AEGEAN CONFERENCES

Linking the international scientific community
Bringing the humanity scholars together

6th International Conference on Pathways, Networks, and Systems Medicine

Minoa Palace Conference Center
June 16-21, 2008
Chania, Crete, Greece

GENERAL INFORMATION

Accommodations
The conference participants will be staying at the Minoa Palace Hotel (A' class), which is located in Platanias, 24 km from Chania International Airport and 12 km west of Chania town center.

Breakfast
Breakfast will be served at the Minoa Palace Hotel during the hours scheduled in this program.

Conference Venue
The Minoa Palace Conference Center is located at the hotel.

Oral Presentations
An LCD projector will be available for the oral presentations. Speakers are asked to bring their USB flash drive or CD to the conference reception desk at least 30 minutes prior to the session. The allocated time for all presentations, unless otherwise indicated, includes five minutes Q&A.

Posters
Posters should be mounted Monday, June 16, from 5:00 pm – 8:00 pm on the designated boards and dismounted at the end of the meeting. The dimensions of the boards are 90 cm wide x 140 cm high. Adhesive tape for mounting the posters on the boards will be available at the poster area.

Internet
Internet will be available for participants during the meeting hours free of charge.

Welcome Reception and Gala Dinner
Badges are **required** for admission.

City Tour
Busses for the city tour will be leaving from the hotel on Thursday, June 19 at 3:00 pm Accompanying persons will also be picked up at the hotel front lobby at that time. ***Badges*** are **required** for participation.

Lunches
Lunches will be served at the indicated times to all registered participants and registered accompanying persons in the hotel. ***Badges*** are **required** for admission.

Tour and Travel Information
A desk operated by the personnel of our official travel agency,
"ERA Ltd.", 8 Alexandrou Soutsou Str., Kolonaki 10671 Athens – Greece
Tel.: 30-210-363-4944, FAX: 30-210-3631690, E-Mail: info@era.gr

will be located at the conference center throughout the meeting.

All Aegean Conferences events are non-smoking.

The organizers gratefully acknowledge the generous help provided by Dimitrios Lambris of Conferex, LLC in managing the organization of this meeting, and designing and publishing this program.

AEGEAN CONFERENCES

6th International Conference on Pathways, Networks, and Systems Medicine

Minoa Palace Conference Center

June 16-21, 2008

Chania, Crete, Greece

CONFERENCE CHAIRS

Joe Nadeau, Ph.D.
Co-Director, Center for Computational Genomics and Department of Genetics Case Western Reserve University School of Medicine Cleveland, OH 44106
Tel: 216-368-0581 | Fax: 216-368-3432
Email: jhn4@po.cwru.edu

Rudi Balling
German Research Centre for Biotechnology (GBF),
Braunschweig, Germany
Email: balling@gbf.de

David Galas, Ph.D.
Vice President and Chief Science Officer
Biology and Life Sciences
Battelle Memorial Institute
Columbus, Ohio 43201
Professor

Institute for Systems Biology
Seattle, WA 98103
Email: GalasD@BATTELLE.ORG

Leroy Hood, M.D. Ph.D.
President and Director
Institute for Systems Biology
Seattle, WA 98103-8904
Tel: 206-732-1201
Email: lhood@systemsbiology.org

Diane Isonaka
Seattle, WA 98119
Tel: 206-285-0521 | Fax: 206-282-0614
Email: disonaka@systemsbiology.org

Fotis Kafatos, Ph.D.
Chair in Immunogenomics
Imperial College: London

Natural Sciences
Division of Cell & Molecular Biology
Tel: +44-(0)20-7594-5360
Email: f.kafatos@imperial.ac.uk

John D. Lambris, Ph.D.
Professor of Pathology & Laboratory Medicine
University of Pennsylvania
Philadelphia, PA 19104
422 Curie Boulevard
Tel: 215-746-5765 | Fax: 215-573-8738
Email: lambris@mail.med.upenn.edu
http://www.lambris.com

Harris A. Lewin, Ph.D.
Professor of Animal Sciences, Gutgsell Chair
Director, Institute for Genomic Biology
Urbana, IL 61801
Tel: 217-244-3034 | Fax: 217-265-6800
Email: h-lewin@uiuc.edu

Laboratory of Mammalian Genome Biology
Urbana, IL 61801
IGB Homepage: http://www.igb.uiuc.edu
Lab Homepage: http://lewinlab.igb.uiuc.edu

Edison Liu, Ph.D.
Genome Institute of Singapore
Singapore, 138672, Singapore
Tel: 65-6478-8007 | Fax: 65-6478-9051
Email: liue@gis.a-star.edu.sg

Shankar Subramaniam, Ph.D.
Department Bioengineering 0412
University of California at San Diego
La Jolla, CA 92093
Tel: 858-822-0986
Email: shsubramaniam@ucsd.edu

PROGRAM OUTLINE

Monday, June 16		
Registration	5:00-8:00 PM	Hotel Lobby
Welcome Reception/Dinner	8:00 PM	Main Restaurant

Tuesday, June 17		
Breakfast	7:00 AM	Main Restaurant
Registration	8:30 AM – 2:15 PM	Conference Center Lobby
Welcome and Opening Remarks	8:30 AM	Conference Center
Session I	8:30 AM	Conference Center
Coffee Break & Poster Viewing	10:45 AM	Conference Center
Session II	11:45 AM	Conference Center
Lunch	2:00 PM	Aldemar Paradise Village
Dinner	8:00 PM	Local Greek Tavern

*The bus for the tavern will depart from the hotel at **7:30 PM***

Wednesday, June 18		
Breakfast	7: 00 AM	Main Restaurant
Session III:	8:30 AM	Conference Center
Coffee Break & Poster Viewing	10:45 AM	Conference Center
Session IV:	11:30 AM	Conference Center

Lunch	2:00 PM	Aldemar Paradise Village
Dinner	8:00 PM	Local Greek Tavern

*The bus for the tavern will depart from the hotel at **7:30 PM***

Thursday, June 19

Breakfast	7:00 AM	Main Restaurant
Session V:	8:30 AM	Conference Center
Coffee Break & Poster Viewing	10:45 AM	Conference Center
Session VI:	11:15 AM	Conference Center
Lunch	1:30 PM	Aldemar Paradise Village
City Tour	3:00 PM	

*The bus for the city tour will depart from the hotel at **3:00 PM***

Dinner	8:00 PM	Local Greek Tavern

Friday, June 20

Breakfast	7:00 AM	Main Restaurant
Session VII:	8:30 AM	Conference Center
Coffee Break & Poster Viewing	10:45 AM	Conference Center
Session VIII:	11:45 AM	Conference Center
Concluding remarks	2:00 PM	Conference Center
Lunch	2:15 PM	Aldemar Paradise Village
Gala Dinner	8:00 PM	Aldemar Paradise Village

Saturday, June 21		
Breakfast	7:30 AM	Main Restaurant

Departure

PROGRAM

Monday, June 16

5:00 - 8:00 PM		Open Registration
8:00 PM		Welcome Reception/Dinner

Tuesday, June 17

8:30 AM		**Welcome and Introductory Remarks** J Nadeau
Session I		**Cancer Pathways and Networks** **Chair:** J Nadeau
8:30 AM	***1***	***Transgenerational gene interactions and susceptibility to inherited disease*** J Nadeau
9:00 AM	***2***	***Protein-based profiling of liver cancer using multi-linear regression and Boolean models*** LG Alexopoulos, J Saez-Rodriguez, B Cosgrove, DA Lauffenburger, and PK Sorger
9:30 AM	***3***	***Drugging the Cancer Epigenome*** Y Qiang
10:00 AM	***4***	***Determination of complex reaction mechanisms*** J Ross
10:35 AM	***5***	***Signaling across cell Bounderies: genetic analysis of the PTEN pathway in the breast tumor microenvironment*** MC Ostrowski, C Cantemir, A Trimboli, F Li, J Wallace, and G Leone
10:45 AM		Coffee Break & Poster Viewing
Session II		**Chair:** V Cheung
11:45 AM	***6***	***Genetics of human gene expression*** R Nayak, D Smirnov, M Morley, T Weber, RS Spielman, and VG Cheung
12:15 PM	***7***	***Computational analysis of biological networks*** M Koyuturk, J Pandey, S Subramaniam, and A Grama
12:45 PM	***8***	***Systems biology of the cell cycle*** L Alberghina

1:15 PM *9* ***Correlation of chromatin modification and genetic regulation***
S Jones, G Robertson, M Bilenky, A Tam, Y Zhao, T Zeng, N Thiessen, T Cezard, A Fejes, E Wederell, R Cullum, M Krzywinski, I Birol, M Hirst, P Hoodless, and M Marra

1:45 PM *10* ***Spatial and temporal control of MAPK signaling during periodic morphogenesis***
Z Hilioti, W Sabbagh, Jr, S Paliwal, A Bergmann, M Goncalves, L Bardwell, and A Levchenko

2:00 PM Lunch

8:00 PM Dinner at a local Greek Tavern
*The bus for the tavern will depart from the hotel at **7:30 PM***

Wednesday, June 18

Session III **Metabolic Disease Networks**
Chair: A Attie

8:30 AM *11* ***A gene expression network model of type 2 diabetes establishes a relationship between cell cycle regulation in islets and diabetes susceptibility***
MP Keller, YJ Choi, P Wang, DB Davis, ME Rabaglia, AT Oler, DS Stapleton, C Argmann, KL Schueler, S Edwards, HA Steinberg, EC Neto, R Klienhanz, S Turner, MK Hellerstein, EE Schadt, BS Yandell, C Kendziorski, and AD Attie

9:00 AM *12* ***Metabolomic: a global biochemical approach to the study of human disease and drug effects***
R Kaddurah-Daouk

9:30 AM *13* ***Integrating gene expression, sequence and proteomics data to identify disease biomarkers***
S Horvath, JE Aten, and AJ Lusis

10:00 AM *14* ***Insulin signaling, adipose tissue and metabolic syndrome***
CR Kahn

10:30 AM *15* ***The genetic architecture of diet-induced obesity***
DA Buchner, LC Burrage, AE Hill, EJ Vazquez, M Rosca, P Solinas, S Yazbek, H Shao, ES Lander, CL Hoppel, and JH Nadeau

10:45 AM		Coffee Break & Poster Viewing
Session IV		**Chair:** H Lewin
11:30 AM	***16***	***Lipid maps approach to lipidomics*** EA Dennis
12:15 PM	***17***	***Multi-tissue profiling of human insulin resistance and pharmacological insulin sensitization*** G Hsiao, A Hsiao, J Chapman, PD Miles, JG Yu, CH Courtney, J Neels, JL Resnik, J Wilkes, D Thapar, JM Ofrecio, S Subramaniam, and DD Sears
12:45 PM	***18***	***A modularized model of apoptosis*** HA Harrington, KHo, Sk Ghosh, KC Tung , CY Kao, and B Aguda
1:15 PM	***19***	***Network analysis identifies affected biological processes in type 2 diabetes models*** M Liu, A Liberzon, SW Kong, WR Lai, PJ Park, IS Kohane, and S Kasif
1:45 PM	***20***	***DGAT1*: *a diet-regulated quantitative trait locus that affects hepatic lipid deposition*** R Oliveira, JJ Loor, NA Janovick Guretzky, JK Drackley, and HA Lewin
2:00 PM		Lunch
8:00 PM		Dinner at Local Greek Tavern *The bus for the tavern will depart from the hotel at **7:30 PM***

Thursday, June 19

Session V		**Organs, Pathways and Stem cells** **Chairs:** S Subramaniam
8:30 AM	***21***	***The systems biology cycle for pathways and networks: modeling and experimentation*** MR Maurya, S Bornheimer, M Farquhar, and S Subramaniam
9:00 AM	***22***	***Transcription factor -mediated global epigenetic remodeling to induced pluripotent stem cells*** K Plath, R Sridharan, J Tchieu, MJ Mason, Q Zhou, W Lowry, N Maherali, and K Hochedlinger
9:30 AM	***23***	***Localization of transcription factors in embryonic stem cells***

X Chen, H Xu, P Yuan, F Fang, M Huss, V Vega, E Wong, Y Orlov, W Zhang, J Jiang, YH Loh, HC Yeo, ZX Yeo, V Narang, K Govindarajan, B Leong, A Shahab, Y Ruan, G Bourque, WK Sung, N Clarke, CL Wei, and HH Ng

10:00 AM ***24*** ***NHLBI programs and resources in genomics, proteomics & other emerging areas***
P Qasba

10:30 AM ***25*** ***A complex systems approach to modeling stem cell decision-making***
JD Halley, DA Winkler, and FR Burden

10:45 AM Coffee Break

Session VI **Chair:** P Hunter

11:15 AM ***26*** **A framework for computational physiology**
PJ Hunter

11:45 AM ***27*** ***Systems biology of kidney development***
S Nigam

12:15 PM ***28*** ***Learning global transcriptional dynamics with the inferelator and cMonkey***
A Madar, P Waltman, T Kacmarczyk, V Thorsson, N Baliga, D Reiss, and R Bonneau

1:45 PM ***29*** ***Transcriptional networks in development and disease***
KP White

1:15 PM ***30*** ***Disease-associated transcription factor modules***
M Wiesinger, P Perco, J Söllner, A Lukas, and B Mayer

1:30 PM Lunch

3:00 PM City Tour
*The bus for the city tour will depart from the hotel at **3:00 PM***

8:00 PM Dinner at a local Greek Tavern

Friday, June 20

Session VII **Inflammation, immunity, microbes and the environment**
Chair: J Lambris

8:30 AM ***31*** ***Evasion strategies of microorganisms***

J Lambris

9:00 AM *32* ***Core diversity profile of human skin microbiome in health and disease***
EA Grice, TC Scharschmidt, HH Kong, AC Young, NISC Comparative Sequencing Program, S Conlan, RW Blakesley, GG Bouffard, ML Turner, ED Green, and JA Segre

9:30 AM *33* ***Microbial community proteogenomics***
J Banfield, V Denef, N VerBerkmoes, P Wilmes, G Dick, C Belnap, D Goltsman, R A Andersson, S Simmons, C Pan, B Baker, C Sun, M Wilkins, S Singer, G Tyson, M Shah, M Thelen, and R Hettich

10:00 AM *34* ***A predictive model fortranscriptional control of physiology in a free living cell***
NS Baliga

10:30 AM *35* ***Modeling interactions of heterogeneous genetic perturbations***
GW Carter and T Galitski

10:45 AM Coffee Break & Poster Viewing

Session VIII **Inflammation, immunity, microbes and the environment**
Chair: M Kellis

11:45 AM *36* ***Interrogating dynamic phenotypes using a high-throughput single cell microfluidic platform***
RJ Taylor, D Falconnet, A Niemisto, S Ramsey, I Shmulevich, T Galitski, and C Hansen

12:15 PM *37* ***Integrative analysis for TNFα-NFκB signaling pathway***
LWK Cheung and M Visvanathan

12:45 PM *38* ***Hepatitis C virus infection protein network***
L Tafforeau, B de Chassey, V Navratil, M S Hiet, A Aublin, S Agaugué, G Meiffren, F Pradezynski, BF Faria, T Chantier, M Le Breton, J Pellet, N Davoust-Nataf, P E Mangeot, A Chaboud, F Penin, Y Jacob, PO Vidalain, M Vidal, P André, C Rabourdin-Combe, and V Lotteau

1:00 PM *39* ***Pathological iron loading among patients with thalassemia: solving the clinical mystery with systems medicine***
JL Miller

1:15 PM *40* ***VelociGene®: an integrated suite of high throughput, customizable genome engineering technologies for***

analysis of gene function and drug target discovery and development
AN Economides

1:30 PM *41* ***Regulatory genomics of drosophila and mammalian species***
M Kellis

2:00 PM Concluding Remarks

2:15 PM Lunch

8:00 PM **Gala Dinner**

Saturday, June 21

7:30 AM Breakfast
Departure

POSTERS

42 ***Pathway enrichment analysis reveals that IL-17 pathway modulation is more closely related to psoriasis disease resolution with effective therapy than IFNγ***
M Suárez-Fariñas, L Zaba, J Fuentes-Duculan, I Cardinale, P Gilleaudeau, M Sullivan-Whalen, and J Krueguer

43 ***Computational synthetic biology***
YN Kaznessis

44 ***A dependency graph approach for analyzing differential gene expression data of B-cell lymphomas***
I Mühlberger, P Perco, A Bernthaler, R Fechete, A Lukas, and B Mayer

