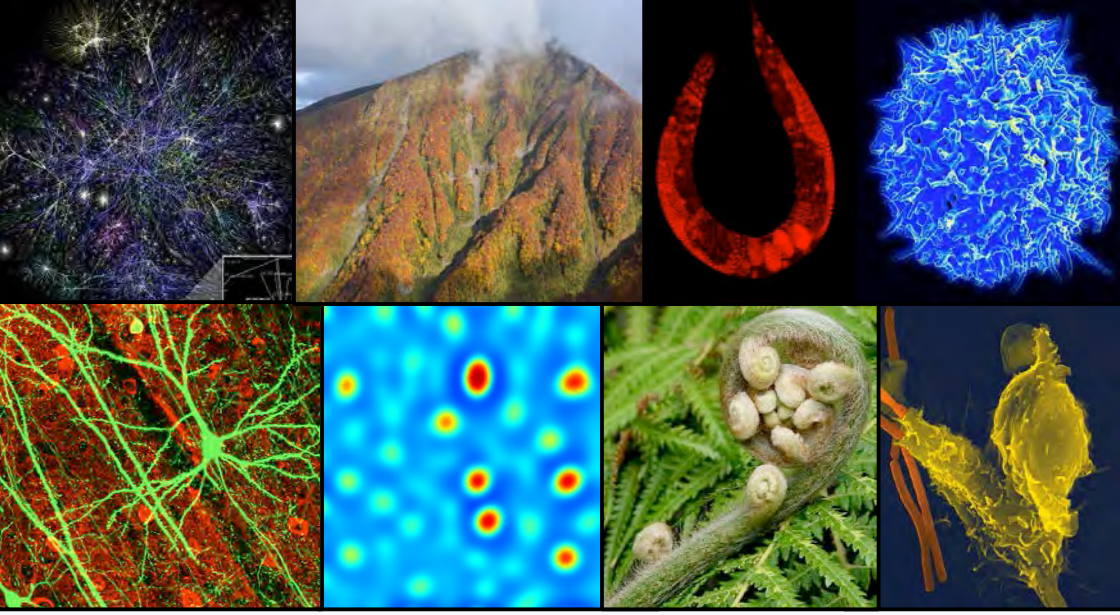

The 2nd Symposium on

COMPLEX BIODYNAMICS & NETWORKS

PROGRAM & ABSTRACTS

TSURUOKA, JAPAN
MAY 11th – 13th
2015

c-bio.org/2015

C-Bio 2015

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2nd Symposium on
Complex Biodynamics & Networks

May 11th–13th, 2015

Lecture Hall

Tsuruoka Metabolome Campus, Japan

Symposium Organizers

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kumar@ttck.keio.ac.jp

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Welcome Message

Dear Friends and Colleagues,
It is a great pleasure and honor to welcome you to the 2nd Symposium on Complex Biodynamics and Networks (cBio), at the Institute for Advanced Biosciences (IAB), Keio University, Japan. The symposium originally started in 2013 at IAB, to gather colleagues who have similar interests in the growing field of complex systems biology.

We all know that living organisms are highly complex, adaptive and self-organizing systems utilizing cell to cell interactions, and triggering thousands of molecular species (genes, proteins, metabolites) to environmental changes or perturbations. Although traditional reductionist approaches using "steady-state" methods have identified crucial single molecular species that control key regulatory functions, understanding the dynamic and evolving characteristics of biology require more sophisticated experimental and theoretical developments to tackle the multi-dimensional information processing. The cBio symposium recognizes the emergent field of systems biology and welcomes participants who have strong desire to make changes to the general mindsets, and take risks in their scientific endeavours. Thus, we welcome people from all backgrounds equally to contribute towards the better understanding of living systems.

This year, we are honored by the presence of world renowned systems biologist Leroy Hood, and Shizuo Akira who is one of the most highly cited immunologist. We would like to thank all invited speakers and participants for supporting the growth of cBio symposium series, and we wish you will have a wonderful time interacting breaking science and forming lasting friendships. Best wishes from Tsuruoka, Japan.

Kumar Selvarajoo
on behalf of the
cBio 2015 symposium committee

INFORMATION

INFORMATION

Oral Presentations
Speakers are encouraged to bring their USB flash drive or SD card to the session chairs during coffee/lunch breaks at least 10 minutes prior to their sessions. Alternatively, we will assist speakers to use their own computers, if preferred.

Session Chairs
Session chairs are required to be present in the lecture hall at least 10 minutes prior to their sessions.

Posters
The poster boards are available within the lecture hall, towards the backend. Presenters are required to obtain pins from the registration desk, and mounting should be done by 11am on May 12th. Posters should be unmounted by 3pm, May 13th.

Biolab Tour
Please gather at 12:50pm in the atrium on May 11th.

Internet
Wireless Internet is available within the symposium venue. Network ID and password will be provided at the registration desk.

Sightseeing
Sightseeing guides are available at the registration desk. An excursion to Haguro (an ancient temple in the forest) is offered for registered participants on May 12th.

Photography or filming of talks and posters is prohibited during the symposium.

Please wear your badge for all events.

Contact Number: +81-(0)90-7948-9515 (Kentaro Hayashi)

INFORMATION

Partner journals

Accepted speakers will be encouraged to submit a commentary or review article of their talk to *Advances in Systems Biology* (http://researchpub.org/journal/asb/asb.html) or In Silico Biology (http://www.iospress.nl/journal/in-silico-biology/).

Acknowledgement:

Keio University

The Japan Society for the Promotion of Science

Advances in Systems Biology journal

In Silico Biology journal

PROGRAM

PROGRAM

Day 1: May 11th

10.00 AM **REGISTRATION**

1.00 PM **BIOLAB TOUR**

OPENING ADDRESS & KEYNOTE SESSION

2.00 PM ***WELCOME TO CBIO 2015***
KUMAR SELVARAJOO, *IAB, Keio University, Japan*

2.15 PM ***FROM CELL SIMULATION TO MULTI-OMICS SYSTEMS BIOLOGY***
MASARU TOMITA, *IAB, Keio University, Japan*

3.00 PM ***SYSTEMS MEDICINE AND PROACTIVE P4 MEDICINE: CATALYZING A REVOLUTION IN HEALTHCARE THROUGH WELLNESS***
LEROY HOOD, *Institute for Systems Biology, Seattle, USA*

3.45 PM ***REGNASE-1, A RIBONUCLEASE INVOLVED IN THE INFLAMMATORY AND IMMUNE RESPONSES***
SHIZUO AKIRA, *WPI Immunology Frontier Research Center, University of Osaka, Japan*

4.30 PM **COFFEE BREAK**

Day 1: May 11th

FUNDAMENTAL METHODS IN BIOLOGY I

Session chairs: CHIKARA FURUSAWA & AMY BROCK

4.45 PM ***SEARCHING FUNCTION OF NON-CODING RNAS IN COMPLEX MAMMALIAN TRANSCRIPTOMES***
PIERO CARNINCI, *RIKEN Center for Life Science Technologies, Japan*

5.15 PM ***THE CELLULAR MECHANICS OF THE IMMUNE RESPONSE***
PHIL HODGKIN, *The Walter and Eliza Hall Institute of Medical Research, Australia*

5.45 PM ***UNIVERSAL RELATIONSHIP IN FLUCTUATION AND RESPONSE IN ADAPTATION AND EVOLUTION***
KUNIHIKO KANEKO, *University of Tokyo, Japan*

7.00 PM **WELCOME DINNER AT *GRAND EL SUN***

Day 2: May 12th

BIOLOGICAL NETWORKS I

Session chairs: KUNIHIKO KANEKO & PHIL HODGKIN

9.30 AM ***PHENOTYPIC CONVERGENCE IN EXPERIMENTAL EVOLUTION OF ANTIBIOTIC RESISTANT BACTERIA***
CHIKARA FURUSAWA, *RIKEN Quantitative Biology Center, Japan*

10.00 AM ***GOMPERTZIAN MODELING OF THE FORMATION OF THYMOCYTE POPULATIONS IN THE PRE-NATAL AND POST-NATAL MURINE THYMUS AND ITS RELATIONSHIP WITH THE BONE MARROW PROGENITORS***
FELIX MIC, *Victor Babes University of Medicine and Pharmacy, Romania*

10.15 AM ***NETWORK DESIGN PRINCIPLES AND RESPONSE SENSITIVITY OF COMPONENTS***
MASAYO INOUE, *AIST, Japan*

10.30 AM ***INVESTIGATIONS ON LONGITUDINAL GENE EFFECTS IN DYNAMICS ASSOCIATION MAPPING BY A RANDOM WAKK KALMAN FILTER MODEL FOR A BACKCROSS MICE DATASET***
BURAK KARACAOREN, *Akdeniz University, Turkey*

10.45 AM **POSTER SESSIONS & COFFEE BREAK**

Day 2: May 12th

SYSTEMS IMMUNOLOGY

Session chairs: MADS KAERN & PIERO CARNINCI

11.30 AM ***ON/OFF SWITCHING MECHANISMS IN SIGNALING NETWORK***
MARIKO OKADA, *RIKEN Center for Integrative Medical Sciences, Japan*

12.00 PM ***A QUANTITATIVE FRAMEWORK FOR ESTIMATING THE IMPACT OF HIV ERADICTION STRATEGIES***
JOHN MURRAY, *University of New South Wales, Australia*

12.15 PM ***THE SELF-LIMITING DYNAMICS OF TGF-Beta SIGNALING, IN SILICO AND IN VITRO***
LISA TUCKER-KELLOGG, *DUKE-NUS Centre for Computational Biology, Singapore*

12.45 PM ***LUNCHEON SEMINAR: DEVELOPMENT AND APPLICATIONS OF QUANTITATIVE AND ISOTOPE-BASED METABOLOMICS FOR SYSTEMS BIOLOGY***
KENJIRO KAMI, *Human Metabolome Technologies Inc.*

1.15 PM ***PROTEINS AS THE NEXT GENERATION OF INDUSTRIAL MATERIALS***
KENJI HIGASHI, *SPIBER INC.*

1.30 PM ***THE REVAMPED IN SILICO BIOLOGY JOURNAL***
MADS KAERN, *Editor-in-chief*

2.00 PM **HAGURO (ANCIENT TEMPLE) EXCURSION**

7.00 PM **DINNER AT *LUNA***

Day 3: May 13th

FUNDAMENTAL METHODS IN BIOLOGY II

Session chairs: MARIKO OKADA & VINCENT RODIN

9.00 AM ***CHROMATIN STRUCTURE AND DYNAMICS IN LIVE MAMMALIAN CELLS***
KAZUHIRO MAESHIMA, *National Institute of Genetics, Japan*

9.30 AM ***OUR SCENARIOS TO PRODUCE DESIGNED GENOMES VIA BACILLUS SUBTILIS GENOME VECTOR***
MITSUHIRO ITAYA, *IAB, Keio University, Japan*

10.00 AM ***DISRUPTED TRNA GENES AND THEIR SPLICING REGULATIONS***
AKIO KANAI, *IAB, Keio University, Japan*

10.30 AM ***ADVANCED CONTINUOUS CULTIVATION METHODS FOR HIGH-RESOLUTION QUANTITATIVE GROWTH SPACE ANALYSIS OF CELLS***
RAIVO VILU, *Tallinn University of Technology, Estonia*

10.45 AM **COFFEE BREAK**

STEM & CANCER CELL DYNAMICS

Session chairs: KAZUHIRO MAESHIMA & DOUGLAS MURRAY

11.00 AM ***PHENOTYPIC PLASTICITY IN TUMORIGENESIS***
AMY BROCK, *The University of Texas at Austin, USA*

11.30 AM ***EVIDENCE FOR NON-GENETIC CAUSES OF EUKARYOTIC DRUG RESISTANCE***
MADS KAERN, *University of Ottawa, Canada*

Day 3: May 13th

12.00 PM ***MATHEMATICAL MODELLING OF BIOLOGICAL NETWORKS HELPS FINDING GENETIC DETERMINANTS OF METASTATIC COLON CANCER***
INNA KUPERSTEIN, *Institut Curie, France*

