Journal of Biochemistry

Vol. 165, No. 3（2019 年 3 月 発 行）

ダイジェスト

JB Reviews
Featured article of the month

Goldfish: an old and new model system to study vertebrate development, evolution and human disease

[1]Yoshihiro Omori and [1]Tetsuo Kon

[1]Laboratory for Molecular and Developmental Biology, Institute for Protein Research, Osaka University, 3–2 Yamadaoka, Suita, Osaka, Japan

The goldfish (*Carassius auratus*) is a domesticated cyprinid teleost closely related to the crucian carp. Goldfish domestication occurred in South China around 1,000 years ago. At least 180 variants and 70 genetically established strains are currently produced. These strains possess diverse phenotypes in body shape, colouration, scales, and fin, eye and hood morphology. These include biologically interesting phenotypes that have not been observed in mutants of zebrafish or medaka. In addition, goldfish strains have been maintained in a non-wild environment for several hundreds of generations, and certain goldfish strains have phenotypes similar to some human diseases. The recent progress in the assembly of the whole-genome sequence of goldfish provides strong tools for a genetic analysis of these phenotypes. The whole-genome duplication (WGD) event occurred in the goldfish genome 8–14 million years ago; this is one of the latest WGD in vertebrates. Goldfish are a useful model for studying genome evolution after the WGD event. This review focuses on the potential for goldfish as a model system in understanding the molecular basis of vertebrate development and evolution and human diseases.

Keywords; chromophore, domestication, goldfish, vertebrate morphology, whole-genome duplication

Molecular function and biological importance of CNNM family Mg^{2+} transporters

[1]Yosuke Funato and [1]Hiroaki Miki

[1]Department of Cellular Regulation, Research Institute for Microbial Diseases, Osaka University, 3–1 Yamadaoka, Suita, Osaka, Japan

Cyclin M (CNNM) family proteins are evolutionarily conserved Mg^{2+} transporters. They extrude Mg^{2+} from cells and maintain intracellular Mg^{2+}levels within the normal range. Moreover, they play an important role in Mg^{2+} (re) absorption in the intestine and kidney by mediating the directional transport of Mg^{2+} across epithelial tissue from the tubular lumen to the body inside. Mg^{2+} efflux is suppressed by the direct binding with phosphatase of regenerating liver (PRL), and the formation of the complex is dynamically regulated by cysteine phosphorylation of PRL. The dysfunction of CNNM family proteins is responsible for inherited hypomagnesemia, as well as various intractable diseases, such as cancer and hypertension. Through multiple functional analyses of CNNM family proteins, the biomedical significance of the proper regulation of Mg^{2+} levels has been elucidated.

Keywords; cancer, cyclin M (CNNM), energy metabolism, Mg^{2+}, phosphatase of regenerating liver (PRL)

BIOCHEMISTRY
Biochemistry General

The N-terminus of EXP2 forms the membrane-associated pore of the protein exporting translocon PTEX in Plasmodium falciparum

[1]Paul R Sanders, [1]Benjamin K Dickerman, [1]Sarah C Charnaud, [1,2,3,4]Paul A Ramsland, [1,2,4]Brendan S Crabb and [1,4]Paul R Gilson

[1]Burnet Institute, Melbourne, VIC, Australia, [2]University of Melbourne, Melbourne, VIC, Australia, [3]RMIT University, Bundoora, VIC, Australia, [4]Monash University, Melbourne, VIC, Australia

In order to facilitate a number of processes including nutrient acquisition and immune evasion, malaria parasites extensively remodel their host erythrocyte. This remodelling is to a large extent accomplished through protein export, a crucial process mediated by the *Plasmodium* translocon for exported proteins (PTEX) translocon which is comprised of three core components, HSP101, PTEX150 and EXP2. EXP2 has been structurally and electrophysiologically shown to form the pore that spans the vacuole membrane enveloping the parasite. Here, we biochemically investigate the structure and function of EXP2. By differential alkylation we provide direct evidence that cysteines C113 and C140 form an intramolecular disulphide bond, while C201 is predominantly in a reduced state. We demonstrate that EXP2 possesses a protease resistant, membrane-associated, N-terminal region of ～20 kDa that does not project into the infected erythrocyte cytosol; however, its C-terminus does project into the vacuole space. We show that a putative transmembrane peptide derived from the N-terminal region of EXP2 is haemolytic and in a polymer-based osmotic protection assay, we demonstrate that this peptide forms a discrete haemolytic pore. This work provides further biochemical insight into the role, function and cellular arrangement of EXP2 as the pore-forming component for protein translocation.