45 ***Neostasis as a therapeutically vulnerable homogeneous component of robust cancer systems***
H Warenius and L Kyritsi

46 ***Predicting targets and risk genes for long-QT syndrome side effects***
SI Berger, A Ma'ayan, and R Iyengar

47 ***CASIMIR, A coordination action focusing on database interoperability and financial sustainability; a step formward***
V Aidinis, C Chandras, M Zouberakis, and the CASIMIR consortium

48 ***Integrated dynamics of the regulatory network***

controlling cell division in Caulobacter
P Brazhnik, S Li, and JJ Tyson

49 ***A comprehensive map of molecular interactions in RB/E2F pathway***
L Calzone, A Gelay, A Zinovyev, F Radvanyi, and E Barillot

50 ***Gene expression responses to endoplasmic reticulum (ER) stress in humans***
BA Dombroski, WM Ankener, K Chapman, KG Ewens, and RS Spielman

51 ***Detection of mRNA transcripts for the ESCRT machinery in Entamoeba histolytica***
I López-Reyes, C Bañuelos-Barrón, and E Orozco

52 ***Cell networks in the pituitary structure, function, development***
M Cassou, N Courtois, N Chauvet, C Lafont, F Molino, P Mollard, J Drouin, I Robinson, and R Smallwood

53 ***Feedback regulation in gene expression and gradient sensing in the yeast pheromone mark pathway***
S Paliwal, Z Hilioti, P Iglesias, and A Levchenko

54 ***Deconvolution of functional association network modules involced in mircrobial iron homeostasis***
R Quatrini, F Lazo, C Oporto, J Ugarte, G Rivera, H Ososrio, and DS Holmes

55 ***Systems analysis of endosomal transport using phenotypic screening and genetic interactions in yeast***
NR Quenneville, M Davey, R White, K Bryan, and E Conibear

56 ***Relational genomic data analysis as an approach to study oldactory endophenotypes of schizophrenia***
L Rioux, M Levy, SJ Kanes, RW Williams, and J Nissanov

57 ***Putative metabolic regulators in nutrient signalling and adaptation: a comparative case study through gene-metabolite correlation networks for plants and humans as two evolutionary distant biological systems***
NN Rudovich and VJ Nikiforova

58 ***Mutagenic analysis of Type I restriction-modification system EcoR124I***
E Šišáková, A Guzanová, and M Weiserová

59 ***Potential role of EF-TU in regulation and cell signalling during morphological and biochemical differentiation in streptomycetes***

J Weiser, M Holub, Š Nezbedová, LD Nguyen, and S Bezoušková

60 ***Analysis of the proteomic profile modulated by the antitumoral peptide CIGB-300***
AR Ulloa, Y Perera, J Gil, Y Ramos, LC Serra, L Betancourt, A Sánchez, LJ González, V Besada, BE Acevedo, D Alonso, DE Gomez, and SE Perea

61 ***Pathways analysis of the proteins encoded by the genes protecting against Huntington's disease mutation identified in a drugable genome-wide RNA intereference screen***
E Gonzalez Couto, A Caricasole, F Heitz, GC Terstappen, and A Kremer

62 ***Cue-signal analysis of U937 cells: a model for inflammation***
CW Espelin, A Goldsipe, JS Rodriguez, P Sorger, D Lauffenburger, D de Graaf, and B Hendriks

ABSTRACTS

TRANSGENERATIONAL GENE INTERACTIONS AND SUSCEPTIBILITY TO INHERITED DISEASE

J Nadeau
Department of Genetics, Case Western Reserve University, OH, USA

Both humans and animal models provide many examples of highly heritable traits where the disease-causing genetic variants elude discovery. Two striking examples are testicular cancer and autism. In both cases heritability is high but the explained genetic variance is low. This 'missing heritability' has also been found in many other genetic diseases. The usual explanation involves undetected genetic variants with weak and heterogeneous actions in affected individuals. Although this explanation is reasonable given the limited statistical power in most genetic studies, high heritability under these conditions is less readily explained. We recently discovered three examples of transgenerational gene interactions (epistasis). In each case, we found pairs of interacting genes that act in different generations, with one of the variants acting in affected individuals and the other in the parents. Individually, these variants have modest phenotypic effects, but their interaction across generation leads to much more dramatic phenotypic outcomes. Remarkably in these cases, the genotype of the parents is a better predictor of phenotypic outcome than genotype of the affected individuals. The interacting genes encode RNAs and proteins that are involved cell signaling, stress response, RNA editing, and miRNA biology. Presumably epigenetic or cytoplasmic inheritance is also involved to account for these transgenerational effects.

PROTEIN-BASED PROFILING OF LIVER CANCER USING MULTI-LINEAR REGRESSION AND BOOLEAN MODELS

LG Alexopoulos[1,2,3], J Saez-Rodriguez[1,2], B Cosgrove[2], DA Lauffenburger[2], and PK Sorger[1,2]

[1]Harvard Medical School, Department of Systems Biology, Boston, MA, USA

[2]Massachusetts Institute of Technology, Department of Biological Engineering, Cambridge, MA, USA

[3]National Technical University of Athens, Department of Mechanical Engineering, Athens, Greece

The goal of this study was to determine cell signaling events which distinguish normal from cancer human hepatocytes. Primary human hepatocytes and 4 hepatocellular carcinoma (HCC) cell lines (HepG2, Hep3B, Huh7, and Focus) were utilized. For normal and HCC hepatocytes we created a cue-signal-response (CSR) protein-based dataset that covers a wide range of hepatocyte phenotype. The dataset is comprised of ~26,000 protein measurements under 88 different perturbations generated by orthogonal co-treatments with a diverse set of ligands and inhibitors. As pro-inflammatory stimuli we chose TNFα, IL1α, IL6, and INFγ; for innate immunity we chose LPS; for the insulin pathway we chose IGF-I; for pro-growth signals we chose TGFα, an EGFR/HER2 ligand. For each stimulus, 7 inhibitors were chosen that target 5 pathways (MEK, p38, NFκB, Akt, and JNK). For each cue+inhibitor perturbation, 17 intracellular phosphoproteins and 50 extracellular cytokines were collected at three time points (phosphoproteins: 0, early-30min, and late-3hr; cytokines: 0, early-3hr, and late-24hr). The resulting dataset ([3 time points] x [7 ligands + control] x [7 inhibitors + control] x [17 intracellular signals + 50 cytokines] x [HepG2 and normal hepatocytes]) was processed with custom made software. The dataset was created using a high-throughput method of bead-based fluorescent readings (Luminex). Assays were optimized for multiplexability and checked for donor-to-donor and preparation-to-preparation variability. The purity of the hepatocyte isolation culture was checked for contamination with nonparenchymal liver cells (NPCs) using either surface biomarkers for NPCs or monitoring responses characteristic of their presence (TNFalpha, IL1beta, IL10). Multi-linear regression (MLR) and Boolean models were used to analyze the dataset. Single step MLR model was used to link phosphoproteins to cytokine release. Two step MLR was implemented to link cues+inhibitors to signals and cytokine release. Transformation patterns among normal and cancer hepatocytes were ranked based on the differences of their correlations values. To visualize differences between cell types, MLR connectivities were overlaid on a literature-curated pathway map where the thickness of the lines correlated with the strength of the

MLR value. Boolean models were implemented in order to compare apriori knowledge of literature-derived pathways with experimentally measured data.
Our goals were two-fold: to quantify transformation patterns present in hepatocellular carcinoma cells, and to correlate those patterns to intracellular protein activities. We found that all transformed cells demonstrated an NF-κB mediated reduction of inflammatory responses, suggesting that this is a common acquired characteristic conferring them with the survival advantage of immune evasion.

DRUGGING THE CANCER EPIGENOME

Y Qiang
Group Leader, Cancer Biology and Pharmacology, Genome Institute of Singapore, Singapore

Transcriptional inactivation of tumor suppressors via aberrant epigenetic events plays an important role in cancer development. Cancer epigenetic therapy aims at reversing the epigenetic process to restore the expression of silenced tumor suppressors. Given the complexity and coordination of multiple epigenetic mechanisms in gene silencing, a drug combination approach that more effectively modulate epigenomic processes needs to be developed. We have developed a novel pharmacological approach that effectively reverses histone modifications in cancer cells by coordinately modulating both histone methylation and deacetylation. This approach is able to reactivate many potential tumor suppressors linked to the control of multiple oncogenic signaling pathways in human cancer. In particular, it abrogates the Wnt signaling pathway and induces massive apoptosis in colorectal cancer cells. Our study points to a potential novel strategy for cancer therapy by targeting multiple epigenetic processes in human cancer.

DETERMINATION OF COMPLEX REACTION MECHANISMS

J Ross
Chemistry, Stanford University, CA, USA

Several experimental and theoretical approaches will be discussed for deducing causal connectivities of species, on elementary reactions among species, on the dimensionality of the reacting system, and on the sequence of elementary reactions that constitute the reaction mechanism. One approach consists of determining correlation functions from measurements of time series of concentrations; a second approach is based on measurements of responses to external perturbations; a therd on the use of genetic algorithms to study the evolutionary development of reaction mechanisms; and a fourth on experiments and theory to determine the mechanisms of oscillatory reactions.

SIGNALING ACROSS CELL BOUNDERIES: GENETIC ANALYSIS OF THE PTENPATHWAY IN THE BREAST TUMOR MICROENVIRONMENT

MC Ostrowski, C Cantemir, A Trimboli, F Li, J Wallace, and G Leone
Departments of Molecular & Cellular Biochemistry, Molecular Virology, Immunology & Medical Genetics and Molecular Genetics
The Comprehensive Cancer Centre, Ohio State University, Columbus, OH, USA

The tumor microenvironment, and in particular tumor stromal fibroblasts, play an active role in tumor initiation, progression and metastasis. However, the genes and pathways within the tumor microenvironment critical for tumor growth and progression remain largely undetermined. The tumor suppressor *PTEN* is inactivated in the germline of Cowden's syndrome patients, and these individuals are predisposed to breast cancer. In addition, genetic analyses of sporadic human breast tumor samples have identified mutations in *PTEN* in tumor stromal fibroblasts. Together, this information leads to the hypothesis that the *PTEN* pathway within stromal fibroblasts may be critical to breast tumor progression. In order to directly test mouse *Pten* action in stromal fibroblasts, we used Cre-loxP technology to conditionally ablate *Pten* specifically in mouse mammary fibroblasts in the context of models of breast cancer. In support of this idea, *Pten* deletion in fibroblasts caused a significant increase in the progression of mammary tumors in the MMTV-her2/neu or MMTV-PyMT breast cancer models. To begin identifying the molecular mechanisms underlying the observed phenotype, we purified primary mammary fibroblasts and subjected them to global gene expression analysis. This analysis revealed that the *Pten* responsive genes in tumor fibroblasts are predominantly involved in inflammation and wound healing. The biological significance of the microarray data was confirmed by histological studies on mammary tumor sections that demonstrated an increase in both the size and composition of the extracellular matrix and a dramatic influx of immune cells, including macrophages. These changes occurred even in the absence of an oncogene in the mammary epithelial cells. Currently, we are performing microarray analysis on two additional cell compartments isolated from the mouse models, macrophages and epithelial tumor cells, in an attempt to begin defining the molecular dialogue between these different cell types during tumor progression. In parallel, bioinformatic approaches that combine imaging and molecular data are being developed that will allow us to take the position of the various cell types relative to each other into account in prioritizing specific pathways identified for further analysis.

GENETICS OF HUMAN GENE EXPRESSION

R Nayak[1,4], D Smirnov[1,5], M Morley[1], T Weber[2], RS Spielman[2], and VG Cheung[1,2,5]
Departments of Pediatrics[1] & Genetics[2], Combined-Degree Program[4], Howard Hughes Medical Institute[5], University of Pennsylvania, Philadelphia, PA, USA

Gene expression determines the function and characteristics of cells. However, it remains unknown how the expression levels of most genes are regulated. The goal of this project is to identify regulators that influence expression level of individual genes and to characterize the gene expression regulatory network in human cells. The expression levels of genes, like other quantitative phenotypes, are highly variable among individuals. Much of this variation is genetically regulated. To identify the genetic variants that influence "gene expression phenotypes", we carried out genome-wide linkage and association analyses. We used SNP genotypes and the expression levels of genes in lymphoblastoid cells from individuals in large families as phenotypes for these analyses. This allowed us to uncover the regulatory regions for ~1,400 genes in cells from normal individuals. As a complementary approach, we also studied cells from patients with ataxia telangiectasia who have mutations in their *ATM* gene, a known master regulator of gene expression, and determined the effects of mutations on gene regulation. I will present results from these studies and describe the cis- and trans-acting regulators that influence expression levels of human genes. I will also discuss the topology of the gene expression regulatory network inferred from results of these analyses.

COMPUTATIONAL ANALYSIS OF BIOLOGICAL NETWORKS

M Koyuturk[1], J Pandey[2], S Subramaniam[3], and A Grama[2]
[1]Case Western Reserve University, OH, USA
[2]Purdue University, USA
[3]University of California, San Diego, CA, USA

As data relating to biochemical pathways and networks becomes increasingly available, there is a need for computational tools and techniques for effectively analyzing and inferring from this data. Unlike algorithms on sequences, even such simple tasks as identification of conservation, differentiation, and modularity in networks have high computational cost, typically exponential in the size and number of networks, in the general case. Consequently, there is a need for utilizing biological underpinnings as well as network structure to derive efficient and effective algorithms. A related problem is the quantification of statistical significance of derived results. Analytical measures of significance, posed in the context of networks, are virtually unexplored. In this talk, we present a number of recent results on algorithms for conservation, differentiation, modularity, and functional characterization of networks. We present modeling and analytical techniques for establishing suitable optimization criteria, algorithms for optimization, and statistical techniques for quantification of significance of results. We demonstrate near real-time performance of our algorithms on large network databases, novel biological insights, and a comprehensive software environment with an intuitive user-interface.

SYSTEMS BIOLOGY OF THE CELL CYCLE

L Alberghina
Dipartimento di Biotecnologie e Bioscienze, Università di Milano-Bicocca, Milano, Italy

Systems Biology is being developed as the new paradigm to better understand complex biological processes. It relies on the integration of molecular analysis (often genome-wide) with mathematical modelling and computer analysis. The recognition of functions as system-level properties and of the robustness of the networks that underlie functions are characteristic of this type of approach.
Cell cycle is a complex biological process that needs to be better understood, both in lower eukaryotes and in mammalian cells. We have developed a modular systems biology approach to model the cycle top-down. The basic network structure of the G1 to S transition has been identified and modelled. The critical cell size to enter S phase, a relevant regulatory function of the yeast cycle, has been shown to be a system-level property of the network. Then the network of the G1 to S transition has been extended to account also for the onset of DNA replication. Since systems biology deals on how dynamic macromolecular interactions generate biological functions, it is relevant to study how the regulatory interactions present in the G1 to S network are modulated by various signalling pathways. Results are reported on the role of nutrients, and of TOR, CK2, Snf1/AMPK signalling on the dynamics of the G1 to S transition.

CORRELATION OF CHROMATIN MODIFICATION AND GENETIC REGULATION

S Jones, G Robertson, M Bilenky, A Tam, Y Zhao, T Zeng, N Thiessen, T Cezard, A Fejes, E Wederell, R Cullum, M Krzywinski, I Birol, M Hirst, P Hoodless, and M Marra

Genomes Sciences Centre and Terry Fox Laboratory, BC Cancer Agency, Vancouver, British Columbia, Canada

Using massively parallel genome sequencing approaches coupled with chromatin immuno-precipitation we have profiled histone modifications and transcription factor binding within both the adult mouse liver and the human cervical cancer derived cell-line, HeLa S3. Specifically, we have looked at the concordance of the Histone H3 lysine 4 mono-methylation (H3K4me1) with the binding of the transcription factors FOXA2 and STAT1. Previously, H3K4me1 has been determined to be associated with regions binding the transcriptional co-activator, p300 and therefore a putative marker of enhancer regions. The level of concordance was determined to be high. For 63,000 STAT1 binding sites determined in interferon gamma stimulated HeLa S3 cells, 81% were associated with H3K4me1 modifications. Similarly, 81% of the 11,000 FOXA2 binding sites determined in the adult mouse liver were found to be closely associated with H3K4me1. Conversely, of the 300,000 regions genome-wide in the HeLa S3 cell-line, associated with H3K4me1, 17% were related to STAT1 binding. This implies that if H3K4me1 is indicative of active regions of regulatory control, then the influence of the activated STAT1 transcription factor on the total repertoire of active regulatory regions is far higher than may have been previously anticipated. We have also determined a spatial pattern of association with H3K4me1 modification, whereby transcription factor binding is flanked by H3K4me1, preferentially modified histones lying 250 base pairs either side of the binding site. This work provides evidence that H3K4me1 represents a robust indicator of active regulatory elements, both distal and proximal to promoters and therefore establishes a framework for the identification of all active regulatory elements within a given cell type.

