The 10th Global COE International Symposium
7th Young Investigators Forum
on
Biochemistry and Cell Biology

Organized by

National University of Singapore
Cancer Science Institute of Singapore

and

Kyushu University
Global COE Program

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December 22 (Thr), 2011

The 10th Global COE International Symposium

CeLS Auditorium @ National University of Singapore

8:30- Registration

9:00-
Welcome Address

Yoshiaki Ito
Cancer Science Institute of Singapore, National University of Singapore

Opening Remarks

Yukio Fujiki
Leader of Global COE Program, Kyushu University

9:20-10:50
Session 1: Cell Sensing
Chair: Yasuhiro Sawada (*DBS, NUS*)

S1-1:Keynote lecture:9:20-10:00
Michael Sheetz ***Mechanobiology Institute and Department of Biological Sciences, National University of Singapore***
Cellular mechanosensing of the microenvironment by actin-dependent stretch-relaxation cycles.

S1-2:10:00-10:25
Sho Yamasaki ***Medical Institute of Bioregulation, Kyushu University***
Sensing “danger” through C-type lectin receptor Mincle.

S1-3:10:25-10:50
Lorenz Poellinger ***Cancer Science Institute of Singapore, National University of Singapore***
Hypoxia and carcinogenesis.

10:50-11:10
Coffee and Tea Break @ CeLS Lobby

11:10-12:50
Session 2: Structural Biology
Chair: Daisuke Kohda (*Kyushu University*)

S2-1:11:10-11:35
Chu-Young Kim ***Department of Biological Sciences, National University of Singapore***
Enzymatic catalysis of anti-Baldwin ring-closure reaction in polyether biosynthesis.

S2-2: 11:35-12:00
Kenji Inaba ***Medical Institute of Bioregulation, Kyushu University***
Structural and mechanistic basis of protein disulfide bond formation in human cells.

S2-3:12:00-12:25
J. Sivaraman ***Department of Biological Sciences, National University of Singapore***
Structure of 8C11 Fab in complexed with Hepatitis E Virus (HEV) E2s domain for understanding the neutralization of HEV.

S2-4:12:25-12:50
Daisuke Kohda ***Medical Institute of Bioregulation, Kyushu University***
Crystallographic and NMR evidence for flexibility in the C-terminal globular domain of oligosaccharyltransferases and its catalytic significance.

12:50-13:50

Lunch @ CeLS Lobby

13:50-14:40
Session 3: Cell Biology on Membrane and Organelle
Chair: **Min Wu** (*DBS, NUS*)

S3-1:13:50-14:15
Go Totsukawa ***Graduate School of Medical Sciences, Kyushu University***
VCIP135 deubiquitinase and its binding protein, WAC, in p97 ATPase-mediated membrane fusion.

S3-2:14:15-14:40
Min Wu ***Department of Biological Sciences, National University of Singapore***
Understanding membrane recycling through reconstitution.

14:40-16:20
Session 4: Omics Biology
Chair: **Christopher Hogue** (*DBS, NUS*)

S4-1:14:40-15:05
Jayantha Gunaratne ***Institute of Molecular & Cell Biology, A*Star and Cancer Science Institute Singapore, National University of Singapore***
Understanding molecular networks and their deregulated hubs in human diseases using SILAC-based mass spectrometry.

S4-2:15:05-15:30
Yasuyuki Ohkawa ***Graduate School of Medical Sciences, Kyushu University***
Cell-fate decision by histone variants.

S4-3:15:30-15:55
Chng Wee-Joo ***Cancer Science Institute of Singapore, National University of Singapore***
System biology approach to dissect PRL-3 function in AML.

S4-4:15:55-16:20
Christopher Hogue ***Mechanobiology Institute and Department of Biological Sciences, National University of Singapore***
Towards a data resource of full-length protein structures.

16:20-16:40

Coffee and Tea Break @ CeLS Lobby

16:40-17:55
Session 5: Transcriptional regulation
Chair: Takehiko Yokomizo (*Kyushu University*)

S5-1:16:40-17:05
Lu Gan ***Department of Biological Sciences, National University of Singapore***
Digital dissection of mitotic picoplankton by electron tomography.

S5-2:17:05-17:30
Xin-Yuan Fu ***Cancer Science Institute of Singapore, National University of Singapore***
Created for control: Functions of STAT proteins in development and diseases.

17:30-
Closing Remarks

Yukio Fujiki
Leader of Global COE Program, Kyushu University

18:00

Bus departure for York Hotel

19:30-

Banquet @ Marie I & II, York Hotel (Invitation Only)

December 23 (Fri), 2011

7th Global COE Young Investigators Forum

CeLS Auditorium @ National University of Singapore

8:25- Explanation of the voting system for the young investigator award
8:30- Opening Remarks

Motomi Osato
Cancer Science Institute of Singapore, National University of Singapore

8:40-10:20 (9 min talk + 3 min discussion)
Session I: Cell fate determination & Signal transduction
Chair: Li Jiang (*Kyushu University*), Jeffrey Robens (*DBS, NUS*)

O1-1
Kohta Miyawaki ***Graduate School of Medical Sciences, Kyushu University***
Identification of the earliest branch point for myelo-erythroid development in adult hematopoiesis.

O1-2
Min Liu ***Graduate School of Medical Sciences, Kyushu University***
Crucial role of the 12-HHT receptor BLT2 in epidermal wound healing.

O1-3
Cheng-han Yu ***Mechanobiology Institute and Department of Biological Sciences, National University of Singapore***
Early integrin binding to fluid membrane RGD activates actin polymerization and contractile moveme nt that stimulates outward translocation.

O1-4
Hirokazu Nakatsumi ***Medical Institute of Bioregulation, Kyushu University***
mTOR regulates transcription through FOXK1 phosphorylation.

O1-5
Jeffrey Robens ***Mechanobiology Institute and Department of Biological Sciences, National University of Singapore***
Intracellular tension regulates bile canaliculi maturation in primary rat hepatocytes.

O1-6
Atsushi Shimada ***Medical Institute of Bioregulation, Kyushu University***
Structural basis of Cdc42-mediated Cdc42-interacting protein 4 (CIP4) recruitment to clathrin-coated pits.

O1-7
Yukio Fujiki ***Graduate School of Life Sciences, Kyushu University***
A Novel Function of AWP1/ZFAND6: Regulation of Pex5p Export by Interacting with Cys-monoubiquitinated Pex5p and AAA ATPase, Pex6p.

O1-8
Li Jiang ***Graduate School of Life Sciences, Kyushu University***
Mechanism of the interaction between Pex14p and LC3-II.

10:20-10:40

Coffee and Tea Break @ CeLS Lobby

10:40-12:35 (9 min talk + 3 min discussion)
Session II: Cancer biology & Immune system
Chair: Tomoaki Koga (*Kyushu University*), Jianbiao Zhou (*NUS*)

O2-1
Hay Hui Sin ***Cancer Science Institute of Singapore, National University of Singapore***
Sumoylation of NEMO and NF-κB activation by a RNA helicase, DP103 defines the metastatic potential of human breast cancers.

O2-2
Tomoharu Kanie ***Medical Institute of Bioregulation, Kyushu University***
Genetic reevaluation of the role of F-box proteins in cyclin D1 degradation.

O2-3
Jianbiao Zhou ***Cancer Science Institute of Singapore, National University of Singapore***
The Pro-metastasis Tyrosine Phosphatase, PRL-3 (PTP4A3), Is a Novel Target of BCR-ABL Signaling Involved in Human Chronic Myeloid Leukemia.

O2-4
Loo Ser Yue ***Cancer Science Institute of Singapore, National University of Singapore***
PPARg regulates tumor-specific repression of MnSOD expression in ER-independent breast cancer: A novel strategy for "oxidation therapy" in cancer.

O2-5
Giselle Sek Suan Nah ***Cancer Science Institute of Singapore, National University of Singapore***
Identification of disease-associated cis-regulatory elements in the Runx3 gene.

O2-6
Yusuke Sakai ***Medical Institute of Bioregulation, Kyushu University***
Regulation of NK cell-mediated cytotoxicity by the atypical Rac activators.

O2-7
Yosuke Harada ***Medical Institute of Bioregulation, Kyushu University***
Identification of a signaling molecule critical for dendritic cell migration in three-dimensional environments.

O2-8
Tomoaki Koga ***Graduate School of Medical Sciences, Kyushu University***
BLT1 as a potential marker of mouse dendritic cell subset.

O2-9
Takuji Yamauchi ***Graduate School of Medical Sciences, Kyushu University***
A new immunodeficient mouse model introduced with defined Sirpa polymorphism for human hematopoietic stem cell assay.

Lunch & Poster presentation @ CeLS Lobby

12:40-13:40
Poster presentation (Odd number)

13:40-14:40
Poster presentation (Even number)

14:40-14:50
Vote for young investigator award

15:10-15:30
Closing Remarks & Award

Yukio Fujiki
Leader of Global COE Program, Kyushu University

December 22 (Thu), 2011

The 10th Global COE International Symposium

Abstract

S1-1 Keynote lecture

Cellular mechanosensing of the microenvironment by actin-dependent stretch-relaxation cycles

○Michael Sheetz
Mechanobiology Institute and Department of Biological Sciences, National University of Singapore. Also, Department of Biological Sciences, Columbia University, U.S.A.

Control of cell growth, death or differentiation involves the integration of microenvironmental signals through cell motile processes to produce the desired cellular responses[1]. In the case of cell-matrix interactions that can control differentiation of stem cells, it seems that there are several different levels of mechanosensing from an early testing of matrix stability, rigidity sensing, adhesion growth-maturation and finally, querying of the adhesions. Recent studies with supported bilayers containing lipid-bound RGD ligands, show that diffusive ligand binding causes clustering of αvβ3 integrins that stimulates actin polymerization and contraction[2]. If contraction is to a barrier in the bilayer, then further actin assembly is stimulated until the cell spreading removes the folds in the membrane and activates contraction to sense rigidity[3]. The process of rigidity sensing involves pulling to a constant displacement of about 130 nm [4] in a local region of the cell [5]. Once a rigid surface is sensed, the cell will assemble a focal adhesion through a force-dependent process. In mature adhesions, the stretching of proteins can unveil binding sites such as the stretching of talin causing the increased binding of vinculin[6]. Recent findings show that talin is stretched by 200-300 nm and relaxed multiple times in vivo with a stochastic period of 6-16s[7]. If the active head of vinculin is expressed, then the stretching is increased to about 400 nm and the oscillations in length are damped. These findings indicate that it is not a single stretch but perhaps the integral of many stretches that defines the cellular response to mechanical aspects of the environment. Because the assembly of talin in adhesion complexes depends upon the clustering of integrins, rigidity of the surface matrix, and formation of a mature adhesion, the integrated signal from the microenvironment through stretch-relax cycles will be a complex function of both the chemical nature of the matrix, rigidity of the matrix and the level of cell motility.

(References)

1 Vogel, V. & Sheetz, M. Local force and geometry sensing regulate cell functions. ***Nat Rev Mol Cell Biol*** 7, 265-275 (2006).
2 Yu, C., Law, J., Suryana, M., Low, H. & Sheetz, M. P. Early integrin binding to RGD activates actin polymerization and contractile movement that stimulates outward translocation. ***Proc Natl Acad Sci U S A*** In Press (2011).
3 Gauthier, N. C., Fardin, M. A., Roca-Cusachs, P. & Sheetz, M. P. Temporary increase in plasma membrane tension coordinates the activation of exocytosis and contraction during cell spreading. ***Proc Natl Acad Sci U S A*** 108, 14467-14472, 1105845108 (2011).
4 Saez, A., Buguin, A., Silberzan, P. & Ladoux, B. Is the mechanical activity of epithelial cells controlled by deformations or forces? ***Biophys J*** 89, L52-54, 105.071217 (2005).
5 Ghassemi, S. *et al.* Cell Rigidity Sensing by Local Contractions. (Submitted).
6 del Rio, A. *et al.* Stretching single talin rod molecules activates vinculin binding. ***Science*** 323, 638-641, 1162912 (2009).
7 Margadant, F. *et al.* Mechanotransduction in Vivo by Repeated Talin Stretch-Relaxation Events Depends Upon Vinculin. ***PLoS Biol*** In Press (2011).

S1-2

Sensing “danger” through C-type lectin receptor Mincle

○Sho Yamasaki
Molecular Immunology, Medical Institute of Bioregulation, Kyushu University

Our body is continuously exposed to “danger” derived from both self and non-self, i.e. tissue damage or infection. Host immune receptors immediately recognize them to evoke appropriate immune responses for the maintenance of homeostasis. However, the receptors that sense DAMPs (damage-associated molecular patterns) and PAMPs (pathogen-associated molecular patterns) have not been fully understood. Mincle (Macrophage-inducible C-type lectin) is a stress-induced C-type lectin mainly expressed in myeloid cells. We found that Mincle is an Fc receptor γ chain (FcRγ)-coupled activating receptor that recognizes dead cells to induce inflammatory cytokines. Mincle also recognizes “non-self” such as mycobacteria. Among mycobacterial components, TDM (trehalose dimycolate; also called cord factor), a glycolipid known as strong adjuvant, was identified as a Mincle ligand. Thus, Mincle may function as a sensor for the “danger” derived from both damaged self (DAMPs) and invading non-self (PAMPs) to evoke innate/acquired immune responses. Constitutive expression of Mincle by transgene resulted in lethal disorder in mice, suggesting that excessive sensing of “danger” breaks homeostatic responses. The physiological advantages and potential risks through the recognition of “danger” will be discussed.