12.15 PM ***CONSERVED PATTERNS OF METABOLIC ACTIVITY SUGGEST GENERAL PRINCIPLES OF REGULATION BASED ON CELLULAR OBJECTIVES AND COMMUNITY CONSTRAINTS***
MARTIN ROBERT, *Tohoku University, Japan*

12.30 PM ***PHLDA1 IS A NEGATIVE FEEDBACK REGULATOR OF ERBB SIGNALING***
SHIGEYUKI MAGI, *RIKEN Center for Integrative Medical Sciences, Japan*

12.45 PM **LUNCH BREAK**

HIGH-DIMENSIONAL BIOLOGY

Session chairs: AKIO KANAI & MITSUHIRO ITAYA

1.30 PM ***SINGLE-CELL TRANSCRIPTIONAL ANALYSIS TO UNCOVER REGULATORY CIRCUITS DRIVING CELL FATE DECISIONS IN EARLY MOUSE DEVELOPMENT***
JIE ZHENG, *Nanyang Technological University, Singapore*

1.45 PM ***EVENT-BASED STOCHASTIC SIMULATIONS FOR BIOCHEMICAL NETWORK***
MIN ROH, *Institute for Disease Modeling, USA*

2.00 PM ***A COMPREHENSIVE PIPELINE FOR THE EPIGENOMIC DATA ANALYSIS OF GENOME-WIDE DNA METHYLATION***
PAO-YANG CHEN, *IPMB, Academia Sinica, Taiwan*

Day 3: May 13th

2.15 PM ***ASSESSMENT OF THE SOMATIC MUTATIONS IMPACT ON CANCER NETWORK DYNAMICS USING DOMAIN-BASED PROTEIN INTERACTION NETWORKS***
MOHAMED HELMY, *University of Toronto, Canada*

2.30 PM **COFFEE BREAK**

BIOLOGICAL NETWORKS II

Session chairs: JIE ZHENG & MARTIN ROBERT

2.45 PM ***A MULTI-AGENT APPROACH FOR VIRTUAL TISSUE MORPHOGENESIS***
VINCENT RODIN, *Université de Brest, France*

3.15 PM ***LOW-FREQUENCY DYNAMICS OF SOME BIOPOLYMERS DURING CONFORMATIONAL CHANGES***
ARTEM DMITRIEV, *Ioffe Institute, Russia*

3.30 PM ***STOCHASTIC FLUX ANALYSIS OF CHEMICAL REACTION NETWORKS***
OZAN KAHRAMANOGULLARI, *The Microsoft Research - University Of Trento, Italy*

3.45 PM ***CLOSING REMARKS***

4.15 PM **BUSES TO AIRPORT/HOTEL**

ABSTRACTS

KEYNOTE SESSION

From Cell Simulation to Multi-omics Systems Biology

Masaru Tomita
Institute for Advanced Biosciences, Keio University, Japan

In 1990's, we started computational systems biology research by constructing "E-Cell", a computer program for whole cell simulation. The first cell model has 127 genes and 495 reactions to sustain its "life", including energy metabolism, transcription, translation and membrane synthesis [1]. We then developed a novel technology for high-throughput metabolome analysis based on capillary electrophoresis mass spectrometry (CE/MS) and it can simultaneously quantify a large amount of cellular metabolites ranged from 70 to 1,000 molecular weights [2]. A large scale multi-omics analyses was first conducted for *Escherichia coli* and its single deletion mutants, and our data include metabolome (541 compounds), proteome (61 enzymes), transcriptome (76 genes), and fluxome (25 paths) [3]. Various biomarkers have been found by CE/MS, including serum biomarkers of hepatotoxicity [4,5] and saliva biomarkers of oral, breast and pancreatic cancer [6]. Cancer-specific energy metabolisms were also discovered [7-10]. Furthermore, metabolome data have been used to successfully confirm simulation results of red blood cell metabolism [11,12] Metabolomics is useful In the area of food science as well; we conducted metabolome analyses of soybeans [13] and sake (rice wine) and its correlation with taste [14]. Intestinal environment is another important topic we have recently focused on and multi-omics analyses including metagenomics and metabolomics are essential to understand metabolism of microflora. We believe systems biology of gut microflora, combined with food metabolomics, will become one of the principal areas of health science [15].

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KEYNOTE SESSION

Biography of Tomita

Masaru Tomita is a Professor and the Director General of the Institute for Advanced Biosciences, Keio University. He received Ph.D. in Computer Science from Carnegie Mellon University (1985), Ph.D. in Electrical Engineering from Kyoto University (1994) and Ph.D. in Molecular Biology from Keio University (1998).

Dr. Tomita is a recipient of Presidential Young Investigators Award from National Science Foundation of USA (1988), Shared University Research Award (2003) from IBM Corporation, Minister for Science and Technology Policy Award (2004), The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology (2007), Recognition of Distinguished Service by International Society of Metabolomics (2009) and various other awards.

URL: http://www.iab.keio.ac.jp/en/

KEYNOTE SESSION

Systems Medicine and Proactive P4 Medicine: Catalyzing a Revolution in Healthcare through Wellness

Leroy Hood
Institute of Systems Biology, Seattle, USA

Systems medicine emerged from a systems-driven approach to disease—to deal in a global or holistic manner with the incredibly complexities of disease. Systems medicine has reached a tipping point and is already beginning to transform the practice of medicine through the emergence of powerful new technologies (e.g. single-cell analyses and peptide protein-capture agents) and systems-driven strategies (e.g., family genome sequencing, dynamical, disease-perturbed network studies of disease, and systems-driven approaches to blood diagnostics). I will discuss recent results from several of these strategies and technologies.

Three converging opportunities—systems medicine, big data (and its analytics) and patient-activated social networks—are leading to a proactive medicine that is predictive, personalized, preventive and participatory (P4). I will contrast P4 medicine with contemporary evidence-based medicine and discuss its societal implications for healthcare. P4 medicine has two central thrusts—quantifying wellness and demystifying disease.

I will discuss how we plan to introduce P4 medicine into the current healthcare system with a P4 pilot program—a longitudinal, high-dimensional data cloud study on each of 100,000 well patients. We have already generated and analyzed the dynamical data clouds for a year on 107 well individuals. The preliminary results from these studies are striking. It is clear that such a wellness approach can be used to optimize wellness for the individual—thus maximizing human potential. It is also clear that wellness to disease transitions can be detected and with systems approaches studied to learn about the earliest disease mechanisms and identify new targets for diagnostics and therapeutics—thus enabling a quick return from a disease to a wellness trajectory. This approach can be used to study any disease. It also poses fascinating opportunities for creating metrics for wellness—to distinguish both the physiological and psychological components. These advances will have profound implications for healthcare and society.

KEYNOTE SESSION

Biography of Hood

Dr. Hood's research has focused on the study of molecular immunology, biotechnology, genomics and systems biology. His professional career began at Caltech where he and his colleagues pioneered four instruments—the DNA gene sequencer and synthesizer, and the protein synthesizer and sequencer—which comprise the technological foundation for contemporary molecular biology. In particular, the DNA sequencer has revolutionized genomics by allowing the rapid automated sequencing of DNA, which played a crucial role in contributing to the successful mapping of the human genome during the 1990s. His studies on antibody diversity transformed our understanding of the mechanisms for producing antibody diversity.

In 1992, Dr. Hood moved to the University of Washington as founder and Chairman of the first cross-disciplinary department in biology--the Department of Molecular Biotechnology. In 2000, he co-founded the first Institute for Systems Biology in Seattle, Washington to pioneer systems approaches to biology and medicine. It was during this time that he began his systems-driven studies on disease and focused on neurodegeneration and cancer.

Dr. Hood was awarded the Lasker Prize for studies of immune diversity in 1987 and the 2002 Kyoto Prize for developing advanced technologies. He received the 2003 Lemelson–MIT Prize for Innovation and Invention and at that time was awarded the 2003 Association for Molecular Pathology (AMP) Award for Excellence in Molecular Diagnostics and the 2004 Biotechnology Heritage Award for lifetime achievements in biotechnology. His lifelong contributions to biotechnology have earned him the 2006 Heinz Award in Technology, the Economy and Employment for his extraordinary breakthroughs in biomedical science at the genetic level. He was elected to the Inventors Hall of Fame for the automated DNA sequencer in 2007. In 2011 he received the NAE's Russ Prize for developing the automated DNA sequencer that revolutionized genomics and medicine. In 2013 he received the National Medal of 'Science from President Obama. In 2015, Dr. Hood was chosen as one of the world's 50 most influential scientists: see: http://www.thebestschools.org/features/50-influential-scientists-world-today/

He has published more than 750 peer-reviewed papers, received 36 patents, and has co-authored textbooks in biochemistry, immunology, molecular biology, and genetics and is just finishing a text on systems biology. Dr. Hood is a member of the National Academy of Sciences, the American Philosophical Society, the American Association of Arts and Sciences, the Institute of Medicine and the National Academy of Engineering. He is one of 10 (of more than 6,000 members) scientists elected to all three academies (NAS, NAE and IOM). Dr. Hood has also played a role in founding more than 15 biotechnology companies, including Amgen, Applied Biosystems, Systemix, Darwin, Rosetta, and the newly formed diagnostic company, Integrated Diagnostics. He has received 17 honorary degrees from national and international institutions. He is currently pioneering systems medicine and the systems approach to disease with the ultimate object of transforming healthcare to a discipline that is predictive, preventive, personalized and participatory—P4 medicine.

KEYNOTE SESSION

Regnase-1, a Ribonuclease Involved in the Inflammatory and Immune Responses

Shizuo Akira
Laboratory of Host Defense, WPI
Immunology Frontier Research Center,
Osaka, JAPAN

The innate immune system is an evolutionally conserved host defense mechanism against pathogens. Innate immune responses are initiated by pattern recognition receptors (PRRs), which recognize specific structures of microorganisms. Among them, Toll-like receptors (TLRs) are capable of sensing organisms ranging from bacteria to fungi, protozoa and viruses, and play a major role in innate immunity. Individual TLRs recognize different microbial components, activate different signaling pathways via selective usage of adaptor molecules, and give rise to different patterns in gene expression. Gene expression is controlled at multiple points, including signal transduction, transcription and mRNA stability. So far, transcriptional regulation has been extensively studied. However, recent studies have revealed that control of gene expression at the mRNA level is as important as transcriptional control in the immune response. We are now focusing on the role of genes induced in response to TLR stimulation, particularly the genes that are rapidly induced in a MyD88-dependent manner within 30 min after LPS stimulation. Among them, we have recently identified a novel gene named Zc3h12a (also called MCPIP1) which has a CCCH-type zinc finger domain. The knockout mice developed spontaneous autoimmune diseases accompanied by splenomegaly and lymphadenopathy. Subsequent studies showed that Zc3h12a is a nuclease involved in destabilization of IL-6 and IL-12mRNA via the stem loop structure present in the 3'UTR of these genes. We renamed it Regulatory RNase-1 (Regnase-1) based on the function. I would like to discuss the role of Regnase-1 in the immune response.

Biography of Akira

Dr. Shizuo Akira is a director and professor of WPI Immunology Frontier Research Center, and also a professor in Institute for Microbial Diseases at Osaka University, Japan. He received his M.D. and Ph.D. from Osaka University. After two years of postdoctoral working in Department of Immunology, University of California at Berkeley, he started to study on IL-6 gene regulation and signaling in the Institute for Molecular and Cellular Biology, Osaka University, and cloned transcription factors, NF-IL6(C/EBP beta) and STAT3. He was a professor in Department of Biochemistry, Hyogo College of Medicine from 1996 to 1999, where he became involved in Toll-like receptors research. By generating TLR family knockout mice, he identified ligands of many TLR members. He also demonstrated that the difference in signaling pathway among TLRs is due to selective usage of adaptor molecules such as MyD88 and TRIF. He demonstrated that pathogen-derived RNA is recognized by cytoplasmic receptor family, besides TLRs, and clarified the molecular mechanism of antiviral response against RNA viruses. His current research interests are molecular mechanisms of innate immunity and inflammation, which are studied mainly by generating knockout mice.