SPATIAL AND TEMPORAL CONTROL OF MAPK SIGNALING DURING PERIODIC MORPHOGENESIS

Z Hilioti[1], W Sabbagh, Jr[2], S Paliwal[1], A Bergmann[1], M Goncalves[1], L Bardwell[23], and A Levchenko[1]

[1]Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD, USA

[2]Department of Developmental and Cell Biology,

[3]Institute for Genomics and Bioinformatics, University of California, Irvine, CA, USA

Cell shape change is the result of complex interactions, between several highly regulated signaling molecules, motor proteins and actin cytoskeleton, occurring at the right time and place. The mating pheromone-induced MAPK signaling regulates the formation of mating projections during the mating response in budding yeast. Activation of the MAPK Fus3 induces mating-specific gene expression which then drives cytoskeleton remodeling. Interestingly, prolonged stimulation of yeast in a uniform field of pheromone leads to periodic formation of mating projections but the mechanism that regulates this response remains unknown. We sought to investigate and characterize the molecular mechanism by which cells change shapes periodically in response to constitutive mating pheromone signals. In doing so, we studied the spatio-temporal aspects of MAPK Fus3 signaling and the mechanisms that regulate it. We also relate the cell signaling dynamics to actin cytoskeleton dynamics and cell shape changes and reveal how the MAPK Fus3 signaling regulates gene expression and how it is regulated by positive and negative feedbacks including ones arising from the cytoskeleton. Genetic and chemical perturbations of the system are used to understand the relationship of cell signaling to actin cytoskeleton and cell shape change. These findings have important implications for control of cell shape in all eukaryotic cells and may potentially accelerate the discovery phase for therapeutic targets in human diseases.

A GENE EXPRESSION NETWORK MODEL OF TYPE 2 DIABETES ESTABLISHES A RELATIONSHIP BETWEEN CELL CYCLE REGULATION IN ISLETS AND DIABETES SUSCEPTIBILITY

MP Keller, YJ Choi, P Wang, DB Davis, ME Rabaglia, AT Oler, DS Stapleton, C Argmann, KL Schueler, S Edwards, HA Steinberg, EC Neto, R Klienhanz, S Turner, MK Hellerstein, EE Schadt, BS Yandell, C Kendziorski, and AD Attie
Depts. of Biochemistry, Biostatistics & Medical Informatics, University of Wisconsin, Madison, WI; Rosetta Inpharmatics, Seattle, WA; KineMed, Emeryville, CA; Dept Nutritional Sciences & Toxicology, University of California, Berkeley, CA, USA

Insulin resistance is necessary but not sufficient for the development of type 2 diabetes. Diabetes results when pancreatic β-cells fail to compensate for insulin resistance by increasing insulin production through an expansion of β-cell mass or increased insulin secretion. Communication between insulin target tissues and β-cells may initiate this compensatory response. Correlated changes in gene expression between tissues can provide evidence for such intercellular communication. We profiled gene expression in six tissues (islet, liver, white adipose, soleus, gastrocnemius and hypothalamus) of mice from an obesity-induced diabetes-resistant (B6) and a diabetes-susceptible (Btbr) mouse strain before (4 wk) and after (10 wk) the onset of diabetes. We examined the correlation structure of mRNA abundance and identified 105 co-expression gene modules, each populated with transcripts showing a highly correlated expression pattern. Cell cycle regulatory modules were identified in all tissues except hypothalamus. In islets and adipose the expression of the genes contained within these modules predicted diabetes susceptibility and obesity, respectively. The islet module predicts islet cellular proliferation; we found a strong correlation between $^{2}H_2O$ incorporation into islet DNA *in vivo* and the expression pattern of genes within the module. The islet cell cycle expression pattern is highly correlated with the expression of several individual genes in insulin target tissues, including *Igf2*, which has been shown to promote β-cell proliferation, suggesting that these genes may provide a link between insulin resistance and β-cell proliferation. To link genetic variance to the regulation of gene expression within the co-expression modules, we generated a segregating cross of 500 B6/Btbr-F2 mice and profiled gene expression in tissues listed above. The results demonstrate that not only do co-expression modules persist in F2 mice, the expression of transcripts within a module strongly map to discrete genomic regions. Causal networks linking genetic variation to changes in gene expression and how each relates to the physiology of diabetes will be presented.

METABOLOMIC: A GLOBAL BIOCHEMICAL APPROACH TO THE STUDY OF HUMAN DISEASE AND DRUG EFFECTS

R Kaddurah-Daouk
Duke University Medical Center, Department of Psychiatry and Behavioral Sciences, Durham, NC, USA

Metabolomics is the comprehensive study of the metabolome, the repertoire of metabolites or small molecules present in cells, tissue and body fluid. The identities, concentrations and fluxes of these metabolites represent the final product of interactions that extend from gene sequence to include gene expression, protein expression and the total cellular environment, an "environment" that in the clinical setting includes drug exposure. Metabolomics has been identified as an important area for development under the NIH Roadmap Initiative. We have developed and used sophisticated metabolomics analytical platforms and informatics tools to define initial metabolic signatures for several central nervous system (CNS) disorders and for response to drugs used to treat these disorders. We will share our experience and early findings from the study of several neurodegenerative and psychiatric disorders. In depression using a gas chromatography-mass spectrometry (GC-MS) based metabolomics platform we find that the depressed state may be associated with alterations in the metabolism of lipids and neurotransmitters, and that treatment with antidepressants adjusts some of the aberrant pathways in disease so that the patients in remission have a metabolic profile more similar to controls than to the depressed population. Using a targeted "lipidomics" platform we mapped global lipid changes in schizophrenia and upon treatment with three antipsychotics demonstrating that schizophrenia is associated with impairments in key phospholipids which are critical for neuronal membrane structure and function.. The abundance of information generated by these high-throughput technologies can enable the exploration of human metabolism as a complex system. The completion of the first genome-scale human metabolic network, Recon 1, is a key tool that will allow systematic analyses of these large data sets to generate biologically meaningful interpretations.

INTEGRATING GENE EXPRESSION, SEQUENCE AND PROTEOMICS DATA TO IDENTIFY DISEASE BIOMARKERS

S Horvath, JE Aten, and AJ Lusis
Human Genetics and Biostatistics
David Geffen School of Medicine and School of Public Health
University of California, Los Angeles, CA, USA

Weighted gene co-expression network analysis (WGCNA) is a systems biologic framework for integrating gene expression, sequence and proteomics data. Here we will describe how WGCNA facilitates the identification of disease pathways and master regulatory genes that control these disease pathways. Systems genetic approaches that incorporate gene expression, clinical outcomes (traits), and sequence data are valuable for identifying the causal drivers of clinical traits. While the pair-wise correlations between gene expression traits and/or clinical traits has been used to define undirected co-expression, genetic markers (and sequence information) can serve as causal anchors for orienting the edges of an undirected network. The availability of hundreds of thousands of genetic markers poses new challenges: how to relate (anchor) traits to multiple genetic markers, how to score the genetic evidence in favor of an edge orientation, and how to weigh the information from multiple markers. We describe and generalize the Network Edge Orienting (NEO) method for inferring directed weighted gene co-expression networks. The networks are oriented by considering each edge separately, thus reducing error propagation. To summarize the genetic evidence in favor of a given edge orientation, we propose several edge orienting scores that compare the fit of several competing causal graphs. Structural equation model fitting indices allow the user to assess local and overall model fit. We illustrate the use of these systems biologic gene screening methods by applying them to mouse cross data and to human cancer data.

INSULIN SIGNALING, ADIPOSE TISSUE AND METABOLIC SYNDROME

CR Kahn
Joslin Diabetes Center and Harvard Medical School, Boston, MA, USA

Following the activation of the insulin receptor kinase, multiple intracellular substrates and signaling proteins form a diverging pathway of signaling. As a result of this combinatorial signaling, there are over 1800 potential pathways of insulin action on metabolism. Three critical questions are to what extent are these pathways redundant versus complementary, which pathways are altered in insulin resistant states such as diabetes, and by what mechanisms are these pathways altered. By knocking-out and knocking-down specific components of the signaling systems in cells and tissues of intact animals, and comparing these knockouts/knockdowns to other insulin resistant or diabetic states, we have attempted to define the specific pathways that lead to each insulin action and determine how they are modified in disease states. In addition, we have determined the effects of these modifications on the pattern of gene expression in each tissue, and how insulin signaling might interact with nuclear receptor signaling in insulin resistance. Tissues of particular importance in development of type 2 diabetes and the metabolic syndrome include the liver, brain and fat. In liver, for example, insulin action through IRS-1 and Akt is involved in control of glucose production, while insulin action through IRS-2 and atypical PKCs is more involved in hepatic lipogenesis. In global insulin resistance, such as observed in the liver-specific insulin receptor knockout (LIRKO) mouse, there is a resistance in both of these pathways leading to hyperglycemia and dyslipidemia, which in the presence of high fat diet can lead to accelerated atherosclerosis. LIRKO mice are also predisposed towards cholesterol gallstone formation, and this involves and interaction between insulin signaling via FOXO1 and altered signaling through FXR created by the insulin resistant state. Insulin signaling in adipose tissue also interacts with developmental genes, such as Hox genes, and nuclear receptor signaling in control of adipose patterning and creation of insulin resistance. This is further modified by protein deacetylases of the sirtuin family. Thus, there is a complex relationship between insulin signaling, adipose tissue and insulin resistance that impacts on many aspects of the development of metabolic syndrome and its ultimate treatment.

THE GENETIC ARCHITECTURE OF DIET-INDUCED OBESITY

DA Buchner[1], LC Burrage, AE Hill[1], EJ Vazquez[2], M Rosca[2], P Solinas[2], S Yazbek[1], H Shao[1], ES Lander[3], CL Hoppel[2], and JH Nadeau[1]
[1]Department of Genetics, [2]Department of Pharmacology, Case Western Reserve University, Cleveland, OH, USA
[3]Broad Institute of MIT and Harvard, Cambridge, MA, USA

Little is known about the genetic basis for diet-induced obesity despite its high heritability. As with many other complex genetic traits, it is thought that a number of genes each contribute a relatively small amount to the overall phenotype. To study the genetic basis of diet-induced obesity, our laboratory has generated a panel of chromosome substitution strains derived from the C57BL/6J and A/J inbred mouse strains. Each strain has a single A/J chromosome on an otherwise entirely C57BL/6J background. We have identified at least 13 A/J derived chromosomes (QTLs) that confer resistance to diet-induced obesity. These QTLs accounted for an average of 53% (for a total of ~700%) of the phenotypic difference between the parental strains, suggesting that there are many large-effect QTLs with evidence for widespread epistatic interactions among them. We have further dissected the architecture of a single chromosome, chromosome 6, by congenic and subcongenic analysis and found a similar pattern of numerous large-effect QTLs that are highly dependent on genetic background. These data suggest that the underlying genetic architecture of diet-induced obesity is dominated by genes with large and non-additive effects rather than the summation of many small effects.

To better understanding the physiological mechanisms underlying the obesity resistance of each strain we sought a more detailed phenotypic analysis. We have completed a survey of traits related to obesity and metabolic syndrome in mice with a single substituted chromosome and in congenic strains derived from the substituted strain. Each obesity-resistant strain analyzed had a distinct phenotype that uniquely modeled different combinations of traits related to metabolic disease. Further characterization of selected congenic strains by genome-wide expression profiling and computational pathway analysis has revealed altered expression of genes in the oxidative phosphorylation pathway that are associated with obesity-resistance. Subsequent studies of *in vitro* mitochondrial function have identified corresponding alterations in mitochondrial structure and function. Understanding the genotype-phenotype relationships of these QTLs may be of great clinical interest given their significant effect on body weight.

LIPID MAPS APPROACH TO LIPIDOMICS

EA Dennis
Department of Chemistry and Biochemistry and Department of Pharmacology, School of Medicine, University of California at San Diego, La Jolla, CA, USA

As part of the LIPID MAPS Consortium (www.lipidmaps.com) [Schmelzer *et al.* (2007) Lipidomics *Methods in Enzymology,* ***432,*** 169-181], we have developed a robust and comprehensive approach to lipidomics analysis [Raetz *et al.* (2006) *J. Lipid Research,* ***47***, 1097-111] of hundreds of fatty acids and eicosanoids, including their numerous metabolites arising from an array of cyclloxygenases, lipoxygenases, cytochrome P450s and non-enzymatic oxidation producing isoprostanes, as well as combinations thereof. The LC/MS approach to eicosanoid analysis [Deems *et al*. (2007) Lipidomics *Methods in Enzymology*, ***432***, 59-82] and GC/MS approach to fatty acid analysis will be summarized as well as the development of standards which facilitates widespread application of the LIPID MAPS approach. We will illustrate the use of these techniques in lipidomics analysis to: a) Discover novel lipids such as the family of dihomoprostaglandins [Harkewicz *et al.* (2007) *J. Biol. Chem,* ***282,*** 2899-2910]; and b) The application to characterize agonist stimulated RAW264.7 murine macrophages. Fifteen specific eicosanoids produced through COX and 5-LO were detected either intracellularly or in the media following stimulation with 16 different agonists including Toll-like receptors (TLR), G protein-coupled receptors, purinergic receptors and combinations thereof. Synergy between Ca^{2+} release and TLR pathways was detected and discovered to be independent of NF-κB induced protein synthesis using lipidomics analysis [Buczynski *et al.* (2007) *J. Biol. Chem*, ***282***, 22834-22847]; and c) We will describe our efforts to integrate the eicosanoid lipidomics results of the other LIPID MAPS lipidomics cores and the genomics core so as to develop a comprehensive picture of the macrophage lipidome. Supported by LIPID MAPS Glue Grant NIH U54 GM069338.

MULTI-TISSUE PROFILING OF HUMAN INSULIN RESISTANCE AND PHARMACOLOGICAL INSULIN SENSITIZATION

*G Hsiao[1], A Hsiao[1], J Chapman[2], PD Miles[1], JG Yu[1], CH Courtney[1], J Neels[1], JL Resnik[1], J Wilkes[1], D Thapar[1], JM Ofrecio[1], S Subramaniam[1], and DD Sears[1]**

[1]University of California, San Diego, La Jolla, CA, USA

[2]Pfizer, Inc, La Jolla, CA, USA

Cellular and tissue defects associated with insulin resistance are coincident with transcriptional abnormalities and are improved after insulin sensitization with thiazolidinedione (TZD) PPARγ ligands. We characterized 72 human subjects by relating their clinical phenotypes with functional pathway alterations. We transcriptionally profiled 364 biopsies harvested before and after hyperinsulinemic-euglycemic clamp studies, at baseline and after 3-month TZD treatment. Insulin resistant subjects exhibited alterations in skeletal muscle glycolytic flux and intra-muscular adipocytes and alterations in adipose tissue inflammation and mitochondrial metabolism. We identified molecular and functional distinctions between TZD treatment responders and non-responder subjects. For example, pre-TZD treatment expression of MLXIP in muscle and HLA-DRB1 in adipose tissue from insulin resistant subjects was linearly predictive of post-TZD insulin sensitization. Osteopontin (OPN) is one of the basally over-expressed, TZD-repressed inflammatory markers we identified in adipose tissue from insulin resistant humans. Using a mouse model, we studied the role of OPN in the early development of insulin resistance. OPN knockout (KO) mice were completely protected from the 2-week high fat diet (HFD)-induced insulin resistance that we observed in wild type (WT) mice. HFD-induced hyperinsulinemia, hyperleptinemia, and increased adipose tissue cytokines were blunted or absent in OPN KO vs. WT mice, consistent with their protection from insulin resistance. Tissue profiling enabled our identification of OPN as a key component of diet-induced insulin resistance. Overall, we have uniquely characterized coordinated cellular and tissue functional pathways that are characteristic of insulin resistance, TZD-induced insulin sensitization, and potential TZD responsiveness.

Support: NIH NIDDK K01-DK62025 (DDS), NIH R01-DK033651 (JMO, JGY, CHC), and the University of California Discovery Program Project #bio03-10383 with matching funds from Pfizer, Inc. (DDS, SS).

