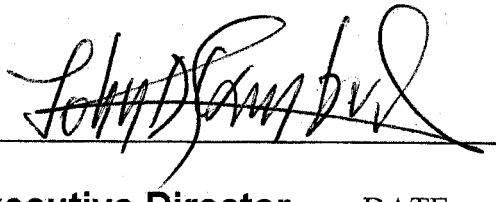
FINANCIAL ASSISTANCE
PROPERTY CERTIFICATION

AWARDEE **Aegean Conferences** AWARD NO. **DE-FG02-03ER63605**

The DOE Assistance Rules, 10 CFR 600, define property acquired under the terms of the award as either exempt (purchased with DOE operating dollars) or non-exempt (acquired with DOE capital equipment dollars, Government-furnished or acquired from the Government excess property lists). Non-exempt property is normally identified in the award document as Government-owned and requires a Semi-Annual Summary Report of DOE-Owned Plant and Capital Equipment to be submitted **annually** pursuant to DOE regulations.

In order to facilitate grant closeout, please review your property records and complete the applicable Item I, for exempt property, or Item II, for non-exempt property, of this certificate and return it to the Contracting Officer as soon as possible.

I. There was no reportable residual exempt property of any description at the completion of this award.

SIGNATURE 

TITLE **Executive Director** DATE **October 7, 2004**

II. A final inventory of residual non-exempt property is enclosed for the category (or categories) checked below:

( ) A. NONEXPENDABLE PERSONAL PROPERTY, Federally-Owned (acquired with Capital Equipment funds, Government-furnished equipment, or through excess)

( ) B. EXPENDABLE PERSONAL PROPERTY (SUPPLIES), if fair market value exceeds $5,000.

Except for the category (categories) checked above, no other reportable residual property of any description remained on this award at its completion.

SIGNATURE _____

TITLE _____ DATE _____

AA-88
01/03

Form Approved
OMB No. 1902-0121

PATENT CERTIFICATION

Aegean Conferences
Awardee

☐ Interim Certification

DE-FG02-03ER63605
DOE Prime and/or Subcontract Nos.

☒ Final Certification

Awardee hereby certifies unless indicated to the contrary, that:

1. All procedures for identifying and disclosing subject inventions as required by the patent clause of the contract have been followed throughout the reporting period.

2. There were no subcontracts or purchase orders involving research, development, and demonstration except as follows: (a separate certification must be provided to DOE for each subcontract or purchase order awarded.)

3. No inventions or discoveries were made or conceived in the course of or under this contract other than the following (Certification includes ☒, does not include ☐ all subcontracts):

TITLE	INVENTOR	DATE REPORTED	DOE "S" NO.*
N/A	**N/A**	**N/A**	**N/A**

4. The completion date of this contract is as follows:

5. The following period is covered by this certification:

July, 8 2003 to **July 31, 2004**
Month Day Year Month Day Year

John D. Lambris
Contractor

Signature

Executive Director
Title

36 Haymarket Lane, Bryn Mawr, PA 19010
Address

July 8, 2003
Date of Certification

* Also include Subcontract No. if available.

NOTE: A positive certification for this Item 3 does not negate the requirement for furnishing to DOE a fully executed Patent Certification from each subawardee identified in Item 2.

FINANCIAL STATUS REPORT

(Short Form)

(Follow instructions on the back)

1. Federal Agency and Organizational Element to Which Report is Submitted	2. Federal Grant or Other Identifying Number Assigned By Federal Agency	OMB Approval No.	Page of
Department of Energy	**DE-FG02-03ER63605**	**0348-0038**	pages

3. Recipient Organization (Name and complete address, including ZIP code)
Aegean Conferences, 36 Haymarket Lane, Bryn Mawr, PA 19010

4. Employer Identification Number	5. Recipient Account Number or Identifying Number	6. Final Report	7. Basis
25-1852984	86-1557-3418	☒ Yes ☐ No	☒ Cash ☐ Accrual

8. Funding/Grant Period *(See instructions)* From: (Month, Day, Year)	To: (Month, Day, Year)	9. Period Covered by this Report From: (Month, Day, Year)	To: (Month, Day, Year)
July 8, 2003	July 31, 2004	July 8, 2003	July 31, 2004

10. Transactions:	I Previously Reported	II This Period	III Cumulative
a. Total outlays			10,000
b. Recipient share of outlays			
c. Federal share of outlays			
d. Total unliquidated obligations			
e. Recipient share of unliquidated obligations			
f. Federal share of unliquidated obligations			
g. Total Federal share *(Sum of lines c and f)*			
h. Total Federal funds authorized for this funding period			10,000
i. Unobligated balance of Federal funds *(Line h minus line g)*			

11. Indirect Expense	a. Type of Rate *(Place "X" in appropriate box)* ☐ Provisional ☐ Predetermined ☐ Final ☐ Fixed			
	b. Rate	c. Base	d. Total Amount	e. Federal Share
	n/a	n/a	n/a	n/a

12. *Remarks: Attach any explanations deemed necessary or information required by Federal sponsoring agency in compliance with governing legislation.*

13. Certification: **I certify to the best of my knowledge and belief that this report is correct and complete and that all outlays and unliquidated obligations are for the purposes set forth in the award documents.**

Typed or Printed Name and Title	Telephone (Area code, number and extension)
John D. Lambris, Executive Director	610-527-7630
Signature of Authorized Certifying Official	Date Report Submitted
John D. Lambris	October 8, 2004

DOE F 4300.3
(09-93)
(All other editions are obsolete)

U.S. Department of Energy

OMB Control No.
1910-0500
OMB Burden Disclosure
Statement on Page 4

Semi-Annual Summary Report of DOE-Owned Plant and Capital Equipment (P&CE)

Contractor Name Aegean Conferences Contract No. **DE-FG02-03ER63605**

Address 36 Haymarket Lane, Bryn Mawr, PA 1901

Location of Property (City, State) _____

Contracting Office _____

1	2	3	4	5	6	7	8	9
Asset Type Code	Beginning Balance As of _____		Acquisitions		Dispositions		Ending Balance As of _____	
	No. of Items	$	No. of Items	$	No. of Items	$	No. of Items	$
n/a								
n/a								
n/a								
n/a								
n/a								
n/a								
n/a								
n/a								
Total Plant and Capital Equipment								

Prepared By John D. Lambris, Executive Director 610.527.7630
name (printed), title, telephone number, signature

Date of Last Physical Inventory of Capital Equipment _____

Contracting Officer Representative Signature _____

(Attach results of latest Physical Inventory if conducted since last reporting period)

Summary of Acquisitions & Dispositions by Type of Transactions

Acquisitions from Column (5) above

1. Purchases:
   a. P&CE Budgeted Items ..... $ n/a
   b. Operating Expense Budgeted Items ..... $ _____
2. Fabrications
   a. Beginning Balance ..... $ _____
   b. Additions - P&CE Budgeted ..... $ _____
   c. Additions - Operating Expense Budgeted ..... $ _____
   d. Completions ..... ($ _____) $ _____
   e. Ending Balance ..... $ _____
3. No-Charge-Transfers from Other DOE Offices or Contractors ..... $ _____
4. No-Charge-Transfers from Other Federal Agencies ..... $ _____
5. Other (Explain) ..... $ _____
6. Total Acquisitions ..... $ _____

Dispositions from Column (7) Above

7. Sales (Salvage Credit of $) ..... $ _____
8. Trade-ins (Salvage Credit of $) ..... _____
9. No-Charge-Transfers to Other DOE Offices or Contractors ..... $ _____
10. No-Charge-Transfers to Other Federal Agencies ..... $ _____
11. Other (Explain) ..... $ _____
12. Total Dispositions ..... $ n/a

Note: Detail lines 1 through 12 above in accordance with the following columnar headings. Attach extra sheets if necessary.

DOE F 4300.3, pg. 2
(09-93)
(All Other Editions Are Obsolete

OMB Control No.
1910-0500

Line No.	Asset Type No. (See DEAR 945.5)	Tag No.	Equipment Item Description (Brief)	In-Service Date	Acquisition Cost
n/a					n/a

CONFERENCE REPORT

Pathways, Networks and Systems: Theory and Experiments (GREECE SYSTEMS I)

Nomikos Center, Santorini, Greece
28 September – 2 October 2003

1. Summary

The international conference provided a unique opportunity for theoreticians and experimenters to exchange ideas, strategies, problems, challenges, language and opportunities in both formal and informal settings. This dialog is an important step towards developing a deep and effective integration of theory and experiments in studies of systems biology in humans and model organisms.

2. Outcome

Conference participants agreed that an annual series of conferences with similar format and venue would make a profound contribution to systems biology research. The program of speakers and topics has been developed for Greece II (see below). Funding applications have been submitted and significant support has already been obtained. An annual conference focusing on cutting-edge systems biology research is envisioned.

Several changes were made to enhance the program:

- We added an additional conference organizer, Magali Roux, to represent European interests in program development and funding opportunities.
- We expanded the opportunities for students to attend the conference, present posters, and make platform presentations.
- We extended modestly the length of the formal presentations each day to accommodate a broader program.

3. Program

A copy of the Greece I program is attached.

The program was divided into eight sessions, each of which addressed a particular aspect of systems biology research. Emphasis was placed on experimental studies and empirical evidence so that attention was focused on the challenging issues of study design and data analysis, and so thattheoretical considerations were placed in a biological context.

Session 1. Defining Experimentally Realistic Biological Models
Session 2. Biological Components of Defined Model Systems
Session 3. Structuring Biological Data, Databases, and Analysis Tools
Session 4. Analysis of Systems Data
Session 5. Interaction between Biological Components; Construction of Networks
Session 6. Methods for Reconstructing Biological Networks
Session 7. Modeling and Simulation of Networks I
Session 8. Modeling and Simulation of Networks II

Each session was chaired by two invited speakers; they presented their work in platform presentations, introduced the short talks, and managed the questions and discussions.

Students contributed actively to the program, in selected platform presentations, in poster sessions (posters were 'up' for the entire conference and were strategically located adjacent to the coffee and lunch area which also served as one of the two passages into the conference hall. Conference participants, including students, shared all meals and coffee together and, with the open afternoon format, considerable opportunity was provided for faculty and students to interact extensively.

The venue and conference organization facilitated numerous informal discussions. All participants stayed at one of two hotels and shared lunch and dinner together. Late afternoons were free for extended opportunities to discuss research topics and to establish collaborations.

4. Organizers

The organizers were:

Joe Nadeau, Case Western Reserve University, Cleveland
David Galas, Keck Graduate Institute, Claremont
Lee Hood, Institute for Systems Biology, Seattle (not able to attend)
Diane Isonaka, Bioconsultant, Claremont
Shankar Subramaniam, UCSD, San Diego

5. Organization

Aegean Conferences (Bryn Mawr, Pennsylvania) sponsored the conference and provide the local logistical support and all of the supporting activities such as managing registrations, arranging the conference facilities and staff, accommodations and meals, and local transportation, and reimbursements.

5. Participants

The conference included 87 participants from 14 countries; US (35), UK (14), Netherlands (5), Greece (8), Finland (4), Japan (4), France (3), Germany (3), Italy (3), Switzerland (3) Denmark (3), Belgium (1), and The Czech Republic (1).

6. Women Participation

Diane Isonaka co-organized the conference by providing assistance with program development and fund raising.

Three women made platform presentations:

- Imam Famili (UCSD; a graduate student in Dr. B. Palsson's laboratory)
- Magali –Roux (Institut Pasteur, Paris).

- Mariko Hatakeyama (RIKEN Genomic Sciences Center, Yokohama)

7. Student Participation

Three students made platform presentations:

- Imam Famili, UCSD
- Toshi Kitami, CWRU
- Mariko Hatakeyama, RIKEN

8. Student travel awards

The Student awardees are listed in Table 1.

Every student who requested assistance with travel expenses and conference fees received an award. This included students from eleven countries; six of whom were males and five were females.

9. Future plans

Based on the tremendous enthusiasm about the topics, program, speakers and venue, the group decided enthusiastically to continue these conferences annually. The consensus was that these conferences provide a unique venue for deep and thoughtful interactions between two important constituencies that do not usually participate in the same systems biology meetings. Progress in systems biology research depends heavily on rigorous analysis of data from carefully designed surveys and experiments. Dialog is needed between these communities to gain understanding of the language and jargon, theoretical, analytical and experimental challenges and opportunities of each discipline. In this way, the hard and important problems that systems biology proposes to tackle are likely to be resolved in a timely, thoughtful and productive manner.