Awards and Honors: 2004 Robert Koch Prize, 2006 William B. Coley Award for Distinguished Research in Basic Immunology, 2007 Imperial Prize and Japan Academy Prize, 2009 National Academy of Sciences of USA, Foreign Associate & Person of Cultural Merit, 2010 Keio International Medical Science Prize & Avery-Landsteiner Prize & EMBO Associate Member & The 2011 Canada Gairdner International Award, 2014 Member of the Japan Academy.

Searching Function of Non-coding RNAs in Complex Mammalian Transcriptomes

Piero Carninci

RIKEN Center for Life Science Technologies, Japan

We have developed cap-analysis gene expression (CAGE) to simultaneously map mRNAs and non-coding RNAs transcription starting sites (TSSs) and measure their expression at each different promoters. Since CAGE shows single nucleotide resolution, we can use this technology to comprehensively measure gene expression at each TSSs. Due to this unprecedented resolution, we have learned that promoters use different regulatory elements in different cells and tissues. Using CAGE, we can also infer the transcriptional networks that regulate gene expression in each different cell type. For its high resolution to map TSSs, CAGE has been used extensively in the ENCODE and modENCODE projects.

In the FANTOM5 project, we have applied CAGE on a comprehensive panel of human and mouse primary cells and other tissues, resulting in a very broad map the promoterome and regulatory networks. Our map reveals the existence of more than 180,000 promoters and 45,000 enhancers, which are often tissue specific. The FANTOM5 database is one of the broadest expression database available to the community (http://fantom.gsc.riken.jp/5/). Additionally, we have determined the pattern of expression of retrotransposon elements (RE), which are likely to have a regulatory role. As example, some families of LTR retrotransposon elements are specifically expressed in ES and iPS cells, where they have a role in maintenance of pluripotency. Future FANTOM projects will be focusing to broadly understand the function and the interaction with cell regulatory networks of these RNAs in several primary cells, with the purpose to create the broadest database of functional lncRNAs.

FUNDAMENTAL METHODS IN BIOLOGY I

The Cellular Mechanics of the Immune Response

Philip D. Hodgkin[1,2], Andrey Kan[1,2], Julia M. Marchingo[1,2], Jie H.S Zhou, John.F. Markham[3], Mark R. Dowling[1,2], Susanne Heinzel[1,2], Ken R. Duffy[4]

[1]The Walter and Eliza Hall Institute of Medical Research, Australia
[2]The University of Melbourne, Australia
[3]Victoria Research Laboratory, National Information and Communications Technology (ICT), Australia
[4]Hamilton Institute, National University of Ireland, Ireland

During the adaptive immune response T and B-lymphocytes receive signals from different sources that eventually determine the strength and type of response they follow. Our laboratory has been studying the rules of signal integration and decision-making under different conditions. By combining studies of population changes over time, both in vitro and in vivo, with direct imaging of single cells making decisions under different stimulation combinations a picture of how T and B cells operate as 'machines' and respond to regulation is becoming clear. Perhaps surprisingly cellular calculation can be described with quite simple quantitative rules, although there are many 'variations' and complex outcomes possible. In the most fundamental response B and T cells simply count through a series of divisions, stop, return to quiescence and die. Here the number of times T cells divide is perhaps the most important variable and is highly influenced by stimulation strength and the unique combination of costimulatory signals. Furthermore we report a role for stochastic variation of timed processes to divide, die and differentiate to manipulate the strength of the population response and to regulate the proportion of cells allocated to different tasks. While perhaps impossible to predict the infinite variety of paths taken by single cells and families, knowledge of the underlying stochastic processes, combined with rules of integration acquired by single cell tracking and analysis enables accurate modelling of the population response over time.

Universal Relationship in Fluctuation and Response in Adaptation and Evolution

Kunihiko Kaneko

Graduate School of Arts and Sciences, University of Tokyo, Japan

Characterization of plasticity, robustness, and evolvability in terms of dynamical systems and statistical analysis is an important issue in biology. First, we present a macroscopic theory of fluctuation and responses in expressions across genes, by assuming that cells undergo steady growth. to sustain each of its internal components. From this steady-growth constraint, expression of all genes is shown to change along a one-parameter curve in the state space, in response to the environmental stress. This leads to a macroscopic law that governs the cellular state, which is confirmed by transcriptome analysis of bacteria.

Second, proportionality between the environmental and evolutionary responses, as well as that between phenotypic variances due to environmental noise and to genetic variation, is demonstrated by bacterial evolution experiments and in-silico evolution of toy cell models, as are theoretically formulated by macroscopic phenomenology theory.

Last, we discuss how consistency between evolutionary and developmental scales leads to the above universal laws

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Phenotypic Convergence in Experimental Evolution of Antibiotic Resistant Bacteria

Chikara Furusawa

RIKEN Quantitative Biology Center, Japan

Laboratory evolution experiments provide valuable information about the nature of adaptive evolution. Here, we performed evolution experiments of *Escherichia coli* under each of 11 antibiotics, and obtained antibiotic resistant strains [1]. Then, we performed gene expression analysis by microarrays and resequencing analysis by next-gen sequencer. Furthermore, for each resistant strain, cross-resistance/sensitivity to other antibiotics were quantified. As results, we demonstrate that phenotypic changes in resistant strains to various antibiotics were similar, and the resistances can be quantitatively predicted by the expression changes of a small number of genes. Several candidate mutations contributing to the resistances are identified, while phenotype-genotype mapping is complex suggesting the phenotypic convergence is based, as least in part, on diverse genetic/epigenetic mechanisms. An experimental verification of theoretically predicted universal law in adaptive evolution [2] will also be discussed.

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BIOLOGICAL NETWORKS I

Gompertzian Modeling of the Formation of Thymocyte Populations in the Pre-natal and Post-natal Murine Thymus and its Relationship with the Bone Marrow Progenitors

Daniela Zaharie, Radu Moleriu and Felix Mic

Victor Babes University of Medicine and Pharmacy, Romania

Thymus cyto-architecture provides a complex micro-environment for the development of T-cells. As a result of many years of investigations, a broad picture of T-cell development has emerged but many interactions remain unclear, mainly in the early stages of fetal thymus, which is difficult to approach experimentally. In this work we used a previously published four-compartment model of the murine thymus based on differential equations (Mehr et al, 1995), that we adapted to model perturbation of thymocyte homeostasis (Moleriu et al, 2014), and turned it into a Gompertz-based model that explains the evolution of the four main thymocyte populations and the formation of the thymus from the appearance of the first fetal thymocytes until the death of the animal. The model has five differential equations, the first four equations describe the rate of change in the number of thymocytes, based on terms that describe the proliferation, death and transfer of thymocytes, while the fifth equation models the compartment of apoptotic cells. Each of the first four equations contains a term which models a proliferation process characterized by a decreasing proliferation rate. The reason for choosing a Gompertz-like proliferation rate is that it allows to model the age-induced thymus involution. We used data from our experimental work, data from our previously published papers and data from literature in creating and testing the model. The results show that the rates of proliferation for the CD4-CD8- double-negative and CD4+CD8+ double-positive thymocyte populations are much higher in the early stages of fetal thymus development and they gradually drop as thymus develops. For the mature, CD4+ and CD8+ single-positive thymocytes, the proliferation rates is higher in the first stages of the post-natal life in agreement with published data. For all thymocyte populations there is a shock in the proliferation rates associated with birth, as these rates are experiencing a sudden and significant discontinuity over the birth period. Models with continuity in the proliferation rates over birth fit poorer than the ones with proliferation discontinuity over the birth period. The model gives us an image of the dynamics of thymocyte generation in the pre-natal and post-natal thymus. The most important finding is that the evolution of the post-natal thymus is independent of the constant inflow of bone marrow progenitors and the post-natal thymus appears to have intrinsic renewal capability.

BIOLOGICAL NETWORKS I

Network Design Principles and Response Sensitivity of Components

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In studies of biological networks, the network structure and the network property are mainly discussed and the properties of their components are less noticed. However, design principles of a network would be different depending on the properties of its components as the network property is affected by the components. Therefore, we studied how characteristic features of an evolved network differ according to the response sensitivity of its components. Here, we investigated the responses of gene regulatory networks containing many genes that have undergone numerical evolution to achieve the following response; among some input genes and target genes, one-to-one correspondences between each are set and only a single corresponding target gene is required to respond while other target genes need not to respond upon input to an input gene. Although we studied under same conditions and same settings except for gene sensitivity, the evolved networks were totally different depending on the sensitivity. Networks with sensitive genes evolved with shortest path structures separated for each input-target pair. A gene responded only to one specific input showing a monotonic response or did not respond at all. On the other hand, networks with non-sensitive genes evolved with a cooperative structure indecomposable to small motifs. In such networks, almost all genes responded to more than one inputs and some genes even responded to all inputs. Moreover, their behaviors were dynamic, i.e. not only monotonic responses but also adaptive responses were observed, albeit often 'partial adaptation' in the sense that expression levels do not always return to original levels perfectly. We also studied their differences in noise robustness and found cooperative networks with non-sensitive genes were more robust. Moreover, we compared the robustness of the two types of structures using networks evolved with genes of same moderate sensitivity. Despite the property of genes was in common, cooperative networks were still more robust than networks of shortest path type. These results have significant implications in designing robust biological networks.

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BIOLOGICAL NETWORKS I

Investigations on Longitudinal Gene Effects in Dynamic Association Mapping by a Random Walk Kalman Filter model for a Backcross Mice Dataset

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Assumptions regarding evaluation of gene and environmental effects might be important in dynamic association mapping. Analyzing longitudinal observations with correct methodology is crucial to detect significant Single Nucleotide Polymorphisms (SNPs) for complex traits in genome wide association studies (GWAS). In this study we assumed that genomic signal over time could be traced by a Bayesian random walk- Kalman filter model in state space form to obtain longitudinal residuals. Because of the Kalman filter we do not have to wait for collecting the whole data set to do estimations hence estimates are available as soon as measurements are taken. For example in order to use functional mapping approach, whole sets of observations needs to be collected in advance. This may include months (if not years) of waiting time depends on nature of phenotypes and experimental designs. And because of longitudinal residuals to employ in association mapping: biological reasoning could also be deduced easily given the signal is genuine. We also extended the random walk model for auto regressive structures by tuning the associated parameters. The advantages of our method are demonstrated by both simulated and real datasets. Both random walk and auto regressive model detected similar genomic signals from the backcross mice dataset. But auto regressive model detected more SNPs and much stronger genomic signals.

SYSTEMS IMMUNOLOGY

On/off Switching Mechanisms in Signaling Network

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Mammalian signal transduction pathways transfer a variety of extracellular information to transcription factors in the nucleus to regulate gene expression. Interestingly, signaling pathways often control these processes in a nonlinear fashion and, in some cases, analogous graded doses of extracellular stimuli result in digital activation of transcription factors. Such digitally activated transcription factors may function to be more easily recognized by their target molecules in a noisy cellular environment.

In this talk, digital activation mechanisms of NF-kB transcription factor, which plays an important role in cellular commitment, will be described. Based on wet-lab experiments and mathematical modeling, we showed that NF-κB activity is controlled by two positive feedback loops within the signaling pathway, from TAK1 to IKK and from IKK to IKK, to produce a switch-like activation of NF-κB. These feedback loops contribute to determine the threshold for NF-kB-mediated B cell proliferation, suggesting that the mechanism is important for B cell lineage commitment.

Our studies suggest that cellular complexity might arise from combinatorial regulation of binary states of transcription factors.

SYSTEMS IMMUNOLOGY

A Quantitative Framework for Estimating the Impact of HIV Eradication Strategies

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New eradication strategies are being investigated for HIV. Although several of these, including the use of histone deactylase inhibitors (HDACi), have shown some experimental benefits their impact in achieving eradication is uncertain. A quantitative framework is needed that can estimate the impact of these strategies and how best to implement them.