A MODULARIZED MODEL OF APOPTOSIS

HA Harrington, KHo, Sk Ghosh, KC Tung , CY Kao, and B Aguda
Imperial College London, Courant Institute of Mathematical Sciences New York University, University of Texas at Arlington, University of Texas Southwestern Medical Center, Mathematical Biosciences Institute, and Department of Mathematics, The Ohio State University Columbus, OH, USA

Background: One of the key physiological mechanisms employed by the cell (during development and for maintenance of homeostasis) in multi-cellular organism is apoptosis, which is characterized by a sequence of well-defined events resulting in cell destruction. Dysregulation of apoptosis is responsible for many physiological health problems and diseases; therefore, it is necessary to understand the responsible signaling pathways and complex interplay of cellularprocesses. **Results:** A combined mathematical model of apoptosis integrating both the extrinsic (type I cells) and intrinsic (type II cells) pathways is constructed through the linking of component modules from the literature. Due to the nature and complexity of these pathways, the combined model is developed using reaction kinetics to capture the molecular interactions (crosstalk between modules, regulation and feedback mechanisms). A system of ordinary differential equations which describe the molecular dynamics controlling cell death is implemented numerically which allows analytical and numerical analysis of the network structure to be conducted. Simulation results are used to investigate the role of each module in triggering apoptosis, and hypotheses of caspase activation are generated. Parameter space explorations demonstrate a potential transition from type I to type II cells based on minor adjustments of two key parameters. **Conclusions:** The model characterizes and provides insight to the role of different modules and predicts the critical variables that lead to a definitive point of no return (where apoptosis is irreversible). In addition, the model motivates future work in characterizing remaining free parameters, and identifies possible experiments to test generated hypotheses and hence further the understanding of apoptosis.

ABSTRACT 19

NETWORK ANALYSIS IDENTIFIES AFFECTED BIOLOGICAL PROCESSES IN TYPE 2 DIABETES MODELS

M Liu[1], A Liberzon[1], SW Kong[2,3], WR Lai[4], PJ Park[3,4], IS Kohane[3,5], and S Kasif[1]

[1]Department of Biomedical Engineering, Boston University, MA, USA
[2]Department of Cardiology, Children's Hospital, Boston, MA, USA
[3]Children's Hospital Informatics Program at the Harvard-MIT Division of Health Sciences and Technology, MA, USA
[4]Harvard-Partners Center for Genetics and Genomics, Boston, MA, USA
[5]Center of Biomedical Informatics, Harvard Medical Schoo, MA, USAl

Impaired insulin signaling is a common feature of type 2 diabetes. It is therefore surprising that the standard hypergeometric enrichment test fails to detect transcriptional dysregulation of this process in a large number of type 2 diabetes and impaired metabolism models. Conversely, a new analysis approach, called Gene Network Enrichment Analysis (GNEA), successfully detects dysregulated insulin signaling, along with nuclear receptor signaling and inflammatory markers, in these same models. In particular, GNEA identifies dysregulated genes such as MAP kinases, STATs, and RAF1 that belong to the cell growth and proliferation branch of insulin signaling. Moreover, it detects various nuclear receptors such as the glucocorticoid receptor and retinoid x receptors that are associated with altered metabolism in addition to known diabetogenes (HNF4A and PPARG). Finally, it detects inflammation in the form of dysregulated interleukins, including IL-6 signaling. GNEA works by combining gene expression data together with a protein-protein interaction network. A maximally dysregulated, connected component is then identified and subsequently tested for over-represented biological processes. The positive results in applying the method to type 2 diabetes mellitus suggest it may hold promise as a useful research tool for complex diseases. Further details on the methodology is available from the following paper: *Liu M, Liberzon A, Kong SW, Lai WR, Park PJ et al (2007) Network-based analysis of affected biological processes in type 2 diabetes models. PLoS Genet 3(6):e96. doi:10.1371/journal.pgen.0030096.*

DGAT1: A DIET-REGULATED QUANTITATIVE TRAIT LOCUS THAT AFFECTS HEPATIC LIPID DEPOSITION

R Oliveira[1], JJ Loor[1], NA Janovick Guretzky[1], JK Drackley[1], and HA Lewin[1,2]
[1]Department of Animal Sciences, University of Illinois, Urbana, IL, USA
[2]Institute for Genomic Biology, University of Illinois, Urbana, IL, USA

Acyl-CoA:diacylglycerol acyltransferase1 (DGAT1) plays an essential role in hepatic triacylglycerol (TAG) metabolism and also the development of nonalcoholic fatty liver disease in humans and mice. In lactating dairy cows, negative energy balance and overfeeding during the prepartum period cause increased accumulation of hepatic TAG and the incidence of ketosis and fatty liver disease during the peripartal period. In the present study, dietary energy intake and genotype for the polymorphism causing a lysine (K) to alanine (A) substitution at codon 232 in the *DGAT1* gene were evaluated for their effects on transcript levels of *DGAT1* and accumulation of TAG and total lipid in liver. This polymorphism has been shown previously to behave as a quantitative trait locus (QTL), being strongly associated with differences in milk and fat yields in dairy cows. Thirty-five cows fed *ad libitum* or restrictively during the peripartum period were genotyped for the *DGAT1* K232A mutation. *DGAT1* mRNA levels, TAG, and total lipid were determined in biopsied liver samples collected at -14, +1 and +14 days relative to parturition. The TAG and lipid concentrations were significantly higher at day +14 in *ad libitum*-fed cows having one or two copies of the *DGAT1 K* allele but not in cows fed the restricted diet. *DGAT1* genotype was shown to affect *DGAT1* mRNA levels ($P < 0.0001$), and an effect of diet on *DGAT1* mRNA levels was found at day +1, thus suggesting a causal effect on liver TAG and lipid accumulation in overfed animals. Our data indicate that nutritional management of animals having the *DGAT1 K* allele can be used to eliminate economic losses due to metabolic disorders that occur as a result of prepartum energy overfeeding. Furthermore, the diet-regulated *DGAT1* QTL represents a unique model system for the study of diseases in which lipid metabolism may play an important role.

THE SYSTEMS BIOLOGY CYCLE FOR PATHWAYS AND NETWORKS: MODELING AND EXPERIMENTATION

*MR Maurya, S Bornheimer, M Farquhar, and S Subramaniam**
University of California at San Diego, La Jolla, CA, USA

The 'state' of a cell is defined by its components – their concentrations and locations, the interactions between components – that are modulated in space and time, and the complex circuitry, that involves a large number of interacting networks. The state represents a snapshot of the dynamical processes – such as gene expression, cell cycle, transport of components, etc, that characterize the cell function. Advances in high-throughput genomic, metabolomic and proteomic technologies now allow the study of the cellular components and their interactions in a quantitative manner. These technologies are aiding in the development of predictive models by combining legacy knowledge and novel data. There are two paradigms in computational systems biology: (1) the iterative cycle of biochemical model – mathematical model – computational model, and (2) integration of novel data and legacy knowledge to develop context specific biochemical, mathematical and computational models. This talk will review the challenges in developing such models through well-characterized exemplar problems in biology. Challenges in building biochemical models include (1) the complexity of proteomic states and interactions, (2) integration of diverse data to infer biochemical interactions, and (3) temporal state of biochemical models. Challenges in building mathematical models include (1) incorporating statistical/probabilistic information into analytical models, (2) utilizing qualitative constraints into mathematical models, and (3) incomplete knowledge and coarse-graining. Challenges in computational modeling are: (1) the absence of knowledge about model parameters such as rate constants, (2) local versus global concentrations of species and multiple length and time-scales, and (3) variation among different cell-types and sub-populational variability or variability among biological repeats. The examples that will illustrate the cycle of modeling and experiment will include the ubiquitous G-protein receptor coupled pathway and the phenotypically significant calcium signaling pathway in mammalian cells. Novel mechanistic insights gained from the model and experimental work that both aids developing and validating the models will be presented.

Supported by funds from the National Institutes of Health and National Science Foundation (SS).

TRANSCRIPTION FACTOR -MEDIATED GLOBAL EPIGENETIC REMODELING TO INDUCED PLURIPOTENT STEM CELLS

K Plath[1], R Sridharan[1], J Tchieu[1], MJ Mason[1], Q Zhou[1], W Lowry[1], N Maherali[2], and K Hochedlinger[2]
[1]University of California at Los Angeles, CA, USA
[2]Harvard University, Boston, MA, USA

Ectopic expression of the four transcription factors Oct4, Sox2, cMyc, and Klf4 is sufficient to confer a pluripotent state to the human or murine somatic cell genome, generating induced pluripotent stem (iPS) cells. iPS cell lines are morphologically and functionally very similar to embryonic stem (ES) cells derived from the inner cell mass of pre-implantation embryos, and can be induced to differentiate into the three embryonic germ layers indicating pluripotency. Nuclear reprogramming by these four factors globally resets the epigenetic and transcriptional programs of the differentiated cell to the pluripotent ES cell state. How the four factors induce global epigenetic remodeling has remained unclear. Our data suggest that all four transcription factors are central regulators of the transcriptional network that specifies ES cell identity.

LOCALIZATION OF TRANSCRIPTION FACTORS IN EMBRYONIC STEM CELLS

X Chen, H Xu, P Yuan, F Fang, M Huss, V Vega, E Wong, Y Orlov, W Zhang, J Jiang, YH Loh, HC Yeo, ZX Yeo, V Narang, K Govindarajan, B Leong, A Shahab, Y Ruan, G Bourque, WK Sung, N Clarke, CL Wei, and HH Ng
Genome Institute of Singapore, Singapore

Transcription factors and their specific interactions with targets are crucial in specifying gene expression programmes. To gain insights into the transcriptional regulatory networks in embryonic stem cells, we use chromatin immunoprecipitation coupled to ultra-high-throughput DNA sequencing (ChIP-seq) to map the locations of multiple sequence specific transcription factors. These factors are known to play different roles in ES cell biology as components of signaling pathways, self-renewal regulators and key reprogramming factors. Our study provides new insights into the integration of the signaling pathways to the ES cell-specific transcription circuitries. Intriguingly, we find specific genomic regions extensively targeted by different transcription factors and these multiple transcription factors generally exhibit two major mode of targeting in ES cells. Collectively, the comprehensive mapping of transcription factor binding sites identifies new features of the transcriptional regulatory networks that define ES cell identity.

ABSTRACT 24

NHLBI PROGRAMS AND RESOURCES IN GENOMICS, PROTEOMICS & OTHER EMERGING AREAS

P Qasba
NIH/NHLBI, MD, USA

This is a presentation that high-lights the recent programs that NHLBI is administering or plans to put forward and that can of interest to the audience. In this case a very select audience with interest in Systems Medicine. I will emphasize our experience with our NHLBI Exploratory Programs in Systems Biology in addition to other genomics and proteomics programs.

A COMPLEX SYSTEMS APPROACH TO MODELLING STEM CELL DECISION-MAKING

JD Halley[1], DA Winkler[1,2], and FR Burden[1,2,3]
[1]CSIRO Molecular and Health Technologies, Private Bag 10, Clayton South MDC 3169, Australia
[2]Monash University, School of Chemistry, Australia
[3]SciMetrics, Victoria, Australia

Despite intense experimental and theoretical research, the ways in which stem cells integrate and process information remains unclear, limiting our ability to manipulate their behaviour. Elegant conceptual frameworks that integrate key components of the stem cell decision-making process are needed to organize increasing data accumulation. We describe such a conceptual model that decomposes the complexity of stem cell decision-making into three layers, using complex systems theory to find an appropriate level of scale. We show how each layer of complexity adds a vital component to the decision-making machinery, allowing stem cells to solve complex problems concerning lineage-specification in a robust and flexible manner. We describe how modules of lineage-specific gene activity are poised in metastable states, where interplay between them, together with external factors, governs whether a stem cell differentiates, self-renews or dies. Decomposition of stem cell complexity into three layers reflects our previous research, which suggests that biological systems necessarily reflect order generated by both natural selection and non-equilibrium sources, particularly self-organization.

A FRAMEWORK FOR COMPUTATIONAL PHYSIOLOGY

PJ Hunter
Bioengineering Institute, University of Auckland, New Zealand

The Physiome Project of the International Union of Physiological Sciences (IUPS) is attempting to provide a comprehensive framework for modelling the human body using computational methods which can incorporate the biochemistry, biophysics and anatomy of cells, tissues and organs. A major goal of the project is to use computational modelling to analyse integrative biological function in terms of underlying structure and molecular mechanisms. A newly formed EU Network of Excellence for the Virtual Physiological Human (VPH) is also contributing and, in particular, addressing clinical applications of the project.

To facilitate model reuse among researchers in computational physiology, two XML markup languages for encoding biological models, CellML (www.cellml.org) & FieldML (www.fieldml.org), are being developed. CellML deals with models of so-called 'lumped parameter' systems, where spatial effects are averaged, and typically involves systems of ordinary differential equations and algebraic equations. FieldML addresses the spatial variations in cell or tissue properties where the models typically rely on partial differential equations. The two standards can be used together. These languages, which define the structure of a model, the mathematical equations and the associated metadata, enable (i) automated checking to ensure consistency of physical units used in the model equations, (ii) models developed by different groups to be combined using commonly agreed ontological terms within the metadata, (iii) models to be modularized and used in libraries to make it easier to create complex models by importing simpler ones. Model repositories based on these standards and implementing a wide variety of models from peer-reviewed publications have been developed (www.cellml.org/models) and open source software tools for creating, visualizing and executing these models are currently available (www.cellml.org/tools) and under continuous development.

This framework and its application to modeling the heart and other organs will be presented.

SYSTEMS BIOLOGY OF KIDNEY DEVELOPMENT

S Nigam
University of California, San Diego, CA, USA

The problem of development of organs like the mammalian kidney is a complex multilayered process well suited to a "systems approach." The kidney plays a key role in homeostatic functions of the body, including the regulation of water balance, blood electrolyte composition and pH. It is also the major route of elimination of many small soluble drugs and toxins. The functional unit of the kidney is the nephron, and in humans there are approximately one million nephrons. Kidney development is an extremely complex multistage process, leading to an organ with two dozen cell types and a complicated three dimensional structure. We have been able to separate many of these processes into in vitro modules, each of which can be interrogated for growth factor and matrix dependence. These modules can be recombined to reform kidney-like tissue which possesses functional characteristics and can also be implanted and partly vascularized. We have performed time series analysis of global gene expression patterns during kidney development and in most of these modules. Current work is focused on computational approaches to identify pathways that regulate the stages of kidney development and on identification of key genes necessary for morphogenesis in each of the modules which, together, can be recombined to form kidney-like tissue.

LEARNING GLOBAL TRANSCRIPTIONAL DYNAMICS WITH THE INFERELATOR AND CMONKEY

A Madar, P Waltman, T Kacmarczyk, V Thorsson, N Baliga, D Reiss, and R Bonneau
New York University, NY, USA

Our system for network inference and modeling consists of two major components: cMonkey and the Inferelator. We describe their integration into a functioning integrated system applied to several prokaryotic organisms.
cMonkey groups genes and conditions into biclusters on the basis of 1) coherence in expression data across subsets of experimental conditions, 2) co-occurrence of putative *cis*-acting regulatory motifs in the regulatory regions of bicluster members and 3) the presence of highly connected sub-graphs in metabolic and functional association networks. We describe the algorithm and the results of extensive tests of several previously described methods, showing that cMonkey has several advantages in the context of regulatory network inference. We will discuss the latest version of the code, which allows for comparative biclustering across multiple species. We will discuss our application of the multi-species code to: 1. A comparative analysis of Human prostate cancer and mouse models for prostate cancer and 2. Integrative biclustering of bacterial groups including pathogen-model pairs. **The Inferelator** is a network inference algorithm that infers regulatory influences for genes and/or gene clusters from mRNA and/or protein expression levels. The procedure can simultaneously model equilibrium and time-course expression levels, such that both kinetic and equilibrium expression levels may be predicted by the resulting models. Through the explicit inclusion of time, and gene-knockout information, the method is capable of learning causal relationships. It also includes a novel solution to the problem of encoding interactions between predictors. We will describe our latest developments that combine methods for simulating and learning explicit global regulatory dynamics (Inferelator 2.0).
For Background on our integrated system see:
http://www.cell.com/content/article/abstract?uid=PIIS009286740701416X
http://genomebiology.com/2006/7/5/R36
http://www.biomedcentral.com/1471-2105/7/280

TRANSCRIPTIONAL NETWORKS IN DEVELOPMENT AND DISEASE

KP White
The University of Chicago Biological Sciences Division and Pritzker School of Medicine, Chicago, IL USA

My research interests are focused on transcriptional network analysis in development, evolution and disease. In the past my research has included the development and application of the first DNA microarrays to study a developmental model organism (Drosophila), the analysis of gene expression evolution in both Drosophila and primates, and the first use of tiling microarrays in Drosophila to map transcription factor binding sites genome-wide. Much of my lab's recent work has involved integrating diverse datasets to build predictive models of transcriptional networks important for human disease processes. This work has recently led to new biomarkers for two different cancers (kidney cancer and breast cancer metastasis). Our recent work has focused on developing a systematic map of nuclear receptor networks in human cells.

