine environments. Results of the expression profiles performed to discover genes regulated by *V. cholerae* growing on crab shell chitin surface or with the soluble chitin oligosaccharide or the chitin monomer provided indications on the global, multistage program for the efficient utilization of chitin. The functional genomic analysis of the hyperthermophilic archaeon, *Pyrococcus furiosus*, was the subject of the presentation by **Mike Adams** (University of Georgia, Athens, GA). The effects of the primary carbon source on the expression of all annotated ORFs as well as the responses of *P. furiosus* to sub-optimal growth temperatures and oxidative stress, based on microarray analysis, were described. Genomic studies to unveil the catabolic power of Rhodococci, an important group of soil bacteria with great potential for applications in bioremediation and green chemistry, were described by **Lindsay Eltis** (University of British Columbia, Vancouver, BC, Canada). At last, **Michael Howard** (University of Maryland, College Park, MD) described the potential strategies by which *Microbulbifer degradans* efficiently degrades complex polysaccharides. Many of the enzymes involved in the depolymerization of complex polysaccharides, emerging from the inspection of the genome sequence, exhibit unusual structural features. For example, polyserine linker domains, located between catalytic, binding and/or anchoring domains, relatively rare among prokaryotes, are present in dozens of secreted carbohydrases and may function as a flexible tether to enhance substrate/enzyme interactions. Several other enzymes have features typical of a lipid-modified surface-anchored lipoprotein which may prevent diffusion of secreted enzymes.

8. The poster sessions

The scientific program of the IMAGE Conference was complemented and enriched by around 80 additional works presented as posters. Posters were left up for the entire conference and presented in two official poster sessions. Among them, it was also possible to view those selected for oral presentation. Yeasts played a minor role in this poster session. However, a very interesting poster provided indications about the rapid and massive reorganization of *C. albicans* metabolism, as a key aspect of the interaction of this pathogenic yeast with mammalian macrophages, using transcription profiling. Globally, the changes were similar to those seen in cells deprived from nutrients in vitro and specific alterations gave important insights into the environment of the phagosome and the nature of the host-pathogen interaction. The widespread metabolic changes observed are almost wholly absent in phagocytosed cells of the non-pathogenic *S. cerevisiae*, suggesting that this is a critical aspect of pathogenesis. Another poster described

the identification, during sequencing of an expressed sequence tag library, of the first gene of the xylose-fermenting yeast *Pichia stipitis* containing an intron. This gene sequence was considered an important tool to verify the absence of genomic DNA in mRNA preparations.

The poster session was dominated by bacteria of diverse species with relevance in infections, biotechnological processes, food industry and the environment. In a large number of studies, comparative genomic, genome-wide expression approaches and other genetic tools supported by bioinformatics were applied to obtain a better understanding of the complexity of metabolism and of the multiple overlapping circuits occurring in model species, as well as of the catabolism of recalcitrant compounds and the regulation of secondary metabolite biosynthesis. Other very interesting studies were presented, aiming at getting clues on the metabolic and molecular adaptation strategies used by extremophiles to live under specific extreme conditions as well as by non-extremophilic microbes when challenged with diverse chemical stresses or other environmental stresses. The fabrication and use of a microbioreactor for high-throughput data analysis and integration of growth physiology and genomics was also reported. A few posters describing the development or application of sophisticated analytical methods for the detection of crucial cell metabolites and the use of metabolomics platforms in combination with multivariate data analysis tools for microbial metabolomic studies completed the session.

9. Final remarks

The contributions presented at the IMAGE Conference made clear that the exploitation of various technologies developed to aid in elucidating the function of the novel genes discovered by systematic genome sequenc-

ing had a tremendous impact in the understanding of how a multitude of biochemical processes are integrated to generate the robust and efficient metabolism of a living cell, in particular the complex and dynamic regulatory networks underlying the cell response to environmental changes. The conference also gave support to the idea that transcriptomic, proteomic, metabolomic and bioinformatic analyses, in combination with classical genetics, biochemistry and molecular biology, are defining the metabolic networks in diverse microorganisms. In addition, the integration of the large amount of information from different levels is helping the move from functional genomics to systems biology. The conference also showed examples of how the massive amount of information emerging from the use of post-genomic approaches provides guidance to the development of enhanced microbial manufacturing processes and to the understanding of the metabolic control of microbial virulence. In summary, it has shown that the integration of metabolism and genomics is a highly fruitful field, generating many new insights and providing a more unified and quantitative description of the full complexity of a living cell. Without pretending to be complete, I have tried to give the flavor of a very exciting meeting. For more information about the scientific program and the abstracts of talks and posters presented at the ASM Conference IMAGE please visit <http://www.asm.org/ASM/files/CCPAGECONTENT/DOCFILENAME/0000027280/Program.pdf>.

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