We develop a mathematical model derived from detailed longitudinal plasma viral levels (pVL) and HIV DNA data obtained from 16 individuals commencing ART containing raltegravir (the PINT Study). The model tracks memory CD4+ T cell numbers, their expansion and loss with and without antiretroviral therapy (ART), their contribution to plasma viral levels (pVL) and to numbers of these cells containing total, 2LTR and integrated HIV DNA within both resting (CD38-) and activated (CD38+) subsets. Dividing cell levels were based on Ki-67 expression. Consistent with published experiments, memory cell depletion is driven by increased activation dependent on viremia.

The model reproduces viral and activated/resting memory CD4+ T cell dynamics prior to and during one year of ART. Model simulations showed that pVL arising from homeostatic proliferation of resting cells with integrated HIV DNA limited the impact of short term ART intensification. Under our calculations long-term ART could eradicate HIV infection but needed to be more effective than this 3-drug regimen and applied over an extensive period determined by 1) the approximate 1 year half-life of resting memory cells and 2) the requirement to reduce integrated HIV DNA by 7 logs. In contrast, HDACi that increased background activation of the latent reservoir by 10%, in combination with ART, decreased integrated HIV DNA by 46% over 1 year, drastically shortening time to eradication. However it also increased pVL from 10 to 600 copies and led to an 8% deterioration of total memory cells compared to no HDACi. If HIV-driven activation is the cause of immune depletion, as suggested by experiments and modelled here, then increased clearance of productively infected cells or virions, eg through infusion of monoclonal antibodies, will have little impact on time to eradication.

We have produced the first mathematical model that can duplicate viral and memory cell dynamics under a number of treatment scenarios. It provides a quantitative framework for estimating the success of different strategies and supports the view that HDACi can significantly impact on HIV eradication.

SYSTEMS IMMUNOLOGY

The Self-Limiting Dynamics of TGF-β Signaling, In Silico and In Vitro

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TGF-β activates the Smad signaling system, but the Smad system can turn itself off over time, even if TGF-β remains abundant in the cellular environment. Several molecular mechanisms have been published to explain how Smad signaling declines over time, but the relative impact of each mechanism on the overall system is unknown.

In this work, we used computational and experimental methods to assess different negative regulators of Smad signaling in HaCaT cells. Models simulated the dynamics of negative regulation, and distinct time-scales were apparent for each mechanism: dephosphorylation of R-Smad is a fast-mode effect, while degradation of phosphorylated R-Smad and I-Smad-induced receptor degradation are slow-mode effects.

Surprisingly, simulations of the known effects, alone and in combination, were not capable of explaining the observed dynamics of TGF-β/Smad signaling, even when all free parameters were adjusted to maximize agreement with the observations. We then proposed a single modification, a negative feedback loop with up-regulation of the phosphatase PPM1A. The resulting model was able to explain the dynamics of Smad signaling, under both short and long exposures to TGF-β1. Cell culture experiments confirmed that this negative feedback does indeed occur, with PPM1A levels significantly increased after TGF-β1 stimulation. Moreover, our model was able to resolve an apparent contradiction in the previous literature concerning the dynamics of phosphorylated R-Smad degradation.

We conclude that the dynamics of Smad negative regulation cannot be explained by the negative regulatory effects that had previously been modeled, and we provide evidence for a novel feedback loop through PPM1A up-regulation. This work shows that tight coupling of computational and experiments approaches can yield improved understanding of complex pathways.

Chromatin Structure and Dynamics in Live Mammalian Cells

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How is the long strand of human genome DNA organized in the cell? The DNA is wrapped around core histones, forming a nucleosome structure. The nucleosome had been assumed to be folded into a 30-nm chromatin fiber and other helical folding structures. However, several recent evidences including our cryo-microscopy (cryo-EM) and synchrotron X-ray scattering analyses have shown that chromatin mainly consists of irregularly folded nucleosome fibers without 30-nm chromatin fiber in the cells (1-4, 7). This irregular folding implies a less physically constrained and locally more dynamic chromatin state. Nucleosome fibers may be constantly fluctuating. Consistent with this notion, recently we uncovered the local nucleosome fluctuation in live mammalian cells (5-7). The obtained results suggested that nucleosome fluctuation increases chromatin accessibility, which is advantageous for many “target searching” biological processes, such as RNA transcription, DNA replication and DNA repair/recombination (5-7).

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Our Scenarios to Produce Designed Genomes via *Bacillus Subtilis* Genome Vector

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Gene networks of cell are well regulated to make cells alive in appropriate environments. All cells/microbes possess genome DNA which seem robust in structure. In contrast to many traditional biological studies on the existing genomes and related gene networks, recent technologies on giant DNA synthesis allows producing cells starting up by synthesized genomes. We have long developed a unique molecular cloning system that allows whole genome synthesis using *Bacillus subtilis* as a cloning host. In addition to the first successful case for *Synechosystis* PCC6803 in 2005, further novel technologies have been under study to construct fully designed genomes and activate them in an appropriate chassis. Along with the challenges, I would like not only to briefly recap our system but also present our recent scenarios and on-going works aiming at production of cells with functional genomes.

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Disrupted tRNA Genes and their Splicing Regulations

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We have been working on regulatory RNAs and their enzymes for more than 15 years, especially focusing on transfer RNA (tRNA). tRNAs are small non-coding RNAs with lengths of approximately 70–100 nt. They are directly involved in protein synthesis by carrying amino acids to the ribosome. In this sense, tRNAs are key molecules that connect the RNA world and the protein world. Thus, study of the evolution of tRNA molecules may reveal the processes that led to the establishment of the central dogma: genetic information flows from DNA to RNA to protein. Thanks to the development of DNA sequencers in this century, we have determined a huge number of nucleotide sequences from complete genomes as well as from transcriptomes in many species. Recent analyses of these large data sets have shown that particular tRNA genes, especially in Archaea, are disrupted in unique ways: some tRNA genes contain multiple introns and some are split genes. Because these tRNAs are encoded as precursor forms (pre-tRNAs) in the genome, they must be processed to yield mature functional tRNAs. Using the hyperthermophilic archaeon *Pyrococcus furiosus* as a model species, we have constructed *in vitro* reconstitution system for the tRNA splicing reaction. Moreover, we found and identified a protease in the *Pyrococcus* whole-cell extract, which specifically cleaves the C-terminal domain of the RtcB tRNA ligase. The RtcB tRNA ligase is the responsible enzyme catalyzing the ligation of spliced tRNA halves into mature-sized tRNA. The tRNA ligase lacking the C-terminal domain showed no detectable tRNA ligation activity. In this meeting, I would like to review the works in the field and discuss the recent progress for the complex tRNA splicing machineries.

Advanced Continuous Cultivation Methods for High-resolution Quantitative Growth Space Analysis of Cells

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Increasing the throughput of systems biology-based experimental characterization of in silico-designed strains has great potential for accelerating the development of cell factories. For this, analysis of metabolism in steady state is essential as only this enables to unequivocally define the physiological state of cells, which is needed for complete description and in silico reconstruction of their phenotypes. In this presentation we summarize the research highlighting that high-resolution characterization of steady state metabolism - growth space analysis - leading to more accurate and quantitative understanding of metabolism can be achieved by using advanced continuous cultivation methods termed changestats. In changestats, an environmental parameter is continuously changed within one experiment while maintaining cells in physiological steady state, similar to chemostats. In addition to the significantly increased resolution and throughput of growth space analysis compared to chemostats, changestats enable to follow dynamics of metabolism, detect metabolic switch-points and optimal growth conditions etc. We also describe the concept and characteristics of changestats in detail. Finally, we propose that such systematic characterization of steady state growth space of cells using changestats has value not only for fundamental studies of metabolism, but also for systems biology-based metabolic engineering of cell factories.

Phenotypic Plasticity in Tumorigenesis

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How can cells maintain the stability of gene expression states over multiple generations, while at the same time retaining sufficient plasticity to permit transitions between different stable phenotypes? This question lies at the heart of normal multicellular development and is also central to understanding how normal cells convert to disease states, such as cancer. The balance between stability and plasticity is a fundamental property of complex biological systems and a key underpinning of cell behavior.

This conceptual framework has important implications for the design of cancer therapies. The primary clinical cause of treatment failure for conventional cytotoxic or targeted cancer therapies is the rise of resistance in rare survivor cells. Our work has demonstrated that cytotoxic chemotherapy treatments, such as vincristine and doxorubicin, trigger a specific phenotype transition in the rare surviving cells and induce gene expression programs that facilitate resistance.

If we can understand what triggers specific phenotype transitions and the rates at which these transitions proceed, we should be able to use these parameters to design novel types of differentiation therapies. Beyond simply avoiding the resistance phenotype, we envision actively 'reprogramming' cells that enter disease states. This strategy co-opts the inherent potential of cells to shift their phenotype. Essentially, defective cells would be rehabilitated instead of eliminated. Here we present a combined computational and experimental approach in which key gene nodes are identified by network inference modeling and then perturbed in vitro and in vivo to block mammary tumor progression.

Evidence for Non-genetic Causes of Eukaryotic Drug Resistance

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Resistance to drug therapy is usually considered an acquired trait that arises through genetic changes. In cancer therapies, one of the most common causes of drug resistance is elevated expression of protein pumps that actively transport anticancer drugs out of the cell. This talk will describe how theoretical studies implicate fluctuations in gene expression and gene regulatory network architecture as potentially significant contributors to drug resistance phenotypes. This hypothesis is supported by experimental studies of a highly conserved multi-drug resistance network, which provides evidence that a 'bet-hedging' survival strategy can facilitate the development of drug resistance in eukaryotes.

Mathematical Modeling of Biological Networks Helps Finding Genetic Determinants of Metastatic Colon Cancer

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Detailed reconstructions of biological networks involved in cancer progression and mathematical modeling of them using high-throughput data helps elucidating the molecular mechanisms involved in carcinogenesis. In colon cancer, the old question formulated in the classical genetic model by Vogelstein on what are the genetic determinants of metastasis still remains unanswered. This is why there is a need in creating mice models prone to early appearance of metastatic tumours. We postulated that Epithelial-to-mesenchymal transition-like process is a necessary condition for appearance of metastasis from gut tumours.

EMT-like is a critical process allowing initiation of metastases during tumour progression. We investigated its role in intestinal cancer, by combining computational network-based and experimental approaches to create a mouse model with high metastatic potential. Construction, analysis and mathematical modeling of the network map depicting molecular mechanisms of EMT regulation based on the literature suggests that Notch activation and p53 deletion have a synergistic effect in activating EMT-like processes. The particular efficiency of such a combination of mutations is confirmed by rigorous mathematical modeling of the signaling networks regulating EMT.

To confirm this prediction, we generated transgenic mice by conditionally activating the Notch1 receptor and deleting p53 in the digestive epithelium (NICD/p53−/−). These mice develop metastatic tumours with high penetrance. Using GFP lineage tracing, we identify single malignant cells with mesenchymal features in primary and metastatic tumours in vivo. The development of such a model that recapitulates the cellular features observed in invasive human colorectal tumours is appealing for innovative drug discovery. In addition to this, mathematical modeling and data analysis using signaling networks unravelled the role of other pathways as well as tumor microenvironment in colon tumorigenesis.

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Conserved Patterns of Metabolic Activity Suggest General Principles of Regulation Based on Cellular Objectives and Community Constraints

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Cells maintain their structure over time and grow by modulating metabolic activity. Multiple studies have shown that a wide variety of cell types display fermentative phenotypes that appear energetically suboptimal. To try to rationalize these responses, we examine how similar metabolic phenotypes shared by rapidly growing microbial cells, stem cells, and cancer cells suggest common strategies connected to cellular activity objectives, differentiation as well as cell community constraints. Cellular needs, whether targeted to growth or other energy-intensive processes, can trigger conserved energy/biomass wasting reactions that reflect fundamental trade-offs between nutrient availability, metabolic demand and costs as well as the interactions between cells. Some counter-intuitive metabolic phenotypes might thus be better interpreted using a cellular economy framework, which in turn may help to formulate novel ways of modulating cellular activity.

PHLDA1 is a Negative Feedback Regulator of ErbB Signalling

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Dysregulation of ErbB signalling network is highly related to incidence of various cancers. The objective of our study is quantitative and system-level understanding of control mechanisms of the ErbB signalling network.