DISEASE ASSOCIATED TRANSCRIPTION FACTOR MODULES

M Wiesinger[1], Paul Perco[2], J Söllner[2], A Lukas[2], and B Mayer[2,3]
[1]Institute for Computer Languages, Theory and Logic Group, Vienna University of Technology
Favoritenstrasse 9,A-1040 Vienna, Austria
[2]emergentec biodevelopment GmbH
Rathausstrasse 5/3,A-1010 Vienna, Austria
[3]Institute for Theoretical Chemistry, University of Vienna, Währinger Strasse 17,A-1090 Vienna,Austria

Background: Understanding the regulatory mechanisms of transcription is an essential component for interpreting differential gene expression profiles aimed at analyzing the pathophysiology of diseases on the molecular level.
We have implemented a computational screening procedure for annotating transcription factor binding sites (TFBS) for all protein coding genes of the human genome, and analyzed their combinatorial association in the context of disease-associated genes. **Methods:** We included all presently annotated protein coding genes as given in the ENSEMBL database release 48, providing about 32.000 genes. For this sequence set we selected +/-5kb around the transcription start site for subsequent analysis. Sequences used for motif scanning were first restricted utilizing a phylogenetic footprinting approach using human-mouse and human-chimpanzee sequence orthologs. Computational screening for TFBS was based on binding matrices resembling a non-redundant motif database including in total 270 TRANSFAC and JASPAR binding matrices. Resulting gene-associated TFBS were further curated involving known experimental data and transcription factor-specific matrix scoring cutoff values. **Results:** We generated a list of disease associated genes by aligning OMIM gene lists (Online Mendelian Inheritance in Man) with the WHO ICD-10 disease categories. Subsequently, disease-associated transcription factor combinations were searched using a genetic algorithm. We present statistics on the distribution of transcription factor combinations in the context of given disease categories, discuss particular motif combinations in the context of cancer, and provide an outlook for using transcription factor modules for searching novel, disease associated genes.

EVASION STRATEGIES OF MICROORGANISMS

John D. Lambris
University of Pennsylvania, Dept. of Path. & lab. Medicine, Philadelphia, USA

CORE DIVERSITY PROFILE OF HUMAN SKIN MICROBIOME IN HEALTH AND DISEASE

EA Grice[1], TC Scharschmidt[1], HH Kong[2], AC Young[4], NISC Comparative Sequencing Program[3,4], S Conlan[1], RW Blakesley[3,4], GG Bouffard[3,4], ML Turner[2], ED Green[3,4], and JA Segre[1, 4]
[1]Genetics and Molecular Biology Branch, NHGRI, NIH;
[2]Dermatology Branch, NCI, NIH;
[3]Genome Technology Branch
[4]NIH Intramural Sequencing Center (NISC), NHGRI, NIH

The recently launched NIH Medical Roadmap Human Microbiome Project aims to comprehensively study aggregate microbial communities in and on the human body with contemporary metagenomic approaches. The skin is a powerful system for microbial studies because of the ease of obtaining multiple samples from diverse niches (moist, dry, sweaty, oily, hairy), that then enable an analysis of both intrapersonal and interpersonal variation. Moreover, many dermatologic disorders are associated with specific microbial infections and affect very specific sub-sites; e.g. the common inflammatory skin disorder atopic dermatitis (AD, eczema) manifests at the antecubital fossa (inner elbow) and is associated with *Stapholococcus aureus* infection. Our initial studies have used direct sequencing and bioinformatic analyses to characterize microbial changes associated with AD. Our analysis of the human antecubital fossa microbiota demonstrated predominance of the genera *Janthinobacteria* and *Pseudomonas* (both *Proteobacteria* division), with less representation from five other bacterial divisions. Our genetically-altered animal model of AD showed a selective shift in the skin microbiota, with constant levels of *Janthinobacteria*, a decrease of the dominant *Pseudomonas*, and an accompanying increase of *Corynebacterium* and *Streptococcus*. We are currently ascertaining samples from ~20 skin sub-sites from healthy humans to comprehensively survey the resident microbiota and to address the fundamental question of whether there is a baseline cutaneous microbiome. These data will provide a foundation for future studies of disease states resulting from underlying gene/environment interactions and ultimately to advance pharmacological treatments of skin disorders.

MICROBIAL COMMUNITY PROTEOGENOMICS

J Banfield, V Denef, N VerBerkmoes, P Wilmes, G Dick, C Belnap, D Goltsman, R A Andersson, S Simmons, C Pan, B Baker, C Sun, M Wilkins, S Singer, G Tyson, M Shah, M Thelen, and R Hettich
University of California Berkeley, Berkeley, CA, USA and Oak Ridge National Laboratory, Oak Ridge, TN, USA

Microbial communities underpin many of Earth's biogeochemical cycles and are critical in human health and disease. Most of the organisms that constitute these communities have not been cultivated, so much remains to be learned about their ecosystem roles. While pure-culture-based studies reveal important aspects of physiology, they generally do not yield insights into inter-species interactions, some of which may profoundly modify microbial activity. New molecular (especially DNA-based) approaches provide ways to circumvent the 'cultivation barrier'. Broadly referred to 'metagenomics', the methods range from surveys using single genes (e.g., 16S rRNA) to whole community shotgun sequence analyses that aim to capture the genetic potential of organisms or environments.

For over a decade, the Richmond Mine at Iron Mountain in northern California has provided a model system for molecular geomicrobiological studies. Early work that assayed the membership and organization of extremely acidophilic biofilm communities by PCR-based clone library analysis and fluorescence in situ hybridization revealed high dominance by a relatively small number of different organism types. This community structure proved key to subsequent community genomic analyses. Using just 76 Mb of shotgun sequence, we reconstructed genomic sequences for the five relatively abundant organisms from one acid mine drainage (AMD) biofilm and uncovered evidence for population heterogeneity that indicated the importance of recombination in shaping population structure (Tyson et al. 2004). Subsequently, the predicted protein sequences were used to identify and qualitatively evaluate the relative abundances of over 2000 proteins in a closely related biofilm community (Ram et al. 2004). Important, abundant hypothetical proteins are now known to be cytochromes involved in iron oxidation, and presumably, acid mine drainage formation (Singer et al. 2008). Changes in protein abundance patterns as biofilms develop highlight the importance of novel proteins in species diversification and niche adaptation.

Microorganisms in natural systems are not clonal. Analyses of genomic data from AMD biofilms revealed the form and distribution of heterogeneity in gene sequence and gene content. Intriguingly, the fastest evolving region in genomes of essentially all biofilm members is the repeat-spacer region of a newly described virus resistance system. As spacers mostly derive directly from viruses, they could be used to 'fish' out viral sequences from unassembled data, enabling near complete genome reconstruction. In addition to providing insights into coupled evolutionary processes, the sequences make it possible to include viruses in community proteogenomic studies.

A PREDICTIVE MODEL FOR TRANSCRIPTIONAL CONTROL OF PHYSIOLOGY IN A FREE LIVING CELL

NS Baliga
Institute for Systems Biology, Seattle, WA, USA

The environment significantly influences the dynamic expression and assembly of all components encoded in the genome of an organism into functional biological networks. We have constructed a model for this process in *Halobacterium salinarum NRC-1* through the data-driven discovery of regulatory and functional interrelationships among ~80% of its genes and key abiotic factors in its hypersaline environment. Using relative changes in 72 transcription factors and 9 environmental factors (EFs) this model accurately predicts dynamic transcriptional responses of all these genes in 147 newly collected experiments representing completely novel genetic backgrounds and environments—suggesting a remarkable degree of network completeness. Using this model we have constructed and tested hypotheses critical to this organism's interaction with its changing hypersaline environment. This study supports the claim that the high degree of connectivity within biological and EF networks will enable the construction of similar models for any organism from relatively modest numbers of experiments.

MODELING INTERACTIONS OF HETEROGENEOUS GENETIC PERTURBATIONS

GW Carter and T Galitski
Institute for Systems Biology, Seattle, WA, USA

Capitalizing on recent advances in genetics will require not only linking individual genes to traits, but also understanding how various alleles of multiple genes interact to affect outcomes. The study of these genetic interactions informs hypotheses of biological network structure and information flow. To date, systems-level genetic interaction studies have been dominated by single and double gene deletion screens. However, genetic interactions can occur between many types of allelic variants. For example, multi-copy suppression is the attenuation of a gene deletion phenotype by the overexpression of a second gene. Understanding these interactions requires techniques that encompass continuous variation in gene activity rather than simplified binary (on/off) gene states. To address this problem we use a computational method that combines molecular interactions, which provide paths for information flow, and genetic interactions, which reveal active information flows and reflect their functional consequences. These complementary data types are integrated to model the transcription network controlling cell differentiation in yeast. Genetic interactions were inferred from linear decomposition of gene expression data and were used to direct the construction of a molecular interaction network mediating these genetic effects. This network included both known and novel regulatory influences, and the model successfully predicted novel combinations of gene deletions. Building on this knowledge, we perform a second round of data collection to refine the model. We then predict the effects of combining overexpression and deletion of gene pairs. These predictions were tested and verified in the laboratory. This iterative process of experimentation and computational analysis produces a network model that enables the inference and testing of genetically complex hypotheses involving diverse genetic variation.

INTERROGATING DYNAMIC PHENOTYPES USING A HIGH-THROUGHPUT SINGLE CELL MICROFLUIDIC PLATFORM

RJ Taylor[1,2], D Falconnet[2], A Niemisto[1], S Ramsey[1], I Shmulevich[1], T Galitski[1], and C Hansen[1,2]
[1]Institute for Systems Biology, Seattle WA, USA
[2]University of British Columbia, Vancouver BC, Canada

Elucidating complex biological networks is of central interest for understanding cellular function and the mechanisms of disease. Genetics and chemical biology have emerged as powerful techniques for dissecting cellular circuits through the controlled perturbations of protein function, and traditional studies have been successful in elucidating the roles of core pathway components. However, current experimental techniques generally lack the ability for precise modulation of environmental stimuli and are limited to averaged measurements of large populations of cells. This lack of precise temporal control of the chemical environment limits the ability to interrogate kinetic information processing circuits or cellular response to temporal stimuli, while the ensemble averaging conceals the ever-present heterogeneity in the cellular response. We have developed high-throughput microfluidic technology for the study of signaling dynamics and cell-to-cell heterogeneity in a prototypical eukaryotic signaling pathway, the pheromone response in *Saccharomyces cerevisiae*, subject to combined time-varying chemical stimuli and genetic perturbations. A combination of scalable microfluidic fluid control and time-lapse live cell imaging was used to implement 256 simultaneous quantitative experiments in which transcriptional output, growth rate, and cell phenotype are measured at single cell resolution across 32 temporal sequences of chemical stimuli and in 8 genetic backgrounds. Using this high throughput technology we conducted over a 1000 live cell imaging experiments investigating the interplay of chemical sequences and genetic perturbations on network response. Dynamic analysis of a collection of 11 mutants compromised for peripheral genes implicated in the regulation of the central MAPK signaling conduit was found to elicit new kinetic phenotypes that are unobserved in static measurements.

INTEGRATIVE ANALYSIS FOR TNFα-NFκB SIGNALING PATHWAY

LWK Cheung & M Visvanathan
Department of Preventive Medicine & Epidemiology and Bioinformatics Core, Loyola University Medical Center, USA

We investigated different signaling pathway models and built a new model for TNF⟨-NFκB signaling via our integrative analytical system. This system has a 3-tier architecture: a Java-based pathway designing/visualization environment (DVE) and a simulation environment (SE) in the front-end application tier, a Java Database Connectivity-Open Database Connectivity in the middle tier, and a relational data management system in the back-end database tier. We first designed the TNF⟨-NFκB pathway model in DVE allowing inclusion of mathematical modeling data, simulation data and biological data from our integrated knowledgebase in the database tier. The designed TNF⟨-NFκB pathway model was exported in an XML format from DVE to SE. The entities of the knowledgebase contain information grouped into three categories: molecular components, reactions and pathways. These entities inherit both, the biological and modeling information concerning specified pathways. Specifically, the knowledgebase incorporates biological knowledge about components, reactions and pathways from three different online external protein databases: Biomolecular Interaction Network Database, Database of Interacting Proteins, and Munich Information Center for Protein Sequences protein-protein interaction database as well as internal experimental verifications and literature studies. Mathematically, our new TNF⟨-NFκB signaling pathway model was based on the use of ordinary differential equations and a detailed protein-protein interaction connectivity map within our system. Using the most detailed mathematical model in the literature as a base model, three new proteins -- TRAF1, FLIP, and MEKK3 -- were identified and included in our new model. Sensitivity analysis of model parameters was performed via simulations. Our results show that our integrative analysis offers the most detailed, stable and consistent mathematical model for TNF⟨-NFκB signaling and further increases the understanding of TNF⟨-NFκB signaling pathway.

HEPATITIS C VIRUS INFECTION PROTEIN NETWORK

L Tafforeau[1,], B de Chassey[1,*], V Navratil[2,*], M S Hiet[1,#], A Aublin[1], S Agaugué[1,$], G Meiffren[1], F Pradezynski[1], BF Faria[1], T Chantier[1], M Le Breton[1], J Pellet[1], N Davoust-Nataf[1], P E Mangeot[1], A Chaboud[3], F Penin[3], Y Jacob[4], PO Vidalain[5], M Vidal[6], P André[1,7], C Rabourdin-Combe[1], and V Lotteau[1,7]*

[1]Inserm Unit 851, IFR128 BioSciences Lyon-Gerland, Lyon, France

[2]INRA, UMR754, rétrovirus et pathologie comparée, BioSciences Lyon-Gerland, Lyon, France

[3]Institut de Biologie et Chimie des Protéines, UMR 5086 CNRS, , IFR128 BioSciences Lyon-Gerland, Lyon, France

[4]Unité Postulante de Génétique, Papillomavirus et Cancer Humain, Institut Pasteur, Paris, France

[5]Laboratoire de Génomique Virale et Vaccination, CNRS URA 3015, Paris, France

[6]Center for Cancer Systems Biology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, USA

[7]Hospices Civils de Lyon, Hôpital de la Croix-Rousse, Laboratoire de virologie, Lyon, France

**These authors contributed equally to this work*

[#]Present address: Department for Molecular Virology, University Heidelberg, Heidelberg, Germany

[$]Present address: Service de Recherches en Hémato-Immunologie, CEA-DSV-DRM, Hôpital Saint-Louis, IUH, Paris, France

Replication of Hepatitis C virus (HCV) relies on multiple interactions with host factors but how these interactions determine infection, pathogenesis and sensitivity to treatment remains largely undefined. To provide a comprehensive view of a HCV mediated cellular infection, we present here a proteome-wide mapping of interactions between HCV and human cellular proteins. A total of 314 protein-protein interactions between HCV and human proteins was identified by yeast two-hybrid, and 170 by literature mining. The dataset was integrated into a reconstructed human interactome and topological analysis of this network showed that cellular proteins interacting with HCV are enriched in highly central and interconnected proteins. The global analysis of these proteins based on functional annotation revealed the enrichment of cellular pathways targeted by HCV. A network comprised of proteins associated with frequent clinical disorders of chronically infected patients was constructed by connecting the insulin, Jak/STAT and TGFb pathways with cellular proteins targeted by HCV. CORE protein appeared as a major perturbator of this network. Focal adhesion was also identified as a new function affected by the virus, mainly by NS3 and NS5A proteins.