Keywords; EXP2, malaria, Plasmodium falciparum, protein translocon, PTEX

Topic: hemolysis erythrocytes alkylation cytosol disulfides endopeptidases malaria tissue membrane osmosis parasites peptides

plasmodium plasmodium falciparum polymers translocation (genetics) vacuole nutrients proteolytic enzymes host (organism) immune evasion

Quantitative proteomic analyses identifi ed multiple sugar metabolic proteins in soybean under shade stress

[1]Yan Li, [1]Hengke Jiang, [1]Xin Sun, [1]Ahsan Asghar Muhammad, [1]Jiang Liu, [1]Weiguo Liu, [1]Kai Shu, [1]Jing Shang, [1]Feng Yang, [1]Xiaoling Wu, [1]Taiwen Yong, [1]Xiaochun Wang, [1]Liang Yu, [1]Chunyan Liu, [1]Wenyu Yang and [1]Junbo Du

[1]Key Laboratory of Crop Eco-physiology and Farming System in Southwest China (Ministry of Agriculture), College of Agronomy, Sichuan Agricultural University, 211 Huimin Road, Wenjiang district, Chengdu, China

Soybean-based intercropping is important for sustainable agricultural practice on a regional and global scale. However, most soybean varieties use shade avoidance strategy to acquire more light absorption when suffered in canopy shade in intercropping systems, thus reduced the yield of the whole population on a farmland. The mechanisms underlying early response of soybean in shade avoidance is still largely unknown. Here we report our identification of differentially accumulated proteins in shade-sensitive soybean seedlings by global quantitative proteome analysis under white light (WL) and shade conditions. By using Tandem Mass Tag (TMT) labelling and HPLC fractionation followed by high-resolution LC-MS/MS analysis, 29 proteins were found up-regulated and 412 proteins were found down-regulated in soybean seedlings by 2-h shade stress than that by 2-h WL treatment. Multiple differentially expressed proteins are enriched in carbohydrate metabolic process especially in the biosynthetic pathways of cell wall polysaccharides in soybean seedlings by shade stress comparing to those in WL growth conditions. Physiological assays showed that saccharides were rapidly declined in shoot apex of soybean seedlings under a short-term shading. Our results would provide new insights into the mechanisms of shade avoidance responses in soybean seedlings.

Keywords; Glycine max, proteome, saccharides, shade avoidance, soybean

Glycobiology and Carbohydrate Biochemistry

Mucin-type glycosylation as a regulatory factor of amyloid precursor protein processing

[1]Naosuke Nakamura and [1]Akira Kurosaka

[1]Department of Molecular Biosciences, Faculty of Life Sciences, Kyoto Sangyo University, Kamigamo-motoyama, Kita-ku, Kyoto, Japan

Mucin-type O-glycosylation is found not only in mucus proteins, but also in a number of cell membrane and secretory proteins. Several recent studies demonstrate that site-specific O-GalNAc glycosylation plays an important role in regulating protein functions by modulating proteolytic processing. Proteolysis of the amyloid precursor protein (APP) is physiologically important, since cleavages at β and γ positions generate amyloid β (Aβ), a major component in the brain of patients with Alzheimer's disease. Akasaka-Manya *et al.* (Excess APP O-glycosylation by GalNAc-T6 decreases Aβ production. *J Biochem* 2017;161:99–111) showed a specific glycosylation at a site proximal to the β-secretase cleavage site and decreased productions of Aβ1-40 and Aβ1-42 in HEK293T cells transfected with a particular mucin-type glycan initiation enzyme, GalNAc-T6, indicating a novel pharmaceutical strategy to inhibit the production of Aβ through the upregulation of mucin-type O-glycosylation.

Keywords; Alzheimer's disease, amyloid β, amyloid precursor protein, GalNAc-T, mucin-type O-glycosylation

Core fucose is essential glycosylation for CD14-dependent Toll-like receptor 4 and Toll-like receptor 2 signalling in macrophages

[1]Kotarosumitomo Nakayama, [1]Kana Wakamatsu, [1]Hironobu Fujii, [1,2]Shinichiro Shinzaki, [1]Shinji Takamatsu, [3]Shinobu Kitazume, [1,2]Yoshihiro Kamada, [2]Tetsuo Takehara, [4]Naoyuki Taniguchi and [1]Eiji Miyoshi

