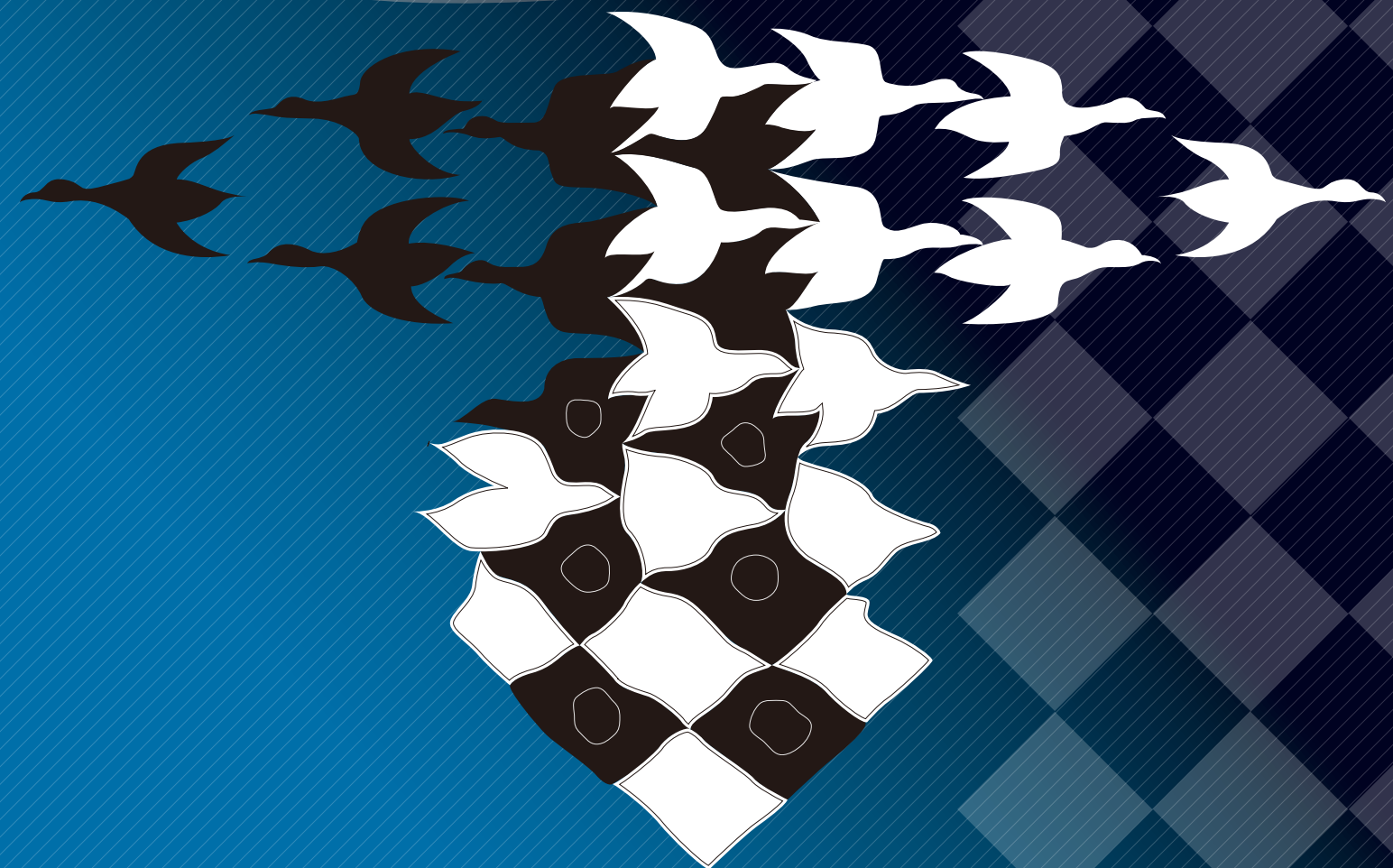


EMERGING APPROACHES and APPLICATIONS in DEVELOPMENTAL BIOLOGY Taking the Next Step

June 24, 2010 13:00
The Clock Tower Centennial Hall, Kyoto University



Speakers:

Eric Wieschaus (*Princeton Univ*)

Roel Nusse (*Stanford Univ*)

Tadashi Uemura (*Kyoto Univ*)

Ryoichiro Kageyama (*Kyoto Univ*)

Norio Nakatsuji (*Director, iCeMS*)

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Message from the Director

On behalf of all my colleagues at Kyoto University's Institute for Integrated Cell-Material Sciences, iCeMS for short, I welcome you to the Seventh iCeMS International Symposium on "Emerging Approaches and Applications in Developmental Biology”.