Transcriptome, proteomics, and high-content imaging cytometry analyses were carried out to measure dynamic activity of ErbB signal-transcription network stimulated by heregulin (HRG) at population and single cell levels. LC/MS analysis was carried out to identify binding partners of a transcriptional product, pleckstrin homology-like domain, family A, member 1 (PHLDA1), of which mRNA is induced upon receptor activation. Mathematical model of this network was constructed by ordinary differential equations.

PHLDA family genes are commonly listed as early responsive genes in many types of mammalian cells stimulated with growth factors, however, its molecular function have remained unclear. We identified that a transcriptionally-inducible PHLDA1 product suppressed phosphorylation of ErbB receptors, ERK and Akt after a few hours of HRG stimulation in MCF-7 breast cancer cells. We investigated binding partners of PHLDA1 by LC/MS analysis, and revealed ErbB3 is one of the binding proteins of PHLDA1. Modulation of PHLDA1 expression affected complex formation of ErbB3 and phosphorylated ErbB2, suggesting that PHLDA1 inhibits the complex formation by binding to ErbB3. To understand the function of PHLDA1 at system level, we constructed a mathematical model of the ErbB receptor-PHLDA1 network in a time-scale of 180 min after HRG stimulation. Our computational simulation showed an excellent agreement with experimental time-course of ErbB receptors, Akt and ERK activities, and PHLDA1 expression at population level. However, there is a discrepancy in single cell level. The analyses explore unknown functions of PHLDA1.

Single-cell Transcriptional Analysis to Uncover Regulatory Circuits Driving Cell Fate Decisions in Early Mouse Development

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Transcriptional regulatory networks controlling cell fate decisions in mammalian embryonic development remain elusive despite a long time of research. The recent emergence of single-cell RNA profiling technology raises hope for new discovery. Although experimental works have obtained intriguing insights into the mouse early development, a holistic and systematic view is still missing. Mathematical models of cell fates tend to be concept-based, not designed to learn from real data. To elucidate the regulatory mechanisms behind cell fate decisions, it is highly desirable to synthesize the data-driven and knowledge-driven modeling approaches.

We propose a novel method that integrates the structure of a cell lineage tree with transcriptional patterns from single-cell data. This method adopts probabilistic Boolean network (PBN) for network modeling, and genetic algorithm as search strategy. Guided by the 'directionality' of cell development along branches of the cell lineage tree, our method is able to accurately infer the regulatory circuits from single-cell gene expression data, in a holistic way. Applied on the single-cell transcriptional data of mouse preimplantation development, our algorithm outperforms conventional methods of network inference. Given the network topology, our method can also identify the operational interactions in the gene regulatory network (GRN), corresponding to specific cell fate determination. This is one of the first attempts to infer GRNs from single-cell transcriptional data, incorporating dynamics of cell development along a cell lineage tree.

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Event-based Stochastic Simulations for Biochemical Network

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With recent increase in affordability and accessibility of high-performance computing (HPC), the use of large stochastic models has become increasingly popular for its ability to accurately mimic the behavior of the represented biochemical system. One important application of such models is to predict parameter configurations that yield an event of scientific significance. Due to the high computational requirements of Monte Carlo simulations and dimensionality of parameter space, brute force search is computationally infeasible for most large models.

In this talk, we present an algorithm-Stochastic Parameter Search for Events (SParSE)-that is specifically developed for event-based stochastic simulations with unknown parameter values. SParSE automatically computes parameter configurations for propagating the system to produce an event of interest at a user-specified success rate and error tolerance. It is highly automated and parallelizable. Moreover, its computational complexity does not scale linearly with the number of unknown parameters; all reaction rate parameters are updated concurrently at the end of each iteration in SParSE. We demonstrate its performance on two simple systems-reversible isomerization and SIRS disease transmission-to show its computational efficiency and accuracy.

A Comprehensive Pipeline for the Epigenomic Data Analysis of Genome-Wide DNA methylation

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DNA methylation is an important epigenetic modification involved in many biological processes. Bisulfite treatment coupled with high-throughput sequencing provides an effective approach for studying genome-wide DNA methylation at base resolution. Libraries such as whole genome bisulfite sequencing (WGBS) and reduced represented bisulfite sequencing (RRBS) are widely used for generating DNA methylomes, to reveal fascinating patterns of DNA methylation in the developmental stages, cancers, plants and animals.

We developed a comprehensive pipeline for the epigenomic data analysis of genome wide DNA methylation. It includes three components: (1) BS Seeker II is a versatile aligner for mapping bisulfite sequencing data and generating DNA methylomes. It largely improves mappability over existing aligners by using local alignment. (2) MethGo was developed with five modules; single nucleotide polymorphism (SNP) calling, epimutation identification, copy number variation (CNV) calling, gene body and promoter methylation level identification, and comparisons of methylation at transcription factor binding sites. It aims to provide important information of epigenome. (3) Hetgene detects heterogeneity of methylation patterns in a cell population. This approach provides insight into epigenetic regulation by identifying potential epigenetic regulatory regions. In cancer research, such information may suggest subsets of cells are progressing differently at certain regions and they are potential targets for early diagnosis.

To demonstrate the effectiveness of our pipeline, we performed whole-genome DNA bisulfite sequencing (BS-seq) and mRNA sequencing (RNA-seq) of different developmental stages of T. melanosporum; namely, fruitbody (“truffle”), free-living mycelium and ectomycorrhiza. The data were processed by BS Seeker 2 and revealed a high rate of cytosine methylation (>44%), selectively targeting TEs rather than genes with a strong preference for CpG sites.

Assessment of the Somatic Mutations Impact on Cancer Network Dynamics using Domain-based Protein Interaction Networks

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Cancer is a complex disease involves wide spectrum of genes, proteins, pathways and molecular interactions that are dynamically regulated. Its association with somatic mutations adds another layer of complication, due to the mutations effects on the protein functions. Thus, understanding cancer requires integrated large-scale approaches that combine experimental and computational methods as well as novel mathematical and statistical models. Despite the efforts done to determine the protein interaction networks in cancer, the current predictions still lacking confidence and experimental validation. Furthermore, most of them do not consider the effects of somatic mutations on the interaction networks topology and dynamics. Here, we present an integrated approach to construct high-resolution protein interaction networks in cancer and predict the effects of somatic mutations on the interactions network dynamics. The protein interaction predictions are based on peptides bind to certain peptide recognition domains (PRDs) and determined in large-scale using phage-display screening experiments and computational and statistical models then supported with several layers of genomic and context information. Using the cancer somatic mutations in COSMIC database and the information of 117 SRC Homology (SH3) domains, we constructed high-resolution interaction networks based on wildtype proteins and cancer mutation variants. By overlaying the two networks, we pointed ~8,900 rewiring events that affect the interaction network dynamics. Our method provides better understanding for cancer biology by elucidating disease mechanisms and help in discovering new cancer genes in unprecedented resolution and large-scale.

BIOLOGICAL NETWORKS II

A Multi-Agent Approach for Virtual Tissue Morphogenesis

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We present here an approach of virtual tissue morphogenesis mainly based on multi-agent modelization and simulation. Computer simulations must be efficient and provide the possibility to simulate large tissue containing thousands of virtual cells. In our work, these cells are represented by agents which are able to modify their membrane shapes thanks to a biomechanical model based on a mass/spring system. These agents are also able to exhibit basic cell behaviors such as cell motility, mitosis and adhesion. They also have the capacity to evaluate the compression/stretching and shearing forces applied to them. Let us note that, in order to achieve the computation of thousands of virtual cells, we propose a parallel implementation of our simulator using the OpenCL framework which allows the use of various architectures such as Graphic Cards or Multi-Core Processors. In order to achieve a specific tissue form during morphogenesis, we set the hypothesis that beyond the influence of mechanical forces and gene expression, spatial constraints applied to the cells over time play a key role in the acquisition of a specific form. We consider that, at the early stage of embryogenesis, these constraints are implied in the fundamental principles of form emergence. In our model, it is due to these constraints that cells choose a particular direction while dividing, migrate or die. Our approach of morphogenesis based on constraints has been used to get effectively all possible phenotypes at any stage of the early embryogenesis and their associated genotypes. Such work allows us to do some pattern prediction and to define novel properties on tissues for the purpose of classification.

BIOLOGICAL NETWORKS II

Low-frequency Dynamics of some Biopolymers during Conformational Changes

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Phase transformations of DNA, proteins, and other biopolymers have been intensively investigated in the last decade. One of the least studied problems is how the vibrational spectrum of a macromolecule behaves when its spatial structure changes, or, in other words, the macromolecular dynamics during a phase transformation. An example of a phase transition is the DNA transition from the A to the B form at hydration. Inelastic light scattering studies of this transition revealed a soft optical mode typical of ferroelectric structures. Experiments aimed at studying the dynamics of egg lysozyme denaturation were performed. The dynamics of biopolymers can be studied by different techniques. Structural analysis employs such physical methods as inelastic neutron scattering, X-ray inelastic scattering, Raman spectroscopy, infrared spectroscopy, NMR, and Brillouin scattering. Inelastic X-ray and neutron scattering have a low resolution; Raman and IR spectroscopy allow one to obtain information on individual bonds but do not give information on the dynamics of a molecule as a whole. The Brillouin scattering technique is able to detect changes in the local structure rather than vibrations of individual bonds in the frequency range from 1 to 1000 GHz. By using this technique, we tried to analyze the behavior of the velocity of sound in biopolymers. Our experience in studies of structural phase transitions in crystals was invoked. This allowed us to come closer to the understanding of the relationship between dynamics and structure. Test objects were solutions of proteins, such as lysozyme and BSA, and also solutions of two portions of the DNA molecule with different nucleotide compositions (obtained from Drosophila melanogaster by polymerase chain reaction) in a sodium phosphate buffer (pH = 7.5). Temperature in the experiment was varied from 290 to 363 K, which allowed us to observe phase transformations at thermal denaturation and solution-gel transitions of proteins and also the evolution of local DNA structures at melting. To analyze the scattered light, a 3-pass piezo-scanned Fabry-Perot interferometer with a free spectral interval of about 22 GHz was used, the 180-degree scattering geometry was employed. A source of exciting light was an argon laser with $\lambda = 488$ nm. Temperature dependences of the shift, intensities and half-width at half-maximum of Brillouin scattering doublets, and intensities of elastic scattering components were obtained.

BIOLOGICAL NETWORKS II

Stochastic Flux Analysis of Chemical Reaction Networks

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Chemical reaction networks provide an abstraction scheme for a broad range of models in biology and ecology. The two common means for simulating these networks are the deterministic and the stochastic approaches. The traditional deterministic approach, based on differential equations, enjoys a rich set of analysis techniques, including a treatment of reaction fluxes. However, the discrete stochastic simulations, which provide advantages in some cases, lack a quantitative treatment of network fluxes. We describe a method for flux analysis of chemical reaction networks, where flux is given by the flow of species between reactions in stochastic simulations of the network (Kahramanogulları and Lynch (2013)). Extending discrete event simulation algorithms, our method constructs several data structures, and thereby reveals a variety of statistics about resource creation and consumption during the simulation. We use these structures to quantify the causal interdependence and relative importance of the reactions at arbitrary time intervals with respect to the network fluxes. This allows us to construct reduced networks that have the same flux-behavior, and compare these networks, also with respect to their time series. We demonstrate our approach on an extended example based on a published ODE model of the same network, that is, Rho GTP-binding proteins, and on other models from biology and ecology, including Oyster reef ecosystem model, an oscillator model, and a phosphorelay model. To summarize our results, we provide a fully stochastic treatment of flux analysis. As in deterministic analysis, our method delivers the network behavior in terms of species transformations. Moreover, our stochastic analysis can be applied, not only at steady state, but at arbitrary time intervals, and used to identify the flow of specific species between specific reactions. Our method provides the means for quantitative comparison of models in terms of flux networks obtained from stochastic simulations. We use the technique for comparing models with respect to their simulations at arbitrary time intervals with varying degrees of accuracy, and for simplifying models when a larger model produces the same behavior as the smaller one. Our cases study of Rho GTP-binding proteins reveals the role played by the cyclic reverse fluxes in tuning the behavior of this network.