[1]Department of Molecular Biochemistry and Clinical Investigation, [2]Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, Osaka, Japan, [3]Disease Glycomics Team, Systems Glycobiology Research Group, RIKEN, Saitama, Japan, [4]Department of Glyco-Oncology and Medical Biochemistry, Osaka International Cancer Institute, Osaka, Japan

Core fucosylation, catalysed by α-1,6 fucosyltransferase (FUT8), regulates growth factor receptors in immune function. Although core fucose regulates many immune cell types, few reports confront the association between core fucose activity and an innate immune reaction. Here, we have investigated the function of core fucose in macrophages *in vivo* and *in vitro* using *Fut8*-deficient mice and cells. Following lipopolysaccharide (LPS) stimulation, inflammatory cytokine production in *Fut8*-deficient ($Fut8^{-/-}$) macrophages was suppressed in both *in vivo* and *in vitro* experiments. Because LPS is recognized by Toll-like receptor 4 (TLR4), which induces the signalling cascade, TLR4 signalling was assumed to be impaired in $Fut8^{-/-}$ cells. Flow cytometry analyses revealed, however, that a lack of core fucose reduced the expression of, not TLR4, but CD14, which is necessary for TLR4 endocytosis. Because CD14 is necessary for TLR2 signalling, the immune response of TLR2 was also impaired in $Fut8^{-/-}$macrophages. Moreover, in the dextran sodium sulphate (DSS)-induced murine colitis model, the mice grafted with $Fut8^{-/-}$ bone marrow cells exhibited higher resistance to inflammation than those grafted with $Fut8^{+/+}$ bone marrow cells. These findings indicate that core fucose is essential for CD14-

dependent TLR4 and TLR2 signalling in murine macrophage activity, leading to DSS-induced experimental colitis.

Keywords; core fucose, glycosylation, inflammatory bowel disease, macrophage, Toll-like receptor

Lipid Biochemistry

Identifi cation and biochemical characterization of a second zebrafish autotaxin gene

[1]Ryoji Kise, [1]Ryohei Okasato, [1,2]Kuniyuki Kano, [1,2]Asuka Inoue, [3]Atsuo Kawahara and [1,2]Junken Aoki

[1]Graduate School of Pharmaceutical Sciences, Tohoku University, 6–3 Aoba, Aramaki, Aoba-ku, Sendai, Japan, [2]AMED-LEAP, Japan Agency for Medical Research and Development, 1–7–1 Otemachi, Tokyo, Japan, [3]Graduate School of Medical Science, Center for Medical Education and Sciences, University of Yamanashi, Shimokato 1110, Chuo, Yamanashi, Japan

Autotaxin (ATX) is a secreted enzyme that produces a bioactive lysophospholipid, lysophosphatidic acid (LPA). ATX plays a role in vascular and neural development in embryos but its mechanisms remain unclear. At the beginning of this study, only one zebrafish *atx* gene (*atxa*) was known and had been investigated. In this study, we generated ATX knockout (KO) fish by TALEN targeting *atxa*. Unexpectedly, *atxa* KO fish showed neither vascular defects nor reduction of ATX activity, implying the existence of one or more other ATXs in the genome. By a BLAST search using ATXa protein fragments as a query, we found a genomic sequence that closely resembled *atxa* exons 13, 14 and 15. Consequently, we cloned a cDNA encoding a second zebrafish autotaxin (ATXb), and found that it was transcribed in various tissues. The *atxb* gene encoded a protein of 832 amino acids (compared to 850 amino acids in ATXa) with 60% amino acid identity to ATXa and clustered with ATXs from other species. A recombinant ATXb protein showed lysophospholipase D (lysoPLD) activities with substrate specificities similar to those of ATXa and mammalian ATXs. These results indicate that ATXb is a second zebrafish ATX, which possibly shares redundant roles with ATXa in embryonic development.

Keywords; autotaxin, lysophospholipid, lysophosphatidic acid, zebrafish

Enzymology

Construction and characterization of ribonuclease H2 knockout NIH3T3 cells

[1]Motoki Tsukiashi, [1]Misato Baba, [1]Kenji Kojima, [1]Kohei Himeda, [1]Teisuke Takita and [1]Kiyoshi Yasukawa