S1-3

Hypoxia and carcinogenesis

○Lorenz Poellinger

Cancer Science Institute of Singapore, National University of Singapore
Department of Cell and Molecular Biology, Karolinska Institute, Sweden

Prof Poellinger's laboratory investigates the mechanisms of regulation of gene expression and the epigenetic landscape in tumour cells in hypoxia (low oxygen concentration) which is one of the pathophysiological signatures of growing solid tumours. These mechanisms determine growth, the metabolic status, the differentiation status and metastasis of the tumours and it is their aim to target these molecular pathways in the development of novel cancer therapy.

S2-1

Enzymatic catalysis of anti-Baldwin ring-closure reaction in polyether biosynthesis

Hideaki Oikawa[1)], Kendall N. Houk[2)], ○Chu-Young Kim[3)]
[1)]*Division of Chemistry, Hokkaido University*
[2)]*Department of Chemistry and Biochemustry, University of California Los Angeles*
[3)]*Department of Biological Sciences, National University of Singapore*

Despite recent advances in the understanding of cyclic polyether natural product biosynthesis (1), the enzymological mechanism of how these stereochemically complex compounds are generated remains elusive. For example, it has been proposed by Nakanishi (2) that the formation of brevetoxin B requires the biosynthesis of an alkene intermediate in which 10 specific carbon–carbon double bonds out of a total of 14 must be formed in an all-*E* configuration and uniformly epoxidated to either all-*S,S* or all-*R,R* polyepoxide. Subsequently, a cascade of 10 epoxide-opening ring closure reactions, all of which are energetically disfavored anti-Baldwin-type *endo* reactions (3), must occur to form the final product with the correct *trans-syn-trans* stereoconfiguration. However, there is currently no structural information available of an enzyme capable of catalyzing such chemistry. Recently, we have determined the X-ray crystal structure of Lsd19, an epoxide hydrolase responsible for the biosynthesis of ionophore polyether lasalocid A (4,5), in complex with substrate and product analogs. This is the first atomic structure of a natural enzyme capable of catalyzing the disfavored epoxide-opening cyclic ether formation. The unique catalytic ability of Lsd19 was analyzed through a combination of crystal structure determination and computational study.

(References)
(1) Vilotijevic and Jamison, ***Angew. Chem. Int. Ed.*** (2009) 48, 5250-5281
(2) Nakanishi, ***Toxicon*** (1985) 23, 473-479
(3) Baldwin, ***J. Chem. Soc. Chem. Comm.***(1976) 734-736
(4) Shichijo Y. *et al.* ***J. Am. Chem. Soc.*** (2008) 130, 12230-12231
(5) Minami, A. *et al.* ***Org. Lett.***(2011) 13, 1638–1641

S2-2

Structural and mechanistic basis of protein disulfide bond formation in human cells

○Kenji Inaba
Division of Protein Chemistry, Medical Institute of Bioregulation, Kyushu University

In the endoplasmic reticulum (ER) of human cells, Ero1α and protein disulfide isomerase (PDI) constitute one of the major electron-flow pathways that catalyze oxidative folding of secretory proteins. Since Ero1 flavoenzymes generate a reactive oxygen species (hydrogen peroxide) as a byproduct during the catalysis, Ero1 activity must be strictly regulated to avoid the futile oxidizing cycle in this organelle. Recently, we have succeeded in crystal structure analysis of human Ero1α in its hyperactive and inactive forms. Our findings reveal that Ero1α modulates its oxidative activity by properly positioning regulatory cysteines within an intrinsically flexible loop, and by fine-tuning its electron shuttle ability through intra-loop disulfide rearrangements. The computational analysis of the Ero1α-PDI binary complex suggested that specific PDI targeting is guaranteed primarily by hydrophobic interactions between the hydrophobic pocket in the PDI β'-domain and the protruding β -hairpin of Ero1α. This complex model was further corroborated by our systematic mutational analyses. Altogether, these results demonstrate the molecular bases of the regulated and specific protein disulfide formation pathway in human cells.

S2-3

Structure of 8C11 Fab in complexed with Hepatitis E Virus (HEV) E2s domain for understanding the neutralization of HEV

○J. Sivaraman[1)], Tang X[1)], Yang C[2)], Gu Y[2)], Song C[2)], Zhang X[2)], Wang Y[2)], Zhang J[2)], Hew CL[1)], Li S[2)], Xia N[2)]
[1)]Department of Biological Sciences, National University of Singapore
[2)]Natioanl Institute of Diagnostics and Vaccine Development in Infectious Disease, School of Life Sciences, Xiamen University, China

Infectious hepatitis viral diseases are major health problems in both developing and developed countries. Parenteral and faeco-oral are two modes of transmission of hepatitis. Hepatitis E virus (HEV) is an important cause of severe hepatitis in humans and is responsible for unusually high rates of mortality in pregnant women by the development of fulminant liver disease. We have demonstrated that HEV capsid is made up of capsomeres consisting of homodimers of a single structural capsid protein (E2) forming a partially enclosed shell and determined its structure at 2Å resolution[1]. This domain protrudes from the viral surface and engages with host cells to initiate infection. Further we have determined the complex crystal structure of 8C11 Fab, a neutralizing antibody, with HEV E2s (I) domain at 1.9Å resolution. The 8C11 epitopes on E2s (I) were identified at Asp[496]-Thr[499], Val[510]-Leu[514] and Asn[573]-Arg[578]. The mutations and cell model assay identified Arg512 as the most crucial residue for 8C11 interaction and for the neutralization of HEV[2]. 8C11 specifically neutralizes HEV genotype I, but not the other genotypes. HEV type I and IV are the most abundant genotypes. To understand the specificity, the structure of E2s (IV) was determined at 1.79Å resolution and an E2s (IV) complex with 8C11 model was generated. The comparison between the 8C11 complexes of type I and IV revealed the key residues that distinguish these two genotypes. In particular the position 497 at the 8C11 epitope region of E2s has distinct amino acids in different genotypes. Swapping of this residue from one genotype to another showed that the 8C11 reactivity was inversed and demonstrated the essential role played by aa497 in the genotype recognition. These studies will lead to the development of antibody-based specific drugs for the treatment against HEV.

References

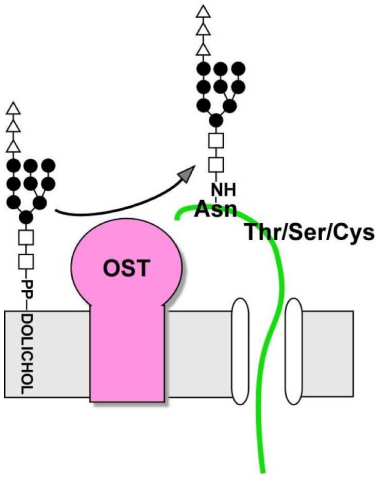
1. Dimerization of hepatitis E virus capsid protein E2s domain is essential for virus-host interaction. Li S, Tang X, Seetharaman J, Yang C, Gu Y, Zhang J, Du H, Shih JW, Hew CL, Sivaraman J and Xia N. ***PLoS Pathogens***. (2009) 5, e1000537.

2. Structural basis for the neutralization and genotype specificity of hepatitis E virus. Tang X, Yang C, Gu Y, Song C, Zhang X, Wang Y, Zhang J, Hew CL, Li S, Xia N and Sivaraman J. ***PNAS*** (2011) 108, 10266-10271.

S2-4

Crystallographic and NMR evidence for flexibility in the C-terminal globular domain of oligosaccharyltransferases and its catalytic significance

James Nyirenda, Shunsuke Matsumoto, Takashi Saitoh, Mayumi Igura, and ○Daisuke Kohda
Division of Structural Biology, Medical Institute of Bioregulation, Kyushu University



Protein Asn-glycosylation is widespread not only in eukaryotes but also in archaea and some eubacteria. The oligosaccharyltransferase (OST) catalyzes the transfer of an oligosaccharide chain from a lipid donor to an asparagine residue in polypeptide chains (Fig shows the eukaryotic N-glycosylation). OST is a multi-subunit membrane protein complex in higher eukaryotes, but a single-subunit protein in lower eukaryotes, archaea and eubacteria. The catalytic subunit is STT3 in eukaryotes, AglB in archaea, and PglB in eubacteria. The STT3/AglB/PglB proteins share a common architecture: A multi-span transmembrane region (10-13 TMs) exists in the N-terminal half, whereas the C-terminal half of the primary sequence forms a globular domain. The STT3 proteins share more than 40% sequence identities, but exhibit limited sequence identities with AglB and PglB proteins, typically less than 20%. Thus, meaningful multiple sequence alignment of STT3/AglB/PglB across the three domains of life is almost impossible. A comparative approach is very effective under this circumstance by finding common structural ground among OSTs.

We determined five crystal structures of the C-terminal globular domains of AglBs from *Pyrococcus* and *Archaeoglobus* and a PglB from *Campylobacter* (1,2). The detailed comparison of the closely and distantly related OSTs revealed the minimal structural unit for the catalysis, a common architecture, and new conserved motifs, beyond sequence comparison. A segment of the minimal structural units of the five structures exhibited large conformational differences, probably due to the crystal contact effects, suggesting an unusual plasticity of the C-terminal globular domain. ^{15}N NMR relaxation study showed that this plastic segment in the *Archaeoglobus* AglB-S2 structure was actually mobile in solution. Finally, we designed engineered disulfide bonds that connected two α-helices to restrict the flexibility in the C-terminal globular domain. These disulfide bonds completely inhibited the enzymatic activity of *Pyrococcus furiosus* AglB-L, while the activity was fully recovered upon the reduction of the disulfide bond. This indicates that the dynamic feature of the C-terminal globular domain is essential for the catalysis.

Recently, a crystal structure of a full-length *Campylobacter* PglB in a complex with an acceptor peptide was reported (Lizak et al, *Nature* (2011) 474, 350-355). This epochal structure revealed a special binding pocket that recognizes +2 Thr/Ser in the Asn-glycosylation consensus, Asn-X-Thr/Ser, X ≠ Pro. Interestingly, the Thr/Ser-pocket is composed of the flexible segment identified in our study. Thus, we propose that the Thr/Ser pocket catches and releases the Thr and Ser residues in the N-glycosylation consensus in synchronization with the catalytic cycle, and its dynamic nature enables the efficient scanning of a nascent polypeptide chain when coupled with the ribosomal protein synthesis.

(References)

(1) Igura M et al, ***EMBO J*** (2008) 27, 234-243.
(2) Maita N et al, ***J Biol Chem*** (2010) 285, 4941-4950.

S3-1

VCIP135 deubiquitinase and its binding protein, WAC, in p97ATPase-mediated membrane fusion

○Go Totsukawa, Yayoi Kaneko, Kaori Tamura, and Hisao Kondo
Department of Molecular Cell Biology, Faculty of Medical Sciences, Kyushu University

Two distinct p97 membrane fusion pathways are required for Golgi biogenesis: the p97/p47 and p97/p37 pathways. VCIP135 is necessary for both pathways, while its deubiquitinating activity is required only for the p97/p47 pathway.

We have now identified a novel VCIP135-binding protein, WAC. WAC localizes to the Golgi as well as the nucleus. In Golgi membranes, WAC is involved in a complex containing VCIP135 and p97. WAC directly binds to VCIP135 and increases its deubiquitinating activity. siRNA experiments revealed that WAC is required for Golgi biogenesis. In an in vitro Golgi reformation assay, WAC was necessary only for p97/p47-mediated Golgi reassembly, but not for p97/p37-mediated reassembly. WAC is hence thought to function in p97/p47-mediated Golgi membrane fusion by activating the deubiquitinating function of VCIP135.

We also showed that the two p97 pathways function in ER membrane fusion as well. An in vitro ER reformation assay revealed that both pathways required VCIP135 but not its deubiquitinating activity for their ER membrane fusion. This was consistent with the finding that WAC is unnecessary for p97-mediated ER membrane fusion.

S3-2

Understanding membrane recycling through reconstitution

○Min Wu
Department of Biological Sciences, National University of Singapore

Endocytosis is the process used by eukaryotic cells to internalize extracellular materials and a small portion of the plasma membrane via the formation of vesicular carriers. The fidelity of this process in vivo is ensured by the concerted action of a large network of cytosolic proteins. While many of these components have been identified and biochemically characterized, information regarding their integration in time and space is still largely lacking. Using a cell-free system that reconstitutes membrane budding and fission reactions [1], we are exploring the shape transformation of the plasma membrane, with a particular focus on the role of clathrin, curvature generating proteins and actin. Information obtained here serves as a link between in vitro reconstitution and in vivo studies, and points to an emerging morphological complexity of the endocytic machinery underlying its plasticity.