TABLE 1. Student Travel Awards

1. Ashwin Kotwaliwale
Beatson Institute for Cancer Research
Glasgow, UK

2. Scott Bornheimer
Dept. of Chemistry & Biochemistry and Cellular & Molecular , University of California San Diego
La Jolla, California, US

3. Vera van Noort
Nijmegen Center for Molecular Life Sciences
Nijmegen, The Netherlands

4. Patrick Lager
Dept. of Molecular Genetics and Microbiology, Duke University Medical Center
Durham North Carolina, US

5. Nils Bluethgen
Institute for Theoretical Biology, Humboldt University
Berlin, Germany

6. Aristotle Chatziioannou
National Technical University of Athens
Athens, Greece

7. Toshimori Kitami
Dept. of Genetics, Case Western Reserve University
Cleveland Ohio, US

8. Nuria Domedel-Puig
Birkbeck College, School of Crystallography, University of London
London, UK

9. Iosifina Pournara
Birkbeck College, School of Crystallography, University of London
London, UK

10. Haifeng Shao
Dept. of Electrical Engineering and Computer Science, Case Western Reserve University
Cleveland, Ohio, US

11. Iman Famili
Dept. of Bioengineering and Bioinformatics, UCSD
La Jolla, California, US

AEGEAN CONFERENCES

Linking the international scientific community
Bringing the humanity scholars together

Aegean Conferences Series - Vol. 12

Nomikos Center, Santorini, Greece

International Conference on Pathways, Networks, and Systems: Theory and Experiments

September 28 - October 2 2003

Aegean Conferences: 36 Haymarket Ln, Bryn Mawr, PA 19010
Phone: 610-527-7630, FAX: 610-527-7631, E-mail: info@aegeanconferences.org
WEB: www.aegeanconferences.org, www.conferex.org

Aegean Conferences Series

AEGEAN CONFERENCES

Linking the international scientific community
Bringing the humanity scholars together

International Conference Pathways, Networks, and Systems: Theory and Experiments

September 28 - October 2, 2003
The Nomikos Center
Santorini, Greece

GENERAL INFORMATION

Accommodations
The conference participants will be staying in the "El Greco" or "Santorini Palace" Hotels (A' class), which are located in Fira, about 7 km from the airport and 8 km from the port. The most convenient way to reach the hotels is to hire a taxi.

Breakfast
Breakfast will be served at your hotel during the hours scheduled in this program.

Workshop Venue
The Nomikos Conference Center is located in Fira, the capital of Santorini, overlooking the Caldera and the Santorini volcano. The "Santorini Palace" Hotel is walking distance from the Conference Center. For those participants staying at the "El Greco" hotel, located 1.5 km from the Nomikos Center, transportation will be provided at the indicated times.

Oral Presentations
An LCD projector, a projector for 2x2 inches slides, and an overhead projector will be available for the oral presentations. Speakers are asked to bring their CD or slides to the slide reception desk at least 30 min. before the beginning of the session.

Posters
Posters should be mounted on Monday morning on the designated boards and dismounted at the end of the meeting. The dimensions of the boards are 120 x120 cm. Adhesive tape for mounting the posters on the boards will be available at the poster area.

Welcome Reception and Banquet
Badges are required for admission.

Island Tour
Buses for the island tour will be leaving from the Nomikos Center on Tuesday, September 30 at 2:00 p.m. The accompanying persons may be picked up at the Nomikos Center or otherwise at their Hotel at 1:45 pm. **Badges** are required for participation.

Lunches and other Events

Lunches will be served at the Nomikos Center during the scheduled lunch hours to participants whose registrations include lunches. For those participants who have not registered for the events, lunch and other event tickets may be purchased on site. ***Badges*** are required for admission.

Tour and Travel Information

A desk operated by the personnel of our official Travel Agency, "ERA Ltd", will be located at the Nomikos Center throughout the meeting.

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

AEGEAN CONFERENCES
Pathways, Networks, and Systems: Theory and Experiments

The Nomikos Center
September 28 - October 2, 2003
Santorini, Greece

ORGANIZING COMMITTEE

Joe Nadeau, Ph.D.
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Chair, Department of Genetics
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La Jolla, CA 92093
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E-mail: shsubramaniam@ucsd.edu

PROGRAM OUTLINE

Sunday, September 28

Registration	6:00-9:00 PM	Nomikos Center
Welcome Reception - Dinner	7:00 PM	Nomikos Center

Transportation to the "El Greco" Hotel will be provided upon the conclusion of the Dinner

Monday, September 29

Breakfast	7:30 AM	Nomikos Center

The bus will depart from the "El Greco" Hotel at 8:30 AM

Registration	8:50 –2:00 PM	Nomikos Center
Organizers: Welcome and Opening Remarks	8:50 AM	Nomikos Center
Session I	9:00 AM	Nomikos Center
Coffee break and poster session	11:00 AM	Nomikos Center
Session II	12:00 Noon	Nomikos Center
Lunch	2:00 PM	Nomikos Center
Dinner	8:00 PM	"Pyrgos", Pyrgos

The bus will depart from the "El Greco" and "Santorini Palace" Hotels at 7:30 PM

Tuesday, September 30

Breakfast	7:30 AM

The bus will depart from the "El Greco" Hotel at 8:30 AM

Session III:	9:00 AM	Nomikos Center
Coffee break and poster session	11:00 AM	Nomikos Center
Session IV:	12:00 Noon	Nomikos Center
Island Tour and Lunch	2:00 PM	

Wednesday, October 1

Breakfast	7:30 AM	

The bus will depart from the "El Greco" Hotel at 8:30 AM

Session V:	9:00 AM	Nomikos Center
Coffee break and poster session	11:00 AM	Nomikos Center
Session VI:	11:30 AM	Nomikos Center
Lunch	1:15 PM	Nomikos Center
Dinner	8:00 PM	"Selene, Fira"

The bus will depart from the "El Greco" and "Santorini Palace" Hotels at 7:30 PM

Thursday, October 2

Breakfast	7:30 AM	

The bus will depart from the "El Greco" Hotel at 8:30 AM

Session VII:	9:00 AM	Nomikos Center
Coffee break	11:00 AM	Nomikos Center
Session VIII:	11:30 AM	Nomikos Center
Lunch	1:30 PM	Nomikos Center

Gala Dinner	8:00 PM	Nomikos Center

The bus will depart from the "El Greco" and "Santorini Palace" Hotels at 7:30 PM

Friday, October 3

Breakfast 7:30 AM

Departure

PROGRAM

Sunday, September 28

6:00 - 9:00 PM	*Open Registration*
7:00 PM	*Welcome Reception - Dinner*

Monday, September 29

Session I. ***Defining Experimentally Realistic Biological Models***
Chair: Joe Nadeau

9:00 AM		***Welcome and Opening Remarks***
9:15 AM	***1***	***Genetic Variation, Homeostasis, and Systems Analysis of Mouse Models of Human Disease*** J Nadeau
10:00 AM	***2***	***Systems Biology and Systems Science*** M Mesarovic
10:30 AM	***3***	***Transcriptional and Metabolic Profiling of Homocysteine Homeostasis After Diet-Induced Perturbations in Genetically Distinct Inbred Strains of Mice*** T Kitami, R Gaspard, J Quackenbush, and JH Nadeau
10:45 AM	***4***	***Kinetic Regulation of the GTPase Cycle of Trimeric G Proteins*** SJ Bornheimer, EM Ross, MG Farquhar, and S Subramaniam
11:00 AM		Coffee Break and Poster Session

Session II ***Biological Components of Defined Model Systems***
Co-Chairs: David Galas and Greg Dewey

12:00 Noon	***5***	***Structure and Evolution of Gene Regulatory Networks: Some Biological Constraints*** D Galas
12:45 PM	***6***	***Network Analysis of Gene Expression Time Series*** G Dewey
1:30 PM	***7***	***Systems Analysis of Bone Fragility*** K Jepsen, C Price, and JH Nadeau
1:45 PM	***8***	**Can Drug Combinations Elucidate Biological Systems?** J Lehár, G Zimmermann, M Lee, J Staunton, A Borisy, and C Keith

2:00 PM		Lunch
8:00 PM		Dinner at "Pyrgos Tavern", in Pyrgos

Tuesday, September 30

Session III		***Structuring Biological Data, Databases, and Analysis Tools*** *Co-Chairs: Shankar Subramaniam and Herb Sauro*
9:00 AM	***9***	***Deciphering Intracellular Networks in Mammalian Cells*** S Subramaniam
9:45 AM	***10***	***The Computational Versatility of the Cascade Cycle*** H Sauro
10:30 AM.	***11***	***An Experimental Systems Approach to Discover MRNAs Encoding Proteins in Related Pathways*** JD Keene and SA Tenenbaum
10:45 AM	***12***	***A Systemic Reference Metamodel for Semantic Data Integration in Systems Biology*** M Roux-Rouquie, N Caritey, L Gaubert, C Auffray
11:00 AM.		Coffee Break and Poster Session
Session IV		***Analysis of Systems Biology Data*** *Co-Chairs: Bruno Dumas and John D. Lambris*
12:00 Noon	***13***	***Total Synthesis of Hydrocortisone from A Simple Carbon Source in Yeast*** B Dumas
12:45 PM	***14***	***A Systems Biology Analysis of Complement Functions*** *JD Lambris, D Morikis, MC Holland, CW Strey, M Winters, M Markieski, and G Sfyoera*
1:30 PM	***15***	***Systems Biology Applied to Organelle Biogenesis and Function*** *JD Aitchison, M Marelli, JJ Smith, E Yi, and D Goodlett*
2:00 PM		Island Tour to Akrotiri (Ancient City), Lunch, and Ia (Sunset)

Wednesday, October 1

Session V	***Interaction Between Biological Components and Construction of Networks***

Co-Chairs: Trey Idekar and Tim Galitski

9:00 AM ***16*** ***Network Genomics: Lessons Learned from the Human Genome Project***
T Ideker

9:45 AM ***17*** ***Modular Structure and Function of Integrated Molecular Networks***
T Galitski

10:30 AM ***18*** ***Reconstructing Biological Pathways from Large-scale Protein Interaction Data***
MP Samanta and S Liang

10:45 AM ***19*** ***Systems Approaches to Understanding Mechanisms of Environmental Responses Using Halobacterium Sp As A Model System***
NS Baliga

11:00 AM Coffee Break and Poster Viewing

Session VI ***Methods for Reconstructing Biological Networks***
Co-Chairs: Bernhard Palsson and Zoltan Oltvai

11:30 AM ***20*** ***Genomes to Life: the Role of in Silico Models***
B Palsson

12:15 PM ***21*** ***Topologic and Dynamical Organization of Cellular Networks***
Z Oltvai

1:00 PM ***22*** ***Modeling the Metabolic and Regulatory Network of the Model Lactic Acid Bacterium Lactobacillus Plantarum***
B Teusink, FHJ van Enckevort, A Wegkamp, D Molenaar, J Hugenholtz, EJ Smid and RJ Siezen

1:15 PM Lunch

8:00 PM Dinner at "Selene" in Fira

Thursday, October 2

Session VII ***Modeling and Simulation of Networks I***
Co-Chairs: Hiroaki Kitano and Andreas Wagner

9:00 AM ***23*** ***Toward the Theory of Biological Robustness***
H Kitano

9:45 AM ***24*** ***The Large-Scale Structure of Genetic Networks: Design,***

		History or Mere Chemistry A Wagner
10:30 AM	***25***	***Probabilistic Boolean Networks As Models of Genetic Regulatory Networks*** I Shmulevich and W Zhang
10:45 AM	***26***	***Protein Complexes and Functional Modules in Molecular Networks*** LA Mirny and V Spirin
11:00 AM		Coffee Break and Poster Session
Session VIII		***Modeling and Simulation of Networks II*** *Co-Chairs: David Fell and Jeff Hasty*
11:30 AM	***27***	***Functional Analysis of Metabolic Networks using Elementary Modes*** D Fell
12:15 PM	***28***	***Engineered Gene Circuits*** J Hasty
1:00 PM	***29***	***Direct and Reverse Simulations of Signal Transduction Pathways in Neuronal Cells*** I Arisi, V Rosato and A Cattaneo
1:15 PM	***30***	***A Computational Model on RAF-AKT Cross-Talk in ERBB Signaling*** M Hatakeyama, S Kimura, T Naka, T Kawasaki, N Yumoto, M Ichikawa, M Shirouzu, S Yokoyama, and A Konagaya
1:30 PM		Lunch
8:00 PM		Gala Dinner at Nomikos Center