[1]Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto, Japan,

Ribonuclease H (RNase H) specifically hydrolyzes the 5′-phosphodiester bonds of the RNA of RNA/DNA hybrid. Both types 1 and 2 RNases H act on the RNA strand of the hybrid, while only type 2 acts on the single ribonucleotide embedded in DNA duplex. In this study, to explore the role of mammalian type 2 RNase H (RNase H2) in cells, we constructed the RNase H2 knockout NIH3T3 cells (KO cells) by CRISPR/Cas9 system. KO cells hydrolyzed RNA strands in RNA/DNA hybrid, but not single ribonucleotides in DNA duplex, while wild-type NIH3T3 cells (WT cells) hydrolyzed both. Genomic DNA in the KO cells was more heavily hydrolyzed than in the WT cells by the alkaline or RNase H2 treatment, suggesting that the KO cells contained more ribonucleotides in genomic DNA than the WT cells. The growth rate of the KO cells was 60% of that of the WT cells. Expression of interferon-stimulated genes (ISGs) in the KO cells was not markedly elevated compared with the WT cells. These results suggest that in NIH3T3 cells, RNase H2 is crucial for suppressing the accumulation of ribonucleotides in genomic DNA but not for the expression of ISGs.

Keywords; genomic DNA, knockout, mammalian RNase H2, NIH3T3, ribonucleotide

MOLECULAR BIOLOGY

Replication and Recombination

Homologous pairing activities of Arabidopsis thaliana RAD51 and DMC1

[1,2]Wataru Kobayashi, [2]Enwei Liu, [2]Hajime Ishii, [3]Sachihiro Matsunaga, [4]Peter Schlögelhofer and [1,2]Hitoshi Kurumizaka

[1]Laboratory of Chromatin Structure and Function, Institute for Quantitative Biosciences, The University of Tokyo, 1–1–1 Yayoi, Bunkyo-ku, Tokyo, Japan, [2]Graduate School of Advanced Science and Engineering, Waseda University, 2–2 Wakamatsu-cho, Shinjuku-ku, Tokyo, Japan, [3]Department of Applied Biological Science, Faculty of Science and Technology, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba, Japan, [4]Department of Chromosome Biology, Max F. Perutz Laboratories, University of Vienna, Vienna BioCenter, A-1030 Vienna, Austria

In eukaryotes, homologous recombination plays a pivotal role in both genome maintenance and generation of genetic diversity. Eukaryotic RecA homologues, RAD51 and DMC1, are key proteins in homologous recombination that promote pairing between homologous DNA sequences. *Arabidopsis thaliana* is a prominent model plant for studying eukaryotic homologous recombination. However, *A. thaliana* RAD51 and DMC1 have not been biochemically characterized. In the present study, we purified *A. thaliana* RAD51 (AtRAD51) and DMC1 (AtDMC1). Biochemical analyses revealed that both AtRAD51 and AtDMC1 possess ATP hydrolyzing activity, filament formation activity and homologous pairing activity *in vitro*. We then compared the homologous pairing activities of AtRAD51 and AtDMC1 with those of the *Oryza sativa* and *Homo sapiens* RAD51 and DMC1 proteins.

Keywords; Arabidopsis thaliana, DMC1, homologous pairing,

homologous recombination, RAD51

CELL
Cytoskeleton, Cell Motility, and Cell Shape

C11ORF74 interacts with the IFT-A complex and participates in ciliary BBSome localization

[1]Mariko Takahara, [2]Masataka Kunii, [1]Kentaro Nakamura, [2]Akihiro Harada, [1]Tomoaki Hirano, [1]Yohei Katoh and [1]Kazuhisa Nakayama

[1]Department of Physiological Chemistry, Graduate School of Pharmaceutical Sciences, Kyoto University, 46–29 Yoshida-Shimoadachi, Sakyo-ku, Kyoto, Japan, [2]Department of Cell Biology, Graduate School of Medicine, Osaka University, 2–2 Yamadaoka, Suita, Osaka, Japan,

Cilia are organelles that serve as cellular antennae. Intraflagellar transport particles containing the IFT-A and IFT-B complexes mediate bidirectional trafficking of ciliary proteins. Particularly, in concert with the BBSome complex, IFT particles play an essential role in trafficking of ciliary G-protein-coupled receptors (GPCRs). Therefore, proteins interacting with the IFT components are potential regulators of ciliary protein trafficking. We here revealed that an uncharacterized protein, C11ORF74, interacts with the IFT-A complex via the IFT122 subunit and is accumulated at the distal tip in the absence of an IFT-A subunit IFT139, suggesting that at least a fraction of C11ORF74 molecules can be transported towards the ciliary tip by associating with the IFT-A complex, although its majority might be out of cilia at steady state. In *C11ORF74*-knockout (KO) cells, the BBSome components cannot enter cilia. However, trafficking of Smoothened or GPR161, both of which are ciliary GPCRs involved in Hedgehog signalling and undergo BBSome-dependent trafficking, was not affected in the absence of C11ORF74. In addition, *C11orf74*/*B230118H07Rik*- KO mice demonstrated no obvious anatomical abnormalities associated with ciliary dysfunctions. Given that C11ORF74 is conserved across vertebrates, but not found in other ciliated organisms, such as nematodes and *Chlamydomonas*, it might play limited roles involving cilia.