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POSTERS

Dynamics of SOCS3 in Skin Inflammation

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The epidermis, which is the outermost layer of the skin tissue, comprises of diverse biological players and cellular processes to maintain its homeostasis. Cytokines are an important intercellular mediator of immune signals that control different cellular and molecular responses through JAK (Janus Kinase) and STAT (Signal Transducers and Activator of Transcription) that tightly regulate epidermal homeostasis. Nevertheless, random perturbation in any of these components can lead to chronic epidermal diseases such as atopic dermatitis and psoriasis.

Recently, numerous groups have been focusing on the role of SOCS (Suppressor of Cytokine Signaling) family proteins that control the cellular responses to multiple cytokines by negatively regulating JAK-STAT signaling pathways, in epidermal homeostasis. One of recent published studies using transgenic mice shown that the dysregulation of SOCS3 in keratinocyte could lead to the abnormally active immune responses and development of hyperplasia in the epidermis, that eventually lead to psoriasis-like phenotype. The result implies that SOCS3 has an effects on various aspects of cellular processes besides immune response, but the exact mechanisms including whether these effects depend on JAK-STAT pathways or any other indirect pathway are still elusive.

In this study, we analyzed the time series data of gene expression in keratinocyte of SOCS3 conditional knockout mice to investigate the role of SOCS3 in epidermal homeostasis. We have identified differentially expressed genes that were mainly involved in signaling pathways that regulate the organization of keratin network at second week after birth. However, at eighth and tenth week after birth, the expression of genes related to signaling pathways controlling cellular proliferation were highly activated, suggesting a possible link between the hyper-activation of these signaling pathways and the development of hyperplasia. Hence, we are now investigating and validating the function of SOCS3 in the regulation of those proliferation-related genes by experimental analyses using a cell line of cultured keratinocyte.

In summary, our results suggest a potential novel function of SOCS3 that controls the expression of genes related to the organization of keratin network or cellular proliferation through some of the specific transcription factors in keratinocyte. We believe that by establishing a regulatory model to explain the dynamics of SOCS3 that controls those cellular processes in keratinocyte will provide a detailed knowledge to elucidate the molecular mechanisms underlying epidermal homeostasis and the development of chronic epidermal diseases. Furthermore, our results raise a possibility that combinational studies including both computational and experimental approaches are

very helpful to study intermolecular regulatory networks that are not easy to be accessed solely by use of experimental approaches.

Community Detection in E. coli Metabolic Network using Markov Stability and Constrain-based Modelling

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Community detection is a main branch in the network analysis field. It is used to detect groups of individuals with similar properties among large structures of linked element. Community detection has been widely used in economy, ecology, sociology and many other disciplines. Biological networks, especially at the molecular level, such as metabolic, protein interaction, signaling or regulatory networks have been subject to network analysis.

On the other hand, constrain-based modelling represents a very popular approach to model metabolic behavior in cells in steady state1. Reactions and metabolites are displayed in stoichiometry matrices and after a series of constrains are imposed a set of feasible solutions for the cell flux distribution are obtained.

In this work we apply techniques of community detection to the metabolic network of the bacteria E. coli. These techniques are Markov Stability2 and Role-Based Similarity3. The bacterial network used was obtained from a reconstruction of the core E. coli metabolic network4. We explore the different representations of adjacency matrix in a given metabolic network and how constrain-based modelling can support additional information to the pure topology, such as reaction stoichiometry, directionality and flux. Then we analyze how different are the detected communities for each representation and how different levels of information define and detect particular communities. Additionally we compare the particular community distributions of each representation of the metabolic network to the classic well-studied biochemical pathways and how they relate and interconnect.

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Systems Biology Strategy Reveals PKCδ is Key for Sensitizing TRAIL-Resistant Human Fibrosarcoma

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Cancer cells are highly variable and largely resistant to therapeutic intervention. Recently, the use of the tumor necrosis factor related apoptosis-inducing ligand (TRAIL) induced treatment is gaining momentum, due to TRAIL's ability to specifically target cancers with limited effect on normal cells. Nevertheless, several malignant cancer types still remain non-sensitive to TRAIL. Previously, we developed a dynamic computational model, based on perturbation-response differential equations approach, and predicted protein kinase C (PKC) as the most effective target, with over 95% capacity to kill human fibrosarcoma (HT1080) in TRAIL stimulation [1]. Here, to validate the model prediction, which has significant implications for cancer treatment, we conducted experiments on two TRAIL-resistant cancer cell lines (HT1080 and HT29). Using PKC inhibitor Bisindolylmaleimide I, we demonstrated that cell viability is significantly impaired with over 95% death of both cancer types, in consistency with our previous model. Next, we measured caspase-3, Poly (ADP-ribose) polymerase (PARP), p38 and JNK activations in HT1080, and confirmed cell death occurs through apoptosis with significant increment in caspase-3 and PARP activations. Finally, to identify a crucial PKC isoform, from 10 known members, we analyzed each isoform mRNA expressions in HT1080 cells and shortlisted the highest 4 for further siRNA knock-down (KD) experiments. From these KDs, PKCδ produced the most cancer cell death in conjunction with TRAIL. Overall, our approach, combining model predictions with experimental validation, holds promise for systems biology based cancer therapy.

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Mathematical Model to Compute Harmonic and Stochastic Gene Expressions

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A central challenge of gene expression analysis during the last few decades has been the characterization of the expression patterns experimentally and theoretically. Today's single-cell analyses clarify that transcriptions and translations are stochastic in time and that clonal population of cells displays cell-to-cell variability in the abundance of a given RNA and protein per cell. Hence, to take into account the cell-to-cell variability, we consider a stochastic model of transcription based on the chemical master equation. Our stochastic analysis and Monte-Carlo simulation show that the limiting distribution of mRNA copy number can be expressed by a Poisson-beta distribution. The distribution represents the four different types of expression patters, which are typically found in various experimental profiles.

Dynamic Modeling of BCR Signaling Pathway to NF-kappaB Activation

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Systems biology aims to understand rational design principles of complex biological systems. Mathematical modeling has become a powerful tool to identify molecular mechanisms of the system and to predict and control the dynamical behaviors. Transcription factor nuclear factor-kappa B (NF-kappaB) plays important roles in proliferation, differentiation and apoptosis of variety of cells, therefore it is important to understand the regulatory mechanism of this particular transcription factor. To shed a light on the regulation of the NF-kappaB signal transduction pathway at molecular level, we quantitatively analyzed the system of B cell receptor (BCR) signaling to NF-kappaB activation by using experiments and ordinary differential equations-based model. In this study, we especially focus on two features of the dynamics of nuclear NF-kappaB; switch-like response and oscillation.

We constructed a model which can reproduce both switch-like response and oscillatory behavior of NF-kappaB activity. Simulation of the model was well consistent with experimental results. In some molecular or reaction parameter conditions of the model, sensitivity analysis and bifurcation analysis showed coupling and decoupling of oscillation and switch-like responses.

The model proposed here is the first model for NF-kappaB signaling pathway which integrates both switch-like response and oscillatory behavior of NF-kappaB, and provides valuable insights for understanding the regulatory mechanisms of NF-kappaB signaling for cell determination.

Does Adaptive Evolution Give an Explanation of Zonal Heterogeneity in Liver?: A Simulation Study

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The liver metabolizes tremendous variety of chemical compounds. Almost all the intermediary metabolisms of carbohydrates, lipid and amino acids take place in the liver. Human liver include 250 billion hepatocytes and it consists of half a million hepatic lobule in which 5 billion hepatocytes are assembled. In the liver lobule, hepatocytes line along a fenestrated capillary vessel termed sinusoid. The blood from the portral vein and hepatic artery flows into the sinusoid and out to the central vein. This construct is referred to as port-central axis (PCA), which is the smallest repetitive unit of liver metabolism. The hepatic lobule is a parallel circuit of PCAs and the liver is also a parallel circuit of hepatic lobules. It is known that there is many heterogeneities of various metabolic aspects in the liver lobule. Gene expression levels, protein activities, and metabolite concentrations between periportal and perivenous zone are distinctly heterogeneous that is referred to as “metabolic zonation”. While many histological facts phenomena of metabolic zonation were reported, their biological significances and/ or functions (essential qualities) are still unclear. Even the present gene engineering technologies cannot such as conditional gene knockout and/or knockdown are insufficient to regulate metabolic zonation at will. In this study, we tried to elucidate what is profit of metabolic zonation of liver ammonia metabolism using mathematical model and computer simulation. We expanded a previous mathematical PCA model by Ohno et al. (Ohno model). Ohno model contains the model of ammonia metabolism in PCA and express the difference in function of upper stream to downstream. By using the mathematical model, it is possible to change any variable in the model feely and produce various virtual PCAs. Through comparing these models, we estimated which elements play a major role to adapt various selective pressures in the actual PCA. We used genetic algorithm (GA) to mimic the process of the evolution. Several hypothetical selective pressures, e.g. elimination rate of ammonia, energy efficiency of urea production, acid-base balance, are used as the evaluation functions for GA. In Ohno model, there are twelve enzymes which are used in urea cycle or transamination reaction, oxidative deamination and so on. In consequence, it was suggested that localizations of some enzymes such as glutamine synthetase (GS) and carbamoylphosphate synthetase I (CPI) were indispensable for maximization of energy efficiency. Our simulation results suggested that GS and CPI should be highly expressed in perivenous (downstream) zone and peripotal (upstream) zone, respectively. These results are roughly consistent with actual enzyme localizations in the liver.

Mathematical Model of Human Gene Regulatory Network

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The cellular state is known to be determined by the gene regulatory network. In order to reveal the dynamics of human cellular state, we constructed a mathematical model of the human gene regulatory network (GRN) based on a Boolean network model.

We first surveyed the human gene regulation information from hundreds of literatures and then constructed the human GRN composed of 241 nodes and 465 links. To reduce the computation time, we identified the core human GRN composed of 73 nodes and 195 links by removing leaf nodes. Second, we obtained 80 tissue attractors composed of 69 Boolean variables from public microarray data [Cell, Vol. 122, 947-956, Sep. 23, 2005]. Each Boolean variable represents each node in the core human GRN. Third, we obtained GRN attractors by simulating the core human GRN using Boolean network model based on a simple rule without any weight. From the simulation, we found that the average of Boolean values in the 80 tissue attractors is positively correlated with the average of Boolean values in the attractors based on the simple rule. Lastly, we constructed 80 Boolean network models optimized for representing each tissue attractor by evolving weights of Boolean rules. We adopted genetic algorithm (GA) to optimize weights of Boolean rules.

We constructed tissue-specific Boolean models using literature-based human GRN and tissue-specific microarray data. Each tissue-specific Boolean model can be used to identify the tissue-specific control kernel. And the tissue-specific control kernels could be potential candidates of tissue-specific differentiation markers. Furthermore, our approach also can be applied to identify novel therapeutic markers which regulate the cellular state to escape disease attractor and reenter to the normal attractor.

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Identifying Key Regulators for TNF-induced Cancer Survival

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Tumor Necrosis Factor (TNF), a well-known proinflammatory cytokine, has been recently observed to be elevated in tumor microenvironment. TNF stimulates normal and cancer cells to the production of key cytokines such as Interleukin-6, Chemokine (C-C motif) ligand 7, Matrix Metalloproteinase-3, which can assist cell growth and proliferation. Hence, the understanding of TNF response in cancer and normal cell is crucial for strategic cancer treatment. Previously, we have shown that TNF-induced proinflammatory gene expressions were suppressed by Receptor-Interacting Protein (RIP) 1 inhibitor, Necrostatin-1 (Nec-1) in mouse normal cells [1]. In addition, we have also demonstrated that Protein Kinase C (PKC) inhibitor, Bisindolylmaleimide I (BIM-I), enhances apoptosis in TNF-Related Apoptosis-Inducing Ligand (TRAIL)-resistant cancer cells [2]. Based on these studies, we hypothesized that PKC and RIP1 can be key targets for cancer suppression. Here, we investigated TNF induced cell death with RIP1 and PKC inhibitors in MCF7, HT1080 and HT29 as cancer cells, and MRC5 as control normal cells. Our results show that, TNF and Nec-1 co-stimulation induced 37% cell death in MCF7 and 46 % in HT29 cells. Next, we tested the effect of BIM-I in MCF7 and HT1080. This time, the cell death was 73 % in MCF7 and 30% in HT1080. Notably, in both cases MRC5 cell survival was not largely affected. From these results, we gather that TNF and BIM-I co-stimulation in MCF7 cells possess the most selective effect. Next, we plan to investigate the mechanism for the cell death by measuring several survival and apoptotic signaling molecules and gene expressions. Overall, we hope to elucidate a single molecular target to optimize the cell death for various cancer cells through TNF signaling.