Reference

1. Wu et al., ***Nat Cell Biol.*** (2010) 12, 902-908.

S4-1

Understanding molecular networks and their deregulated hubs in human diseases using SILAC-based mass spectrometry

○Jayantha Gunaratne
*Institute of Molecular & Cell Biology, A*Star and Cancer Science Institute Singapore, National University of Singapore*

Modern mass spectrometry (MS) technology offers exciting new strategies for advances in virtually all areas of biology. Among the quantitative MS strategies, stable isotope labeling of amino acids in cell culture (SILAC) has emerged as a simple and robust approach to solve a wide range of biological problems. SILAC eliminates false positives in protein-interaction studies, and reveals large-scale dynamics of proteomes and protein turnover kinetics by adding a temporal dimension to proteomics. It has become a prime assay for measuring changes in the posttranslational modifications from the yeast pheromone pathway to stem-cell differentiation.

Our research mainly covers protein-, peptide- and RNA-protein interactions, and global changes in proteome, phosphoproteome and ubiquitylome in perturbed cells using SILAC and its improved formats. We have built high quality protein interactome catalogues of two key tumor suppressors, PTEN and p53, and two of their cancer associated mutants, PTEN-G20E and p53-R273H, using improved strategy named SILAC-based parallel affinity purification (PAP-SILAC). We found several known wild-type interacting proteins as well as new interactors in each catalogue. New interactors of wild-type and its respective mutant can be divided into three different categories: proteins common to wild-type and the mutants, mutation-specific interactions and those unique to wild-type. We have validated the specificity of selected novel interactors using biological assays. Functional studies of some of these interactions showed that such interactions are essential in tumor progression and metastasis. We have expanded our SILAC-based protein interactomics to capture the host cell proteins binding to dengue virus (DENV) RNA. Using RNA chromatography of DENV-2 5' and 3' untranslated regions that have been known to form secondary structures necessary for virus replication, we identified several proteins implicated in P body and stress granule function, and not previously known to bind DENV RNAs. The colocalization study of these proteins with sites of DENV replication further implicated these proteins in either a pro- or anti-viral role in the host cell.

SILAC-based global proteome quantification is now ready to tackle comprehensive protein expression analysis comparable to mRNA quantification (transcriptome analysis). We have acquired crucial roles of Annexin 1 (ANXA1) through proteome quantification of mouse mammary gland epithelial cells. Besides mediating inflammation, ANXA1 is involved in important physio-pathological implications including cell proliferation, differentiation, apoptosis and metastasis. However, with controversies in ANXA1 expression in breast carcinomas, its role in breast cancer initiation and progression remains unclear. To elucidate how ANXA1 plays roles in breast cancer initiation, we carried out SILAC analysis on normal mammary gland epithelial cells from ANXA1- heterozygous (ANXA1+/-) and ANXA1-null (ANXA1-/-) mice. Among over 5000 quantified proteins, we observed over 200 up- and down-regulated proteins in ANXA1-/-. Bioinformatics analysis of these protein clusters followed by appropriate functional assays revealed that ANXA1 is potentially implicated in DNA damage, apoptosis, cell adhesion and migration pathways. For further understanding of such roles associated with ANXA1 we have carried out SILAC-based phosphoproteomics analysis using the same cell lines. We have quantified more than 4000 phospho sites of which

~250 sites were either up- or down- regulated in ANXA1-/- . The relevant biological studies are in progress to uncover connection of the detected changes of phosphorylation with the biological roles of ANXA1.

S4-2

Cell-fate decision by histone variants

○Yasuyuki Ohkawa
Department of Advanced Medical Initiatives, Graduate School of Medical Sciences, Kyushu University

Cell differentiation is achieved through the actions of lineage-determining transcription factors. MyoD is a regulatory factor that determines skeletal muscle cell fate, yet its function in undifferentiated cells remains unknown. We show that chromodomain helicase DNA-binding domain 2 (Chd2), a member of the SNF2 family of chromatin remodeling enzymes, cooperates with MyoD and is required to determine cell fate. Chd2 interacts with MyoD and with myogenic gene regulatory sequences to specifically mark these loci via deposition of the histone variant H3.3 prior to cell differentiation. Directed and genome-wide analysis of endogenous H3.3 incorporation demonstrates that knockdown of Chd2 prevents H3.3 deposition at myogenic genes, does not affect H3.3 deposition at housekeeping genes, and results in inhibition of myogenic gene activation during differentiation. The data indicate that MyoD determines cell fate and facilitates differentiation-dependent gene expression through a Chd2-dependent deposition of H3.3 at myogenic loci prior to the onset of differentiation.

S4-3

System biology approach to dissect PRL-3 function in AML

○Chng Wee-Joo
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Much of intracellular signaling are mediated by tight control and regulated interplay between kinase and phosphatase activity. While deregulated kinase activity in malignancy is well described, the role of deregulated phosphatase activity is only starting to be unraveled. We recently found that PRL-3, a phosphatase originally described to be associated with metastasis in solid tumors, is upregulated in a significant proportion of acute myeloid leukemia and mediates drug resistance. We further showed that AML samples with higher PRL3 expression were associated with poor prognosis and that knockdown of PRL-3 in higher expressing cell lines lead to significant cell death. PRL-3 therefore appears to be both biological and clinical relevant in AML. We went on to dissect the oncogenic role of PRL3 and the upstream and downstream signaling network in AML using an integrative analysis of phenotypic, genomics, and proteomics correlation. This could potentially lead to better understanding of the role of this molecule in AML and also provide important avenues for therapeutic intervention.

S4-4

Towards a data resource of full-length protein structures

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Large-scale protein model databases including the SwissModel Repository (1) and MODBase (2) have provided high quality 3D structural models for large numbers of folded domains from complete genomes. While the conventional view is that the missing portions of 3D structure may represent folded domains with no homologous structure, the rate of deposition of new folds into the PDB database has been shrinking, and appears to be reaching an asymptote. There is a significant fraction remaining, up to 30% of protein coding regions, where we may expect that no single stable folded structure may ever be found. These are classically considered to be unfolded regions, linkers, or intrinsically disordered domains, some of which contain compositional sequence biases. Advances in detecting and classifying these intrinsically disordered domains have been made, however we are still left with the problem of representing and modeling these as 3D structures. In order to achieve a goal of a resource of full-length 3D structures, we must start considering the expansion of 3D structure into large ensemble representations, which are able to capture a diversity of random and non-random protein conformers. The TraDES (3) software system has been in use in my group and by NMR scientists(4) to generate large samples (10^5-10^9) of protein conformers, and then use experimental structures, barriers, or energetic constraints to narrow these populations down to more representative sizes (4,5). The resulting plausible ensemble sizes for disordered domains that can represent structural heterogeneity is still very large in the case of long disordered regions, yet this ensemble space can be reduced in size by constraints by a few orders of magnitude. A database infrastructure will be required to represent all of the full-length protein structures in the Human Genome, and estimates are that approximately 10 petabytes of storage will be required to hold the representative ensemble structures for one eukaryotic genome. This suggests, that very large scale database systems are required if we are to build a system capable of holding and representing the folded and non-folding portions of protein structures. We are developing a new software architecture and workflow tools for 3D structure management capable of growing to this scale of resource.

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S5-1

Digital dissection of mitotic picoplankton by electron tomography

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The picoplankton *Ostreococcus tauri* is a tiny unicellular eukaryote, just 1 - 2 µm wide. *O. tauri* has a highly simplified cytological organization: early interphase cells have just one chloroplast, one mitochondrion, one Golgi body, and one nucleus. This simplification even scales down to the macromolecular level because each cell has just one or two nuclear pore complexes and one or two cytoplasmic microtubules. *O. tauri* cells nevertheless have 20 linear chromosomes, and are therefore expected to use a spindle with more than 40 microtubules to segregate the 40 mitotic chromosomes. To test this hypothesis, we need to simply image and count the spindle microtubules. The only method that can resolve these tightly packed microtubules is electron tomography. Our electron tomograms revealed that mitotic *O. tauri* cells have less than ~ 10 spindle microtubules. We propose that these picoplankton undergo either multiple independent rounds of anaphase or a single anaphase using a highly adapted kinetochore. To test these new hypotheses we will need to determine how mitotic chromatin interacts with spindle microtubules.

S5-2

Created for control: Functions of STAT proteins in development and diseases

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It was twenty years ago the STAT proteins were first revealed as p91 and p113 (Stat1 and Stat2) proteins in an interferon induced complex term ISGF3 (Fu and Darnell et al, PNAS, 87:8555-8559, 1990). After this discovery, many other cytokines that activate JAK kinases were shown to be capable of activating STAT proteins and thus the JAK-STAT pathway for cytokine signaling was proposed. However, other protein tyrosine kinases (PTKs) such as EGFR receptor tyrosine kinase and Src kinase may activate STAT proteins directly, indicating that STAT proteins might be substrates of these kinases and serve as functional mediators of many kinds of PTKs. In this study we have first tried to reveal the original role of STAT during metazoan evolution through analyzed the evolution of STAT proteins and its activators, JAK, EGFR and Src Kinase from unicellular ancestral organisms to multicellular modern humans. Second, we asked whether this original function of STAT during early metazoan evolution is replayed during early embryonic development. We examined whether STAT proteins, STAT3 in particular, has any role during mouse embryogenesis from zygote to the early embryo. Using specific STAT3 deficient mice as a model, we have shown that STAT3 has an essential function during early embryogenesis, such as in stem cell development. The details will be further presented and discussed.

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Abstract

O1-1/P-1

Identification of the earliest branch point for myelo-erythroid development in adult hematopoiesis

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In murine hematopoiesis, hematopoietic stem cells (HSCs) have been identified within the LSK fraction of bone marrow cells, which is defined by the absence of lineage-associated surface markers (Lin) and high expression of Sca-1 and c-Kit (1). The first commitment step at the myeloid versus lymphoid bifurcation has been proposed outside the LSK fraction, where myeloid or lymphoid lineage-committed progenitors such as common myeloid progenitors (CMP) and common lymphoid progenitors (CLP) are prospectively isolated (2). However, recently, by utilizing mice harboring a fluorescent reporter for GATA-1 transcription factor, we found that the upregulation of GATA-1 occurs within the LSK population; The GATA-1+ LSK population was capable of generating only myelo-erythroid cells but lacks lymphoid potential (3), suggesting strongly that the earliest myeloid development occurs at the LSK stage.

To isolate the earliest myelo-erythroid LSK progenitors in normal mice without utilizing the GATA-1 reporter system, we conducted expression profiling of GATA-1+ LSK cells by cDNA microarray analyses, and identified a cell-surface antigen (X) specifically expressed in GATA-1+ LSK cells. By using this new surface marker X, we could successfully purify a cell population from normal C57B6 mice, which gives rise exclusively to granulocyte-macrophage (GM) and megakaryocyte-erythroid (MegE) colonies, but lacks lymphoid potential. The gene expression analysis of the X+ LSK population revealed that this population expresses GM and MegE-associated genes at a high level, but not lymphoid genes. Thus, the X+ LSK population might represent the earliest stage for myelo-erythroid development in normal mice, which should be a useful tool for investigating the molecular mechanisms for hematopoietic lineage fate decision.

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O1-2/P-2

Crucial role of the 12-HHT receptor BLT2 in epidermal wound healing

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BLT2 is a G-protein coupled receptor that is activated by 12(S)-hydroxyheptadeca-5Z, 8E, 10E-trienoic acid (12-HHT) and leukotriene B4 (LTB4). Mouse BLT2 is expressed highly in small intestine and skin. In skin, BLT2 expression was detected in follicular and interfollicular keratinocytes. Keratinocytes are the major population of epidermis and the first barrier of the body. In the process of tissue injury and repair, keratinocytes rapidly migrate and form epithelial sheets. This step is one of the key events in the process of wound healing.

To clarify the role of BLT2 in skin wound healing, full-thickness excisional (3 mm diameter) skin wounds were made on the dorsum of 7-10-week-old BLT2 -/- and WT mice. BLT2 -/- mice exhibited a delay in skin wound healing in comparison to WT mice.

As the production of 12-HHT is cyclooxygenase (COX)-dependent, a COX inhibitor aspirin was applied in drinking water to the mice. Aspirin delayed skin wound healing only in WT mice, but not in BLT2 -/- mice.

To address the mechanism of delayed wound healing in BLT2 -/- mice, we performed DNA microarray analysis of mice skin. Several genes known to enhance cell migration were downregulated in both intact and punched skin of BLT2 -/- mice.

We also investigated the roles of BLT2 in keratinocyte migration in vitro. Spontaneously immortalized human skin keratinocytes, HaCaT cells stably transfected with human BLT2 exhibited more efficient migration than mock cells in the scratch assay. Quantitative real-time PCR was performed to address the responsible genes for the enhanced migration of BLT2 overexpressing HaCaT cells. The amount of mRNA of several MMPs, cytokines and chemokines was increased in BLT2 overexpressing cells under 12-HHT stimulation.