PATHOLOGICAL IRON LOADING AMONG PATIENTS WITH THALASSEMIA: SOLVING THE CLINICAL MYSTERY WITH SYSTEMS MEDICINE

JL Miller
Molecular Medicine Branch, NIDDK, National Institutes of Health, Bethesda, MD, USA

For the last decade, human erythroid biology has provided a robust model for exploring systems medicine approaches for a variety of disease states involving red blood cells. Clinical problems are studied using a combination of bedside, bench, and browser-based information to generate novel diagnostic tools and therapies. Recently, this research path was taken to explore the unsolved clinical problem of iron-related toxicity and death among patients with ineffective erythropoiesis and thalassemia syndromes. Iron overload is a major cause of morbidity and death among these patients due to inappropriate suppression of the iron regulator, hepcidin. To determine whether hepcidin-suppressing proteins are secreted during erythropoiesis, transcriptomes were generated from developmentally-staged populations of human erythroblasts that were cultured and isolated from the hematopoietic stem cells of 15 healthy adult human donors. Informatics analyses were focused upon the TGF-beta/BMP signal transduction superfamily since SMAD4 regulates hepcidin expression. Fifty-four genes were identified according to this screen, and high-level expression of a gene named growth differentiation factor 15 (GDF15) was discovered within the transcriptome profiles. GDF15 is an apoptosis-associated protein expressed primarily by the placenta. Quantitative PCR, Western, and ELISA analyses confirmed expression and secretion of GDF15 during erythroblast maturation. GDF15 levels were also measured directly from clinical samples from a variety of patients with hemoglobinopathies or other anemias. Compared with GDF15 levels among the healthy volunteers (mean: 650 ± 50 pg/ml), patients with beta thalassemia (mean: 24,600 pg/ml; range 9,000-75,100 pg/ml) showed significantly elevated levels of GDF15. To determine whether GDF15 serves as a regulator of hepcidin expression, quantitative PCR for hepcidin mRNA expression were performed using primary human hepatocytes. When titrated to the concentrations in healthy volunteer blood, GDF15 produced a 1.5-fold increase in the expression of hepcidin compared to matched cultures containing no supplemental GDF15. However, GDF15 dosed to levels above 5,000 pg/ml resulted in a dosed suppression of hepcidin. These novel findings further validate systems approaches for translational and clinical research and strongly suggest high-levels of GDF15 contribute to the pathological suppression of hepcidin among thalassemia patients.

VELOCIGENE®: AN INTEGRATED SUITE OF HIGH THROUGHPUT, CUSTOMIZABLE GENOME ENGINEERING TECHNOLOGIES FOR ANALYSIS OF GENE FUNCTION AND DRUG TARGET DISCOVERY AND DEVELOPMENT

AN Economides
Regeneron Pharmaceuticals, Inc, NY, USA

VelociGene®, is an integrated suite of technologies for high throughput gene targeting. In contrast to traditional gene targeting technologies, it utilizes BACs as targeting vectors, which in turn necessitate the use of quantitative PCR-based genotyping assays to determine which ES cell clones are correctly targeted. The process of generating targeted ES cells has been increasingly automated, partly as a result of Velocigene's participation in the Knockout Mouse Project (KOMP: http://www.komp.org/). Still, VelociGene's production process supports the engineering of highly customized alleles. Several technological advances will be discussed: (a) VelocImmune, a project culminating in the generation of mice whose Ig loci have been humanized via the replacement of ~6 Mb of mouse genomic sequence with ~1.5 Mb of human sequence; (b) VelociMouse, a method for generating mice derived entirely from ES cells, which result in either minimizing breeding or even generating experimental cohorts directly by microinjection; and (c) Conditionals by Inversion (COIN), a new method for generating conditional alleles, which addresses some of the limitations of traditional methods. Examples of uses of these technologies for drug discovery, drug development, and pathway analysis will be presented.

REGULATORY GENOMICS OF DROSOPHILA AND MAMMALIAN SPECIES

M Kellis
Massachusetts Institute of Technology, MA, USA

A systematic understanding of gene regulation in animal genomes requires the ability to determine pre- and post-transcriptional regulatory elements, the regulatory networks they define, and their dynamics across development. Our lab is developing computational methods to address these challenges in flies and mammals, in collaboration with large-scale experimental efforts. We have used comparative genomics of 12 Drosophila genomes and of 24 mammalian genomes to discover regulatory motifs associated with promoter and enhancer regions, as defined by their chromatin marks. We have also used comparative methods to discover and characterize microRNA (miRNA) genes and their targets in Drosophila, revealing many novel miRNAs and miRNA families, which lead to a denser miRNA targeting network with increased potential for combinatorial control. We have recently demonstrated that both arms of a miRNA hairpin can be functional, and both strands of a miRNA gene can be transcribed and lead to functional miRNA regulators. In the case of the Hox-encoded miRNA miR-iab-4, we have shown that its anti-sense miRNA can lead to homeotic transformation of halteres into wings, establishing it as a new Hox gene, and the first functional anti-sense miRNA, Lastly, we have used comparative genomics to infer regulatory networks based on individual conserved instances of regulatory motifs, which show functional enrichments similar and sometimes higher to genome-scale experimental methods such as ChIP-chip. As part of the ENCODE and modENCODE projects, we are now studying dynamics of developmental and cell-differentiation networks in Drosophila and human, and the tissue-specificity of the establishment and maintenance of chromatin state.

PATHWAY ENRICHMENT ANALYSIS REVEALS THAT IL-17 PATHWAY MODULATION IS MORE CLOSELY RELATED TO PSORIASIS DISEASE RESOLUTION WITH EFFECTIVE THERAPY *THAN IFNγ*

M Suárez-Fariñas, L Zaba, J Fuentes-Duculan, I Cardinale, P Gilleaudeau, M Sullivan-Whalen, and J Krueguer
Laboratory of Investigative Dermatology, The Rockefeller University, NY, USA

Important Psoriasis is an inflammatory skin disease that affects 2% of North American populations. Until recently, psoriasis was considered to be a disease of pathogenic IFNg-producing T helper cells (Th1 cells). Some single gene studies suggest that that IL-17-producing Th17 cells are also present in lesional psoriatic plaques but the relative contribution of these two T cell subsets is still unknown. In this study, we first defined IL-17, IFNγ and TNFα Pathways by culturing primary human normal keratinocytes with these cytokines and measuring mRNA using Affymetrix gene array. Genes differentially expressed in each condition are considered in the respective Pathway. We then analyzed temporal profiles of these pathways in lesional biopsies of 15 patients treated with etanercept, a TNFα blocking immune modulating drug, at baseline, and weeks 1, 2, 4, and 12. Gene Set Enrichment analysis of those pathways was conducted to test if those pathways were enriched with genes having a special time profile. Here, we extended the original GSEA statistics, proposed for the two groups case, to the time course framework to account for the correlations among time measurements from the same patient. Results show that genes modulated by TNFα were downregulated rapidly with etanercept treatment. Excitingly, genes from the IL-17 Pathway were also rapidly downmodulated and correlated closely with histological markers of disease resolution. Although both Pathways show similar velocity of recovery to NL levels after treatment, IL17 pathways have higher expression values at lesional levels than TNFα pathways. In contrast, IFNγ genes were not down modulated until late in disease resolution suggesting that Th1 cells alone cannot perpetuate the psoriatic phenotype.

COMPUTATIONAL SYNTHETIC BIOLOGY

YN Kaznessis
Department of Chemical Engineering and Material Science, University of Minnesota, USA

The nascent field of synthetic biology offers the promise of designer gene networks that precisely control the expression of protein molecules. Biomedical and biotechnological applications abound: from protein production optimization to biosensing and to gene therapies, engineering novel synthetic gene regulatory networks is taking advantage of an ever-expanding toolbox of molecular components becoming known thanks to genome projects. For example, regulatable gene networks are becoming important components of effective gene therapies. Ideally, an effective gene therapy would be one based on regulatable gene expression, where the therapeutic gene expression is turned on and off on demand. Generally, although currently used designs of regulatable gene networks are ingenious, there are limitations that need to be addressed. Indeed, there is a need for improving existing inducible regulatory elements and for engineering novel regulatable gene networks that can be used in numerous biotechnological and biomedical applications. In the presented work we will describe how to rationalize synthetic biology using model-driven, molecular-level engineering principles. In the presentation we will focus on the theoretical effort to develop an algorithm for simulating biomolecular systems across all relevant time and length scales; from stochastic-discrete to stochastic continuous and deterministic continuous, we are developing the theoretical foundation for accurately simulating all biomolecular interactions in transcription, translation, regulation and induction and how these result in phenotypic probability distributions at the population level. We are simulating and develop design principles for tetracycline-inducible networks. These are being used in gene therapy applications, among other important biomedical applications, and we are attempting to address current, known practical limitations. Armed with supercomputers, we are predicting the relation between synthetic DNA sequences and important, biomedically relevant, physiologic behavior. We are engineering these networks in E.coli, establishing an integral connection between experimental expression systems and multiscale models.

Y N Kaznessis, Models for synthetic biology, BMC Systems Biology 2007, 1:47

A DEPENDENCY GRAPH APPROACH FOR ANALYSING DIFFERENTIAL GENE EXPRESSION DATA OF B-CELL LYMPHOMAS

I Mühlberger[1], P Perco[2], A Bernthaler[3], R Fechete[3], A Lukas[2], and B Mayer[1,2]
[1]Institute for Theoretical Chemistry,University of Vienna
Währinger Strasse 17,A-1090 Vienna,Austria
[2]emergentec biodevelopment GmbH
Rathausstrasse 5/3,A-1010 Vienna,Austria
[3]Institute for Computer Languages,Theory and Logic Group,Vienna University of Technology, Favoritenstrasse 9,A-1040 Vienna,Austria

Background: Analysis of differential gene expression data for reaching a conclusion on the functional dependencies displayed by omics profiles resembles a major challenge. We have established an interaction network scheme for a general molecular object definition merging genomics, transcriptomics and proteomics data resulting in an object dependency graph for analyzing specific transcriptomics profiles. **Methods:** Our annotation of molecular objects integrates given data on biological categories, tissue specific gene expression; protein sub-cellular location, and protein interaction. This parameterization allows for the definition of an object dependency function by computing pair-wise object relations in the context of functional similarity / procedural dependency, providing a dependency matrix for pair-wise object dependency. In turn, this matrix can be interpreted on the level of an undirected graph, where the edges are characterized by weights resembling different degrees of dependency. This reference graph can now be used for mapping and interpreting gene expression profiles performed for a particular experimental setting. **Results:** We present our object annotation, the functional for computing the dependency matrix, and show topological features of the reference graph strongly resembling characteristics of scale free biological networks. We have analyzed gene expression profiles characterizing B-cell lymphomas on the level of this dependency graph and identified sub-graphs where differentially regulated cancer signatures culminated, allowing a functional interpretation particularly in the molecular processes involving interleukins, cell cycle, and protein modifications along apoptosis.

NEOSTASIS AS A THERAPEUTICALLY VULNERABLE HOMOGENEOUS COMPONENT OF ROBUST CANCER SYSTEMS

H Warenius[1] and L Kyritsi[2]
[1]Visiting Professor of Anticancer Drug Development, University of Southampton School Of Chemistry UK
[2] Oncology Research Unit, University of Liverpool Department of Medicine, Liverpool UK

An important question in cancer biology is whether cancer cells should be regarded as "supercells" compared to their normal counterparts or are more deranged and disorganised. Human cancer cells exhibit impaired differentiation and programmed cell death and suffer from genetic instability. These observations suggest that an important consequence of transformation from normal to cancer is functional disorganisation within the cell. Alternatively the functional heterogeneity of protein networks in cells has been proposed to confer robustness to tumours[1]. A recent model of cancer in terms of systems science, complexity and chaos theory[2], regards cancer as a system-in-failing due to the destabilization of the human body as a system by the unrestricted exponential growth of the cancer. This model conceptualizes zones of order and chaos in carcinogenesis and places cancer on the outer edge of chaos in such a system. Cancer cells can be serially propagated for many years outside the human host in which they initially arose. In this situation, although the majority of cells continue to divide successfully, a proportion of cancer cells undergo spontaneous death. We propose that in order that the tumour can continue to propagate at the edge of chaos, new or latent functional protein connections emerge and render the tumour robust. This functional redundancy provides neostasis, or re-balancing of cancer cells which are intrinsically unstable as a result of malignant transformation and ongoing genetic instability. Failure of neostasis results in continued progression of the tumour mass along the direction of entropy. This can make the molecular agents of neostasis potential drug targets in an otherwise robust cellular milieu.

References: 1. Kitano H, Cancer as a robust system: implications for anticancer therapy. Nature Reviews Cancer. 2004; 4: 227 – 235. 2. Janecka IP. Cancer control through principles of systems science, complexity, and chaos theory: A model. Int J Med. 2007; 4 (3): 164 – 173.

PREDICTING TARGETS AND RISK GENES FOR LONG-QT SYNDROME SIDE EFFECTS

SI Berger, A Ma'ayan, and R Iyengar
Mount Sinai School of Medicine, Department of Pharmacology and Systems Therapeutics, NY, USA

Long-QT Syndrome (LQTS) is a congenital or acquired prolongation of the QT interval observed by electrocardiogram due to the cardiac ventricles taking too long to repolarize after depolarization. This causes symptoms ranging from syncope to sudden death by fatal arrhythmias such as Torsades De Pointes (TdP). The congenital form of LQTS has been linked to mutations in several different cardiac ion channels and associated proteins. The acquired form can be induced by metabolic and ion disturbances and can be a side effect of more than 70 FDA approved drugs. While acquired LQTS and drug induced TdP are uncommon, they can be life threatening in genetically susceptible individuals and have led to drugs being withdrawn from the market. The ability to design drugs with decreased risk for these adverse events and screening patients for genetic susceptibility will increase the safety of many medications. This project is based on the hypothesis that genes products involved in the pathogenesis of LQTS form a functional neighborhood within the intracellular regulatory interaction network. To identify the boundaries of the LQTS neighborhood, we developed a method based on random walks for computing functional distance between sets of proteins in protein-protein interaction networks (PIN). Using gene products known to be involved in LQTS as landmarks, this method was used to identify the boundaries of a neighborhood in protein interaction space and rank the gene products based on how central they are in this neighborhood. The resulting subnetwork contained several gene products known to be involved in modulation of the QT interval and was enriched for targets of drugs associated with acquired LQTS and drug-induced TdP. This neighborhood provides insight into the pathogenesis of congenital and acquired LQTS and provides hypothesis generation tools for finding novel susceptibility genes and drug targets with risk of LQTS.

CASIMIR, A COORDINATION ACTION FOCUSING ON DATABASE INTEROPERABILITY AND FINANCIAL SUSTAINABILITY; A STEP FORWARD

*V Aidinis, C Chandras, M Zouberakis, and the CASIMIR consortium**
B.S.R.C. Al. Fleming, 34 Fleming Street, Vari, Athens, Greece
**CASIMIR Partners*
Univeristy of Cambridge, Cambridge, UK; MRC Harwell, Oxfordshire, UK; MRC, Edingburgh, UK; EBI, Cambridge, UK; EMBL, Monterotondo, Italy; BSRC Fleming, Vari, Greece; GSF, Neuherberg, Germany; Helmholtz-Zentrum, Braunschweig, Germany; CNR, Monterotondo, Italy; Geneservice Limited, Cambridge, UK

Modern advances in biological sciences, have given rise to increased data production and the generation of numerous, scattered biological resources for the storage and sharing of data and biological material. These informatics infrastructures have subsequently become an important tool in assisting scientists to further their understanding of the biology of human disease. The wide range of data types, both genomic and phenomic, together with their world wide distribution among many differently specialised databases, makes it potentially difficult and frequently impossible to ensure preservation and consistency of information, data quality and future retrieval. It is therefore essential to look closely on the validated growth and accessibility of databases and the way that data is stored, recovered and processed. As European efforts aim toward the recent challenges of database interoperability, additional data standardisation, representation and transfer is required to facilitate and promote the interconnectivity among existing and new databases. Underlying the organizational and technical challenges of database integration is that of database infrastructure funding across Europe. For databases to retain their value to the community they need to be sustained both financially and scientifically over time. Examination of database funding patterns and strategies, together with the legal implications of publishing data on public databases, is long overdue. CASIMIR (www.casimir.org.uk), a co-ordination action of the 6th Framework Programme of the European Commission, focuses on the integration and interoperability of databases containing biological collections, relevant to the use of the mouse as a model organism for human disease. The overall aim of the project is to establish a framework of interconnected databases with concomitant added value to the scientific community, which should additionally become self-sustained in terms of data deposition, usage, development and financial support, thus endorsing the ultimate goal to create a ‘one-stop-shop’ where the user may find everything in one database and paving the way towards systems biology.