The iCeMS is founded as a response to the Japanese government initiative of World Premier International Research Centers (WPI Program). The initiative is meant to establish globally visible research centers here in Japan, which will attract top-notch researchers from around the globe, particularly talented young scientists -- ones expected to become world's leading investigators in the future. The proposal to establish the iCeMS was funded as one of the five such centers throughout Japan. The iCeMS places a strong emphasis on international collaborations, and the iCeMS international symposium, to be held in a series, is one of our major means to develop our ties with international scientific communities.

We at the iCeMS strive to develop the fundamental understanding and control of molecular complexes in the meso-scale of 5-100 nm (meso-control), as the cell appeared to develop them through evolution. They are also very important to design and create various novel “smart materials” by understanding the molecular events in such materials. We consider these efforts critical for creating the science and technology of the next generation. For this purpose, we make cross-disciplinary approaches to create new sciences focusing on 1) meso-control of functional architectures and 2) meso-control of stem cell systems.

I hope that this symposium will provide a unique opportunity for researchers from various fields to meet and develop closer relationships, exchanging their expertise and new ideas to push back the frontiers of sciences.

Thank you very much again for joining us at the Seventh iCeMS International Symposium. I hope you will enjoy this meeting.

Norio Nakatsuji, D.Sc.

Director and Professor

Institute for Integrated Cell-Material Sciences (iCeMS) Kyoto University

Welcome to the Seventh iCeMS International Symposium
“Emerging Approaches and Applications in Developmental
Biology: Taking the Next Step”

June 24, 2010
Clock Tower Centennial Hall
Kyoto University, Kyoto

It is my pleasure to welcome you to the Seventh iCeMS International Symposium.

The past several decades have witnessed an explosion in the discovery of molecules involved in specific developmental processes. *Drosophila* has been critical in the discovery of these genes, most of which are now known to be fundamental in the development of other organisms including vertebrates. In recent years, many have striven to create a holistic view of how these genes and proteins work as a system and together make up complete organisms. These approaches have been enabled by advancing technologies to decompose and modularize high-resolution biological data. On the other hand, translational approaches are also progressing, aiding in the manipulation of developmental processes and aiming toward the diagnosis, prevention and cure of genetic and developmental disorders.

This symposium will present an overview of recent challenges faced by developmental biology with a central emphasis on technologies and applications. The program includes six distinguished presentations covering broad areas from *Drosophila* genetics to human disease mechanisms. In particular, we are especially honored to feature keynote lectures by Drs. Eric Wieschaus and Roel Nusse.

We fully expect that this symposium will provide everyone with key opportunities to discuss new discoveries as well as ideas for future cutting-edge research in the field.

Mineko Kengaku, Ph.D.
Organizer of the symposium
Associate Professor
Institute for Integrated Cell-Materials Sciences (iCeMS)
Kyoto University

Program

13:00-13:10	Opening Norio Nakatsuji (iCeMS, Kyoto University)
Session 1 13:10-13:40	Chair: Mineko Kengaku Norio Nakatsuji Human pluripotent stem cell lines as valuable tools for research and application
13:40-14:20	Roel Nusse (Stanford University) Wnt signals during stem cell self-renewal and tissue repair
14:20-14:40	coffee break
Session 2 14:40-15:20	Chair: Takashi Hiiragi Akihiro Kusumi (iCeMS, Kyoto University) Meso-scale membrane domains as studied by single-molecule tracking
15:20-16:00	Tadashi Uemura (Kyoto University) Linking global tissue asymmetry to cell polarity on the plane
16:00-16:20	coffee break
Session 3 16:20-17:00	Chair: Akihiro Kusumi Ryoichiro Kageyama (Kyoto University) Uladian oscillations in somite segmentation and other biological events
17:00-17:40	Eric Wieschaus (Princeton University) The mechanics of shape change in the Drosophila embryo
17:40	Closing

Introduction of iCeMS, and Human Pluripotent Stem Cell Lines as Valuable Tools for Research and Application

Norio Nakatsuji

*Professor and Director, Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto
University, Kyoto 606-8507, Japan.*

Our iCeMS was founded in 2007 as one of five research centers selected by Japan's education and science ministry (MEXT) under its World Premier International Research Center (WPI) Initiative. It aims to advance the integration of cell and material sciences by creating a uniquely innovative global research environment. The iCeMS seeks to integrate the biosciences, chemistry, material science, and physics to capture the potential power of **meso-control** of **functional architectures** and **stem cells**. Between the nano-scale (the realm of atoms and simple molecules) and the micron and larger scales lies the **meso-scale world**: between 5 and 100 nanometers dominated by interactions among large molecules within cells and cell systems. Understanding these processes and ultimately learning to control or even mimic them should lead to breakthroughs in medicine, drug delivery, and novel chemical technologies.