Taken together, these results strongly suggest a crucial role of BLT2 in epidermal wound healing *in vivo* and keratinocyte migration *in vitro*.

O1-3/P-3

Early integrin binding to fluid membrane RGD activates actin polymerization and contractile movement that stimulates outward translocation

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Integrin-mediated adhesions are critical for stem cell differentiation, cancer metastasis, and immune response (1). However, hindered by immobile ligands in conventional matrices, mechanisms of early adhesion formation, especially lateral sorting of integrins and associated proteins remain unclear. Here we show unreported long-ranged lateral movement of ligated integrins in a sequence of force-dependent events. We utilized mobile RGD ligands over supported lipid membrane system to trigger integrin activation and investigate lateral reorganizations of associated molecules (2). In addition, nano-fabricated physical barriers that partitioned continuous fluidic supported membranes provided local confinement of ligated integrin clusters and rigidity for cytoskeletal force generation. Initially, RGD-activated integrin clusters stimulate actin polymerizations in proximity and form contractile pairs that move toward each other against barriers via myosin-II activity. Src kinase-dependent stimulation triggered by contractile-assembled RGD-integrins results in lamellipodial extension and outward movement of clusters. Subsequent retraction by myosin-II causes inward movement of clusters, and cell spreading area is modulated by various spacing between periodic barriers. Integrin clustering recruits adhesion proteins, talin, paxillin and FAK, irrespective of force generation. However, greater recruitment of vinculin is observed upon inward movement. The early steps in cell adhesion of integrin clustering, actin polymerization, and contraction of clusters provide new targets for manipulating adhesion formation that precede the normal extension, contraction and polarization processes.

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O1-4/P-4

mTOR regulates transcription through FOXK1 phosphorylation

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The serine/threonine kinase mTOR senses the nutritional and energy status of cells and initiates its downstream responses. Only several substrates of mTOR have been identified, although mTOR has been shown to control divergent biological processes. To explore unidentified downstream molecules of mTOR, we examined the effect of rapamycin on serum-induced phosphorylation with the use of quantitative phosphoproteome analysis. On the basis of this analysis, twenty novel downstream molecules of mTOR were identified including a transcription factor, FOXK1. We identified a novel protein, designated as K1BP1 (FOXK1 binding protein 1) that specifically interacts with phosphorylated FOXK1. To examine whether the function of FOXK1 is regulated by mTOR-induced phosphorylation, we performed microarray analysis for FOXK1-overexpressing cells. We found that expression of many genes affected by FOXK1 expression was downregulated by the treatment of rapamycin. Furthermore, the transcription activated by FOXK1 was inhibited by the expression of dominant-negative form of K1BP1. We thus effectively identified a novel signaling pathway that is composed of mTOR/FOXK1/K1BP1 to regulate transcription on the basis of multi-omics approach.

O1-5/P-5

Intracellular tension regulates bile canaliculi maturation in primary rat hepatocytes

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A major obstacle in tissue engineering is the formation of in vivo-like structures in in vitro cultures. Once removed from their organized 3D tissue architecture, many primary epithelial cells lose their differentiated functions. This is major obstacle for hepatocytes, which quickly lose many of their liver-specific functions upon isolation. As the generation of quality in vitro hepatocyte cultures are critical for many drug-testing applications, as well as hepatitis viral studies, we have begun studies aimed at understanding how the in vitro environment regulates the polarity of hepatocytes.

Hepatocytes have a unique epithelial topography whereby the apical domains are located along the lateral sides of the cells sandwiched between two basolateral domains. These apical domains form elongated lumen called bile canaliculi that merge with neighboring bile canaliculi to form an interconnected network within the monolayer. The mechanical regulation of these mesoscale tissue-like structures have yet to be elucidated. To this end, we have cultured primary rat hepatocytes in vitro in the presence of actomyosin inhibitors that decrease intracellular tension. We have demonstrated that bile canaliculi maturation is dependent on actomyosin contractility, which can be modulated by substrate rigidity. Additionally, this actomyosin contractility is required to offset the osmotic pressure within the lumen of the bile canaliculi. Lastly we have identified a subset of actin stress fibers associated with the bile canaliculi that may help balance the tension generated by these structures to maintain the proper cell shape.

Together, our results demonstrate that actomyosin contractility regulates the intracellular tension generated by osmotic pressure in the developing bile canaliculi. Further this tension may be transmitted to the substratum to generate a subset of actin stress fibers to balance these forces and maintain proper cell shape during hepatocyte polarization and maturation.

O1-6/P-6

Structural basis of Cdc42-mediated Cdc42-interacting protein 4 (CIP4) recruitment to clathrin-coated pits

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Cdc42-interacting protein 4 (CIP4) and formin-binding protein 17 (FBP17) are Cdc42 effectors implicated in clathrin-mediated endocytosis. CIP4 and FBP17 contain an evolutionarily conserved extended FCH (EFC)/FCH and BAR (F-BAR) domain. We previously solved the crystal structure of the EFC/F-BAR domain dimer of CIP4 and FBP17 and revealed that it is an elongated helical-bundle dimer with shallow curvature (1). The EFC/F-BAR domain of CIP4 and FBP17 interacted with the negatively charged phospholipid membrane with its positively charged concave surface and oligomerized to deform membranes into a tubular shape. We also showed that this mechanism plays an important role in invagination of clathrin-coated pits (CCPs). On the other hand, the mechanism how CIP4 and FBP17 are recruited to CCPs was obscure.

A guanine-nucleotide exchange factor for Cdc42 is an important component of CCPs and CIP4 and FBP17 interact with Cdc42 with their Cdc42-specific HR1 (cHR1) domains. Thus, we hypothesized that activated Cdc42 recruits CIP4 and FBP17 to CCPs. To investigate this hypothesis, we first solved the crystal structures of the cHR1 domains of CIP4 and FBP17 in complex with Cdc42•GMPPNP. The antiparallel coiled coil of the cHR1 domain bound the switch regions of Cdc42 and extensively recognized the Cdc42-specific phenylalanine residue. Thus, we revealed the mechanism that ensures specific recognition of Cdc42 among other Rho-family GTPases by the cHR1 domain.

We previously showed that FBP17 was transiently recruited to CCPs in cells (1). CIP4 was also transiently recruited to CCPs, while Cdc42 was co-localized with CCPs. In line with our hypothesis, CIP4 recruitment to CCPs was blocked by Cdc42-binding defective mutations in the cHR1 domain or by Cdc42 knockdown. Interestingly, Cdc42 knockdown selectively blocked the internalization of CCPs with relatively large sizes. Thus, we propose that the recruitment of CIP4 and FBP17 to CCPs is important for invagination of relatively large CCPs.

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O1-7/P-7

A Novel Function of AWP1/ZFAND6: Regulation of Pex5p Export by Interacting with Cys-monoubiquitinated Pex5p and AAA ATPase, Pex6p

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In biogenesis of peroxisome, a subcellular organelle, Pex5p is the shuttling receptor for peroxisomal matrix proteins harboring peroxisome-targeting signal 1 (PTS1). Several peroxins are involved in the Pex5p shuttling between the cytosol and peroxisomes. However, the precise mechanism underlying the PTS1 receptor shuttling remains elusive. We herein suggest that liver cytosol contains at least two distinct factors involved in the Pex5p export. We isolate one of the factors by biochemical fractionation and in vitro Pex5p export assay and identify it as AWP1/ZFAND6 (termed p40), a ubiquitin-binding NF-κB modulator. In in vitro Pex5p export assay, recombinant p40, stimulates Pex5p export, whilst anti-p40 antibody interferes with Pex5p export. p40 interacts with AAA ATPase Pex6p, but not Pex1p-Pex6p complex. p40 binds Cys-ubiquitinated form of Pex5p more preferentially than unmodified Pex5p, apparently via its A20 zinc-finger domain. RNA interference for p40 significantly affects the PTS1 protein import into peroxisomes. Furthermore, in the p40 knocked-down cells Pex5p is unstable, as in fibroblasts from patients each defective in Pex1p, Pex6p, and Pex26p, all prerequisite to the Pex5p export. Taken together, p40 is a novel cofactor of Pex6p involved in the regulation of Pex5p export in peroxisome biogenesis.

O1-8/P-8

Mechanism of the interaction between Pex14p and LC3-II

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Pexophagy, the selective degradation of peroxisomes by autophagic machinery, has been well studied in yeast (1,2). In mammalian culture cells, it can be also induced experimentally by switching the culture medium to the starvation condition. Pex14p, which is identified as an essential peroxisomal membrane protein for matrix proteins in binding to soluble receptor Pex5p, is involved in the mammalian autophagic degradation of peroxisomes and interacts with the lipidated form of LC3, termed LC3-II, an essential autophagy marker, under the starvation condition in CHO-K1 cells, whereas the binding of Pex5p to Pex14p under the starvation condition was greatly reduced (3). However, the molecular mechanism of interaction between Pex14p and LC3-II is largely unknown.

To verify whether Pex14p directly binds LC3-II, We reconstituted a conjugation system for synthesis of LC3-II *in vitro*. LC3-II was successfully synthesized and then pulled down by GST-Pex14p, suggesting that Pex14p directly binds to LC3-II. We also found that Pex5p negatively regulates the binding of LC3 to Pex14p *in vitro*. These results agree well with those *in vivo* under the autophagic condition.

We further show that the region encompassing amino-acid residues at 106-140 of Pex14p, which contains the transmembrane domain (110-138), is important for LC3-II binding. Notably, this domain is not the binding region consisting of amino-acid residues at 31-70 for Pex5p (4). Accordingly, our data suggest that LC3-II and Pex5p do not directly compete in binding to Pex14p. We are now analyzing how Pex14p preferentially binds Pex5p under the nutrient-rich condition, whilst Pex14p interacts with LC3-II under the starvation condition *in vivo*.

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O2-1/P-9

Sumoylation of NEMO and NF-κB activation by a RNA helicase, DP103 defines the metastatic potential of human breast cancers

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Mortality from breast cancer is almost entirely the result of invasion and metastasis of neoplastic cells; therefore, understanding gene products involved in breast cancer metastasis is an important research goal. We discovered DP103, a RNA helicase, to be correlated with the level of maglinancies and its suppression led to the decrease in the ability of highly invasive breast cancer cells to migrate and invade. MMP9 is found to be a major matrix metalloproteinase involved in the degradation of the cell's extracellular membrane to facilitate metastasis. We screened a panel of breast cancer cell lines for MMP9 expression. Interestingly, our screen shows cell lines that are highly metastatic such as MDA-MB-231 and BT549 display high expression levels of MMP9 and they are highly correlated to DP103 levels. When grouping patients with positive DP103 expression to positive and negative MMP9 expression, Kaplan-Meier correlation analysis show patients with positive DP103 and MMP9 expression have poorer survival outcomes (p=0.029). We next show via EMSA and luciferase reporter assay that this decrease in MMP9 levels is regulated by NF-κB and not AP-1. Additionally, under the stimulation of known NF-κB genotoxic agents, NF-κB activity was also decreased when DP103 was knockdown. We also discovered that DP103 coimmunoprecipitated with NEMO, an essential NF-κB modulator, and that it is required for the SUMOylation of NEMO upon genotoxic stress. Downregulation of DP103 abrogated this modification to occur. Additionally, we observed that DP103 enhances the interaction of NEMO and PIASy, an E3 SUMO ligase, while decreasing that of NEMO and the specific desumoylating enzyme, SENP2. Together, our study identifies DP103 as the "missing" key regulator of NF-κB activation under genotoxic stress. Importantly, DP103 is a novel prognostic marker that identifies patients who are at risk for developing metastases, thus enabling oncologists to begin tailoring treatment strategies to individual patients.

O2-2/P-10

Genetic reevaluation of the role of F-box proteins in cyclin D1 degradation

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Progression of the cell cycle in eukaryotic cells depends on the activity of a series of protein complexes composed of cyclins and cyclin-dependent kinases (CDKs). Transition from G_1 phase of the cell cycle to S phase is promoted by D-type cyclins (cyclins D1, D2, and D3), which form complexes with CDK4 or CDK6 that phosphorylate and inactivate the product of the retinoblastoma tumor suppressor (1). Consistent with their functions, D-type cyclins, especially cyclin D1, are overexpressed in various types of malignant tumors such as breast cancer (2) and esophageal cancer (3). Such overexpression of cyclin D1 is thought to result from up-regulation of gene transcription or mRNA translation or from impaired protein degradation. Whereas transcriptional regulation of the cyclin D1 gene has been extensively studied and is well understood, the mechanism of cyclin D1 degradation has remained unclear. Four SCF-type ubiquitin ligases (SCF^{Fbxo4} (4), SCF^{Fbxw8} (5), SCF^{Skp2} (6), and SCF^{Fbxo31} (7) have independently been shown to contribute to cyclin D1 ubiquitylation so far. However, it remains unclear which F-box protein plays the key role in regulation of cyclin D1 stability.