Friday, October 3

7:30 AM	Breakfast
11:00 AM	**Departure**

POSTERS

31 MAPPIT : A versatile method to study protein-protein interactions in mammalian cells.
J Tavernier, S Eyckerman, I Lemmens, S Lievens, Joël Vandekerckhove, J Van Der Heyden, A Verhee, and E Vertenten

32 Plant defense signaling network inference using knowledge-based perturbations and global gene expression analysis.
T Genoud, T Kulisic, M B Trevino Santa Cruz, J-P Métraux, and J Pufky

33 Eukaryotic mRNPs as operational networks regulating post-transcriptional gene expression
PJ Lager, SA Tenenbaum, and JD Keene

34 Simple model explains small-world, scale-free architecture of gene co-expression network.
V van Noort, B Snel, and MA Huynen

35 Mode content and distribution: Discovering details of differences in gene array responses to perturbation
H Shao, Y-H Pao, S Ernest, and J H Nadeau

36 Step vs. ramp induction of myocardial ischemia: Comparing in vivo and in silico results.
JE Salem, ME Cabrera, MP Chandler, TA McElfresh, H Huang, JP Sterk, and WC Stanley

37 Dissecting gene networks influencing body weight by QTL analysis and text mining.
S Dietmann, HJ Thiesen, and G Brockmann

38 Gene network reconstruction in mouse from microarrays and gene interaction information in fly.
K Evans, N Domedel-Puig, I Pournara, L Wernisch

39 Simulation and reconstruction of small scale genetic network
T Vu and J Vohradsky

40 Zero-Order ultrasensitivity limited by sequestration
N Bluethgen, H Sauro, S Legewie, I Axmann, F Bruggemann, and H Herzel

41 Inference of gene function in probabilistic graphical models.
S Skoulakis, I Pournara, S Soneji, M Edwards, and L Wernisch

42 Performance of learning algorithms for regulatory networks.

I Pournara and L Wernisch

43 ***Toward brain systems biology: Synapse proteomes show scale-free network properties underlying plasticity, cognition and mental illness.***
SGN Grant, H Husi, J Choudhary, LYu, M Cumiskey, W Blackstock, TJ O'Dell, P M Visscher, and JD Armstrong

44 ***Theoretical study of homeostatic and developmental gene networks dynamics.***
AV Ratushny., VA Likhoshva, YuGMatushkin, and NA Kolchanov

45 ***Bioinformatic methods for longitudinal metabolomics data***
AK Smilde, J Jansen, HCJ Hoefsloot, HFM Boelens, R-J AN Lamers, S Bijlsma, J van der Greef, and ME Timmerman

46 ***Cross-talk of different receptor systems in GDNF signalling***
H Sariola, A Popsueva, and M Saarma

47 ***Managing and Extracting Information From Proteomics Data to Model Pathways***
A Kotwaliwale, K Vass

48 ***"Homology of Function" in Human Cellular Systems: A Unified Systems Biology Approach for the Identification of Gene Networks and Drug Action***
E Hytopoulos, E Kunkel, E L Berg, I Plavec, A Ebens, and E C Butcher

49 ***Delineating Functional Modules from Genomic Context***
B Snel, C von Mering, P Bork, and MA Huynen

50 ***Knowledge Discovery and Dynamic Modeling in Complex Gene Regulatory Networks***
X Qian, S Godbole, A Gupta, A Ray

51 ***Gene-Metabolite Correlation Network Reflects Flow of Information along the paths of Propagating Concentrational Changes in Response to External System Perturbation***
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ABSTRACTS

GENETIC VARIATION, HOMEOSTASIS, AND SYSTEMS ANALYSIS OF MOUSE MODELS OF HUMAN DISEASE

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A major problem in studying biological traits is understanding how genes work together to provide organismal structures and functions. Conventional approaches usually involve reductionist studies where specific functions are attributed to particular genes, motifs and amino acids. The equally important but harder problem involves the synthesis of information to understand functionality at higher levels. We have developed a computational method that uses assays of component traits to learn about higher level systems. We used subtle, naturally-occurring, multigenic variation of cardiovascular (CV) properties in the A/J and C57BL/6J strains and the AXB / BXA RI strains to perturb CV functions in non-pathologic ways. In this proof-of-concept study, computational analysis correctly identified the known functional relations among CV properties and revealed key aspects of CV functions. This functional network was then used to account for pleiotropies and homeostasis in single gene mutant mice and the effects of pharmacologic treatments. These networks account for functional dependencies in ways that compement genetic networks of complex traits and molecular pathways. These networks are therefore an important and general approach for defining and characterizing functional relations in complex biological systems in health and disease.

SYSTEMS BIOLOGY AND SYSTEMS SCIENCE

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Systems biology is based on a triad: Experimentation of the biological phenomena in a holistic manner rather than reductionism, computer software tools to unravel information from a wealth of data, and system science to help transform information into understanding. The role of systems science is best illustrated by a quote attributed to Charles Darwin: *"How odd it is that anyone should not see that all observation must be for or against some view if it is to be of any service?"*
Systems science provides the hypothesis to help interpret information as well as design experiments.
The weakest link in the triad is that of systems science and experimentation. On one hand, systems biology has not fully used the concepts which already have been developed in systems science—in particular in the multilevel, hierarchical, systems area—while on the other hand systems science has been traditionally developed for the issues in physical science and has not as yet addressed sufficiently the specific issues vital for biology. To illustrate this point we consider two case studies: Use of the coordination principles for understanding how the regulators are regulated from the second level in transcriptional pathways; and formulation of resistance and resilience for launching systems science research specifically to serve biology.
A language for systems biology is proposed using logic and categorical, mathematical branches rather than precise analytical tools more appropriate for classic physical phenomena. As recognition that vital signs—such as heart rate—are chaotic and have a fractal structure is a sign of health illustrates that the system concepts beyond homeostasis and traditional dynamic analysis are needed. For example, for system's parameters identification there should be a search for upper and lower limits rather than a precise numbers. Mathematical Theory of General Systems provides the foundation for making systems science more integrated into systems biology.

TRANSCRIPTIONAL AND METABOLIC PROFILING OF HOMOCYSTEINE HOMEOSTASIS AFTER DIET-INDUCED PERTURBATIONS IN GENETICALLY DISTINCT INBRED STRAINS OF MICE

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Abnormalities in the folate and homocysteine metabolic pathways are associated with increased risk for atherosclerotic vascular disease, stroke, neural tube defects, and certain forms of cancer in humans. These pathways are involved in a variety of metabolic reactions including the biosynthesis of purines and pyrimidines, methylation of DNA, RNA, proteins and lipids, and the metabolism of several amino acids. Dietary supplementation with folic acid decreases the level of homocysteine in serum as well as the risks for birth defects and certain adult diseases. While association studies in humans are in progress, mouse models offer powerful opportunities for exploring the impact of specific dietary perturbations on the dynamics of these key pathways in health and disease. Genomic tools such as microarrays enable systematic analysis of molecular components, allowing for studies of pathways and networks that contribute to elevated homocysteine level and the contribution of anomalies in these pathways to these common but complex diseases. We used two inbred strains of mice which under the same folate perturbation, show significant difference in serum homocysteine and folate levels. We monitored the transcription and metabolite profiles of these two strains as folate level was lowered and raised with dietary restriction and supplementation. We found that these dietary perturbations induced dramatic changes in folate and homocysteine levels and in gene expression profiles within weeks. We showed that disruption of homocysteine homeostasis leads to changes in the expression level of specific genes and pathways and offer new insight to the association between hyperhomocysteinemia and human complex diseases and their mouse models.

KINETIC REGULATION OF THE GTPase CYCLE OF TRIMERIC G PROTEINS

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The GTPase cycle is an essential and ubiquitous biochemical module in eukaryotic signal transduction pathways. To understand and simulate its behavior, we present and analyze a kinetic model of the GTPase cycle for a trimeric G protein. The model includes freely associating G-protein-coupled receptor (GPCR), G protein, GTPase-activating protein (GAP), GTP and GDP. We study the effects of protein and nucleotide concentrations on fractional G protein activation and GTPase activity at steady state, and on the kinetics of approach to steady state. The m1 muscarinic cholinergic receptor, G_q, and RGS4 as GAP constitute our test GTPase cycle. Kinetic parameters come from experimental data or parameter optimization and the model reproduces experimental results well. A rich variety of G protein activity is predicted, with four limiting regimes. To change limiting regimes, specific ~100-fold changes in [GPCR] or [GAP] are required. In one regime, GAP activity promotes GPCR catalyzed GDP/GTP exchange, allowing a GAP to accelerate signal turn-off upon removal of agonist without attenuating signal while agonist is present. This explains the paradoxical observation that GAPase deactivator of G proteins—speeds the activation of some ion channels without inhibiting current amplitude. Our kinetic modeling technique is generally applicable to other GTPase cycles and can be extended to independent functions of G proteins such as effector activation, enabling the study of larger pathway networks.

STRUCTURE AND EVOLUTION OF GENE REGULATORY NETWORKS: SOME BIOLOGICAL CONSTRAINS

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Gene regulatory networks are at the heart of the information processing function of both the individual cell and of the developmental process. Since networks evolve and grow it is useful to examine the possible mechanisms of growth and to study the relationship between the evolved structures and the processes that give them form. The networks that have evolved in biological systems have some features in common with other, non-biological networks, but also some important differences. We will examine some of the mathematical relationships that constrain the overall growth patterns of networks under different growth models and discuss the graph parameters that may be relevant to considering biological networks. We will examine some of these models and these constraints, particularly those based on gene duplication.

The molecular interactions involved during transcription of genes are the molecular embodiments of these networks and themselves constrain and facilitate their evolution by their very nature. We will discuss some recent experimental analyses of several systems - yeast and *E. coli* and an example of network evolution from the echinoderm developmental pathway – and compare them. These will be discussed with respect to the insights gained and questions raised about the structure and evolution of genetic regulatory networks.

NETWORK ANALYSIS OF GENE EXPRESSION TIME SERIES

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There has been considerable interest in developing computational techniques for inferring genetic regulatory networks from whole-genome expression profiles. When expression time series data sets are available, dynamic models can, in principle, be used to infer correlative relationships between gene expression levels, which may be causal. Network models derived from simple rate laws offer an intermediate level analysis, going beyond simple statistical analysis, but falling short of a fully quantitative description. This talk discusses how such network models can be constructed and describes the global properties of the networks derived from such a model. These global properties are statistically robust and provide insights into the design of the underlying network. Several whole-genome expression time series datasets from yeast microarray experiments were analyzed using a Markov-modeling method to infer an approximation to the underlying genetic network. We found that the global statistical properties of all the resulting networks are similar. The overall structure of these biological networks is distinctly different from that of other recently studied networks such as the Internet or social networks. These biological networks show hierarchical, hub-like structures that have some properties similar to a class of graphs known as small world graphs. Small world networks exhibit local cliquishness while exhibiting strong global connectivity. In addition to the small world properties, the biological networks show a power law or scale free distribution of connectivities. An inverse power law, $N(k) \sim k^{-3/2}$, for the number of vertices (genes) with k connections was observed for three different data sets from yeast. We propose network growth models based on gene duplication events that closely mimic the experimental derived networks.

SYSTEMS ANALYSIS OF BONE FRAGILITY

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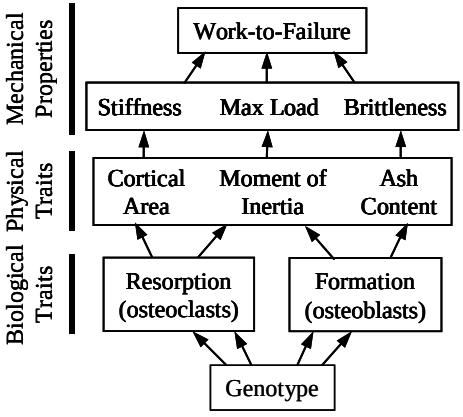
The use of genetic information to reduce fracture risk associated with osteoporosis, a disease attributed to low bone mass, requires an understanding of how genes work together to create a mechanically functional structure.