Our research group has been carrying out various aspects of basic and application research using pluripotent stem cells such as human embryonic stem cells. For example, our group has been creating normal and disease model cells for disease mechanism research and drug discovery tools. Also, we are still the only group in Japan to have derived and distributed human ES cell lines to many biomedical researchers. At iCeMS, we are focusing on the following multidisciplinary research projects using ES and iPS cell lines by collaboration with other research groups of iCeMS.

- 1) Creation and analysis of model cells for biomedical research, such as neurodegenerative disease model cells produced by genetic modification of stem cell lines and differentiation into relevant cells in each disease.
- 2) Control of stem cells by screening of synthetic small molecules to control differentiation of ES/iPS cells.
- 3) Development of synthetic artificial transcription factors to regulate important genes for pluripotency and differentiation into specific cell lineages.

Wnt signals during stem cell self-renewal and tissue repair

Roel Nusse

Department of Developmental Biology and Howard Hughes Medical Institute, Stanford University, School of Medicine, Stanford CA 94305, USA

In many contexts, the self-renewal and differentiation of stem cells is influenced by signals from their environment, constituting a niche. It is postulated that stem cells compete for local growth factors in the niche, thereby maintaining a balance between the numbers of self-renewing and differentiated cells. A critical aspect of the niche model for stem cell regulation is that the availability of self-renewing factors is limited and that stem cells compete for these factors. Consequently, the expression, range and concentrations of the niche factors are critical importance.

We study the role of Wnt signaling and stem cells by purifying active Wnt proteins and applying them to cells and tissues, thereby being able to assess the consequences of Wnt signaling in a direct way. Wnt proteins are modified by lipid attachment, to make them hydrophobic in nature. We found that isolated Wnt proteins are active on a variety of stem cells, including neural, mammary and embryonic stem cells. In general, Wnt proteins act to maintain the undifferentiated state of stem cells, while other growth factors instruct the cells to proliferate. The combination of Wnts and those factors has allowed stem cells to clonally expand and propagate in an undifferentiated state for multiple passages. By subsequent in vivo transplantation, we find that the cells have retained their developmental potential.

During the diffusion and transport of the lipid-modified Wnt proteins between cells, the interaction with a novel Wnt binding protein, called SWIM, may be essential in maintaining the solubility of Wnt, at least in *Drosophila*.

Meso-Scale Membrane Domains As Studied by Single-Molecule Tracking

Akihiro Kusumi

*Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University,
Kyoto 606-8507, Japan.*

Techniques that allow researchers to track and manipulate single molecules in living cells are revolutionizing our understanding of the plasma membrane dynamics, structure, and signal transduction functions. In the plasma membrane, single-molecule tracking approaches have revealed three types of meso-scale membrane domains that are critically important for many functions.

[1] 30-200 nm compartments made by the partitioning the entire plasma membrane by the membrane-associated actin-based meshwork (membrane skeleton) and its associated transmembrane proteins.

[2] Protein oligomers of various sizes and lifetimes.

[3] Meta-stable 1-5 nm raft domains that can be turned into stable ~10-nm domains by ligand-induced clustering of glycosylphosphatidylinositol (GPI)-anchored receptor (as pointed out in the item [2]), followed by the assembly of raft-associated molecules (receptor-cluster rafts). Intracellular lipid-anchored signaling molecules, such as G α i2, Lyn, and PLC, were then transiently recruited, via both protein-protein and lipid-lipid interactions, leading to enhanced interactions among them, eventually resulting in large intracellular responses such as IP $_3$ -calcium signaling. One of the critical observations made here was that the recruited periods for these signaling molecules are generally short (~0.1 s).

In this presentation, I will first talk about [1], and then [3], emphasizing short periods of recruitment/interaction of intracellular signaling molecules, using the signaling of CD59, a GPI-anchored receptor, as an important paradigm. Transient formation of signaling complexes would give rise to digital, pulse-like signals, implying that long, analog-like cellular signals are generated by simple summation of pulse-like signals of individual molecules rather than the complex integration of the long-term (e.g. a minute) signals of individual molecules. Such a method for generating signals is advantageous from systems engineering point of view. First, simple summation is much easier than complex integration. Second, because the default state for individual molecules is always off and the molecule is automatically turned off by thermal fluctuation (signaling complexes disintegrate), the runaway of the signal is less likely to occur. Third, like any digital circuit, it is less prone to the problem of thermal noise.

It might sound paradoxical, but single-molecule tracking will become a valuable tool for systems biology of cellular signaling.