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A-to-I RNA editing fine-tunes miRNA function in human cells

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Adenosine deaminases acting on RNA (ADARs) mediate the hydrolytic deamination of adenosine (A) into inosine (I) on double-stranded RNA (dsRNA). Like guanosine (G), I forms base pairs with cytosine (C), so that A-to-I editing can regulate RNA structure. By overexpressing miRNAs containing either A, I or G at a single nucleotide position in the seed region, we show that A-to-I editing not only redirects miRNAs to a completely new set of genes, but also demonstrate for the first time *in vivo* that the extent of the downregulation is different between I and G. Finally, past studies have shown a role of A-to-I editing with the regulation of miRNA processing, but these results were limited to only a few examples. In order to get the complete picture of ADAR-mediated miRNA regulation, we performed RIP-seq in HeLa cells overexpressing the isoforms ADAR1-p110 and ADAR2. Our results suggest that ADAR isoforms selectively bind and edit different populations of pre-miRNAs, and uncover the possibility of editing-independent processing regulation.

Spatially Distributed RhoGTPase Interactions Coordinate Cell Migration

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Cell motility involves dynamic and spatially regulated changes to the cytoskeleton. The RhoGTPases, including RhoA and Rac1, control cellular responses required for cell migration in time and space. Here combining experiments and mathematical modeling, we elucidate the spatial profile and the intricate dynamics of RhoA-Rac1 interactions in highly motile MDA-MB231 breast cancer cells. RhoA can activate Rac1 via mouse Diaphanous related formin (mDia1) and inhibit Rac1 via Rho kinase (ROCK1). Using proximity ligation assay, we show that in single cells RhoA-mDIA1 interaction strength is relatively weak at the cell rear and markedly increases towards the leading edge, whereas RhoA- ROCK1 interactions are the weakest at the cell front. Based on these findings, our model demonstrates that in migrating cells, RhoA and Rac1 activities can oscillate at the leading edge, whereas at the cell rear there are bistable switches between high and low RhoA and Rac1 activities, coordinated with their behaviour at the cell front. Thus, our computational results can explain different cell dynamics during cell motility and how a migrating cell reconciles these dynamics in space and time.

Transcriptome-wide Variability in Single Embryonic Development Cells

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Molecular heterogeneity of individual molecules within single cells has been recently shown to be crucial for cell fate diversifications. However, on a global scale, the effect of molecular variability for embryonic developmental stages is largely underexplored. Here, to understand the origins of transcriptome-wide variability of oocytes to blastocysts in human and mouse, we examined RNA-Seq datasets. Evaluating Pearson correlation, Shannon entropy and noise patterns (η^2 vs. μ), our investigations reveal a phase transition from low to saturating levels of diversity and variability of transcriptome-wide expressions through the development stages. To probe the observed behaviour further, we utilised a stochastic transcriptional model to simulate the global gene expressions pattern for each development stage. From the model, we concur that transcriptome-wide regulation initially begins from 2-cell stage, and becomes strikingly variable from 8-cell stage due to amplification and quantal transcriptional activity.

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Age-related Changes in Ionic Systems Evoke Ectopic Electrical Activity in Pulmonary Vein Myocardium: A Simulation Study

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The pulmonary vein contains a myocardial layer which is capable of generating spontaneous or triggered action potentials, which is considered to play a central role in the generation and maintenance of atrial fibrillation. The pulmonary vein myocardial layer is extending from the left atrium, but has less negative resting membrane potential due to a lower density of the inwardly rectifying K(+) current. Although electrophysiological and pharmacological characteristics of the pulmonary vein myocardium are compiled in various literatures, a comprehensive understanding of the spontaneous action potentials generated in the myocardial layer is yet to be assessed. Here, we integrated electrophysiological properties of the pulmonary vein myocardial layer on the basis of the Kyoto model in order to simulate effect of changes in individual ionic systems attributed to various risk factors for atrial fibrillation. Based on the preceding research which reported that approximately half of the isolated pulmonary vein myocardial layer exhibited spontaneous action potential and the remaining half was quiescent, we constructed various combinations of the pulmonary vein myocardial models in order to represent the variation of the action potential tracings. Age is one of the well-known risk factor of atrial fibrillation. In order to assess the effect of age-related changes in individual ionic systems to generation of the action potentials, we expanded the combinations to represent the aged pulmonary vein myocardial layer on the basis of the transcriptome data from between young and aged myocardial tissues. As a result, we predicted that the spontaneous action potentials, including burst-like action potentials, are more likely to be observed in “aged” combination than “young” combinations. We further modified the model to represent late component of Na(+) current (I(NaL)) in order to predict antiarrythmic effect of ranolazine, a selective I(NaL) blocker, on the predicted spontaneous action potentials.

Development of a New Method for Combinatorial Drug Discovery using a Genome-scale Metabolic Model and Database

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Antibiotics are indispensable to modern medical care. Recently, it is a serious problem that bacteria become resistant to multiple drugs by the overuse of antibiotics. Most of the current drug discovery approaches are finding out critical target and creating effective compounds to inhibit its functionality. However, the conventional approaches are facing the limit of defense against multiple drug resistant bacteria because the number of significant targets for drugs is quite limited. Therefore, in contrast to the conventional way, combinatorial drug discovery approach increases in importance. In the combinatorial drug discovery approach, it is quite important to find out effective combinations of available drugs and specific resistant cells based on the cellular network. Further, according to the development of various high throughput measurement techniques and accumulation of omics data, the technology for cellular modeling and simulation has progressed dramatically too. Many large-scale models of bacteria have been published, such as the whole cell model of Mycoplasma genitalium (Karr et al., 2012) and the genome-scale model of Escherichia coli (E. coli) metabolic pathway named iJO1366 (Orth et al., 2011). iJO1366 includes 1,366 metabolic genes out of all 4,289 genes in E. coli, and it can reproduce single knockout phenotypes observed experimentally with more than 90% accuracy. Prediction by such genome-scale models is indispensable to understand the systemic behavior of large-scale cellular networks and to develop new drug combinations. In this study, we developed a new analysis method, named the "combinatorial knock-down", to suggest the effective combinations of drug targets by using the genome-scale metabolic model and detailed drug database. In the combinational knock-down method, the limit of multiple fluxes in the metabolic pathway is decreased relative to the wild-type fluxes, and the synthetic effects on growth rate are indexed. This method was developed by modifying a module in COBRApy (The COnstraints Based Reconstruction and Analysis for Python, Ebrahim et al., 2013). COBRApy is the tool for Flux Balance Analysis (FBA) that evaluates biomass synthesis as growth rate and enables one to analyze the iJO1366 model. Compared to the knock-out analysis, our knock-down method can reveal the synergetic effect between the pair of targets (genes or reactions) in metabolic pathways. As a result, we found 150 sets of gene pairs indicating synergy. In addition, we acquired the data of approved drugs by the detailed drug database, DrugBank (http://www.drugbank.ca/), and suggested synergetic pairs related to these drug targets by the double knock-down analysis, though the model was incomplete for drug discovery and knowledge for drug target and action

mechanisms on the database were insufficient. The application of comprehensive simulation and metabolic engineering will be one of the major techniques to discover novel drug combinations efficiently.

Modeling a Comprehensive DNA Repair Signaling Network from ACSN Collection Predicts Genotoxic Drug Sensitivity from Cancer Cell Lines and Patient Data

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Studying reciprocal regulations between cancer-related pathways is essential for understanding signaling rewiring during cancer evolution and in response to treatments. With this aim we have constructed the Atlas of Cancer Signaling Network (ACSN, http://acsn.curie.fr), a resource of cancer signaling maps and tools with interactive web-based environment for navigation, curation and data visualisation (http://navicell.curie.fr). The content of ACSN is represented as a seamless 'geographic-like' map browsable using the Google Maps engine and semantic zooming. The associated blog provides a forum for commenting and curating the ACSN maps content. The atlas contains multiple crosstalk and regulatory circuits between molecular processes implicated in cancer. Genomic instability is one of the cancer hallmarks and the DNA repair machinery represents a promising reservoir of therapeutic targets. Perturbations in checkpoints and DNA repair in cancer are exploited in genotoxic treatments that are not always efficient due to backup mechanisms in DNA repair signaling machinery. A comprehensive map of cell cycle and DNA repair signaling network, from the ACSN collection, has been used for deciphering genetic interactions of drug resistance. This map is composed of three interconnected core cell cycle, DNA repair and checkpoints layers covering the most recent knowledge on molecular mechanisms implicated in these processes. To overcome the resistance to drugs we looked for synthetically interacting combinations of genes on the comprehensive map of cell cycle and DNA repair signaling network by: (1) deriving a state transition graph from the map and retrieving all paths from a damaged DNA to the repaired DNA state; (2) applying an algorithm for searching the minimal cut sets to model the effect of invalidating a set of genes involved in regulating DNA repair on the overall efficiency of DNA repair machinery; (3) considering genes that regulate each state transition as potential target for interference. Using this approach we have retrieved all possible gene sets whose knock-out halts DNA repair. Integrating expression and mutation data from drug resistant breast cancer cell lines and patients resistant to genotoxic treatment allowed prioritizing synthetically lethal gene sets and predicting specific intervention set for restoring sensitivity to drugs in each individual drug resistant cell line or patient. Our approach using the comprehensive DNA repair map reconstruction and cancer patient or cell lines data, is relevant for complementing genotoxic chemotherapy by targeting specifically cancer cells harboring certain DNA repair defects. This approach provides opportunity

to develop personalized cancer treatment strategies and improve patient stratification considering in the future the extended cancer signaling represented in the ACSN resource.

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Modelling and Simulation of Interaction between Murine Gut Microbiota and Host Immune System

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The mammals including humans are exposed to various microbes immediately after birth, which gradually form the complex gut microbial community. The gut microbiota is well known as an important factor for maintenance of various systems including the immune system, but the process of forming gut microbiota is barely understood. Here, we orally administrated Escherichia coli (E. coli) and Bifidobacterium longum (B. longum) to the gut of germ-free (GF) BALB/c mice as a minimum microbial ecosystem; we aimed to examine and simulate the interaction among E. coli, B. longum, and host immune response via production of intestinal immunoglobulin A (IgA). Intestinal IgA covers mucosal surfaces for protection against pathogens that contact the mucous membranes and restrains microbial growth and colonization to the gut of mammals. We mathematically modeled host-microbe interactions in order to clarify the mechanisms underlying the process of forming microbial ecosystem under the host immune system. We measured populations of two bacteria and quantity of IgA in the murine feces. On the basis of the experimental data, we constructed a mathematical model based on exponential growth model of microbes and generalized Lotka-Volterra equation, and estimated the parameters using genetic algorithm. In order to model the host immune system in response to gut microbes, both T-cell dependent and independent pathways of IgA secretion were considered. Subsequently, we simulated the process of forming gut microbial community through the interaction between IgA and microbes. Furthermore, we determined the parameters that represent differences in various characteristics of E. coli and B. longum with common set of mathematical equations. The model well reproduced the dynamic changes in intestinal IgA production, and the populations of E. coli and B. longum. The simulation of the host-microbe interaction enabled us to quantitatively describe the contributions of various parameters to formation of gut microbiota and interaction among E. coli, B. longum, and intestinal IgA.