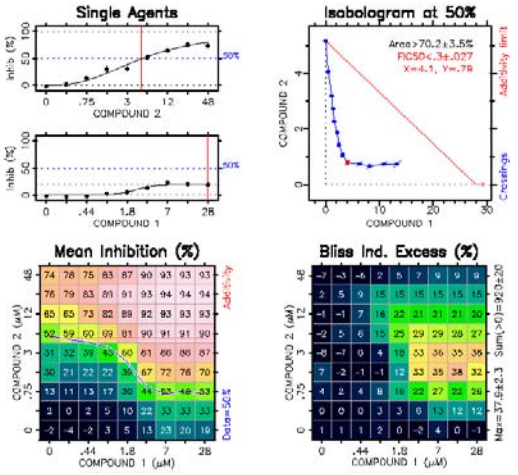


Correlative studies which utilized variation in long bone traits among inbred mouse strains revealed a hierarchical relationship among traits and whole bone mechanical properties, including measures of fragility (Fig). These relationships, which accounted for 66-88% of the genetic variability in bone mechanical properties, were consistent with mechanical theory and provided a guide for understanding the heritability of fragility-related mechanical properties. We next sought to identify the biological processes (formation, resorption) that contribute to variation in adult bone traits. An examination of traits from embryonic day E16.5 to 1 year of age revealed that mechanically relevant traits 'matured' at different times during postnatal development; thus, each trait is subject to different genetic and environmental factors. We show that for a systems analysis of bone fragility, functional networks take on a hierarchical order; this approach allows us to step down the hierarchy systematically and move closer to the genetic level for a more comprehensive understanding of how genotype relates to phenotype.

CAN DRUG COMBINATIONS ELUCIDATE BIOLOGICAL SYSTEMS?

J Lehár, G Zimmermann, M Lee, J Staunton, A Borisy, and C Keith.
CombinatoRx, Inc.

Biological networks involve complex connections between many components, so even agents with specific molecular targets can have pleiotropic effects. One consequence is that drug mixtures can produce very different outcomes from the additive response of their components.



A synergistic combination in a proliferation assay using HCT116 cancer cells. The isobologram shows that to achieve 50% effect, much higher concentrations are required for single agents than for the combination. Moreover, there are large regions of combination space where the inhibition exceeds the expectation from Bliss independence.

At CombinatoRx, we are examining the effects of various compound mixtures in our disease-model assays as candidates for novel combination therapeutics. As part of our screening effort, we have compiled synergy and antagonism data for half a million combinations in several assays.

These data could provide important information on biological systems: (1) Synergistic effects involving compounds with very different known target pathways can reveal previously unknown interactions between networks. (2) Correlations between the synergy of many mixtures and the pathways targeted by their components provide constraints on the connectivity of disparate networks. (3) Using an assay which probes a well-understood pathway, we can determine how strongly the endpoint synergy or antagonism depends on the proximity of the components' known targets in that pathway.

We present some initial results and describe plans for future investigations.

DECIPHERING INTRACELLULAR NETWORKS IN MAMMALIAN CELLS

S Subramaniam
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Cells respond to input by invoking signaling and regulatory networks in a context-dependent manner. Spatio-temporal measurements of genes, proteins and other molecules after input provide quantitative information that can help in the reconstruction of the cellular networks that link input to response. In the Alliance for Cellular Signaling project, primary cells were subject to varied input of distinctive ligands singly and in combination to study signal transduction response. The response was quantitatively monitored through independent measurements of free intracellular calcium, cAMP, a few proteins and their interactions and several genes as a function of time. In this talk, I will present the challenges faced in combining and analyzing heterogeneous data from intracellular measurements in reconstructing cellular networks in a context-specific manner. Specifically, I will discuss how the use of biological knowledge in combination with the cellular data can lead to a systemic picture of the cellular response.

STRUCTURING BIOLOGICAL DATA, DATABASES, AND ANALYSIS TOOLS

H Sauro
Keck Graduate Institute, Claremont, CA, USA

The bulk of all signaling networks in both prokaryotes and eukaryotes are based on the simple phosporylation/de-phosporylation cycle; from this simple unit it is possible to construct a huge variety of control and computational circuits, both analog and digital. In this talk I will summarize the computational versatility of this simple unit and point out it's close similarity to certain man-made devices.

AN EXPERIMENTAL SYSTEMS APPROACH TO DISCOVER mRNAs ENCODING PROTEINS IN RELATED PATHWAYS

JD Keene and SA Tenenbaum
Duke University Medical Center, Durham, NC, USA

Every mRNA that is detected on a microarray has proteins associated with it in the cell. RNA-binding proteins regulate mRNAs post-transcriptionally, including their splicing, export, stability and translation. Regardless of the precision of transcriptional control, these post-transcriptional events must be executed properly to attain the intended gene expression outcome. We have found that post-transcriptional expression pathways are not singular events, but can function together in a parallel and coordinated manner. The organization of these messenger RNP complexes is poorly understood but our findings indicate that multiple mRNAs can be regulated as groups or functional classes in mRNPs. Our in vivo procedures to identify the biochemical components of mRNPs have focused on the mRNAs themselves and their relationships to one another. We will describe experiments in which we recover mRNPs using antibodies followed by identification of the mRNAs using various microarray formats. The presentation will focus on the La RRM protein that regulates the production and processing of both small RNAs and a class of mRNAs that encode proteins involved in translation. For example, mRNAs that encode ribosomal proteins are bound to La protein via sequences in their 5' UTR. We will describe a microarray format that allows identification of novel spliced variants of ribosomal protein mRNAs, as well as small noncoding RNAs that associate with La protein. These data will be interpreted in terms of the post-transcriptional operon model of eukaryotic gene regulation that was recently posited (Keene and Tenenbaum, Molecular Cell, 2002).

A SYSTEMIC REFERENCE METAMODEL FOR SEMANTIC DATA INTEGRATION IN SYSTEMS BIOLOGY

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One of the main issues in Systems Biology is to deal with technological and semantic data integration, including data quality and interoperability, and data organization in models of systems for producing new knowledge. We examined the requirements for a reference metamodel to guide semantic integration, based on the systemic principles. We used the Unified Modeling Language (UML) to design a systemic metamodel that makes it possible unambiguous representation of biological systems and suitable for translation into mathematical and computational formalisms enabling analysis, simulation and prediction of systems behavior.

Motivation: For decades, molecular biology accumulated knowledge on biochemical components of living systems, allowing understanding a large variety of individual cellular mechanisms and functions. More recently, high-throughput technologies have made it possible to assess quantitative and qualitative variations of the whole system's components according to controlled changes of experimental conditions, i.e., internal (directed-mutagenesis) and/or external (growth culture). To understand the functioning of these components within systems, the challenge is to integrate data from both approaches.

Semantic integration aims at producing models of the real world. However the abstraction level may vary as some models represent interacting molecules as a set of "reactions" whereas less abstract models figure the molecules by themselves. Models or even the goals for designing models may be very different: in database design, the main purpose is to handle many data and the database models must specify the structure of the records; in contrast, if the goal is to operate on data, models specify the details of the methods of operations as well as the events that are necessary to trigger them.

To date, a large variety of models have been designed to serve in systems biology and there is a need for a reference model of an ideal biological system able to integrate their useful aspects.

Furthermore, such a reference model would be needed:

* to clarify the appropriate concepts and terminology that describe biological systems and thus developing a common language that can be used;

* to establish a reference for biologists when describing a new component system that may be a part of the system of systems;

* to provide a basis for formalism implementation, thus linking description to analysis and simulation.

Results: We designed a conceptual metamodel of an ideal biological system by taking into account the systemic principlesi. In the systemic paradigm, the basic concept is not the object or the combination of stable objects (i.e. the structure) but the action. The characterization of an action (or a function) relies on the general concept of process, which is defined recursively by its occurrence and its result. A process occurs when the modification over time of an object's position is identifiable in a reference "Space-Form" frame: the conjunction of a temporal transfer (a displacement over time in a particular space; for example transport between subcellular compartments) and a form modification (a morphological change such as a post-translational modification by phosphorylation). A process is recognized through its result: a displacement in the "Time, Space, Form" reference frame. An entity identified as unique will be described differently according to its relationships to the context (organized) including actions onto the environment (organizing) as well as onto itself (self-organizing). This allows us to introduce the concept of complex system essential to the description and the representation of the functioning of living systems: any complex system is represented by a system of multiple actions or a process or a tangle of processes. As tangled as these actions are, one can always represent them by the composition of temporal, space (transfer) and morphological (transformation) modifications.

Accordingly, our metamodel allows description of any functional units of biological systems. This reference model deals with all features, which are important from the point of view of integration with precise characterization of components system's structure and evolution in a time-space-form frame, especially in terms of modularity and scalability.

This conceptual model was transcribed into semi-formal terms within the object-oriented paradigm, using the Unified Modeling Languageii. UML is a standard diagrammatic, semi-formal language developed by the software developer community for designing the structure and the behavior of complex systems of which larger ones have mobile, concurrent and/or distributed components. Models in UML consists in class diagrams that capture the static structure of the system by describing the different entities gathered into classes and composing that system as well as their static relationships (inheritance: "Is-a" relation; composition: "Has-a" relation, etc.). The dynamic behavior of a particular object is recorded using state diagrams and sequence diagrams are used to describe how the objects interact.

Because of these general features, UML can be used in systems biology to design the structure (using the class diagrams) and the behavior (using sequence and state diagrams) of functional entities (from molecules to organisms). Using UML, Functional Units (FUs) of biological systems are described as active objects (i. e. which have their own life cycle), which exchange messages

(domains) as they interact. These FUs are embedded into a supra-FU with which they can communicate. Otherwise, these FUs are compound with other Fus (infra-Fus), according to the abstraction level that commands the “deepness” of the model. Alternatively, FUs communicate with other FUs within their direct environment. Specific extensions of UML can be considered to adapt this language to systems biology; then, providing a Systems Biology-Unified Modelling Language (SB-UML).

Models derived from our metamodel will be fed with global datasets on the genome, transcriptome and proteome, produced under quality assurance with high throughput functional genomics platforms in the framework of pilot projects focusing on cancer and immunity and using, respectively, the cell cycle and the HLA complex as “integrators”.

Perspectives: During the last years, many efforts of translating UML diagrams into formal models suitable to carry out analysis on firm grounds, have been developed in the computer science fieldiii. As our UML metamodel can be used to design biological representation of systems is a non-ambiguous way, automatic devices for translating any biological process into mathematical formalisms can be implemented. Once the translation is extracted from SB-UML descriptions, the properties of the described systems, relying on tools that have been already developed to study for instance complex distributed and mobile systems in the computer science field, can be established. Then, simulation can be carried out, as the various mathematical formalisms available can be equipped with probabilistic distribution yielding stochastic system descriptions.

TOTAL SYNTHESIS OF HYDROCORTISONE FROM A SIMPLE CARBON SOURCE IN YEAST

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Aventis Functional Genetics, France

We report on the production of hydrocortisone, the major adrenal glucocorticoid of mammals and an important intermediate of steroidal drug synthesis, from a simple carbon source by recombinant Saccharomyces cerevisiae strains. An artificial and fully self-sufficient biosynthetic pathway involving 13 engineered genes was assembled and expressed in a single yeast strain. Endogenous sterol biosynthesis was rerouted to produce compatible sterols to serve as substrates for the heterologous part of the pathway. Biosynthesis involves eight mammalian proteins (mature forms of CYP11A1, adrenodoxin (ADX), and adrenodoxin reductase (ADR); mitochondrial forms of ADX and CYP11B1; 3b-HSD, CYP17A1, and CYP21A1). Optimization involved modulating the two mitochondrial systems and disrupting of unwanted side reactions associated with ATF2, GCY1, and YPR1 gene products. Hydrocortisone was the major steroid produced. This work demonstrates the feasibility of transfering a complex biosynthetic pathway from higher eukaryotes into microorganisms. This complex engineering could be used as a model to study the network of proteins responsible for steroidogenesis. In particular, we could assess the fine regulation of steroid synthesis from a simple set of enzymes.

A SYSTEMS BIOLOGY ANALYSIS OF COMPLEMENT FUNCTIONS

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The complement system is a key player in innate defense. In addition it has been shown that complement functions as a bridge between innate Immunity and aquired Immunity. Recent studies, however, have indicated that complement proteins might exert novel functions that are distinct from their well-established inflammatory role, by modulating cellular responses and cell-cell interactions that are crucial to early development and cell differentiation. Accumulating evidence suggests that complement might have important roles in diverse biologic processes, ranging from early hematopoiesis to skeletal and vascular development and normal reproduction. Furthermore, it is now becoming evident that complement-regulated pathways interact with other signaling networks and influence the outcome of complex developmental programs, such as limb regeneration in lower vertebrates and organ regeneration in mammals. These findings highlight a previously under-appreciated role of complement and might have important implications in the context of normal development by helping to elucidate the rather obscure role of innate immunity in such cell modulatory pathways. In addition, in recent years was well established that complement functions like a double-edged sword: on the one hand it promotes phagocytosis, supports local inflammatory responses against pathogens, and instructs the adaptive immune response to select the appropriate antigens for a humoral response; on the other hand its unregulated activation leads to host cell damage. In addition, its interactions with the proteins of foreign pathogens may provide a mechanism by which these microorganisms evade complement attack. Therefore, a systems knowledge of the complement system and the networked interactions of its components is necessary in the rational design of molecular adjuvants, complement inhibitors, and new therapeutic agents against infectious diseases. Here, we will present a systems biology approach to study the structure and functions of the complement components and receptors. We will review our current work on the structural-functional aspects of complement protein-protein interactions and the rational design of small-sized complement inhibitors using *in vitro*, *in vivo*, and *in silico* approaches. We will present our proteomics-based approaches to studying protein-protein interactions within the complement system and discuss our progress in the study of viral immune evasion strategies. Furthermore we will discuss the evolution and the involvement of complement proteins in organ regeneration and hematopoietic development.