We have now adopted a genetic approach to test which of four F-box proteins is most relevant for cyclin D1 degradation. We have generated mice deficient in Fbxo4 and found no differences between these animals and wild-type mice. Accumulation of cyclin D1 was thus not observed in tissues of *Fbxo4*$^{-/-}$ mice. We also examined the stability of cyclin D1 in mouse embryonic fibroblasts (MEFs) prepared from *Fbxo4*$^{-/-}$, *Fbxw8*$^{-/-}$, and *Fbxo4*$^{-/-}$*;Fbxw8*$^{-/-}$ mice and again found that the half-life of cyclin D1 in these cells did not differ from that in wild-type MEFs. Furthermore, additional depletion of Skp2 and Fbxo31 in *Fbxo4*$^{-/-}$*;Fbxw8*$^{-/-}$ MEFs by RNA interference did not affect the stability of cyclin D1. Although the depletion of Fbxo31 in MEFs resulted in an increase in the basal expression of cyclin D1, this effect appeared to be attributable to an increase in the amount of cyclin D1 mRNA. Furthermore, the inhibition of SCF complexes by the expression of dominant negative Cul1 did not alter the half life of cyclin D1.

Our genetic analyses thus do not support a role for any of F-box proteins in cyclin D1 degradation during normal progression of the cell cycle. They therefore suggest the existence of other ubiquitin ligases that are responsible for cyclin D1 proteolysis.

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O2-3/P-11

The pro-metastasis tyrosine phosphatase, PRL-3 (PTP4A3), is a novel target of BCR-ABL signaling involved in human chronic myeloid leukemia

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Chronic myeloid leukemia (CML) is the best and most successful disease model for tyrosine kinase inhibitor (TKI). The mechanism of BCR-ABL leading transformation and signaling transduction networks have been intensively characterized. However, resistance to TKIs remains a challenge in management of patients with CML. A better understanding BCR-ABL signaling network will lead to a better therapy.

Here we report the discovery of a novel downstream target of BCR-ABL signalling, PRL-3 (*PTP4A3*), an oncogenic tyrosine phosphatase. Analysis of CML cancer cell lines and CML patient samples reveals the upregulation of PRL-3. A search of Gene Expression Atlas (http://www.ebi.ac.uk/gxa/gene/ENSG00000184489) identified the expression level of PRL-3 was highest in CML among 950 human cancer cell lines crossing 32 different types of cancers (Dataset code: E-MTAB-37), suggesting a potential role of PRL-3 in CML pathogenesis. Inhibition of BCR-ABL signalling either by Imatinib or by RNAi silencing BRC-ABL in CML cell line K562, KCL-22 and primary patient samples reduces PRL-3, in parallel with suppression of signal transducer and activator of transcription (STAT) pathway activities and increased cleavage of PARP, a hallmark of apoptosis. In contrast, the amount of PRL-3 protein remains constant or even increased in response to Imatinib treatment in drug resistant cells expressing BaF3-P210 T315I. Finally, analysis with specific shRNA demonstrated K562-shPRL-3 (shP) cells proliferated as much as 2-time lesser than K562-shControl (shC) at day 8 ($p < 0.001$). Colony-forming efficiency is an indicator of self-renewal capacity of leukemic cell. K562-shP cells also showed significantly impaired colony generating capacity by 3-fold compared to K562-shC ($p < 0.001$). These results indicate a critical role for PRL-3 in CML cell expansion and self-renewal. These data support a functionally important role of PRL-3 in CML biology downstream of BCR-ABL and maybe a viable therapeutic target in BCR-ABL positive cells even in those with Imatinib resistant mutations.

O2-4/P-12

PPARγ regulates tumor-specific repression of MnSOD expression in ER-independent breast cancer: A novel strategy for “oxidation therapy” in cancer

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Ligand induced peroxisome proliferator-activated receptor gamma (PPARγ) activation has been reported to inhibit the proliferation of malignant cells, possibly through reactive oxygen species (ROS) production. An increasing number of studies have demonstrated that elevation of cellular ROS can indeed kill cancer cells more effectively. Compelling evidence suggests that cancer cells are generally under oxidative stress, which renders them more dependent on superoxide dismutases (SOD) to protect them. Manganese superoxide dismutase (MnSOD) is one of the major antioxidant enzymes that could regulate ROS-mediated cell death induced by PPARγ activation. Recently, a strategy of ‘oxidation therapy’ has evolved using ROS-inducing approaches to target cancer cells and increase their chemo-sensitivity to anti-cancer drugs.

We report the identification of human MnSOD as a PPARγ target gene and that activation by PPARγ agonists led to downregulation of MnSOD mRNA and protein levels. Importantly, normal breast cells were completely refractory to this effect. Furthermore, MDA-MB-231 xenograft model in nude mice treated with PPARγ ligands showed significant reductions in tumor size, and tumor tissues stained by immunohistochemistry showed a decrease in MnSOD protein levels. A histopathologic analysis of breast cancer biopsies obtained from patients treated with synthetic PPARγ agonists showed MnSOD repression. Alongside MnSOD repression, a corresponding increase in intracellular superoxide production in breast cancer cells was observed upon PPARγ activation, which did not occur in normal breast cells. Suppression of MnSOD levels by small-interfering RNA or PPARγ agonists in breast cancer cells increased oxidative stress and enhanced chemo-sensitivity to ROS-promoting drugs such as docetaxel and doxorubicin.

Together, our data not only identifies MnSOD as a novel target of PPARγ but also provides a molecular mechanism for ROS-manipulation therapy in the clinic through the intelligent use of PPARγ ligands in combination with ROS-promoting drugs such as doxorubin or docetaxel.

O2-5/P-13

Identification of disease-associated *cis*-regulatory elements in the Runx3 gene

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The *RUNX3* gene encodes a Runx family transcription factor that functions as a developmental regulator and is involved in a variety of human cancers and immunological disorders. Despite such widespread involvement in diseases, the transcriptional regulation of *Runx3* remains elusive due to the huge ~350-kb *Runx3* locus and the complexity of the use of two distinct promoters for transcription. To identify functional *cis*-regulatory elements of *Runx3*, we employed an *in silico* approach that combines comparative genomics to determine conserved non-coding elements (CNE), and chromatin immunoprecipitation-sequencing (ChIP-seq) data from publicly available databases. This led to the discovery of elements capable of driving *in vivo* spatial-temporal reporter expression in the zebrafish. Of these, one element, Runx3 mCNE-L reflected endogenous *runx3* expression in early hematopoietic tissues. To further characterize this element, mCNE-L-EGFP transgenic (Tg) mouse lines were generated. Analyses of the Tg mouse demonstrated that Runx3 mCNE-L enhancer drives reporter gene expression in multiple lineages of the hematopoietic compartment, particularly in cells involved in immunity, such as T cells, B cells, NK cells and macrophages. In good agreement with the established roles of Runx3 in immunity and the fact that Runx3 deficient status leads to intestinal inflammation, this immune cell-specific mCNE-L enhancer was found to be regulated by potent anti-inflammatory glucocorticoids (GC), through its binding to a glucocorticoid response element (GRE) within the enhancer. GC-induced Runx3 expression regulated the differentiation and/or function of anti-inflammatory regulatory T (Treg) cells that are responsible for immunological homeostasis in the intestine. Therefore, Runx3 CNE-L enhancer activity appears to play a central role in the intestinal immunological homeostasis and its deregulation due to genetic variegation(s) may be associated with human inflammatory bowel disease (IBD), one of the most common intestinal inflammatory diseases which increase the risk of developing colorectal cancer.

O2-6/P-14

Regulation of NK cell-mediated cytotoxicity by the atypical Rac activators

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Natural killer (NK) cells play important roles in host immunity by killing virus-infected and tumor cells. This function of NK cells needs to be precisely regulated not to attack normal autologous cells. To achieve this regulation, NK cells control killing function by a coordinated signal generated from a number of inhibitory and activating receptors.

NKG2D is one of the most well-known activating receptor, and its ligands on target cells are MICA/B, ULBP 1, 2, 3, and 4 in humans, and Rae-1, H60, and MULT in mice. Stimulation of NKG2D causes the target cell cytotoxicity, by secreting lytic granules toward the target cells. These granules contain perforin, granzymes, Fas ligand, and other hydrolytic enzymes which ultimately induce target cell apoptosis. However, the mechanisms controlling NK cell functions are not fully understood.

We found that the atypical Rac activators coordinately regulate NK cell-mediated cytotoxicity. In my talk, I will discuss its implication and the regulatory mechanism.

O2-7/P-15

Identification of a signaling molecule critical for dendritic cell migration in three-dimensional environments

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Dendritic cells (DCs) are specialized antigen-presenting cells that essential for the initiation of adaptive immune response, during which they capture and present antigen, and migrate from peripheral tissues to draining lymph nodes to activate T cells. To perform these functions, DCs constantly adapt their shape to the given structure of the interstitial extracellular matrix (ECM) and follow the path of least resistance. This amoeboid migration of DCs occurs independently of adhesion to specific substrates and ECM degradation, yet its regulatory mechanisms are poorly understood.

Here, we have identified a novel signaling molecule that regulates interstitial DC migration. I will discuss the mechanism of how this molecule regulates interstitial DC migration.

O2-8/P-16

BLT1 as a potential marker of mouse dendritic cell subset

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Dendritic cells (DCs) are highly specialized antigen-presenting cells that regulate Th1 and Th2 immune responses by releasing cytokines and interacting directly with T cells. BLT1 is a high-affinity receptor for leukotriene B4 (LTB4), a classical pro-inflammatory lipid mediator for neutrophils. Recently, LTB4 was also identified as an important attractant for effector $CD4^+$ and $CD8^+$ T cells. Although there is growing numbers of evidence on the role of BLT1 in immune responses, its role in dendritic cells remains largely unknown.

In this study, we describe distinct subsets of $CD11c^+$ MHC class II^+ DCs characterized by different expression levels of BLT1, namely $BLT1^{bright}$ and $BLT1^{dim}$ DCs. $BLT1^{bright}$ and $BLT1^{dim}$ DCs are present in spleens and bone marrow-derived DCs from C57BL/6 mice. We next separated these subsets and analyzed the differences in characters including morphology and cytokine production. Microscopic studies show that $BLT1^{bright}$ cells are adherent, but $BLT1^{dim}$ cells are not. Interestingly, $BLT1^{bright}$ DCs stimulated by various PAMPs produced pro-inflammatory cytokines such as IL-12p70, MCP-1, TNF-α, and IL-6, whereas $BLT1^{dim}$ DCs produced almost no IL-12p70 and other cytokines to lesser amounts. On the other hand, $BLT1^{dim}$ DCs induced more efficient proliferation of T cells than $BLT1^{bright}$ cells.

In summary, we propose BLT1 as a new marker of distinct mouse DC subsets. Future study will focus on the detailed differences between $BLT1^{bright}$ and $BLT1^{dim}$ DCs, and the role of LTB4-BLT1 signaling in regulating immune responses in DCs.

O2-9/P-17

A new immunodeficient mouse model introduced with defined Sirpa polymorphism for human hematopoietic stem cell assay

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To evaluate stem cell potential of human cells in xenotransplant models, a variety of immunodeficient mouse lines have been developed. It has been shown that depletion of lymphoid cells including T, B and NK cells by introducing with γc^{null}, and SCID or RAG^{null} mutations are necessary to avoid rejection of human cells in these models. Interestingly, in mice having these immunodeficiencies, the NOD strain shows even better engraftment of human cells as compared to B6 or Balb/c strains, although the NOD mouse lines are not easy to maintain partly because of their infertility. Recently, we have shown that the engulfment of human cells by mouse macrophages plays a critical role in xenograft rejection (Nat Immunol 2007). In the setting of xenotransplantation from human to mouse, the innate phagocytic reaction of mouse macrophages occurs because murine signal regulatory protein alpha (mSIRPA) on macrophages cannot bind to human CD47 (hCD47). We found that the NOD-specific polymorphism of mSIRPA allows NOD-type SIRPA to bind hCD47, resulting in inhibition of phagocytic reaction against human cells in this strain at least *in vitro*. Here we test whether the efficient engraftment of human cells in the NOD strain could be ascribed simply to the NOD-type SIRPA polymorphism *in vivo*. To test this hypothesis, we have established a new immunodeficient $Rag2^{null}\gamma c^{null}$ B6 mouse line with NOD-type SIRPA ($Sirpa^{NOD/NOD}$). As compared to the $Rag2^{null}\gamma c^{null}$ B6 stain, the introduction of $Sirpa^{NOD/NOD}$ significantly improved the engraftment of human hematopoietic stem cells. This result strongly suggests that to achieve successful engraftment of human cells, the introduction of NOD-type SIRPA alone is sufficient, instead of backcrossing mice to obtain complete NOD background. This mouse might be very useful in xenotransplant experiments since the B6 background is well characterized, and is a common healthy strain with good fertility.