Linking global tissue asymmetry to cell polarity on the plane

Tadashi Uemura

Graduate School of Biostudies, Kyoto University; CREST, JST

Cells sense global axes of the tissue to which they belong and manifest polarity for specialized functions. One such example is planar cell polarity (PCP), which is seen in many animals and tissues such as some epithelia that develop unidirectionally beating cilia. To date, underlying mechanisms of PCP have been best studied in the *Drosophila* wing, where epidermal cells somehow sense the cue along the proximal-distal (P-D) axis, localize an assembly of actin filaments at the distal cell vertex, and produce single wing hairs. The pertinent molecular players have been classified into at least the 2 following categories: The first group includes atypical cadherins Dachsoous and Fat that are thought to contribute to the tissue patterning information across the axis. Second, members of the “core group,” including Frizzled and the seven-pass transmembrane cadherin Flamingo, assemble into asymmetric complexes that straddle the proximodistal junctions between adjacent cells; and they specify the intracellular location of the wing hair formation.

Unsolved questions include how the above 2 categories of regulators are functionally related to each other and why Frizzled is relocalized at distal cell borders in the first place. We previously proposed that cellular mechanisms underlying this relocalization include polarized transport of Frizzled-containing vesicles along P-D-oriented non-centrosomal microtubules (MTs). We have been analyzing dynamics of the MTs and movements of the vesicles to elucidate 2 critical questions: First, how do the MTs become aligned along the P-D axis? Second, why are the Frizzled vesicles transported distally? Our quantitative *in vivo* imaging has shown that Dachsoous and Fat control alignment and asymmetry of the MT growth, and it has also revealed statistical properties of the vesicle movements, which will give us insight into the asymmetric relocalization of the core group.

Ultradian Oscillations in Somite Segmentation and Other Biological Events

Ryoichiro Kageyama

Institute for Virus Research, Kyoto University; CREST, Japan

In mouse embryos, somite formation occurs every two hours by segmentation of the anterior ends of the presomitic mesoderm. This periodic event is regulated by a biological clock called the segmentation clock, which involves cyclic expression of the basic helix-loop-helix gene *Hes7*. Both loss of expression and sustained expression of *Hes7* result in severe somite fusion, suggesting that *Hes7* oscillation is required for proper somite segmentation. *Hes7* oscillation is regulated by negative feedback, and this process has been mathematically simulated by differential-delay equations, which predict that this dynamic expression depends on both rapid degradation of *Hes7* gene products and substantial transcriptional and translational delays. We experimentally evaluated these predictions and showed that both the stabilization of *Hes7* protein and shortening of the delays abolished *Hes7* oscillations, thus proving the validity of the mathematical modeling.

A related gene, *Hes1*, plays an important role in maintenance of neural stem cells. *Hes1* expression oscillates with a period of about two hours in neural stem cells, and persistent *Hes1* expression inhibits proliferation and differentiation of these cells, suggesting that *Hes1* oscillation is required for their proper activities. We further found that the proneural gene *Neurogenin2* (*Ngn2*) and the Notch ligand *Delta-like1* (*Dll1*) are expressed in an oscillatory manner by neural stem cells but persistently by *Hes1*-negative differentiating neurons. These results suggest that *Hes1* oscillation regulates *Ngn2* and *Dll1* oscillations, which in turn lead to maintenance of neural stem cells by mutual activation of Notch signaling.

We also found that *Hes1* expression oscillates in embryonic stem (ES) cells. Those expressing low and high levels of *Hes1* tend to differentiate into neural and mesodermal cells, respectively. Furthermore, inactivation of *Hes1* facilitates neural differentiation more uniformly at earlier time, indicating that *Hes1*-null ES cells display less heterogeneity in both the differentiation timing and fate choice. Thus, it is likely that the cyclic gene *Hes1* contributes to pluripotent responses of ES cells. Taken together, these results suggest that oscillatory expression with short periods (ultradian oscillation) is important for many biological events.

The mechanics of shape change in the *Drosophila* embryo

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With the first three hours of development, the *Drosophila* embryo establishes a precise pattern of transcription factors that divides the blastoderm into groups of cells destined to form different organs and tissues in the adult. Along the dorsal ventral axis, the first and perhaps most important of these cell fate decisions is the establishment of mesoderm controlled by expression of the Twist and Snail transcription factors. The immediate response of these cell fate decisions is the formation of the ventral furrow and involves re-organization of the cytoskeleton, adhesion and motor activities to achieve distinct shape.

In my talk I will discuss the relationship between the initial transcription profiles and a novel pulsating reorganization of the Actin/Myosin cytoskeleton in the apical region of cells that will make the ventral furrow. We show that the resultant contractile pulses drive cell shape changes in the entire mesodermal primordium. The individual contractions appear to be unpolarized but they result in polarized wedge-like constrictions because global tension in the sheet is polarized along the AP axis. We analyze the force distributions in the mesodermal primordia using a combination of genetics and RNAi to lower adhesive strengths between cells, and laser dissections to locally disrupt the cytoskeleton.