SYSTEMS BIOLOGY APPLIED TO ORGANELLE BIOGENESIS AND FUNCTION

JD Aitchison, M Marelli, JJ Smith, E Yi, and D Goodlett
Institute for Systems Biology

Systems biology approaches are being employed to comprehensively understand all the cellular events involved in the formation, maturation and turnover of functional yeast peroxisomes. The approach is divided into three phases. In the first phase proteins we are applying novel large-scale proteomics techniques to provide an exhaustive list of peroxisomal proteins and microarray analysis to define the differential expression of genes underlying peroxisome assembly in yeast. Results from these approaches have yielded large lists of candidate proteins, which are being subjected to "high throughput" phenotypic screens, computational clustering and visualization tools to illuminate high priority proteins for the third phase which demands "low throughput" cell biological characterization. This approach has permitted the identification of several novel proteins required for normal peroxisomal function and biogenesis.

NETWORK GENOMICS: LESSONS LEARNED FROM THE HUMAN GENOME PROJECT

T Ideker
Whitehead Institute for Biomedical Research, Cambridge, MA, USA

Computational cellular models are becoming crucial for analysis of a variety of complex biological systems, including cellular response to DNA damage, toxicity, and stress. An important new paradigm for cellular modeling that involves building a comprehensive scaffold of protein-protein, protein-DNA and other protein interactions, and then mining this scaffold to reveal a hierarchy of signaling, regulatory and metabolic pathways. As more and more interaction data become available across species, this 'pathway mapping' approach is also being used in a comparative sense, to shed light on pathway evolution and function at the network structural level. Recent experimental and computational trends enabling the pathway mapping paradigm will be discussed, as well as how the protein interaction scaffold is spurring the development of models at multiple levels of abstraction. Current applications of pathway mapping will be highlighted, including construction of a large-scale network model of DNA-damage response circuitry in yeast.

ABSTRACT 17

Modular Structure and Function of Integrated Molecular Networks

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The availability and maturation of high-throughput biotechnologies is transforming the way cellular systems are studied. Our expanding genome-scale understanding suggests a hierarchical view of the cell in which groups of interacting molecules form biological modules, and biological modules interact in complex networks that control the properties of a cell. By traversing this hierarchy, biologists can discover properties of cells responding to perturbations or stimuli, and formulate molecular hypotheses on the control of cell properties such as metabolic capabilities, cell-cycle control, and cell morphology.

To bridge the gap between molecules and complex cell properties, we have studied the structure and function of a complex network controlling the differentiation of budding yeast cells into a filamentous form, also known as pseudohyphal growth. Budding-yeast strains grow in the familiar yeast form in liquid medium with plentiful nutrients; they grow in the filamentous form on solid medium with low ammonium. Dimorphism is common among fungal pathogens and involves several cell properties. We assembled an integrated filamentation network and quantitatively explored its hierarchy of structure and function. This exploration generates hypotheses on the control of complex cell properties.

RECONSTRUCTING BIOLOGICAL PATHWAYS FROM LARGE-SCALE PROTEIN INTERACTION DATA

MP Samanta and S Liang
NASA Advanced Supercomputing Division, NASA Ames Research Center, Moffet Field, CA, USA

In this work, we present a reliable computational method to reconstruct functional modules and pathways from large-scale protein-interaction data. First, we show that if two proteins share significantly larger number of common interaction partners than random, they have close functional associations. Analysis of publicly available data from *S. cerevisiae* reveals more than 2800 reliable functional associations involving 852 proteins. Next, we reconstruct the functional modules of the interaction network by clustering these associations based on the derived probabilities. The modules are subsequently connected in the form of a network using inter-module associations. By manually comparing more than 200 top modules with the information provided in the SGD database, we find that they are truly parts of functional complexes or pathways. From the derived modules, we are able to predict functions for 81 unannotated proteins with high certainty. It has been an encouraging sign that the functions of some of these proteins were recently annotated by the SGD database from other sources after the completion of our work, and all but one (22 out of 23) of our predictions proved to be correct. Our method is not overly sensitive from the false positives widely present in the two-hybrid data. Even after adding 50% randomly generated interactions to the measured dataset, we are able to recover almost all (90%) of the original associations.

SYSTEMS APPROACHES TO UNDERSTANDING THE ENVIRONMENTAL RESPONSE USING *HALOBACTERIUM SP.* AS A MODEL SYSTEM

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

The mechanisms by which organisms sense and respond to changes in environmental factors are particularly challenging to characterize given that biological systems have learned to simultaneously sense, integrate and appropriately respond to infinite combinations of environmental perturbations. We are applying a systems approach to decipher the cellular response of the halophilic archaeon *Halobacterium sp. NRC*-1 to well-defined environmental perturbations. This halophile has evolved the capacity to withstand up to 4.5M salinity, over 280J/m^2 ultraviolet radiation (UV), and oxidative stress among other extreme factors in its natural environment. We have conducted the systems analysis by systematically perturbing this archaeal system genetically and environmentally in elements of the oxygen and UV response. The consequential mRNA and/or protein level changes for all of the 2400 predicted genes in its genome were subsequently integrated with protein-protein interactions inferred through comparative genomics approaches. The integration of the genome-wide measurements has provided several systems level insights into the oxygen- and UV-response of *Halobacterium sp*.

In response to anaerobic stress *Halobacterium sp.* coordinately regulates phototrophy and arginine fermentation, the two major sources for anaerobic energy production. In response to UV irradiation, on the other hand, it up regulates DNA and protein repair modules. Both perturbations caused changes in many other aspects of metabolism that, at first glance, did not have any apparent relation to the environmental perturbation. These genome-wide changes are believed to be a consequence of the necessity to maintain homeostasis during the stress response so the available energy and metabolite resources are appropriately allocated.

Finally, the systems approaches have also facilitated by sequence- and structure-based methods the functional annotation of many genes that were previously of unknown function. This in turn has enabled the formulation of many systems level hypotheses by providing information on previously unidentified enzymes and regulators.

BRINGING GENOMES TO LIFE: THE USE OF GENOME-SCALE *IN SILICO* MODELS

BO Palsson
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High throughput data generation in biology has led to the availability of vast amounts of genomic and biochemical data on living cells. These developments have led to the emergence of systems biology that is widely viewed as being comprised of four steps: 1. information about cellular components, 2. reconstruction of biochemical reaction networks, 3. formulation of *in silico* models of network functions (i.e. phenotypes) and 4. measurement of phenotypic responses and their comparison to computed properties. Disagreement leads to an iterative model building procedure. High throughput phenotyping is one of the limiting steps in this process.
Reconstruction of genome-scale networks for metabolism and regulation in single cellular organisms is now possible, and efforts in reconstructing networks in human cells have begun. *In silico* models that characterize their function can be used to analyze, interpret, and predict the genotype-phenotype relationship. These models integrate a wide variety of high-throughput data. We have reconstructed genome-scale models for *E. coli* and Yeast that include metabolism, regulation and transcription/translation.

TOPOLOGIC AND DYNAMICAL ORGANIZATION OF CELLULAR NETWORKS

ZN Oltvai
Department of Pathology, Northwestern University, Feinberg School of Medicine, Chicago, IL, USA

Cellular metabolism, the integrated interconversion of hundreds of metabolic substrates through enzyme-catalyzed biochemical reactions, is perhaps the most studied example of the complex intracellular web of molecular interactions. While the topological organization of metabolic networks is increasingly well understood, the dynamical principles governing their activities remain largely unexplored. In this lecture, we present a flux balance-based analysis on the conversion rate properties of the *Escherichia coli* metabolic network. The results of systematic experimental verification of this framework by global transposon mutagenesis and the evolutionary consequences of such organization will also be discussed.

MODELING THE METABOLIC AND REGULATORY NETWORK OF THE MODEL LACTIC ACID BACTERIUM *LACTOBACILLUS PLANTARUM*

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The complete genome of *Lactobacillus plantarum WCFS1* has recently been sequenced (PNAS USA 2003;100:1990). Putative biological functions could be assigned to 2,120 (70%) of the 3,052 predicted protein-encoding genes. This makes the genome of *Lactobacillus plantarum* one of the largest *Lactobacillus* genomes and it will serve as a model for genome annotations and genome-scale modeling of Lactic Acid Bacteria.

We are currently in the process of pathway reconstruction. *LacplantCyc* was generated from the annotated genome and the MetaCyc database developed by Peter Karp (Bioinformatics 2002 Suppl 1:S225-32). KEGG and ERGO (Integrated Genomics) are also being used as they contain alternative routes or enzymes. The predicted metabolic network is being manually adjusted based on literature and experimental data.

Biosynthetic capacities of amino acids and vitamins have been compared with their requirement in growth medium. Six out of 28 comparisons were incorrect (e.g. pathway incomplete but compound not required; pathway complete but compound could not be omitted). This simple analysis has already resulted in the identification of missing enzymes.

We are also setting up a systems biology infrastructure comprising data generation (several high-throughput technologies), data warehousing and data analysis (visualization, stoichiometry analysis, modeling). In an iterative process this should lead to an improved reconstructed metabolic and regulatory network, and to improved annotation of individual components thereof.

TOWARD THE THEORY OF BIOLOGICAL ROBUSTNESS

H Kitano

One of the most salient features of biological systems are robustness. While intrinsic robsutness protects systems from external and internal perturbations, it often causes numerous diseases. At the same time, systems that are highly robust against specific type of perturbations may be extremely fragile for unexpected perturbations. Scientifically, one of the fundamental goal of the systems biology research is to understand biological robustness, and apply insights for medical practices and drug discovery. Robustness as exemplified by adaptation, parameter insensitivity, noise tolerance, and graceful degradation are often implemented by feedback control, redundancy, modular architecture, and structural stability. Despite enormous interests on robsutness of biological systems, only a limited research has been carried out to create theory of biological robustness --- a fundamental principles of how and why bioloigcal systems are robust. This talk attempt to address issues on biological robustness, and how we may strive to create the theory of biological robustness.

THE LARGE-SCALE STRUCTURE OF GENETIC NETWORKS: DESIGN, HISTORY OR MERE CHEMISTRY

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Functional genomics is generating much information about the structure of genetic networks, information that is largely qualitative. How much biology can we learn from such qualitative information? I will address this question in the context of the two well-studied networks of metabolism and protein interactions. Specifically, I will ask whether these networks have their observed structure because this structure provides robustness against mutations. I will also ask whether this structure contains information about the history of these networks and of life itself.

PROBABILISTIC BOOLEAN NETWORKS AS MODELS OF GENETIC REGULATORY NETWORKS

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Mathematical and computational modeling of genetic regulatory networks promises to uncover the fundamental principles governing biological systems in an integrative and holistic manner. It also paves the way toward the development of systematic approaches for effective therapeutic intervention in disease. We discuss the Boolean formalism as a building block for modeling complex, large-scale, and dynamical networks of genetic interactions, along with the goals of modeling genetic networks and the data requirements. The Boolean formalism is justified from several points of view. We then introduce Boolean networks and discuss their role in understanding cell differentiation and cellular functional states. The inference of Boolean networks from real gene expression data is considered from the viewpoints of computational learning theory and nonlinear signal processing, touching on computational complexity of learning and robustness. Then, a discussion of the need to handle uncertainty in a probabilistic framework is presented, leading to an introduction of probabilistic Boolean networks and their relationships to Markov chains. Methods for quantifying the influence of genes on other genes are presented. The general question of the potential effect of individual genes on the global dynamical network behavior is considered using stochastic perturbation analysis. This discussion then leads into the problem of target identification for therapeutic intervention via the development of several computational tools based on first-passage times in Markov chains. Finally, we will briefly discuss steady-state analysis of probabilistic Boolean networks.