Modeling and Simulation of Developmental Changes in Contractile Apparatus of Ventricular Cells

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The heart develops and acquires new functions, all while continuously pumping blood. Meanwhile, heart abnormalities that develop early in this process progress to congenital heart malformations. Accordingly, the developmental program of the heart, including expression of genes encoding ion channels, is likely to be tightly regulated. We previously modeled developmental changes in action potentials (AP) in rodent ventricular cells by integration of quantitative changes in ionic components of the cellular membrane and sarcoplasmic reticulum (SR) throughout the course of development using the Kyoto model, a comprehensive model of guinea pig ventricular cells (Kuzumoto et al., 2008). We then incorporated a glycolysis model (Lambeth and Kushmerick., 2001) to represent developmental changes in contribution of glycolysis and mitochondrial oxidative phosphorylation to ATP production in ventricular cells. Here, we further modified the model to represent developmental changes in contractile apparatus of ventricular cells. During the course of development, the ventricular cells utilize different isoforms of both myosin heavy chain and troponinI; the differences in isoforms affect Ca(2+) sensitivity, ATPase activity, and velocity of contraction. In order to consider the differences in isoforms, we integrated a new contraction model (Niederer et al., 2006) to the Kyoto model; briefly, the original contraction model (Negroni and Lascano., 1996) in the Kyoto model assumed 4 states of troponin C that represent cross-bridge dynamics; on the other hand, Niederer et al., (2006) considered tropomyosin which inhibits formation of cross-bridge by actin and myosin filaments. We varied the level of Ca(2+) sensitivity in order to obtain similar traces for contractile force between the original Kyoto model and the modified model. We also modified the new contraction model to consider ATP consumption by myosin-ATPase in order to simulate the changes in ATPase activity caused by the difference in MHC isoforms. The modified model enabled us to compare the contribution of developmental changes in the ATP consumption via contraction to excitation contraction coupling, which are regulated differently between fetal and adult guinea pigs.

Prediction of Changes in Electrophysiological Properties of Human Embryonic Stem Cell Derived Cardiomyocytes Attributed to Coupling with Fibroblasts

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Regenerative medicine is a novel therapy for heart critical damage. And the methods towards clinical application have been confirmed (Limor Zwi et al. 2009, K. Shimoji et al. 2010, H. Uosaki et al. 2011). In particular, tissue engineering approach, constructing a cardiac tissue in vitro, showed much therapeutic efficacy (M. Kawamura et al. 2013). And It is suggested that appropriate intercellular networks and extracellular matrix (ECM) created by cardiac fibroblasts are important for cardiac regeneration (K. Matsuura et al. 2012, T. Iwamiya et al. 2014.).

The computer simulation is a powerful approach for confirming experimental data as well as providing new insights that could not be obtained from experiment by changing various parameters in the mathematical models. Jonsson et al., (2012), for example, combined molecular biology and computer simulations to demonstrate that human embryonic stem cell-derived cardiomyocytes (hESC-CMs) have an immature electrophysiological phenotype, based on insufficient function of inward rectifier K+ current (IK1) channels and a shift in the activation of sodium channels. In addition, Paci et al., (2012) integrated electrophysiological recordings of various ionic systems in hESC-CMs on the basis of a human ventricular cell model (Ten Tusscher et al., 2004) to reproduce action potential recordings of the hESC-CMs at 15–40 days of differentiation (“early” stage) and those at 50–110 days of differentiation (“late” stage); the constructed model was further modified by electrical coupling with fibroblasts. Here, we utilized the models developed by Paci et al., (2012) for simulation of increasing number of fibroblasts electrically coupled with myocardial cells, in both “early” and “late” stages. We also simulated the influence of changing current densities of various K(+) channels on fibroblast membrane to hESC-CMs. As a result of simulations, we demonstrated that both the number of fibroblasts and current densities of various K(+) channels on fibroblast membrane affect electrophysiological properties of hESC-CMs and the effects are different between early and late stages of differentiations.

Simulation of Changes in Ionic Mechanisms Underlying Contraction of Ventricular Cells During Embryonic Development

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The heart develops and gains new functions while continuously pumping blood, and heart abnormalities progress to congenital heart malformations; therefore, the developmental program of the heart, including the expression of the genes responsible for various ionic channels, is likely to be tightly regulated. The quantitative changes in ionic channels, pumps, exchangers, and sarcoplasmic reticulum Ca2+ kinetics are responsible for the changes in electrophysiological properties of the developing cardiomyocytes. Previously, we demonstrated that the developmental changes in action potentials of ventricular myocytes were well represented, as Na+ current (INa) increased before the disappearance of and funny current (If), followed by a 10-fold increase in inward rectifier K+ current(IK1) via simulation (Okubo et al., 2013). In the previous study, however, the relative current densities of the 9 components were switched between early embryonic (EE) and late embryonic (LE) values and all the components were assumed to change equally during the course of embryonic development. Here, we constructed a model that represents “middle” embryonic (ME) stage of guinea pig ventricular cell on the basis of experimental data in order to consider differences in increment of developmental changes of the 9 ionic component. We changed the relative current densities of the 9 ionic components by increment of 10% both from EE to ME stages and from ME to LE stages, and compared the simulated results with the simulation in which the relative current densities were shifted equally from EE to LE stages without assuming ME stage. As a result, we determined that the balance of INa and IK1 densities are important for spontaneous firing of action potentials when all components are shifted from EE to ME stages. In addition, we demonstrated that 80% decrease in If density by ME stage was necessary in order to evoke action potential by various amplitudes of external stimulation and maintain proper excitation-contraction coupling during ME to LE stages.

Comparative Characterization of Human Dental Pulp Stem Cells between Different ages

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Human dental pulp stem cells (DPSCs), mostly found in the cell-rich zone of the pulp, belong to mesenchymal stem cells (MSCs). Because of their potent self-renewal and multilineage differentiation capacities, DPSCs were thought to be a promising candidate for tissue regeneration and wound healing. In this study, DPSCs were isolated from pulps of different ages including child (7- to 9-year-old; SHED), youth (20- to 29-year-old; DPSC-Y), and elder (60- to 65-year-old; DPSC-E). By using flow cytometric analysis, all of these cells were CD73, CD90, and CD105 positive and CD14, CD34, and CD45 negative. The cell size and intracellular complexity among these cells were not significantly different. The attached cells were fibroblast-like morphology, however, DPSCs from elder were quantified more “round” than cells from child or youth. A major difference among these cells was found in the assessment of proliferation rate. The growth rates were: SHED > DPSC-Y > DPSC-E. It was noted that the growth of DPSC-E was nearly ceased, and the stop growing of the cells was identified as the G2-M arrest by cell cycle analysis. In addition, the chondrogenic potentials of these cells were assessed, and all had significant expression of glycosaminoglycan under chondrogenic induction. In conclusion, the stemnesses among DPSCs form different disparity age were quite different, and SHED showed the most potential for cell-based therapeutic applications.

Dynamic Proteome Analysis of Cisplatin-Resistant Cancer Cells

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Drug resistance is a major setback during cancer treatment. Previous studies have identified several proteins and certain cellular functions, such as drug transport, DNA repair, anti-apoptosis and drug metabolism that contribute to cancer drug resistance. Nevertheless, detailed understanding of resistance mechanisms remains poorly understood. To uncover global resistance mechanisms, from the standpoint of intracellular protein aberrations and the corresponding network dysfunction, dynamical analysis of highthroughput proteomic data of resistant cancer cells is important. Here, we investigated the temporal proteome and phosphoproteome response of cisplatin - sensitive (A2780) and –resistant (A2780cis) ovarian cancer cells using liquid chromatography tandem mass spectrometry (LC-MS/MS) at several time points (0, 1, 3, 5, 10, 30, 60 min, 3, 24, 48 h) after cisplatin treatment. Using statistical pathway analysis (Metacore), we identified differentially activated proteins and their biological functions. Our results showed phosphorylated proteins involved in DNA-damage response and apoptosis pathways were differentially expressed between A2780 and A2780cis. As these differences may likely contribute to cisplatin resistance, we screened for novel candidates of resistance proteins that are specifically upregulated in cisplatin-resistant A2780cis cells, and are currently investigating the different temporal profiles using statistical clustering approaches. Subsequently, we plan to investigate the expression of candidate proteins using western blotting and test their effect on cisplatin resistance through genetic knock-down approaches. Overall, from a global perspective, we hope to shed light into novel intracellular protein aberrations leading to drug resistance.

DNA-neutral Liposomes-Mg^{2+} Complexes: Role in Nuclear Pore Assembly and Gene Expression

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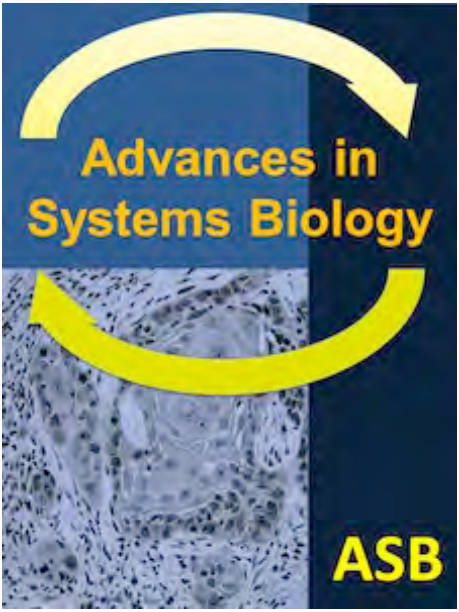
The ternary complexes (TC): DNA- phosphatidylcholine (PC) liposomes- divalent metal cations unlike lipoplexes are only lately has received attention. We proposed their involvement in the nuclear pore assembly. The formation of TC accompanied by the aggregation and fusion of PC liposomes was shown by freeze-etching and cryo- TEM technique.

At the same time DNA double helix unwinds in the region of liposomes fusion that enhances initiation of DNA transcription. Membrane vesicles forming the nuclear pores in a cell are analog of PC liposomes. In our last nuclear pore model TC arises in the chromatin areas with three-stranded hybrids: DNA – small nuclear RNA (snRNA) at their interactions with two small membrane vesicles (~70 nm in diameter). The thermo stability of DNA/snRNA triple helix is considerably lower than the same sequence of double-stranded DNA. That specifies preferential attachment of three-stranded hybrids to membrane vesicles. The triple helical hybrid unwinding during fusion of two membrane vesicles results in pre-pore formation: double-stranded DNA/snRNA hybrid and a single-stranded DNA (ssDNA) located on the outer diameter of fused “big vesicle”. This vesicle during interaction with double nuclear membrane can form channel between membranes. During this fusion ssDNA and hybrid DNA/snRNA shifts to pore annulus center. The ssDNA in pore annulus is the reason for the enhanced transcriptional activity of the genes neighboring nuclear pore. The number of pores in a nucleus specifies chromosome territory and number of chromosome loops.

Thereby snRNA (U1-, U2- types), causing chromatin attachment to a membrane influences on gene expression, so on a cell differentiation. In fast dividing embryonic cells the greatest number of active genes is expressed, nucleus content maximum number of pores. During differentiation number of active genes and pores in a nucleus are decreased. Besides snRNA, got to dividing embryonic cell from initial egg the set of snRNAs is defined by their synthesis de novo, and also by snRNA transferred to this cell from neighboring cells. Thus, transfer of snRNAs between cells and information about actively working genes promotes cells survival, but may cause of cancer. The ssDNA located along the outer diameter of “big vesicles” serve as sites of transcription initiation and their aggregates can be considered as transcription factories. Aggregates of “big vesicles” from different chromosomes can be responsible for “chromosomes kissing” effect.

We confirmed existing of pore clusters on molecular level as tandem repeats of “pore DNA” isolated by us from HeLa cells not long ago.



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cBio 2015

The cBio Symposium brings together scientists with multidisciplinary interests in biology, from budding young scientists to well established leaders. It was founded at the Institute for Advanced Biosciences, Keio University in 2013. The 2nd Symposium has grown to include a bigger and more international program committee and invited speakers. The participants of the event are encouraged to form deep scientific collaborations and friendships, not only across their diverse research fields, but also surpassing global borders.

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