P-18

ER retention of Ero1α is regulated by the pH–dependent C-terminal tail movement of ERp44

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Many secretory and membrane proteins undergo disulfide bond formation in the endoplasmic reticulum (ER), where Ero1 (ER oxidoreductin-1) and PDI (protein-disulfide isomerase) constitute a major disulfide-introducing oxidative pathway. We recently reported that human Ero1α binds to the hydrophobic pocket of PDI, leading to the effective Ero1α-PDI catalytic cycle (1). While Ero1α is an ER-resident oxidase, the enzyme lacks an ER retention signal. ERp44, a multifunctional chaperone of the PDI family member, is known to play a critical role in retrieving Ero1α from the cis-Golgi to the ER. Despite of the functional importance of the Ero1α-ERp44 interaction, its mechanism remains unclear.

ERp44 is composed of three thioredoxin-like domains called a-, b-, and b'-domains, respectively, and a C-terminal tail (C-tail). The N-terminal a-domain has a CxxS motif at the active site, near which a hydrophobic patch concealed by the C-tail is present (2). We hypothesized that the hydrophobic patch in the a-domain of ERp44 is important for interaction with Ero1α, in which the C-tail functions as a key regulator.

To investigate how the C-tail regulates the ERp44-Ero1α interaction, we focused on the pH gradient between cis-Golgi (pH6.5) and ER (pH7.2). The pH-dependent movement of the C-tail was assessed using a fluorescent hydrophobic probe, ANS. Its fluorescence peak in the presence of ERp44 was significantly enhanced and blue-shifted when buffer pH was decreased from 7.5 to 6.5. This result suggests that the C-tail opens to expose the hydrophobic patch in a pH-dependent manner. Moreover, surface plasmon resonance measurements revealed that ERp44 has much higher affinity for Ero1α at lower pH, indicating the pH-regulated interaction between ERp44 and Ero1α. Together with several *in vivo* data, the present study demonstrates that ERp44-mediated ER retention of Ero1α is ensured by enhanced association in the cis-Golgi and facilitated dissociation in the ER, of ERp44 and Ero1α, in which the ER-Golgi pH gradient is the essential element.

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P-19

Discovering stage specific mechanisms through the dynamical genome-wide C/EBPα chromatin occupancy during granulocytic differentiation

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Given the essential role of C/EBPα in controlling granulocytic differentiation and its role in myeloid leukemia, it is crucial to elucidate the transcriptional mechanism of C/EBPα. Expression profiling studies during granulocytic differentiation following inducible expression of C/EBPα suggest that hundreds of genes may be regulated by C/EBPα. The most elementary goal is to establish the ensemble of target genes that C/EBPα regulates genome-wide. Cell fate is specified by lineage-determining transcription factors (e.g. C/EBPα, Pu.1), whose expression is often not limited to a single cell type. Comparisons of the genome-wide binding patterns of different transcription factors in a variety of species and cell types have generated two major insights regarding transcription factor binding patterns: (1) different factors in the same cell type tend to co-localize on a genome wide scale and (2) the same factor in different cell types or at different stages of development exhibits different genome-wide binding patterns. Here, we discuss our approach of ChIP-Seq and gene expression microarray to define the C/EBPα *cistrome* in mouse hematopoietic system using bone marrow stem cells, progenitors (LSK, CMP, GMP) and differentiated cells (Granulocytes). This approach defined a binding dynamic for C/EBPα during granulocytic differentiation and highlighted potential stage specific regulatory modules. Identification of this gene signature profile for C/EBPα within the hematopoietic system would provide insights into gene regulations patterns in leukemic patients with C/EBPα aberrations.

P-20

Development and application of a novel technique for microscopic visualization of locus-specific DNA methylation in individual cells

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DNA methylation at CpG sites is a major epigenetic modification of genome DNA that plays important roles in normal development, aging, and a variety of disease conditions. Immuno-fluorescence microscopy with anti-5-methylcytosine antibodies has been used to visualize global DNA methylation patterns. However, methods that allow visualization of DNA methylation at specific loci have been lacking to date. Recently, it was reported that reactivities of methylated and unmethylated cytosines for interstrand complex formation with osmium and bipyridine-containing nucleic acid (ICON) are markedly different *in vitro*. Based on this chemistry, we have developed an experimental protocol, named MeFISH, to observe the DNA methylation status of specific loci in individual cells *in situ*. Cell nuclei or chromosomes were hybridized with fluorescence-labeled ICON probes for major and minor satellite repeats, treated with osmium and then deprobed. Specific retention of fluorescent signals was observed in wild type ES cells but not in Dnmt1/3a/3b triple knockout ES cells, which had virtually no DNA methylation. Therefore, the signals observed in wild type ES cells most likely resulted from specific interstrand crosslinks formed between the ICON probes and methylated cytosines of the target sequences. MeFISH was also successfully used to detect DNA hypomethylation of satellite repeats in lymphoblast cells of ICF syndrome patients. Moreover, MeFISH was used to detect DNA methylation of satellite repeats in mouse germ cells during embryonic and postnatal testis development, with a combination of immunostaining. MeFISH will have a wide application in epigenetics research.

P-21

DNA methylation differences between humans and chimpanzees in chromosome 21 and 22

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Humans (Homo sapiens) and chimpanzees (Pan troglodytes) show many phenotypic differences despite the low genomic sequence divergence. Besides the changes in protein structure and function, differences in gene expression likely have an important role in evolution of complex traits. DNA methylation is a major epigenetic modification that can cause stable changes in gene expression and thus may contribute to inter-species phenotypic differences.

We here present a survey of DNA methylation differences between humans and chimps in chromosomes 21 and 22, which identified fifteen species-specific differentially methylated regions (S-DMRs). Most S-DMRs are tissue-specific and arise late in development, and some of them are associated with genes and located within CpG island (CGI) shores. Moreover, there are cases where the S-DMR is clearly correlated with the difference in promoter activity or alternative splicing pattern.

Interestingly, some of the S-DMRs are found in disease-related genes such as APP (Alzheimer's disease), KCNJ15 (diabetes) and MN1 (tumor). By comparing the methylation status and genomic sequence of the S-DMRs with those of other great apes, we find evidence supportive of both genetic and epigenetic origins of the species-specific methylation changes. Our findings show that epigenetic changes that arise with or without genetic changes are associated with transcriptional divergence in hominoid evolution.

P-22

Identification of Runx as centrosome-associated proteins involved in regulation of cell division

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Abnormal chromosome number (aneuploidy) is one of the most common characteristics in cancer cells. While it was proposed that aneuploidy drives tumorigenesis, the significance of aneuploidy in cancer initiation remains unclear. Moreover, with a myriad of genes regulating mitosis, understanding the molecular mechanisms which give rise to aneuploidy has proved challenging. Strong experimental evidence has demonstrated a causal link between centrosome dysfunction, mitotic abnormalities and aneuploidy. Centrosomes facilitate the formation of the bipolar mitotic spindle and as such, play a critical role in ensuring faithful chromosome segregation in metazoan cells. It was previously observed that the tumor predisposition exhibited by Runx3+/- mice is reminiscent of other animal models with gene defects in components of the mitotic process. Runx3+/- heterozygous mouse develop intestinal adenomas after very long latencies of 15-16 months. Moreover, upon carcinogenic insult, the Runx3+/- and Runx3-/- mice, relative to wild type, show accelerated tumor development. Here, we assess the role of RUNX3 in mitosis. Immortalized gastric epithelial cells derived from Runx3+/+ and Runx3-/- mice were subjected to treatment with paclitaxel, a common chemotherapeutic agent that kills cancer cells by targeting mitotic division. We found that depletion of Runx3 results in a significant increase in resistance to paclitaxel. This is indicative of a role for Runx3 in mitosis. These studies were further reinforced by the findings that a subset of RUNX proteins localize to the centrosome and that RUNX3 interacts with BRCA1, a protein which regulates centrosome function through polyubiquitination events.

P-23

Functional role of NS3-NS5 interaction in dengue virus replication

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Dengue virus (DENV), a member of the *Flaviviridae* family, is a causative agent of dengue fever and its more severe forms, dengue haemorrhagic fever and dengue shock syndrome. The RNA genome of DENV encodes a single strand polyprotein, which is processed into the three structural proteins and seven non-structural (NS) proteins by both host and viral proteases. Of the NS proteins, NS3 and NS5 are the best characterized, and both are essential for DENV replication. NS3 is multifunctional protein possessing protease, helicase, 5'-nucleoside triphosphatase and 5'-terminal RNA triphosphatase activities. NS5 is also multifunctional, which is structurally and enzymatically divided into two distinct domains, methyltransferase and RNA-dependent RNA polymerase. Several studies have reported that NS3 and NS5 proteins interact *in vitro* as well as *in vivo*, while the biological relevance of the interaction in virus replication has not been clarified.

In order to investigate molecular function of the NS3-NS5 interaction, we mapped the binding domain of NS3 with NS5 by employing a luminescent-based interaction assay (AlphaScreen assay). Full-length NS3 and NS5, and their mutants could be produced as soluble protein by wheat-germ cell-free protein production system *in vitro*. The AlphaScreen interaction assay revealed that a sub-domain of the helicase region of NS3, which referred as domain II, appeared to be one of the interaction surfaces of NS5. In addition, preliminary data showed that overexpression of NS3 domain II in 293T cells significantly inhibited DENV type 2 replication. These results support the importance of NS3-NS5 interaction in DENV replication.

P-24

Anchoring NuMA to the spindle poles by NuBP1 is required for the chromosome alignment

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Formation of a bipolar spindle in mitotic cells is a prerequisite to ensure the symmetrical distribution and segregation of chromosomes to each daughter cell. Spindle microtubules are tethered at minus ends to ether of the two spindle poles and at plus ends to chromosomes. The NuMA (nuclear mitotic apparatus) protein, exclusively present in the cell nucleus during interphase, is recruited during mitosis to the spindle poles and plays a crucial role in spindle assembly by focusing and physically tethering minus-end microtubules. Here we have identified a novel protein that directly binds to NuMA, tentatively designated NuBP1 (NuMA-binding protein 1), using proteomics approach and various binding assays. Immunoblot analysis using lysates of synchronized cells reveals that endogenous NuMA interacts with NuBP1 in a mitosis-specific manner. Consistent with this, NuBP1 colocalizes with NuMA in the vicinity of the spindle poles in mitotic cells. Depletion of NuBP1 by RNA interference prevents the assembly of bipolar spindles, which results in cell cycle arrest in prometaphase, indicative of its crucial role in bipolar spindle formation. In these cells, NuMA is much less targeted to the resultant monopolar spindle pole, suggesting an important role of NuBP1 in recruitment of NuMA. On the other hand, in cells where NuBP1 expression is significantly but slightly suppressed, the bipolar spindle is formed but with unaligned chromosomes; a similar defect is also observed in NuMA-depleted cells. These findings indicate that NuBP1 functions not only in bipolar spindle formation but also in chromosome alignment by anchoring NuMA to the spindle poles.

P-25

Live imaging of TCF/LEF activity in developing, growing, and regenerating tissues of the highly responsive Wnt/β-catenin signaling reporter-transgenic zebrafish

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Wnt/β-catenin signaling plays multiple roles in early embryogenesis, organogenesis, adult tissue homeostasis, and tissue regeneration. However, the spatiotemporal dynamics of Wnt/β-catenin signaling in these events has not been fully characterized. Live imaging in zebrafish can be a powerful tool for unraveling it. Although a transgenic zebrafish line carrying Wnt/β-catenin signaling reporter has been already generated, to date, definite reporter activity is observed only at a part of Wnt/β-catenin signaling-active sites in this line. Here we show a new responsive Wnt/β-catenin signaling reporter Tcf/Lef-miniP:dGFP and new transgenic zebrafish lines carrying this reporter. In the new lines, the reporter expression was observed in known Wnt/β-catenin signaling-active sites during embryogenesis, organ development and growth, and tissue regeneration. Inhibition of Wnt/β-catenin signaling blocks the reporter activity. Thus, this new line is a highly useful tool for studying Wnt/β-catenin signaling dependent processes.

We are now challenging to visualize both spatiotemporal pattern and activity strength of several cellular signaling pathways in zebrafish using our own self-developed system. We will also introduce this.

P-26

The transcriptional regulatory mechanism of *Wnt3a* expression in the primitive streak and tail bud

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Wnt3a, a member of the Wnt family of secreted signaling molecules, is required to form the caudal structures during the mouse development. Thus, *Wnt3a* homozygous null mutants lack entire structures caudal to the forelimb. On the other hand, *vestigial tail* (*vt*) mice, carrying an unidentified mutation in *Wnt3a* locus, fail to form the tail.

Although *Wnt3a* is expressed in the primitive streak during gastrulation and in its descendant, the tail bud, the mechanism regulating such expression remains unknown.

In this study, we identified two transcriptional enhancers within *Wnt3a* locus by transgenic reporter assay. Moreover, we uncovered the mutation responsible for *vt* phenotype in one of the two enhancers.