INTEGRATED DYNAMICS OF THE REGULATORY NETWORK CONTROLLING CELL DIVISION IN CAULOBACTER

P Brazhnik, S Li, and JJ Tyson
Virginia Tech, Blacksburg VA USA

To understand how the cell division is controlled by underlying molecular interactions is of fundamental importance to science and to human health. Protein interaction networks regulating the cell division cycle have been intensively studied genetically and biochemically. The mechanism of the cell cycle 'engine' is most well known for yeast, and theoreticians have created comprehensive, quantitative (mathematical) models that reproduce a large amount of experimental data and productively guide further experimental studies. Recently, a few simplified models of mammalian cell cycle controls have also been proposed. Progress in understanding cell cycle regulation in bacteria has lagged behind eukaryotes. Here we fill this gap by developing a realistic, experimentally verified, computational mathematical model of the gene-protein network governing DNA replication and cell division in an α-proteobacterium, *Caulobacter crescentus*. *Caulobacter* undergoes asymmetric division producing two progeny cells with identical genome but different developmental programs: the "swarmer" cell is flagellated and motile, and the "stalked" cell is sessile (attached to a surface by its stalk). Only stalked cells undergo chromosome replication and cell division. A swarmer cell must shed its flagellum and grow a stalk before it can enter the replication-division cycle. Our quantitative model reconciles large amount of experimental data and predicts detailed temporal dynamics of regulatory gene expression during the cell division cycle of the Caulobacter wild-type cells as well as several mutant strains. It helps to interpret phenotypes of known mutants and predict novel ones. *Caulobacter* has been recently detected as a human pathogen, which makes the study of its reproduction directly related to human health. Since many genes and mechanisms discovered in *Caulobacter* are evolutionarily conserved among other α-proteobacteria, our computational model of cell replication in *Caulobacter* is expected to be extendable to other family members, in particular to the α-proteobacteria that cause brucellosis in cattle and Rocky Mountain spotted fever in humans. Insights gained into the temporal and spatial control of gene expression and protein interactions in *Caulobacter* could provide new clues for rational design of antibacterial agents and bacteria-based drug delivery technologies.

GENE EXPRESSION RESPONSES TO ENDOPLASMIC RETICULUM (ER) STRESS IN HUMANS

BA Dombroski, WM Ankener, K Chapman, KG Ewens, and RS Spielman
Department of Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA, USA

Endoplasmic reticulum (ER) stress, the cellular response to unfolded or misfolded proteins, causes many responses, including changes in gene expression. These changes are crucial to cell function; if ER homeostasis cannot be restored, the cell undergoes apoptotic cell death. Understanding relationships among responses to ER stress and identifying determinants of genetic variation in the response, will contribute greatly to understanding of the process and its role in health and disease. In this study we address two questions: 1. Which genes show the greatest gene expression responses to ER stress and how are they related, and 2. Which gene expression responses show heritable variation? Experiments were carried out with lymphoblastoid cell lines from 26 pairs of monozygotic (MZ) twins. We induced ER stress with thapsigargin and measured gene expression with Affymetrix U133 Plus 2.0 arrays. Among the ~1500 transcripts with response (up or down) greater than 1.5-fold, some were previously known to be involved in ER stress, and serve as "proof of principle" for the experimental system; examples are CHOP (fold-change 3.2), and TRIB3 (4.6). In addition, there were very strong gene expression responses not known before; examples are INHBE (14.4 fold), VLDLR (10.9), CTH (7.7). The network of gene expression responses clearly distinguishes at least two functional categories of genes that respond to ER stress induced by thapsigargin. For assessment of heritable variation, the gene expression changes in the MZ twins were analyzed by analysis of variance (ANOVA). From the ANOVA we also calculated the intraclass correlation coefficient (ICC), a standard measure of twin similarity and evidence for a genetic contribution. We used the ICC to rank the genes for heritable variation in expression response to ER stress. Among those with high ICC were several known to be involved in ER stress: IRE1/ERN1 (ICC= 0.82), HERPUD1 (0.97), ATF4 (0.89), ATF3 (0.86), and IL8 (0.72). There are also genes with high ICC that have not previously been implicated in ER stress, for example PSPHL (ICC=0.81). This analysis provides the evidence for genetic variation in gene expression response to ER stress, a key cellular process. We will discuss the possible connections between genetic variation and network relationship.

DETECTION OF mRNA TRANSCRIPTS FOR THE ESCRT MACHINERY IN *ENTAMOEBA HISTOLYTICA*

I López-Reyes, C Bañuelos-Barrón, and E Orozco
Departamento de Patología Experimental. Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN). Av. IPN 2508. Col. San Pedro Zacatenco. Del. Gustavo A. Madero. México, D. F. México

The EhCPADH complex is a key molecule for *Entamoeba histolytica* virulence, participating in adherence, phagocytosis and cytolisis to target cells. EhCPADH is formed by a cysteine protease (EhCP112) and an adhesin (EhADH). EhADH is located at trophozoite plasma membrane and cytoplasmic vacuoles and undergoes translocation during erythrophagocytosis. EhADH displays structural similarity to mammalian Alix and yeast Bro1 proteins, due to the presence of the conserved Bro1 domain at their N-terminus. Alix, Bro1 and other Bro1-domain containing proteins participate in several processes that include multivesicular bodies (MVB) formation, apoptosis, virus budding and pH regulation, among others. During MVB biogenesis, Bro1 domain-containing proteins interact with proteins of the Endosomal Sorting Complex Required for Transport (ESCRT) machinery to perform the sorting and transport of transmembrane proteins through endosomes. In yeast and human, around 20 proteins named Vacuolar Proteins Sorting (Vps) are required for the assembly of ESCRT-0, -I, -II and -III. Although *E. histolytica* is a highly endocytic organism, we do not know if the endocytic pathway functions through ESCRT proteins. In this work, we examined the presence of the ESCRT protein machinery in *E. histolytica*. We initiated this study by screening of *E. histolytica* genome through an *in silico* analysis. We identified 18 *Vps* protein-encoding genes related to conserved yeast and human ESCRT. Moreover, during erythrophagocytosis and RT-PCR experiments, we detected mRNA transcripts for almost all *E. histolytica* putative *Vps* genes, raising the possibility that ESCRT proteins participate in the *E. histolytica* endosome pathway.

CELL NETWORKS IN THE PITUITARY STRUCTURE, FUNCTION, DEVELOPMENT

M Cassou[1], N Courtois[1], N Chauvet[1], C Lafont[1], F Molino[1], P Mollard[1], J Drouin[2], I Robinson[3], and R Smallwood[4]
[1]Institut de Génomique Fonctionnelle, Montpellier
[2]Institut de Recherches cliniques de Montréal (IRCM), Montréal, Canada
[3]MRC, National Institute for Medical Research, London, UK
[4]Department of Computer Science, Kroto Research Institute, North Campus, University of Sheffield, Broad Lane, Sheffield, UK

Recent discoveries in the pituitary gland from our group have uncovered an unexpected degree of integration among the secreting cells, emphasizing the importance of their network organisation in space for the secretory function (Bonnefont X. et al., 2005, PNAS 102, 16880). This organisation displays changes, which correlate with the hormone levels in the blood. This observation leads to two questions. First, to characterise this spatial organisation for the different cell types present in the pituitary, and then to describe its development across embryogenesis. The first question can only be addressed in quantitative terms through a large scale map of the gland, in which the five different cell types are positioned in space. From these data spatial correlation function defining typical geometrical signatures are extracted, for the different cell networks. This goal is accessible using transgenic animals expressing different fluorescent proteins in the five different cell types present in this 'rainbow pituitary', coupled to 3D imaging of the whole gland and automatic cell positioning and identification from the image stacks. This method gives access to a high-throughput characterisation of the large-scale organisation of the cell types in the gland, in different physiological and pathological conditions. Genetic engineering will also give access to specific manipulations of this geometrical structure, and give opportunity to associate geometrical and functional changes.
The second question concerns the development of the gland. A large amount of work has been devoted to the identification of genetic factors responsible for the successive cascade of differentiation and proliferation events taking place in the developing gland, factors which can be manipulated to hinder normal development of the network and to observe the associated structural changes. Coupling these methods with large-scale histology data from glands at different stages of the development will be the key to raise important questions: how are the space position, the neighbouring cells structure, correlated with the expression of different genetic factors in the cells? To assess these questions, the use of an agent-based-model to test different development rules will be coupled to the histology data in order to simulate the development of a substructure of

the gland, the so-called Pit-1 lineage generating the GH GnH and PRL cells. We intend to demonstrate the role of direct couling between neighbouring cells in growth/differentiation of the tissue, as well as the rôle of the developping capillary network. We thus propose to couple high-throughput data at the genetic and the structural level with large-scale simulations to better understand the spatio-temporal dynamics of this regulatory network for the first time in a whole mammalian organ.

FEEDBACK REGULATION IN GENE EXPRESSION AND GRADIENT SENSING IN THE YEAST PHEROMONE MAPK PATHWAY

S Paliwal, Z Hilioti, P Iglesias, and A Levchenko
The Johns Hopkins University, Baltimore, Maryland, USA

Haploid cells of the budding yeast have two mating types, MATalpha and MATa, which release pheromones detected by the opposite mating type. Pheromone binding to the receptor leads to activation of a MAPK cascade resulting in cell cycle arrest in the G1 phase and polarized morphogenesis leading to formation of a mating projection. Projection formation occurs in the direction of the source of pheromone when the cell is exposed to a pheromone gradient. However, following uniform pheromone exposure, the cell forms multiple projections sequentially, with the first projection being formed at the presumptive budding site. The pheromone MAPK pathway has been very well studied, yet several aspects of its regulation are not well-understood. We present an integrated systems biology approach to study the role of feedback regulation in the pheromone response. We have developed a high-throughput microfluidics-based experimental platform to study both, gradient and uniform pheromone response in yeast using quantitative fluorescence single cell imaging. We have also developed a mathematical model for gene expression in the pathway. A combined modeling and experimental approach has uncovered the important roles of Kss1, a MAPK thought to be redundant in the pheromone pathway, as well as autoregulation of the transcriptional factor Ste12. The combination of positive and negative feedback at the transcriptional level has been shown to regulate gene expression in the pathway, and we have correlated it to the pheromone gradient sensing response. Additionally, we have investigated the role of negative feedback in the upstream MAPK signaling pathway in regulating the cellular response to graded and uniform pheromone stimuli.

DECONVOLUTION OF FUNCTIONAL ASSOCIATION NETWORK MODULES INVOLVED IN MICROBIAL IRON HOMEOSTASIS

R Quatrini[1], F Lazo[2], C Oporto[2], J Ugarte[1], G Rivera[1], H Ososrio[1], and DS Holmes[1]
[1]Departamento De Ingeniería Informática, USACH; [2]Center For Bioinformatics And Genome Biology, Fundación Ciencia Para La Vida-MIFAB-Universidad Nacional Andrés Bello, Santiago, Chile

Structure and function of a living cell strictly depend on molecular interactions at multiple levels (e.g. proteins complexes, metabolic pathways, signaling cascades and transcriptional circuits). These interactions are not independent from each other and together create an extremely complex and highly ordered molecular network. A major challenge for biology is to understand the structure and the dynamics of this complex intracellular web of interactions. Network theory offers novel possibilities to begin to unravel it. Several mathematical approaches have been developed to identify highly connected protein clusters in these networks that correspond to functional modules of major biological interest, especially useful in automatic metabolic reconstruction. We have implemented a new cluster recognition strategy that overcomes the need for a priori knowledge of the number of existing clusters or their size, and assesses the statistical significance of the cluster obtained. Using pre-computed protein interaction data from *Escherichia coli* we have calibrated the strategy and applied it to functional association data calculated for a set of five reference acidophilic bacteria for which genome sequence data is currently available. Herein, we apply this approach to the deconvolution of network modules involved in iron homeostasis. Species-specific genes have been identified with distinctive functional roles in iron management as well as genes shared by several species in the microbial consortia. Their analysis contributes to our understanding of the general survival strategies in acidic and iron loaded environments and suggests functions for genes with currently unknown functions. Novel and unexpected aspects of the iron response in acidophiles that emerged from the reconstruction of the metal management subsystem will be discussed. Comprehensive examination of the occurrence and conservation of regulatory functions and regulatory sites also allowed the prediction of the metal regulatory networks that link the iron management functions in this group of microbes. The existence of a number of parallel iron management modules suggests that these organisms are versatile in performing the function and could better survive in diverse environments or conditions where the function is needed.
Acknowledgements: Fondecyt 11060164 & 1050063, DI-UNAB 34-06 & Microsoft Sponsored Research Award.

SYSTEMS ANALYSIS OF ENDOSOMAL TRANSPORT USING PHENOTYPIC SCREENING AND GENETIC INTERACTIONS IN YEAST

NR Quenneville[1], M Davey[2], R White[3], K Bryan[3], and E Conibear[1,2]
Center for Molecular Medicine and Therapeutics, Child and Family Research Institute, [2]Dept. of Medical Genetics, [1]Dept. of Biochemistry and Molecular Biology, [3]Dept. of Statistics, University of British Columbia, Vancouver, British Columbia, Canada.

Endocytic transport involves coats, adaptors, tethering complexes, SNAREs, and small GTPases that mediate transport of specific cargo in both an anterograde (secretion) and retrograde (recycling) direction. Defective retrograde transport has been implicated in neurological disease including Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease and Down syndrome. Deciphering how trafficking machinery coordinates, regulates and establishes the multitude of endosomal transport pathways in yeast and higher eukaryotes is a complex problem. We are using systems biology techniques to dissect the complex regulatory networks of endosomal transport. Our techniques utilize quantitative phenotypic mapping to create and analyze networks of genetic interactions relating to vesicle transport pathways. This work relies on a high-throughput, quantitative, biochemical assay we developed to measure the capacity of cells to selectively transport cargo between the Golgi, plasma membrane and endosomes. Localization of our chimeric reporter, GFP-Snc1-SUC2 (GSS), is conferred by sorting signals present in the secretory vesicle SNARE, Snc1, a homolog of mammalian synaptobrevin/VAMP. Like Snc1, wild-type yeast localize a proportion of GSS to the cell surface, exposing an enzymatic portion of the reporter (SUC2) to the extracellular space. Enzymatic activity of GSS can be directly quantified from yeast colonies growing on agar plates, enabling high-throughput analysis. Defects in endosomal transport and secretion are characterized by low levels of GSS activity. We have screened the haploid yeast-deletion collection to identify ~350 gene deletions that are defective in endosomal transport. The dataset is highly enriched for vesicle transport machinery but is also enriched for metabolism, signaling, and chromatin remodeling. To delineate the relationships between pathways in this gene set, we have performed genetic interaction analyses recording two phenotypes: biochemical changes in endosomal transport, and growth. Preliminary analyses of a network involving 51 single mutant phenotypes and 2 550 corresponding double mutant phenotypes, suggest the two datasets provide different but complimentary types of information. Identification of high-confidence genetic interactions in the biochemical dataset reveals both expected and novel co-equal and epistatic relationships. Further evaluation of these relationships is expected to reveal novel insights into the regulation of retrograde endosomal transport.

RELATIONAL GENOMIC DATA ANALYSIS AS AN APPROACH TO STUDY OLFACTORY ENDOPHENOTYPES OF SCHIZOPHRENIA

L Rioux[1], M Levy[2], SJ Kanes[2,3], RW Williams[4], and J Nissanov[1]
[1]Drexel University College of Medicine, Philadelphia PA, USA
[2]University of Pennsylvannia, Philadelphia PA, USA
[3]Currently at Clinical Research-Neuroscience, AstraZeneca, Greater Philadelphia PA, USA
[4]University of Tennessee Health Center, Memphis TN, USA

Schizophrenia is a neurodevelopmental disease resulting from complex interactions between genetic and environmental factors. Endophenotypes, quantitative traits characterizing the disease, include abnormalities in structure and function of the olfactory system. The present work focuses on the olfactory bulb (OB), a valuable area in which to examine genetic control of development and plasticity mechanisms in schizophrenia because continuous neurogenesis and re-innervation occur there throughout life. There is also ample evidence that genes modulate variation in OB size and structure. Four quantitative trait loci (QTL), accounting for 20% of the variance in bulb weight, have already been identified in mice. To identify QTLs modulating OB morphology, we examined mice strain variability in OB volumes using parental inbred strains, C57BL/6J (B6) and DBA/2J (D2), as well as 30 BXD recombinant inbred strains. Studies were conducted entirely through the use of the Mouse Brain Library, a large public collection of sectional brain images from recombinant inbred mice strains suitable for unbiased stereological measurements, and web-based QTL analysis tool (www.webqtl.org). Total OB volume was mapped to a significant linkage peak at chromosome 16, LRS of 19.7, and a suggestive peak at chromosome 2, LRS of 15.5. HES1 (Hairy/Enhancer of Split), a potential candidate gene for the QTL of olfactory bulb volume is located at the linkage peak on mouse chromosome 16. Current work dissects those differences further and focuses on significant volumetric differences in OB substructures. In addition to mapping of susceptibility loci determining OB gene expression, microarray assays will be used for genetic correlation analysis of candidate gene expression with morphometric traits evaluated in these strains. Gene-to-gene correlations will also be performed to reveal pathway members. Animal procedures were in accordance with National Institutes of Health guidelines and University Animal Care and Use Committees. This work was supported by grants P20 MH62009 (RWW), KO8 MH067091 (SJK) and RO3 MH072875 (LR).