PROTEIN COMPLEXES AND FUNCTIONAL MODULES IN MOLECULAR NETWORKS

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Proteins, nucleic acids, and small molecules form a dense network of molecular interactions in a cell. Molecules are nodes of this network and the interactions between them are edges. The architecture of molecular networks can reveal important principles of cellular organization and function, similarly to the way that protein structure tells us about the function and organization of a protein. Computational analysis of molecular networks has been primarily concerned with node degree or degree correlation, and hence focused on *single/two-body* properties of these networks. Here, by analyzing the *multi-body* structure of the network, we discovered molecular modules that are densely connected within themselves but sparsely connected with the rest of the network. Comparison with experimental data and functional annotation of genes showed that such modules correspond either to protein complexes (splicing machinery, transcription factors, etc.) or to dynamic functional units (signaling cascades, cell-cycle regulation, etc.). These modules are highly statistically significant, as is evident from comparison with random graphs, and are robust to noise in the data. Our results provide strong support for the network modularity principle introduced by Hartwell et al, suggesting that the found modules constitute the “building blocks” of molecular networks.

FUNCTIONAL ANALYSIS OF METABOLIC NETWORKS USING ELEMENTARY MODES

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What is a metabolic pathway? There are so few formal definitions that 'a chapter in a biochemistry book' is effectively the only criterion in wide use. But if we want to assess the metabolic potential of an organism, it is clear that we cannot regard it as a simple sum of the set of classical pathways for which it possesses the enzymes. The complement of enzymes expressed by an organism specifies a metabolic network, and we need to determine the characteristics of the routes available through this net. Elementary modes analysis is a method for finding all the feasible routes through metabolic network from nutrients to outputs.

It has potential as a tool for the functional interpretation of genomics data by allowing a metabolic reconstruction based on the enzyme set inferred from gene sequencing data and by detecting inconsistencies or possible gaps in the annotation. However, with a fully-specified biochemical network, it can be used to assess hypotheses about metabolic function, as will be illustrated with analyses of photosynthesis in C3 and CAM plants. Another application is in assessing the efficiency of formation of particular metabolic end-products, which will be illustrated with an investigation of the potential for the synthesis of polyhydroxybutyrate in recombinant *Saccharomyces cerevisiae*. Yet another use is in providing a functional interpretation of the metabolic shifts occurring during the course of a fermentation, as a complement to metabolic flux analysis approaches. This aspect will be illustrated with results from lactic acid fermentations.

ENGINEERED GENE CIRCUITS

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Uncovering the structure and function of gene regulatory networks has become one of the central challenges of the post-genomic era. Theoretical models of protein-DNA feedback loops and gene regulatory networks have long been proposed, and recently, certain qualitative features of such models have been experimentally corroborated. This talk will focus on model and experimental results that demonstrate how a naturally occurring gene network can be used as a "parts list" for synthetic network design. The model formulation leads to computational and analytical approaches relevant to nonlinear dynamics and statistical physics, and the utility of such a formulation will be demonstrated through the consideration of specific design criteria for several novel genetic devices. Fluctuations originating from small molecule-number effects will be discussed in the context of model predictions, and the experimental validation of these stochastic effects underscores the importance of internal noise in gene expression. Potential biotech applications will be highlighted within the framework of cellular control schemes. Specifically, the coupling of an oscillating cellular process to a synthetic oscillator will be considered, and the resulting model behavior will be analyzed in the context of synchronization. The underlying methodology highlights the utility of engineering-based methods in the design of synthetic gene regulatory networks.

DIRECT AND REVERSE SIMULATIONS OF SIGNAL TRANSDUCTION PATHWAYS IN NEURONAL CELLS

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We formulated a mathematical model of intracellular signal transduction in neurons, in response to extracellular signals, based on protein-protein (P2P) interaction information from existing databases. We described the propagation of a signal through a model network activated by the binding of extracellular ligands to P75, Trk, EGFR, Fas receptors. The network is composed by N protein species, the P2P interactions can be unary, binary or multiple and belong to different typologies. The model is composed by a set of 2N first-order non-linear differential equations in time, in the variables x(i) and n(i), concentration of the active form and of the total amount of the protein/complex (i). Space is neglected in this approximation. Most of the kinetic parameters of the model are unknown, thus to estimate them we realized a reverse engineering of the network by implementing a Genetic Algorithm (GA) on a parallel computational platform. The set of kinetic parameters are considered to be the "genome" of the system. The GA exploits the laws of natural selection to generate the "genome" able to better fit the given constraints in the system, usually experimental data. The GA is able to reasonably estimate the unknown parameters, generating an ensemble of different solution sets {K(i,j)} all acceptable from the biological point of view. Thus the model can be reliably used for direct simulations such as receptor induced activation of the network, modeling of protein knock-out and d of the output steady state.

A COMPUTATIONAL MODEL ON RAF-AKT CROSS-TALK IN ERBB SIGNALING

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ErbB receptor tyrosine kinases play essential roles in cellular proliferation and differentiation, and their deregulated expression or mutation highly correlates with the incidence of human cancers. Among ErbBs, the signaling pathway of EGF receptor has been extensively analyzed and studied by experiments and mathematical modeling. In this study, we examined cross-talk between Raf-ERK pathway and PI3K-Akt pathway in heregulin (HRG)-stimulated ErbB4 receptor signaling and developed a computational simulation model for this signaling pathway. The simulation results raised the possibility that HRG signaling is regulated by a protein phosphatase 2A (PP2A) as well as Raf-Akt cross-talk by modulating the kinase activities in both PI3K-Akt and Raf-MEK-ERK pathways. Furthermore, in our model ERK and Akt showed maximum activity and behaved as if there were no cross-talk under certain conditions where the catalytic activity or the concentration of PP2A is extremely low. Thus, the system behavior in the model which contains Raf-Akt cross-talk is influenced significantly by the concentration and kinetic character of kinases and phosphatases. We therefore assume that such mechanisms may contribute to cellular machinery for the induction of Raf-Akt cross-talk in ligand-specific or stage-specific signal transduction of cells.

MAPPIT : A VERSATILE METHOD TO STUDY PROTEIN-PROTEIN INTERACTIONS IN MAMMALIAN CELLS

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Protein interactions underlie the structural and functional integrity of many subcellular processes. The notion that most of the proteins within a cell are part of higher order complexes regulating signal transduction, gene expression, apoptosis and other crucial events, becomes generally accepted. Existing methodologies for large-scale protein interaction mapping include yeast two-hybrid, phage display and mass spectrometric analysis. Although these approaches have found wide application, each of them suffers from intrinsic limitations, not at least from the fact that interactions are analyzed in a non-physiological context.
We have developed a cytokine receptor-based two-hybrid method in mammalian cells. Incorporation of an interaction trap in a signalling-deficient receptor allows identification of protein-protein interactions using a STAT-dependent complementation assay. Interaction between 'bait' and 'prey' leads to ligand-dependent STAT activation, which is detected by the use of STAT-dependent reporter/selector genes. Using this Mammalian Protein-Protein Interaction Trap (MAPPIT) we were able to demonstrate both modification-independent and phosphorylation-dependent interactions. MAPPIT can be used as screening tool, either to detect novel protein-protein interactions, or to search for inhibitors of known interactions.
This MAPPIT procedure places protein-protein interactions in their normal physiological context and may be particularly instrumental for the *in situ* analysis of signal transduction pathways.

PLANT DEFENSE SIGNALING NETWORK INFERENCE USING KNOWLEDGE-BASED PERTURBATIONS AND GLOBAL GENE EXPRESSION ANALYSIS.

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In Arabidopsis three main signaling compounds activate the plant responses to pathogen attack: salicylic acid (SA), ethylene (ET), and jasmonic acid (JA). These substances and their different combinations can modulate the activation of groups of particular and/or similar genes. In order to determine the complexity of stimuli interpretation and to reconstitute the structure of the defense signaling network in Arabidopsis, we performed global analysis of gene expression (using microarray technology) of plants submitted to exogenous treatment with SA, ET, and JA, as well as their combinations (SA+JA, SA+ET, JA+ET, SA+JA+ET). Our concept of clustering is founded on preestablished signaling/genetic knowledge coupled with a set of highly significant perturbations allowing us to group genes in regulons with a high biological connectivity. The resulting classes determine the circuits of interpretation/combinations of cell defense signals. They have been inferred as minimal logical circuits, and represented in a Boolean formalism. In order to produce a representation of these statistical circuits inside a single graphical model, and to perform qualitative digital simulation, we developed a method for bio-digital circuits concatenation. As a first interesting result, signaling network analysis suggests that virulent pathogens block part of the defense signaling network in a semi specific manner.

EUKARYOTIC MRNPS AS OPERATIONAL NETWORKS REGULATING POST-TRANSCRIPTIONAL GENE EXPRESSION

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The study of gene expression networks has focused primarily on transcriptional control. Recent findings by our group and others suggest that post-transcriptional mechanisms coordinately regulate the expression of multiple genes and profoundly affect biological programs. Messenger RNA binding proteins (mRNA BPs) are essential regulators of post-transcriptional gene expression and are responsible for much of the diversity in the proteome. Combining immunological and biochemical techniques with genomic methodologies, we have begun dissecting the infrastructure of *in vivo* mRNA BPs and associated mRNA targets which form messenger ribonucleoprotein (mRNP) complexes. Our findings demonstrate that unique subsets of mRNA are associated with specific mRNA BPs *in vivo* and that single mRNAs may associate with multiple mRNA BPs. The composition of mRNA subsets in an mRNP can be dynamic during induction of biological programs and does not simply reflect concomitant changes in the transcriptome. We will present examples of post-transcriptional network architecture that include: ***(i)*** autoregulation; ***(ii)*** single input motif; ***(iii)*** multi-input motif; ***(iv)*** regulator chain; and ***(v)*** feedforward loop. We propose that operational networks consisting of specific mRNA BPs and associated mRNA subsets regulate post-transcriptional gene expression and may function in a dynamic and combinatorial manner to achieve a given biological outcome. Analysis of the proteins encoded by these unique associated mRNA subsets will allow us to elucidate the organization of post-transcriptional networks and the flow of genetic information on an integrative systems level.

SIMPLE MODEL EXPLAINS SMALL-WORLD, SCALE-FREE ARCHITECTURE OF GENE CO-EXPRESSION NETWORK

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Cellular systems function through the concerted action of a variety of interlocked processes, which are regulated in response to changes in the environment, as cells progress in cell cycle or during development. To gain knowledge of the functioning of complete cells, these processes are often translated into networks. We here regard the genes of *Saccharomyces cerevisiae* as nodes and co-expression as links between the nodes and show that the resulting network has an architecture characteristic for many biological networks: a scale-free distribution ($N(k) \sim k^{-\gamma}$) of number of connections (k) per node (N) (Figure 1), a small average shortest path length (L) and a high clustering coefficient (c), thus a small-world, scale free architecture. Furthermore, there is a positive correlation between the likelihood of a connection between two paralogs and their sequence similarity. Previous network models cannot account for all these features. We therefore introduce a simple, neutralist model for the evolution of the co-expression network. We show that our model reproduces the observed network properties without the need for selection on the global network structure.

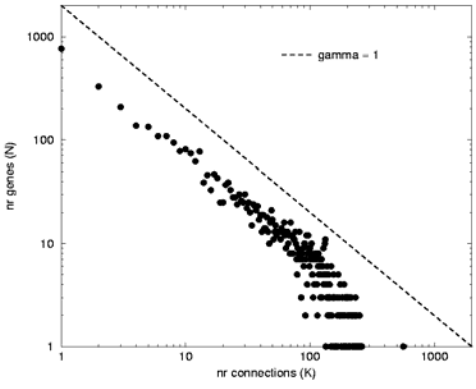

Figure 1. Scale-free distribution of connectivity in the co-expression network.

MODE CONTENT AND DISTRIBUTION: DISCOVERING DETAILS OF DIFFERENCES IN GENE ARRAY RESPONSES TO PERTURBATION

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Analysis of responses of genomic/genetic systems to perturbations, which might be evolutionary, genetic or chemical in nature, often involve segregation operations based on self-organization, or clustering or formation of trees. Such actions are helpful but are also subject to certain limitations. In this paper, we report on methods which are suitable for capturing information on further details of the characteristic modes in the distribution of such data in response space. Comparisons of changes in mode contents and modal distribution provide means for indexing changes in responses to perturbations and for describing differences in changes between experiments. Methodology is described in the context of comparison between gene expression profiles for different inbred mutant mice populations in response to a set of perturbations.