P-27

Re-activation of the stem cell factor SALL4 and its role in hepatocellular carcinoma

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SALL4, one of the critical factors in the embryonic stem cell (ESC) transcriptional regulatory network, is important for the maintenance of cell self-renewal and pluripotency. Besides its physiological function, SALL4 is a novel oncogene. It is constitutively expressed in acute myeloid leukemia and *SALL4B* transgenic mice developed leukemia, suggesting a role of SALL4 in leukemogenesis. In murine liver, Sall4 expression gradually diminishes during liver development and is eventually silenced in adult hepatocytes. In this study, we reveal for the first time that SALL4 is expressed in human fetal liver, silenced in adult liver and re-expressed as an oncofetal protein in hepatocellular carcinoma (HCC). The expression of SALL4 in HCC correlates with unfavorable prognoses. We identify gene amplification as one of the mechanisms underlying *SALL4* re-activation in HCC. Overexpression of SALL4 *in vivo* causes liver tumor formation, as demonstrated in our *SALL4B* transgenic mice. Knocking down of *SALL4* reduces HCC cell viability as well as tumorigenecity, suggesting its functional role in HCC. HCC is the third leading cause of cancer-related deaths globally. Despite advances in treatment for HCC, prognosis remains bleak, with most patients eventually succumbing to the disease within 6 to 20 months. While the epidemiological risk factors for HCC are well known, the molecular mechanisms underlying hepatocarcinogenesis are not well characterized. Elucidating these mechanisms will enable identification of novel candidates for therapeutic targeting. Our findings propose SALL4 to be an attractive target for the development of more effective targeted therapies for HCC.

P-28

3-Dimensional imaging of whole midgestation murine embryos shows an intravascular localization for all hematopoietic clusters

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Hematopoietic cell clusters associated with the midgestation mouse aorta, umbilical and vitelline arteries play a pivotal role in the formation of the adult blood system. Both genetic and live-imaging data indicate that definitive hematopoietic progenitor/stem cells (visualized as clusters) are generated from hemogenic endothelium. A 3-dimensional whole embryo immunostaining and imaging technique has allowed quantitation and cartographic mapping of intravascular hematopoietic clusters. During this period the vitelline artery is most extensively remodelled, and several reports have suggested that vitelline remodelling leads to extravascular hematopoietic cluster emergence. Whether the earliest definitive progenitors/stem cells are intra or extra vascular could influence the process by which these cells migrate to the next hematopoietic territory, the fetal liver. Hence, by 3-dimensional imaging we more closely examined hematopoietic clusters in the vitelline and associated connected small vessels and show here that hematopoietic clusters (particularly large clusters) are intravascular during the period of vascular remodelling.

P-29

A critical role of CD30L in maintenance and activation of a subset of naturally occurring IL-17A-producing gd T cells in mice

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Naturally occurring IL-17A producing gd T cells are differentiated in the fetal thymus and present in the peripheral tissues, such as uterus, peritoneal cavity (PEC), lamina propria lymphocyte (LPL) of intestine, liver and lung of naïve mice and participate in the innate immune response against the bacterial pathogen, such as *Mycobacterium bovis*(1) or *Listeria monocytogenes*(2)at an early stage of infection. CD30 and CD30 ligand (CD30L, CD153) are included in TNFR and TNF superfamily, respectively (3). Although CD30L/CD30 function is clearly important in mycobacterial infection (4) and inflammatory colitis (5-7), its specific relevance to the gd T cell response *in vivo* remains to be elucidated.

In this study, we investigated the roles of CD30L/CD30 signal in thymic differentiation and maintenance in peripheral tissues of gd T cells, using CD30L knock out (KO) or CD30KO mice. There were no differencesin the number and V gamma repertoire of IL-17A-producing gd T cells in fetal thymus between WT mice and CD30KO or CD30LKO mice, suggesting no role of CD30L/CD30 signaling in thymicdifferentiation of IL-17A-producing gd T cells. The number of IL-17A-producing gd T cells in PEC, LPL and liver of naive CD30KO or CD30LKO mice were significantly lower than those in WT mice but not in the lung and spleen of CD30LKO or CD30KO mice.V gamma repertoire analysis revealed that IL-17A producing $Vg1^{-}Vg4^{-}$gd T cells, presumably bearing Vg6, were selectively reduced in PEC, LPL and liver in CD30LKO or CD30KO mice as compared with that in WT mice.gd T cells, mainly $Vg1^{-}Vg4^{-}$gd T cells in PEC and LPL, but not spleen from naïve mice expressed CD30L.We further found that activation of gd T cell producing IL-17A was significantly attenuated, especially $Vg1^{-}Vg4^{-}$gd T cell in PEC of CD30KO or CD30LKO mice compared with WT mice at the early stage of BCG or Listeria infection. The bacteria clearance was markedlyimpaired in PEC and Liver of CD30LKO mice after Listeria infection.

Taken together, it appears that CD30L/CD30 signaling plays an important role in maintenanceand activation of $Vg1^{-}Vg4^{-}$gd T cell subset of naturally occurring IL-17A-producing gd T cells in the periphery, and contributes to IL-17A-producing gd T cell-mediated protection against mycobacterial infection.

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P-30

Regulation of FcεRI-mediated mast cell degranulation by the atypical GEF

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Mast cells are best known as the primary effector cells in type I hypersensitivity reactions, such as allergy and anaphylaxis. Their reaction is mediated through the allergen-specific IgE bound to the high affinity IgE receptors (FcεRI) expressed on the surface. Cross-linking of FcεRI leads to degranulation of mast cells and the release of chemical mediators that trigger allergic reactions. Although many molecules, such as Fyn, Syk, LAT, PLC-γ, and SLP76, have been reported to be involved in the signal transduction mediated by FcεRI, the mechanisms controlling mast cell degranulation are poorly understood.

The CDM (*Caenorhabditis elegans* CED-5, mammals DOCK180, and *Drosophila melanogaster* Myoblast city) family guanine nucleotide exchange factors (GEFs) catalyze the GTP-GDP exchange reaction by means of its DHR-2 domain. We found that one of the CDM family proteins plays a critical role in mast cell functions. I will discuss the mechanism of how this molecule acts downstream of FcεRI in controlling degranulation process.

P-31

Structural basis of functional complex formation between DOCK2 and ELMO1

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DOCK2 is a guanine nucleotide exchange factor belongs to CDM family (*Caenorhabditis elegans* CED-5, mammals DOCK180 *Drosophila melanogaster* Myoblast City). It predominantly expressed in haematopoietic cells and plays critical role in lymphocyte chemotaxis and activation by regulating the actin cytoskeleton through Rac activation. DOCK2, like DOCK180, is tightly bound to ELMO1 through its N-terminal region including SH3 domain. Although complex formation with ELMO1 is essential for the function of DOCK2 and DOCK180, the mechanism by which ELMO1 regulates these GEFs are controversial.

In this study, we present the crystal structure of the complex of the interacting regions of DOCK2 and ELMO1. The C-terminal proline-rich tail of ELMO1 winds around the SH3 domain of DOCK2. Moreover, the α-helical region following the SH3 domain forms an intermolecular five-helix bundle with ELMO1. On the basis of the structure of the complex, we mutated several of the ELMO1-interacting residues in the DOCK2 and identified two residues critical for the complex formation; one was located in the SH3 domain and another in the helix bundle.

To examine the functional significance of the DOCK2-ELMO1 complex, we expressed wild-type DOCK2 and two point mutants that failed to interact with ELMO1 in BW5147$\alpha^-\beta^-$ (a thymoma cell line lacking endogenous DOCK2 expression) and primary T cells derived from *DOCK2*$^{-/-}$ mice. As compared with non-transfected cells, the expression of wild-type DOCK2 significantly increased the migration speed both in BW5147$\alpha^-\beta^-$ and primary T cells. However, the expression of either mutant DOCK2 failed to restore the motility. We will discuss the mechanism of how ELMO1 contribute to the function of DOCK2 in the presentation.

P-32

Functional significance of DOCK2 dimerization in lymphocyte migration

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DOCK2, a guanine nucleotide exchange factor (GEF) predominantly expressed in hematopoietic cells, plays critical roles in lymphocyte migration and activation by rearranging the actin cytoskeleton through Rac activation. DOCK2 belongs to the CDM family (*Caenorhabditis elegans* CED-5, *mammals* DOCK180, and *Drosophila melanogaster* Myoblast city) of atypical GEFs. DOCK2 encodes a unique GEF domain named DHR-2, which is unrelated to the tandem DH/PH domain typically found in GEFs. Other than GEF activity, a part of the DHR-2 domain provides an interface for homodimer formation. However, the functional significance of the dimerization remains unclear. We show here that dimer formation is critical for DOCK2 mediated-cellular functions and will discuss the underlying mechanism.

P-33

New strategy to capture crystal contact-free structures of a ligand as bound to proteins

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Most mitochondrial proteins are encoded in the genomic DNA in the nucleus. After mitochondrial proteins are synthesized in the cytosol, they are imported into mitochondria by protein assemblies, translocase of outer membrane (TOM) complex and translocase of inner membrane (TIM) complex. A subunit of the TOM complex, Tom20, functions as a general protein import receptor by recognizing N-terminal signal sequences (presequences) of mitochondrial proteins. The broad specificity for presequences enables Tom20 to import a wide variety of mitochondrial proteins. We have been studying the promiscuous interaction between Tom20 and presequences using structural biology approach. Based on the past crystallographic and NMR studies[1,2], we proposed that substantial mobility occurred on the sub-millisecond time scale at the interface between Tom20 and presequence peptides was the molecular mechanism to realize the promiscuous recognition of presequences by Tom20. To characterize the residual mobility at an atomic resolution, we are now trying to capture the presequence peptide structure free from the crystal-contact artifacts in Tom20 protein crystals.

To realize contact-free states in protein crystals, we take advantage of fusion with maltose binding protein (MBP). We expect that insertion of linker residues between MBP and Tom20 changes the relative orientation of the two proteins in the fusion protein, and, in a special case, presequence peptides are not involved in the crystal-packing contacts when bound to Tom20. First, we searched an appropriate length of the linker. After a systematic search, we obtained one crystalline form of the fusion protein with two residues inserted in the linker region. This crystal contains two fusion protein molecules in the asymmetric unit. In one molecule, the binding site of Tom20 is blocked by crystal contacts, but the binding site of the other Tom20 is open. At the moment, we are testing the soaking of presequence peptides into the crystal, and co-crystallization with presequence peptides.

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P-34

Regulation of Force Generation by p53 and NF-κB: Mechanical Implication of Cancer Progression

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Tumor suppressor p53 is reported to suppress activity of NF-κB pathway (1). Consistently, NF-κB is constitutively activated in p53-deficient cells as well as numerous malignant cells (2). However, it remains unclear how NF-κB regulates tumor progression such as metastasis. We first examined whether NF-κB was involved in the regulation of force generation that is associated with cell migration, hence invasiveness and metastasis. Using traction force microscopy, we found that the traction forces generated by p53-deficient mouse embryonic fibroblast (*p53*$^{-/-}$ MEFs) were significantly decreased upon NF-κB knockdown or introduction of p53. Since focal adhesion proteins such as integrins, FAK and p130Cas are involved in the transmission of traction forces (3,4), we tested whether NF-κB modulated the functions of these molecules. Knockdown of NF-κB expression affected integrin β3 phosphorylation, while it did not lead to significant alteration of the expression/phosphorylation of p130Cas and FAK. In addition, NF-κB knockdown in *p53*$^{-/-}$ MEFs resulted in a decrease of integrin β3 at focal adhesion sites. Furthermore, traction forces generated by *p53*$^{-/-}$ MEFs were decreased when treated with the integrin β3 blocking antibody. These results suggest that NF-κB up-regulates traction force generation through modification of integrin β3, which is related to metastasis.

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P-35

Cytoplasmic translocation of retinoblastoma protein inhibits sarcomeric organization in human skeletal muscle

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Skeletal muscle wasting, characterized by the progressive depletion of muscle strength, occurs in a variety of chronic diseases such as advanced cancer, congestive heart failure, and AIDS (1). The intercellular mechanism responsible for skeletal muscle wasting is presently thought to be multifactorial and inflammatory cytokines, TNF-α in particular, have been shown to be key mediators of it (1). Elevated level of TNF-α precedes the onset of skeletal muscle wasting and leads to it through several cancer-related signaling pathways including p53 and nuclear factor kappa B (2, 3). Tumor suppressor retinoblastoma protein (Rb) is implicated in cancer progression as well as cancer initiation (4) and plays a pivotal role in the completion of the skeletal muscle differentiation (5). Inactivation of Rb is induced by TNF-α in apoptotic signaling pathways (6), but it is unknown whether alteration of Rb might be caused or effect on skeletal muscle wasting.