PUTATIVE METABOLIC REGULATORS IN NUTRIENT SIGNALLING AND ADAPTATION: A COMPARATIVE CASE STUDY THROUGH GENE-METABOLITE CORRELATION NETWORKS FOR PLANTS AND HUMANS AS TWO EVOLUTIONARY DISTANT BIOLOGICAL SYSTEMS

NN Rudovich[1,2] and VJ Nikiforova[3,4]
[1]Campus B. Franklin, Charite´ University of Medicine, Berlin, Germany
[2]German Institute of Human Nutrition, Potsdam-Rehbrücke, Germany
[3]Max Planck Institute of Molecular Plant Physiology, Golm, Germany
[4]Timiryazev Institute of Plant Physiology RAS, Moscow, Russia

A living organism consists of many highly diverse molecular entities organized in a functional dynamic system, capable of simultaneously maintaining homeostasis and reacting to changes within its environment. In order to perform both functions the systemic response to perturbation constitutes a branched chain of consecutive changes of cellular entities. Information concerning these changes propagates along this chain, forming a dense network of causally directed interactions - starting from an exciter (a cause) and its perception through transduction and transformation to an endpoint response (an effect). In order to attempt these chains of response reactions and to elucidate regulatory points within them, time series of transcript and metabolite profiling data from plant and human study, allowing cross-correlation analysis, were conducted. We focused on a particular aspect of the transmission of information from a metabolite to a gene, with the goal to identify putative metabolic regulators in nutrient signaling and adaptation. We compared network topologies regarding three different groups of system elements: transcription factors, other genes and metabolites. In plants, implementing causality into the network allowed classification of metabolite-gene associations into those with causal directionality from metabolite to gene. Several metabolites were positioned relatively early in the causal hierarchy and possessed many connections to the downstream elements. Such metabolites were considered to have higher regulatory potential. For the biological example of hypo-sulfur stress response in plants, the highest regulatory potential scores were established for fructose and sucrose, isoleucine, methionine and sinapic acid. Thus, besides elucidation of metabolic markers of a stressed state, the approach allows revealing of putative regulators of stress response. To check the universality of inferred regulatory capacities of the particular metabolites, we undertake cross-systems comparison of gene-metabolite correlation networks for plants and humans as two evolutionary distant biological systems.

MUTAGENIC ANALYSIS OF TYPE I RESTRICTION-MODIFICATION SYSTEM EcoR124I

E Šišáková, A Guzanová, and M Weiserová
Institute of Microbiology, v.v.i., Academy of Sciences of the Czech Republic, Videnska, Czech Republic

Recent years have witnessed a renaissance of interest in restriction-modification (R-M) systems Type I. The massive ongoing sequencing programmes revealed that these enzymes are widely distributed in a broad range of microorganisms including pathogenic bacteria. Since bacteria frequently exchange DNA among each other by horizontal gene transfer; formation of a barrier against an unregulated uptake of foreign DNA seems to be one important biological function of R-M systems, in addition to the classical function of protection of bacteria against bacteriophage infection. R-M systems provide the host bacteria with immunity to infection by foreign DNA and protect cellular DNA from restriction by methylation of adenosyl residues within the sequence recognised by the restriction enzymes. In the complex Type I R-M systems, restriction and modification activities are catalysed by one enzyme composed of three different subunits, which are encoded by the *hsdR, hsdM* and *hsdS* genes. These enzymes are able to read the methylation status of the recognition sequence on the substrate DNA and as a consequence switch to restriction-mode when the recognition sequence is un-methylated. Many efforts are still focused on elucidating the control mechanism of restriction versus modification. Assembly of these complex enzymes is a strong candidate for that role. The HsdS subunit plays a key role in the function of Type I R-M enzymes being responsible for both the specific recognition of the target DNA and interaction with HsdM and HsdR subunits, while the HsdR subunit is representing the motor component of this enzyme being responsible for ATP-binding, ATP- hydrolysis and subsequent DNA translocation.We focus on identification of amino acids residues of both the HsdR and HsdS subunits involved in subunit assembly of the Type I R-M enzyme EcoR124I

POTENTIAL ROLE OF EF-TU IN REGULATION AND CELL SIGNALLING DURING MORPHOLOGICAL AND BIOCHEMICAL DIFFERENTIATION IN STREPTOMYCETES

J Weiser, M Holub, Š Nezbedová, LD Nguyen, and S Bezoušková
Institute of Microbiology v.v.i., Prague 4, Czech Republic

Protein synthesis elongation factor Tu (EF-Tu), as an important component of translation system in bacteria, participates on correct positioning of the incoming aminoacyl-tRNA during elongation on the ribosome. In recent years accumulated experimental evidence indicates that this protein might be involved in other parts of the cell metabolism as well. There have been described several post-translation modifications of the protein some of them playing role in translation, others important for its potential functions outside of the elongation cycle. In several bacteria it was shown that a part of EF-Tu population, located on the membrane, can be methylated in response to starvation for essential nutrients. In *Mycoplasma pneumoniae* EF-Tu was identified as a protein mediating binding to fibronectin which is a multifunctional protein interacting with molecular motor like structures in eukaryotes. Very recently, it was shown that EF-Tu participates also in communication between plants and bacteria by activation of a set of signalling events and defence responses in the plant. In our studies we concentrated on EF-Tu in filamentous bacteria from genus *Streptomyces*, inhabiting mainly soil ecosystems, undergoing a complex developmental cycle and known to produce many diverse secondary metabolites including antibiotics. We described previously a spontaneous polymerisation of EF-Tu from *Streptomyces aureofaciens*, which might serve as a protective mechanism for EF-Tu present in spores. EF-Tu in aggregates preserves its functionality and we have developed a simple and effective method for purification of the protein in aggregates. When cloned and over-expressed in *E. coli*, the protein showed similar charge heterogeneity as EF-Tu isolated from *S. aureofaciens* mycelium and all its isoforms were phosphorylated. We demonstrated association of EF-Tu with cell membrane and showed its presence in spores. We have found that Streptomycetes membrane contains protein kinase(s) catalyzing phosphorylation of both, its own, and an externally added EF-Tu, whereas non-differentiating *Mycobacterium* membranes contained protein kinase phosphorylating only its own EF-Tu. When studying the effect of primary metabolism alteration on antibiotic production in *Streptomyces lividans* we observed developmentally dependent changes in expression and stability of EF-Tu. All the specific features of EF-Tu structure and function reviewed in our presentation imply potential role of this protein in complex regulation of Streptomycetes life cycle.

ANALYSIS OF THE PROTEOMIC PROFILE MODULATED BY THE ANTITUMORAL PEPTIDE CIGB-300

AR Ulloa[1], Y Perera[1], J Gil[1], Y Ramos[1], LC Serra[1], L Betancourt[1], A Sánchez[1], LJ González[1], V Besada[1], BE Acevedo[1], D Alonso[2], DE Gomez[2], and SE Perea[1]
[1]Center for Genetic Engineering and Biotechnology, Havana, Cuba
[2]National University of Quilmes, Buenos Aires, Argentine

CIGB-300 is a cyclic peptide able to inhibit the phosphorylation by casein kinase 2 (CK2), a serine/threonine kinase up-regulated in cancer that has been proposed as an attractive therapeutic target. To better understand the cytotoxic effect of CIGB-300 we study the proteomic profile modulated by the peptide in a non-small-cell lung cancer (NSCLC) cell line H125. The proteome of H125 exposed to CIGB-300 during 40 minutes was determined by two experimental techniques: electrophoresis bi-dimensional and LC MS/MS, in both cases a control condition was included for comparison and proteins with a fold change greater than two were identified. In this work we analyze a set of proteins modulated by CIGB-300. Based in the function of the identified proteins and their relation with cancer we give an approach to the molecular basis for CIGB-300 anti-tumoral activity. To archive this goal the information from different biological databases was integrated. We found modulation in processes such as cellular proliferation, apoptosis, energetic metabolism, proteosomal degradation and cytoskeletal organization. Also, these findings provide information about proteins that could be used as markers response to CIGB-300. Finally the results suggest that CIGB-300 may be combined with other anti-cancer drugs in the treatment of tumors.

PATHWAYS ANALYSIS OF THE PROTEINS ENCODED BY THE GENES PROTECTING AGAINST HUNTINGTON'S DISEASE MUTATION IDENTIFIED IN A DRUGABLE GENOME-WIDE RNA INTERFERENCE SCREEN

E Gonzalez Couto, A Caricasole, F Heitz, GC Terstappen, and A Kremer
Siena Biotech, Via Fiorentina 1, Siena, Italy

Huntington's Disease (HD) is a devastating neurodegenerative disease with total unmet patient needs. The genetics of HD is characterized and involves the expansion of a polyglutamine tract at the amino-terminus of the protein encoded by the Huntingtin (HTT) gene. Nevertheless, little is known about the pathological mechanisms and there are no effective ways to slow or prevent the neurodegeneration caused by the HTT mutation. In order to identify novel targets and new pharmacological therapeutic approaches, as well as improved means of determining their efficacy in preclinical and clinical studies, a European team of 5 different laboratories contributing state-of-the art molecular, cellular, bioinformatics, data mining and metabonomics approaches was coalesced into the TAMAHUD consortium, funded through the FP6 program (http://www.tamahud.eu). For target identification, a high throughput-RNAi assay was performed on a novel and robust inducible HD cellular disease model. The assay was designed to identify proteins whose inhibition of expression is protective against mutant HTT. A library of 16926 siRNAs targeting 5642 human genes encoding pharmacologically tractable proteins, specially enriched with genes associated to HD and expressed in the two major brain areas affected by HD was used. In addition, genes associated to HD were included as controls, providing low, intermediate and high rescue upon knock down. The first pass of the high content screen of the whole siRNA library tested on the induced versus non-induced mutant HTT cells allowed identifying a subset of specific protective hits. Initial bioinformatics analysis of this subset of genes protective upon RNAi knock down shows a clear enrichment towards pathologically relevant functions. Additional data integration approaches, combined with pathway analysis and text mining are applied to characterize signaling networks of interest. This detailed dissection of cellular pathways highlighted by the hits reveals the subtle mechanistic aspects of the disease.

CUE-SIGNAL ANALYSIS OF U937 CELLS: A MODEL FOR INFLAMMATION

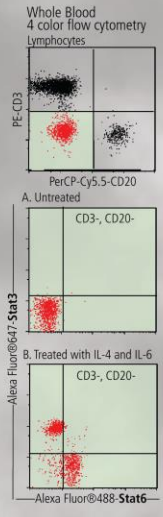
CW Espelin[1], A Goldsipe[2], JS Rodriguez[3], P Sorger[3], D Lauffenburger[2], D de Graaf[1], and B Hendriks[1]
[1]Pfizer Research Technology Center, Cambridge, MA USA
[2]MIT Division of Biological Engineering, Cambridge, MA USA
[3]Harvard Medical School Dept. of Systems Biology, Boston, MA USA

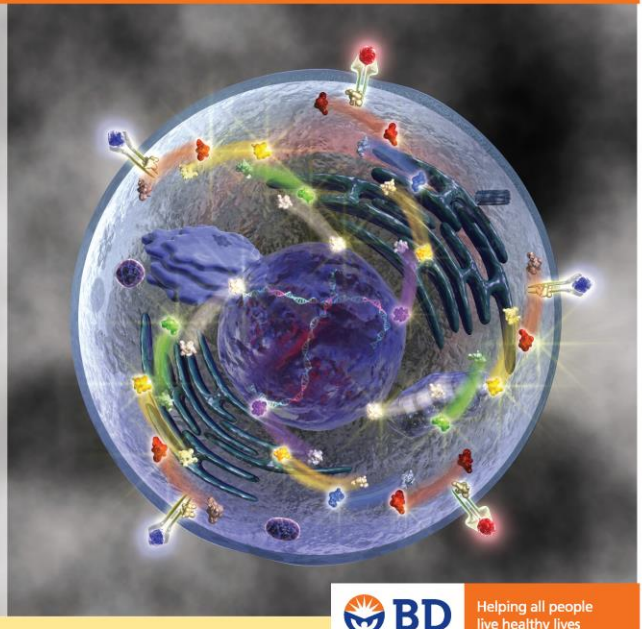
The human inflammatory response involves a wide range of cell types, signaling and responding to each other in an orchestrated process meant to respond to cellular insult and restore immune homeostasis. Failure to maintain a proper balance can result in inflammatory diseases such as Rheumatoid Arthritis and Crohn's Disease. A number of targeted therapies have been developed to limit the effects of an unchecked immune reaction, notably modulators of TNFα. However, substantial percentages (50-70%) of patients do not experience clinical relief with TNFα inhibitors and adverse side effects (including opportunistic infections) are a common cause for cessation of treatment.

The human leukemic U937 cell line serves as an experimental model with which to study the role of the monocyte/macrophage in the inflammatory process. Using the Meso-Scale Discovery (MSD) and Luminex/xMAP platforms, we have collected largescale, dynamic datasets (100,000+ data points) evaluating the status of 23 phosphoproteins and 50 cytokines in U937 cells in response to multiple stimulatory conditions. We have further incorporated inhibitors targeting multiple distinct pathways under different stimulatory conditions in order to evaluate the context-dependent response of the cell to these inhibitors. A range of analytical techniques including PCA, PLSR and Boolean Logic are being been employed to provide an overview of the cellular landscape in response to the various stimuli. This combination of high content data and multiple modeling approaches will allow us to make predictions and test the global effects of perturbing specific intracellular pathways, with an eye towards better understanding the efficacy and consequences of moderating the immune response.

BD™ Phosflow

Phospho-Specific Flow Cytometry Reagents





BD

Helping all people live healthy lives

Analyze Signaling Networks in Single Cells

BD™ Phosflow Advantages:

- Collect statistically significant data for individual cells
- Analyze the phosphorylation state of multiple proteins simultaneously
- Study rare cell types directly in complex primary samples (ie, whole blood)
- Perform rapid and scalable screens in 96-well plates using BD FACS™ flow cytometers
- Choose validated, directly conjugated cell-surface marker antibodies and industry leading flow cytometry instrumentation from one source

For a list of BD™ Phosflow reagents, protocols, and sample data visit **bdbiosciences.com/phosflow**

BD Biosciences
Tel.: (32) 2 400 98 95
Fax: (32) 2 401 70 94
help.biosciences@europe.bd.com
bdbiosciences.com

SPONSORS

Aegean conferences and the conference organizers wish to gratefully acknowledge the following sponsors for their generous support of this meeting.

BD

Case Western Reserve University (CWRU)

Department of Energy

Genome Institute of SIngapore

Helmholtz Zentrum für Infektionsforschung

Human Genome Organization (HUGO)

Institute for Genomic Biology

Institute for Systems Biology

Regeneron Pharmaceuticals

Wiley Publishers

First International Conference: From Deserts to Monsoons
Aldemar Knossos Royal Village, Crete, Greece. June 1-6, 2008

2nd International Conference on Osteoimmunology: Interactions of the Immune and Skeletal Systems
Hilton Hotel, Rhodes, Greece. June 8-13, 2008

6th International Conference on Pathways, Networks, and Systems Medicine
Minoa Palace Conference Center, Chania, Crete, Greece. June 16-21, 2008

5th International Conference on Innate Immunity
Minoa Palace Conference Center. Chania, Crete, Greece. June 21-26, 2008

2nd International Conference on Ovarian Cancer: State of the Art and Future Directions
Hilton Conference Center, Rhodes, Greece. June 26 - July 1, 2008

3rd International Conference on Tissue Engineering
Aldemar Paradise Village, Rhodes, Greece. September 21-26, 2008

4th International Conference on Gene Regulation in Lymphocyte Development
Aldemar Paradise Village, Rhodes, Greece. October 11-16, 2008

First International Conference on Inflammation and Retinal Disease: Complement Biology and Pathology
Aldemar Knossos Royal Village, Crete, Greece. June 28 - July 3, 2009