STEP VS. RAMP INDUCTION OF MYOCARDIAL ISCHEMIA: COMPARING IN VIVO AND IN SILICO RESULTS

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The robustness of our computer model of myocardial metabolism was tested by comparing model responses to 2 inputs with data obtained in our lab under similar conditions. An abrupt and a gradual reduction in flow of similar magnitude were implemented and used as model input. After flow reached 40% of normal, ischemia was kept constant for 60 min in both groups. Our hypotheses were that: (1) these two flow-reduction profiles would result in different transients (concentrations and flux rates) with similar steady-state values and (2) model simulations would predict experimental results in an anesthetized swine model of myocardial ischemia. The 2 different ischemia-induction patterns resulted in the same decrease in steady-state MVO2 and in similar steady-state values for metabolite concentrations and flux rates at 60 min of ischemia. While simulated and experimental results showed decreased glycogen concentrations, accumulation of lactate, and net lactate release with ischemia, the onset of glycogen depletion and the switch to lactate efflux were more rapid in the experiments than in the simulations. Thus, valid and abundant experimental data is needed to develop robust models that predict metabolic dynamics under various conditions. On the other hand, a validated model can aid in experimental design by simulating the experiments prior to their performance and giving insight into the experimental outcome. This study demonstrates the major advantages of using computer models combined with experiments for investigating metabolic regulation under physiological and pathological conditions.

DISSECTING GENE NETWORKS INFLUENCING BODY WEIGHT BY QTL ANALYSIS AND TEXT MINING

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We present a text-mining approach for dissecting the complex gene networks influencing growth traits obtained by QTL analysis. Genes influencing body weight/composition and serum concentrations of leptin, insulin, and IGF-I in non-fasting animals were mapped in an intercross of the extreme high growth mouse line DU6i and the inbred line DBA/2. Significant loci with major effects for body weight, obesity and muscle weight were found on chromosomes *1*, *4*, *5*, *7*, *11*, *12*, *13*, and *17*, for leptin on chromosome *14*, for insulin on chromosome *4*, and for IGF-I on chromosome *10* at the *Igf1* gene locus itself and on chromosome *18*. Significant interaction between different QTL positions was observed. Evidence was found that loci having small direct effect on growth or obesity contribute to the obese phenotype by gene gene interaction. For a further functional characterization of these genes, we use a comprehensive dictionary of ~80,000 words characterizing biochemical function, subcellular location, mouse developmental stages and anatomical terms. Based on the occurrence of dictionary words and gene names in Medline abstracts, genes are represented as word vectors, and the selected sets of genes as word-gene matrix. Functional associations between genes on different QTL's are computed by vector decompositon of the word-gene matrix and subsequent sparse matrix reordering. The results enable us to evaluate the functional context of interacting gene pairs and to score candidate genes for further analysis.

GENE NETWORK RECONSTRUCTION IN MOUSE FROM MICROARRAYS AND GENE INTERACTION INFORMATION IN FLY

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Microarray analysis has emerged as an important tool for finding networks of interactions of transcription factors and their regulated genes. Our goal is to use single cell microarrays to unravel gene regulatory networks determining cell differentiation in the neuronal development in the embryo of mice and chicken. Our basic tools are Bayesian networks. In addition to the immediate experimental data there is also a variety of other information which should improve our understanding of the gene interaction network. This information ranges from sequence analysis of promoter regions to gene interaction data for other species. A comparatively well developed area of knowledge is that of genetic interactions in the fly (Drosophila melanogaster). We have set up statistical models to transfer knowledge of fly interactions to mouse taking into account the strength of the homology and uncertainty about coverage and reliability of the databases in a principled way. Much of the information on gene interaction is still in form of textual description. We therefore established an automated parsing procedure to extract information from text sources such as the database Interactive Fly. We reduce the complexity of natural language interpretation by applying a part-of-speech tagger looking for patterns that are characteristic for descriptions of interactions. The probabilities obtained in this way are used as priors in the construction of a probabilistic network representing the gene interactions. This form of model is well suited for expansion to include other sources of information.

SIMULATION AND RECONSTRUCTION OF SMALL SCALE GENETIC NETWORK

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With the emergence of technologies for large scale monitoring of parallel processing cell regulatory networks, construction of a model of the cell becomes more realistic. In the example case of small scale genetic network we suggest an approach for in silico model and analysis of behavior of the real system. The lambda phage decision circuit, which operates within single *E. coli* cell and controls one phase of single phage life cycle, is probably the most completely characterized complex genetic network. Nevertheless the quantitative dynamic behavior of the switch was not experimentally measured in sufficient details. Therefore the analysis of the model of the system becomes the only alternative. The dynamics of the network was simulated, starting immediately after the infection of single cell, and the results were compared with known observations. The behavior around transition from lytic to lysogenic state was analyzed by simulation for different stability of key regulatory protein CII. It has been found that the network works as very robust bistable switch. In the transition region, the change in the stability, proportional to the amount of single molecule, causes inversion of the switch, while outside this region changes several orders higher do not influence the state of the network.
General scheme for reconstruction of small scale genetic networks from experimentally measured time series is suggested.

ZERO-ORDER ULTRASENSITIVITY LIMITED BY SEQUESTRATION

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Ultrasensitivity is believed to be important in signaling networks for various reasons: It suppresses sub threshold stimuli and therefore filters noise. Incorporated in a positive feedback it can be a tool for generating all-or-none decisions and hysteresis. Within a negative feedback, it can drive oscillations. There are several molecular mechanisms known to generate and amplify ultrasensitivity such as multi-site phosphorylation and cascade like structures. Simple substrate circles, however, can also generate an ultrasensitive response, if the enzymes operate under saturation, an effect termed "zero-order ultrasensitivity".

Here we explore the limits of zero-order ultrasensitivity. By utilizing both dynamical modeling and control theory we show that sequestration due to enzyme-substrate complexes limits ultrasensitivity. If the concentration of the enzyme and substrates are comparable, which is believed to be the usual case in signaling cascades, ultrasensitivity vanishes and the stimulus-response curve gets more linear. We also illustrate this effect by first experimental results obtained from a simple isolated substrate which we analysed in vitro.

Finally, we compare the robustness of ultrasensitivity generated by zero-order effects with respect to random parameter variations to models of the MAPK-cascade, where ultrasensitivity is essentially generated by double-phosphorylation and the cascade structure.

INFERENCE OF GENE FUNCTION IN PROBABILISTIC GRAPHICAL MODELS

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Functional annotation of proteins is a key problem in the post-genomic era. High-throughput experimental data from microarray experiments or screens for protein-protein interaction combined with bioinformatics data about sequence homology, genome structure or domain composition, for example, are invaluable for identifying possible functional roles of proteins. High-throughput data differ in their quality from data obtained in more specialised experiments on specific systems in several respects in that they are charaterised by comparatively large false positive or false negative rates and their interpretation requires careful statistical modelling.

More recently, probabilistic graphical models such as Markov networks and Bayesian networks have been suggested to model functional relationship and gene interaction networks. One attraction of probabilistic models is that they can combine a variety of information sources. The same network linking functional knowledge with knowledge on gene or protein interaction, for example, can be used to infer function from interaction or interaction from function, or actually solve a mixture of both inference tasks. Bayesian networks in particular have the advantage that false positive and negative rates can be incorporated easily.

We use Bayesian networks to link information on gene expression profiles, protein-protein interactions, protein complexes, domain structures, and gene locations in Escherichia coli and Mycobacterium tuberculosis to gene function. An obstacle to efficient inference is the evaluation of the partition function. We employ a range of methods such as pseudolikelihood and MCMC for the estimation of parameters and for inference.

PERFORMANCE OF LEARNING ALGORITHMS FOR REGULATORY NETWORKS

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Gene networks give insight into the complex relationships of genes and the ability to predict or generate new hypotheses for experimental testing. For reconstruction of regulatory networks probabilistic networks have been suggested. One algorithm, the PC algorithm, is based on partial correlations (constraint based), while others have suggested Bayesian learning using scoring schemes in order to learn Gaussian networks (scoring based).
Since the underlying gene network is hardly ever known in the case of real data, it is difficult to evaluate the performance of learning algorithms; simulated data have the advantage that the true network is known. The data can be drawn from the multivariate Gaussian distribution, for example. However, the distribution of biological data is rarely Gaussian, and results on the performance of the algorithms might not be representative. A further assumption of the learning algorithms is that the variables are linearly dependent, which is an oversimplification of the intricacies of regulation by complex cis-regulatory modules.
Here we propose a way of generating data that resemble realistic data from microarray experiments. Algebraic functions for cooperative or inhibitory relationships are easily inferred from rate equations and steady-state assumptions. Another source of simulated data is from stochastic simulations. Based on such data we compare the performance of constraint based and scoring based algorithms. We also discuss transformations of data that improve the performance of algorithms based on multivariate Gaussians. Such evaluation is crucial in order to understand how reliably gene networks can be reconstructed by probabilistic models.

TOWARD BRAIN SYSTEMS BIOLOGY: SYNAPSE PROTEOMES SHOW SCALE-FREE NETWORK PROPERTIES UNDERLYING PLASTICITY, COGNITION AND MENTAL ILLNESS

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Identification of the molecular mechanisms for processing information is an important goal of biology. The synapse not only transmits information between cells, but also processes information by detecting patterns of neural activity that activate intracellular biochemical pathways changing the properties of the neuron. How the synapse regulates this plasticity and its importance in cognition is a central problem in neuroscience. In recent years it has become clear that synapses, like other cell-cell interactions in metazoans, involve signal transduction complexes and pathways with a high degree of molecular complexity and cross-talk.

Genetic[1,2] and proteomic studies[3] show that 2MDa multiprotein complexes found at the postsynaptic terminal of brain synapses detect patterns of nerve activity and process the signals into intracellular changes. The complexes are comprised of neurotransmitter receptors (e.g N-methyl-D-aspartate, NMDA receptors), adaptors, signalling and cytoskeletal proteins[3]. These complexes are embedded in the postsynaptic terminal of excitatory synapses with other postsynaptic proteins in a structure known as the Post Synaptic Density (PSD).

Toward the goal of a developing a systems biology of the synapse, we now report the identification of 698 proteins from the postsynaptic terminal of mouse central nervous system synapses. We have used a variety of bioinformatics strategies to address the higher order organisation and function of these proteins. We created a manually curated mammalian protein-protein interaction database of approximately 1700 proteins and 4000 interactions (www.PPID.org). We found that the protein-protein interactions in mammals followed a scale-free network topology as previously reported in yeast. Moreover, the synapse proteome and the proteome of the signalling complexes also showed scale-free architecture. The synapse network topology was evolutionarily conserved from yeast but enriched with signaling proteins associated with the emergence of multicellularity. The network model predicted the molecular complexity and

robustness of synaptic plasticity to mutations and drug interference. Moreover, the network predicted the involvement of proteins in rodent and human cognitive functions. A surprisingly high number of network proteins are altered in mental retardation and schizophrenia. We propose that synapse molecular networks with simple design principles underpin cognition and its disorders.

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THEORETICAL STUDY OF HOMEOSTATIC AND DEVELOPMENTAL GENE NETWORKS DYNAMICS

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Computer dynamic models of complex nonlinear gene networks are important for obtaining dynamic characteristics of biosystems behavior under different conditions, study of various mutations in gene networks, investigating their possible function modes and determining optimal strategies of their correction including pharmacological and therapeutic methods.
The computer dynamic models simulating two gene networks regulating (1) cholesterol homeostasis in the cell and (2) erythroid cell differentiation developed earlier with more precisely described regulatory mechanisms and additionally verified values of their parameters were used for computer analysis of the effects of various mutations in these gene networks.
Mutational portraits of the gene networks in other words sensitivities of their components to mutational changes in the rates of molecular processes running within the ones were analyzed. It was demonstrated that the mutations hitting regulatory processes changed the key components of the gene networks such as free cholesterol in the cell to a largest degree. Analysis of mutational portraits of gene networks is an approach allowing to develop optimal strategies for correcting various pathologies and detection of the targets for pharmacological regulation.