We found that TNF-α induced up-regulation of cyclin-dependent kinase 4 (CDK4) and concomitant phosphorylation of Rb in human skeletal muscle myotubes. Phosphorylated Rb translocated into cytoplasm and impaired sarcomeric organization, which is important for biomechanical and contractile properties of skeletal muscle. Human diaphanous-related formin 1 (hDia1), a potent actin nucleation factor, was identified as a binding partner of cytoplasmic Rb and binding of Rb to hDia1 inhibited its actin polymerizing activity. These findings reveal a potential mechanism of skeletal muscle wasting by which TNF-α causes disruption of sarcomeric organization.

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P-36

Mutant p53 regulates p130Cas via cellular mechanical properties in transformed cells

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The tumor suppressor p53 is the most frequently mutated gene in human cancer cells and the mutation is observed especially in the late-stage of cancer, indicating loss of p53 plays integral role in tumoregenesis and malignant progression(1). p130Cas, the Src family kinase substrate, is known to play a crucial role in cancer metastasis and acts as a direct mechano-sensor that converts physical cues into biochemical events (2,3). We hypothesized that biochemical signal induced by mutation of p53 can regulate p130Cas via cellular mechanical properties and participate in tumor progression including metastasis.

Oncogenic Ras induces transformation of NIH3T3 mouse fibroblasts that have wild-type p53 gene, and the cells exhibit feeble spreading with less lamellipodium formation. Since it has been reported that the average cellular traction forces was reduced upon transformation (4) , we examined whether p130Cas was modulated by the transformation. We found that p130Cas phosphorylation was decreased and its proteolysis was excessive in Ha-Ras-transformed cells compared with wild type. To test whether alteration of cellular traction forces modulate p130Cas in transformed cells, biaxial cell stretching was applied to the transformed cells. It was shown that p130Cas proteolysis was attenuated and its phosphorylation was elevated upon cell stretching. Moreover, we found that, in transformed cells, introduction of cancer-related mutant p53 increased the cellular traction forces and partially recovered the down regulation of p130Cas, indicating that alteration of p130Cas was associated with cellular mechanical properties modulated by transformation. These findings support that mutant p53 may promote tumor progression through p130Cas via cellular mechanical properties.

We have identified the candidate protease which is associated with alteration of p130Cas by transformation. Currently, we are investigating the molecular mechanism of p130Cas metabolism by the candidate protease and the link between mutant p53 and the candidate protease during tumor progression.

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P-37

Turnover dynamics of the mechano-sensing protein p130Cas at focal adhesions

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There is accumulating evidence that mechanically induced signal transduction, termed as mechanotransduction, is involved in the regulation of a variety of physiological cell functions. Since focal adhesions (FAs) are the sites where actomyosin-derived tensile forces are concentrated, FA proteins are thought to play significant roles in mechano-sensing.
We recently reported that the FA protein p130Cas (Cas: Crk-associated substrate) acts as a mechano-sensor through mechanical extension-dependent phosphorylation (Sawada et al., 2006, Cell). To further define the role of Cas in the mechanosensation, we analyzed the dynamic localization and turnover of Cas molecules at FAs. Time-lapse fluorescence imaging of mCherry-paxillin demonstrated longer lifetime of FAs in Cas-deficient mouse embryonic fibroblasts (MEFs), compared with those in the control MEFs. FA lifetime was completely rescued by the expression of exogenous Cas (wild type, WT), but not by the expression of the phosphorylation-defective and the Src-binding (SB)-attenuated mutants of Cas, Cas15YF and CasmPR, respectively. Considering the significant decrease in the phosphorylation of CasmPR compared with CasWT, these results suggest that phosphorylation of Cas is involved in the FA dynamics.

Both the N-terminal SH3- and C-terminal SB-domains have been reported to be required for Cas to be localized to FAs (Nakamoto et al, 1997, Mol Cell Biol), suggesting that Cas molecules are anchored to FA complex via those two domains. Although the lifetime of individual FAs was longer than 10 minutes in MEFs, the fluorescence recovery after photobleaching (FRAP) analysis showed that the average recovery time of GFP-CasWT at FAs was shorter than 30 seconds. These results suggest that Cas molecules are constantly subject to rapid turnover within the individual FAs. Furthermore, FRAP analysis using Cas-deficient cells demonstrated lower recovery rate and longer recovery time of GFP-Cas15YF compared with GFP-CasWT, suggesting that release of Cas molecules from FAs depends upon their phosphorylation status. In addition, we found that the recovery time of GFP-CasmPR was not significantly shorter, despite the tempered SB-domain anchoring to FA complex, than that of GFP-CasWT. These results suggest that the disruption of the SH3-domain anchor of Cas to FA complex is dependent on the phosphorylation status of Cas. Together with the exclusive localization of phosphorylated Cas at FAs, phosphatase(s) of Cas may regulate the disruption of the association of Cas SH3-domain with the FA complex.

Thus, the mechano-sensing protein Cas plays a significant role in the regulation of FA dynamics through its phosphorylation-dependent rapid turnover.

P-38

Molecular Basis for Targeting of C-tail-anchored Proteins to Peroxisomes

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C-tail anchored (TA) proteins are a distinct class of membrane proteins that harbor a single transmembrane domain at the extreme C-terminal region and expose their N-terminal functional domains to the cytosol. Although TA proteins are found in all of subcellular membranes facing the cytosol and play pivotal roles in various biological processes, the pathways by which they are targeted to and inserted into specific organellar membranes, including peroxisomal membranes, are not fully defined.

We herein show that knockdown of Pex19p, a predominantly cytosolic protein that functions as a chaperon and/or soluble receptor for newly synthesized peroxisomal membrane proteins (PMPs), eliminates the import of peroxisomal TA proteins (P-TAs) *in vivo*. Pex19p forms complexes with P-TAs in the cytosol. These results indicate that Pex19p is involved in the import of P-TAs as well as non-TA-type PMPs. We also show that P-TAs, which form complexes with Pex19p, are specifically targeted to peroxisomes, onto Pex3p, even under ATP-depleted condition in an import assay using semi-intact mammalian cells. The targeting of P-TAs to peroxisomes is driven by Pex19p-Pex3p interaction. Collectively, these results strongly suggest that P-TAs are, like most PMPs, targeted to peroxisomes in a Pex19p- and Pex3p-dependent manner. Thus, P-TAs share the import pathway with non-TA-type PMPs, in contrast to the TA proteins directed to the ER and mitochondrial outer membrane that do not share the import pathways with non-TA proteins.

P-39

Lipidomics of peroxisome-deficient Chinese hamster ovary cells and fibroblasts from patients with Zellweger syndrome

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Peroxisome is a subcellular organelle that functions in lipid metabolisms including β-oxidation of very long chain fatty acid (VLCFA), plasmalogen biosynthesis, and bile acid synthesis. Zellweger syndrome (ZS) is the most severe disease in peroxisome biogenesis disorders, manifesting the defect of neuronal migration and dysfunction in central nervous system. Deficiencies in peroxisomal metabolisms are thought to be responsible for peroxisome biogenesis disorders including ZS phenotype.

In this study, we analyzed the phospholipid compositions in peroxisome deficient Chinese hamster ovary (CHO) mutant cells and skin fibroblasts from ZS patients using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Plasmalogens were remarkably reduced and phosphatidylethanolamines were inversely increased in CHO mutants and ZS fibroblasts. The level of phophatidylcholine containing VLCFA (VLCPC) was elevated in the mutants. Peroxisomal β-oxidation is required for the biosynthesis of docosahexaenoic acid (DHA, C22:6n-3). LC-MS/MS analysis revealed that total amount of DHA-containing phospholipids containing DHA was decreased in ZS fibroblasts, apparently mainly due to the marked reduction of plasmalogen.

We also performed the lipidomic analysis of ZPEG251 mutant cell defective in alkyl-dihydroxyacetonephosphate synthase (ADAPS), the second step enzyme of plasmalogen biosynthesis localized in peroxisomes. Plasmalogen level severely was decreased in ZPEG251, where VLCPC was highly accumulated, to our surprise. Upon expression of *ADAPS* or by addition of hexadecylglycerol, a plasmalogen precursor, the level of plasmalogens and VLCPC was normalized. The β-oxidation activity for VLCFA was not altered in the ZPEG251 cells. Taken together, these results suggested that plasmalogens likely regulate the biosynthesis of VLCFA.

P-40

Pex11p mediates peroxisomal proliferation in living cells

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Peroxisomes increase the number by growth and division. As the progression of peroxisomal maturation, peroxisomes are to be elongated and then cleaved by the dynamin-like protein1 (DLP1) to form new peroxisomes. In the proliferation process, peroxisomal membrane peroxin Pex11p proteins act as a key player. Three isoforms of Pex11p proteins, Pex11pα, Pex11pβ and Pex11pγ, are identified in mammalian cells. Of the three isoforms, constitutively expressed Pex11pβ is better demonstrated to function in peroxisomal proliferation. In fibroblasts from Pex11pβ-knockout mouse, peroxisomes are decreased in number and morphologically elongated (1). Conversely, overexpression of Pex11pβ gives rise to significant increase in peroxisome number likely by proliferation of peroxisomes in mammalian cells (2). Our group earlier showed that Pex11pβ promotes peroxisomal proliferation by forming a ternary complex with DLP1 and Fis1 (3). We also showed that the *N*-terminal domain of Pex11pβ is involved in the homo-oligomerization of Pex11pβ and is indispensable for the peroxisome-proliferating activity. However, the molecular mechanisms underlying how Pex11pβ functions remains unclear. Here, we show that overexpression of Pex11pβ forms small vesicles from peroxisomes, apparently in a manner dependent of DLP1 and oligomerization of Pex11pβ. We verified the function of endogenous Pex11pβ in cells where peroxisomal fission step was blocked by knocking down of DLP1. In the DLP1-knocked down cells, peroxisomes were elongated and translocated along the endoplasmic reticulum. Pex11pβ formed membrane sub-domain structures on the elongated peroxisomes where matrix PTS1 proteins were discernible like beads on strings. Each compartment appeared to be separated with Pex11pβ, and such segregation became more distinct by the overexpression of Pex11pβ. Taken these results together, it is more likely that in an early step of peroxisomal proliferation, Pex11pβ oligomerizes and forms a sub-domain in peroxisomal membrane that partially segregates luminal space of peroxisome, presumably ready to proceed to fission by DLP1.

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P-41

Functional annotation of small RNAs associated with bacterial Argonaute protein by deep sequencing

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Similar to small RNA playing important role in defending against foreign nucleic acids in eukaryotic organism, prokaryotes have also developed a nucleic acid-based immune system called CRISPR (clustered regularly interspaced short palindromic repeats) that might be functionally analogous to RNA interference in eukaryotes. The CRISPR system, consisting of multiple copies of a short repeat (24-48 nt) sequences separated by similar-sized spacer sequences, incorporates sequences derived from the foreign elements into a small-RNA-based repertoire to recognize and destroy the invader. Central to this immune system is the production of CRISPR-derived RNAs (crRNAs) after transcription of the CRISPR locus (1). Simultaneously, many archaea and bacteria do encode homologs of the major protein components of eukaryotic RNAi machineries, in particularly, Argonaute-PIWI family proteins and Dicer-RNAase III family proteins. T. thermophilus HB8 is one of the model organisms for system biology and structure biology research. Bioinformatics analysis on T. thermophilus genome showed T. thermophilus encodes one Argonaute homolog and no apparent Dicer homolog. The recent crystal structure of T. thermophilus Argonaute protein displays the similar structural fold to other known Argonaute proteins with robust RNAse H activity in vitro (2,3). However, the function of T. thermophilus Argonaute in vivo is largely unknown. Here, after investigation of the small RNA (16-30 nt) profiles derived from the wild-type and Ago-defective lines of T. thermophilus by deep sequencing , several classes of small RNAs are especially abundant in the wildtype compared to its mutant line, especially small RNAs derived from CRISPR loci were dramatic reduced in Ago mutant line. Last but not least, a new class of bacteria small RNA called 19A-RNAs were discovered. 19A-RNA are precisely 19 nucleotides long, begin with a adenylate at 5 terminus but are diversie in their remaining 18 nucleotides. Hence we suggest that T. thermophilus may posse a piwi-like pathway and prokaryotic Argonaute-like protein might bind piwi-like RNA to target and degrade invading mobile elements**.**

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Oral

session1

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session2

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Poster

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SESSION 1: STRUCTURAL BIOLOGY
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Daisuke Koda *(Kyushu-U)*

SESSION 2 : CELL SENSING
Michael Sheetz *(MBI,NUS)*
Lorenz Poellinger *(CSI, NUS)*
Sho Yamasaki *(Kyushu-U)*

SESSION 3 : MEMBRANE & ORGANELLA
Wu Min *(DBS, NUS)*
Go Totsugawa *(Kyushu-U)*

SESSION 4 : OMICS BIOLOGY
Jayantha Gunaratne *(IMCB/CSI, NUS)*
Yasuyuki Ohkawa *(Kyushu-U)*
Chng Wee Joo *(CSI, NUS)*

SESSION 5 :TRANSCRIPTION
Lu Gan *(DBS, NUS)*
Fu Xin-Yuan *(CSI/Dept. Biochem., NUS)*
Kenichiro Morohashi *(Kyushu-U)*

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