BIOINFORMATIC METHODS FOR LONGITUDINAL METABOLOMICS DATA

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Metabolomics is a technique that enables quantification and qualitative analysis of metabolites in biological fluids. There is an increasing awareness in the systems biology community that time-resolved metabolomics measurements contain important information regarding biological organisms. This is, obviously, related to the dynamic nature of organisms resulting in biorhythms. Such biorhythms can be disturbed by external causes (e.g. drug intake, food intake) or internal causes (e.g. developing diseases). Such disturbances affect the metabolism of organisms and are expected to show up in properly measured longitudinal metabolomics data (e.g. in the urine or blood of the organism).
Longitudinal metabolomics analysis can serve several goals. In normality studies, the goal is to establish biorhythms under homeostasis which serve as a reference point to detect future deviating dynamic behavior. Another goal is to detect early biomarkers for developing diseases; this calls for dynamic models based upon which biomarker selection can take place. Yet another goal is to model the dynamic response of an organism to external stress which gives insight in the way such an external stress influences the system. All these goals require a different data analysis method. The type of method depends also on the set-up of the metabolomics data set.
An overview will be given of different longitudinal modeling strategies for metabolomics data. These methods are based on principal component analysis and extensions thereof. Examples will be given using i) a normality study of monkey urine and ii) a longitudinal metabolomic study with urine of guinea pigs developing osteoarthritis during aging. The strengths and limitations of the methods will be illustrated with these example studies.

CROSS-TALK OF DIFFERENT RECEPTOR SYSTEMS IN GDNF SIGNALLING

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Growth factor signaling is commonly considered as a one-way event, in which the binding of a ligand to its specific receptor triggers downstream events leading to changes in gene expression. We show that horizontal interplay between receptor systems takes place, when Glial cell line-derived neurotrophic factor (GDNF) binds to its GPI-linked GDNF family receptor α1 (GFRα1). GDNF and hepatocyte growth factor (HGF) are multifunctional signaling molecules. HGF binds to and activates Met receptor tyrosine kinase, whereas the receptor complex for GDNF typically includes both GFRα1 and Ret receptor tyrosine kinase. GDNF also signals independently of Ret through GFRα1. We first noticed that GDNF partially restores ureteric branching in *ret*-deficient mice with severe renal hypodysplasia. We then studied the mechanism of this Ret-independent effect of GDNF by the MDCK cell model. In MDCK cells expressing GFRα1, but not Ret, GDNF stimulates branching. Thus, the action of GDNF on these cells mimics that of HGF on wild-type MDCK cells. Indeed, Met is phosphorylated by GDNF/GFRα1 complex. It does not however bind to Met, but its activation is mediated by Src-type kinases. Our data show a mechanism for GDNF-induced branching in non-Ret signaling (Fig. 1). This kind of horizontal cross-talk between receptor systems might be more common than assumed.

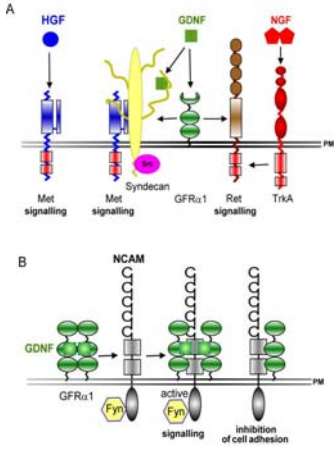

Fig. 1. Cross-talk in GDNF/GFRα1/Ret signaling. A. GDNF/GFRα1 complex promotes phosphorylation of Met through Src-type kinases. GDNF is locally concentrated by heparan sulphate proteoglycans, such as syndecan. On the other hand, RET is phosphorylated upon activation of TrkA by nerve growth factor (NGF). B. NCAM is an alternative signaling receptor for GDNF. It is also activated by the GDNF-GFRα dimer.

MANAGING AND EXTRACTING INFORMATION FROM PROTEOMICS DATA TO MODEL PATHWAYS

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MAPK pathways are ubiquitously expressed signalling modules involved in cell proliferation and differentiation. We discuss the potential use of proteomics data for modelling the pathway.2D gels identify a large number of proteins ideal for protein expression studies; but 2D gels are not comprehensive as one has to account for undetectable proteins(Raf1),protein modifications affecting Mass spec results and co-detection algorithms. We use Amersham DIGE and P^{32} labelled gels with Decodon software. DIGE is able to detect precursor proteins with relative quantiation whereas P^{32} labelled gels only detect phosphorylated proteins; in fig4 we see many spots only present in one sample. MAPK pathway proteins are represented as spots on 2D gels. We have developed a relational database management system to allow data from the 2 experimental systems for

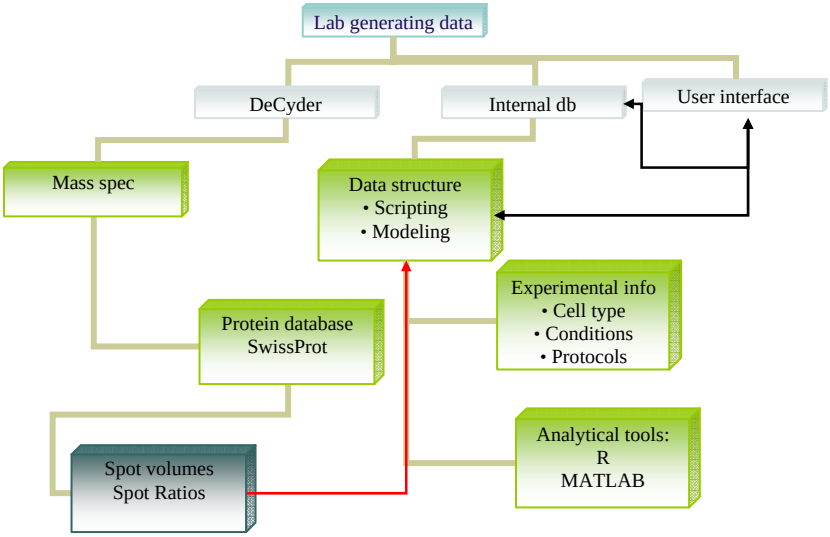

Figure 1: General schema of data portal for proteomics

use in modelling. Graphs of protein interactions and differentially expressed proteins of the pathway can be studied with the database coupled to a adapted query tool. Bioinformatics approach can help understand regulation+cross-talk

and overcome problems associated with poor quantitation of gels. Modelling is done with SIMULINK in conjunction with such a database. The database has proved very useful to the biologists as it a quick means to store, retrieve,query and compare data uniformly. Our understanding of 'properties of the system' can improve using such approaches.

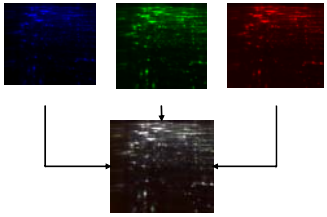

Figure 2: Gel images DIGE

Figure 3: User Interface

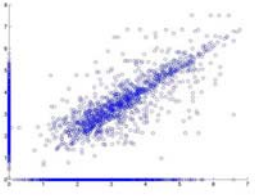

Figure 4: Statistical Analysis

HOMOLOGY OF FUNCTION" IN HUMAN CELLULAR SYSTEMS: A UNIFIED SYSTEMS BIOLOGY APPROACH FOR THE IDENTIFICATION OF GENE NETWORKS AND DRUG ACTION

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The completion of the human genome in combination with modern approaches to chemical diversity has opened unparalleled opportunities for advances in biology and medicine. Realization of this potential, however, will require rapid and practical approaches to discovering the functions of novel genes and understanding their interactions with molecules in the context of human biology. We propose here a practical approach to systems biology using complex primary human cell-based disease models in scalable assay formats to probe drug and gene function. Measuring a limited number of readout parameters optimally selected for their information content and relevance to the process of interest, in multiple complex systems, we produce characteristic signature profiles for genes and drugs. Combining these functional response profiles with appropriate statistical methods we are able to gain insights into the mechanisms of drug action and obtain systematic reconstruction of higher order biological networks including the incorporation of novel participants in known pathways. As examples, we report 1) the functional classification of chemically diverse anti-inflammatory drugs, correlating with their mechanisms of action, and 2) the clustering of genes by functional involvement in common cellular signaling pathways. Generation of a comprehensive database of such functional signatures can accelerate the pace of cell biology, functional genomics, and drug discovery.

DELINEATING FUNCTIONAL MODULES FROM GENOMIC CONTEXT

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Functional links between proteins can often be inferred from genomic associations between the genes that encode them: groups of genes that are required for the same function tend to show similar species coverage, are often located in close proximity on the genome, and tend to be involved in gene-fusion events. We have developed the database STRING as a resource for the exploration of these associations. It features a unique scoring-framework based on benchmarks of the different types of associations against a common reference set, which allows us to integrate all these scores into a single score per prediction. The graphical representation of the network of protein interactions provides a high-level view of functional linkage, facilitating the analysis of modularity in biological processes. In addition we have studied the properties of the network of pairwise interactions between proteins, as predicted by the conserved co-occurrence of their genes in operons, in order to identify functional modules: sets of proteins that operate together. Analysis the network reveals that it is a scale-free, small-world network with a high degree of local clustering (C = 0.6). Comparative genome analysis, thus, allows identification of a level of functional interaction between that of pairwise interactions, and of the complete genome.

KNOWLEDGE DISCOVERY AND DYNAMIC MODELING IN COMPLEX GENE REGULATORY NETWORKS

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Towards a systems level description of a complex gene regulatory network, the network of meiosis and sporulation in yeast, we have developed a tool for supervised machine-aided identification of knowledge gaps in gene networks and for dynamic simulation of their behavior in response to changes in arbitrary variables. In particular we have built a dynamic database model, PathSys, which is an information system for biological interaction networks. It can interoperate with prediction engines to implement query driven simulation. In its core is a graph-based data model that captures the inter-connectivity and the nature of genetic or physical interactions between elements of a biological network. This knowledge discovery tool is helping us construct striking new hypothesis for testing through laboratory experiments. Hypotheses generated by PathSys on yeast data have been shown to be extendible to other organisms. In addition to the static network model in PathSys, we have developed a dynamic model of gene regulatory network as a Petri Net, where numerous descriptive and semi-quantitative data on genes and gene products are modeled through their logical relations. In Petri Net models, genes and gene products have states that undergo transition to other states given the arrival of certain information packets (tokens) with adjustable probabilities. We show that our Petri Net model can simulate the dynamic behavior of a complex gene regulatory network in yeast. These methods of modeling gene regulatory networks can be extended to any biological system.

GENE-METABOLITE CORRELATION NETWORK REFLECTS FLOW OF INFORMATION ALONG THE PATHS OF PROPAGATING CONCENTRATIONAL CHANGES IN RESPONSE TO EXTERNAL SYSTEM PERTURBATION

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To reveal a communication network, involved in the development of plant response to environmental alteration, the system in steady-state was perturbed by the external exciter, i.e. depleted sulfur. Non-biased correlation analysis was targeted to the combined dataset of transcript and metabolite profiles obtained in experiments with sulfur starved *Arabidopsis thaliana* plants. From similarities in transcript and metabolic pattern alterations we reconstructed the communication network, into which the dynamics in response development was incorporated. By centralizing sulfur as starting point of system excitement the course 'cause-to-effect' was implemented to the originally non-directed correlation paths. The robustness and stress tolerance as consequences of the scale-free network topology, and hubs as critical cites of system vulnerability were revealed. The communication paths constituting the flows of the diffusion of system excitement, from sulfur to physiological endpoints, such as anthocyanins accumulation and enforced root formation, were dissected from the network. Cross-talk of biochemical pathways in the response development was reflected in the network by the positioning of common metabolites, as shown for the elements 'sulfur – serine – tryptophan'. This positioning strongly supports also directionality from cause to effect, implemented by centralising sulfur. The gene-metabolite network reconstruction provides an useful extension towards development of systems biology.

REPRESENTING METABOLIC NETWORKS BY THE SUBSTRATE-PRODUCT RELATIONSHIPS

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The substrate-product structure relationships are indispensable information in reliable pathway reconstruction. Given a sequence of reactions, its validity as a pathway depends on two factors: 1) the atom under focus, and 2) the conserved structural moiety in the reactions. In deducing or predicting pathways from reactions, it is therefore essential to consider the atomic level correspondence of metabolites to guarantee that some moiety is conveyed through the reactions.
We introduce a software system that computationally reproduces biochemical radioisotope-tracer experiments. It consists of 3 main components: a mapping database of substrate-product atomic correspondents derived from known reaction formulas, a tracing engine that can compute all pathways between 2 given compounds by using the mapping database, and a graphical user interface. The system can facilitate the display of all possible pathways between any 2 compounds and the tracing of every single carbon, nitrogen, or sulfur atom in the metabolism. The curated and precompiled data-set represents the metabolism of more than 2700 reactions in 1700 EC sub-subclasses. It is essentially a subset of reactions from the Enzyme Nomenclature and was prepared to cover reactions in the basic metabolism. The software tools and data are available at http://www.metabolome.jp/.

Sponsors

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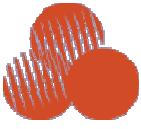

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