Keywords; BBSome, C11ORF74, cilia, IFT-A complex

Vol. 165, No. 4（2019 年 4 月 発 行）

ダイジェスト

JB Reviews
Featured article of the month

Comprehensive analysis of kinase-oriented phospho-signalling pathways

[1]Mutsuki Amano, [1]Tomoki Nishioka, [1]Daisuke Tsuboi, [1]Keisuke Kuroda, [1]Yasuhiro Funahashi, [1]Yukie Yamahashi and [1]Kozo Kaibuchi

[1]Department of Cell Pharmacology, Graduate School of Medicine, Nagoya University, 65 Tsurumai, Showa-ku, Nagoya, Aichi, Japan

Accumulating information on eukaryotic protein phosphorylation implies a large and complicated phospho-signalling network in various cellular processes. Although a large number of protein phosphorylation sites have been detected, their physiological consequences and the linkage between each phosphorylation site and the responsible protein kinase remain largely unexplored. To understand kinase-oriented phospho-signalling pathways, we have developed novel substrate screening technologies. In this review, we described the *in vitro* and *in vivo* screening methods named kinase-interacting substrate screening analysis and kinase-oriented substrate screening analysis, respectively.

Keywords: kinase, phosphoproteomics, protein phosphorylation, signal transduction, substrate

Roles of angiopoietin-like proteins in regulation of stem cell activity

[1,2]Tsuyoshi Kadomatsu and [1,2,3]Yuichi Oike

[1]Department of Molecular Genetics, Graduate School of Medical Sciences, Kumamoto University, 1–1–1 Honjo, Chuo-ku, Kumamoto, Japan, [2]Center for Metabolic Regulation of Healthy Aging (CMHA), Graduate School of Medical Sciences, Kumamoto University, 1–1–1 Honjo, Chuo-ku, Kumamoto, Japan, [3]Core Research for Evolutional Science and Technology (CREST), Japan Agency for Medical Research and Development (AMED), 1–7–1 Otemachi, Chiyoda-ku, Tokyo, Japan

Various types of stem cells reside in the body and self-renew throughout an organism's lifetime. Such self-renewal is essential for maintenance of tissue homeostasis and is co-ordinately regulated by stem cell-intrinsic signals and signals from stem cell niche. Angiopoietin is a niche-derived signalling molecule well known to contribute to maintenance of haematopoietic stem cells (HSCs). Angiopoietin-like proteins (ANGPTLs) are structurally similar to angiopoietin, and recent studies reveal that they function in angiogenesis, lipid and energy metabolism and regulation of inflammation. However, unlike angiopoietins, activities of ANGPTLs in stem cell maintenance have remained unclear. Recently, several studies have reported an association of ANGPTL signalling with stem cell maintenance. Here, we summarize those findings with a focus on HSCs, intestinal stem cells, neural stem cells and cancer stem cells and discuss mechanisms underlying ANGPTL-mediated stem cell maintenance.

Keywords: angiopoietin, angiopoietin-like protein, niche, stem cell, stem cell maintenance

BIOCHEMISTRY

Lipid Biochemistry

The role of intracellular anionic phospholipids in the production of *N*-acyl-phosphatidylethanolamines by cytosolic phospholipase $A_2\varepsilon$

[1]Smriti Sultana Binte Mustafiz, [1]Toru Uyama, [1]Zahir Hussain, [2]Katsuhisa Kawai, [3]Kazuhito Tsuboi, [2]Nobukazu Araki and [1,2]Natsuo Ueda

[1]Department of Biochemistry, [2]Department of Histology and Cell Biology, Kagawa University School of Medicine, 1750–1 Ikenobe, Miki, Kagawa, Japan, [3]Department of Pharmacology, Kawasaki Medical School, 577 Matsushima, Kurashiki, Okayama, Japan

N-Acyl-phosphatidylethanolamines (NAPEs) represent a class of glycerophospholipids and serve as the precursors of bioactive *N*-acylethanolamines, including arachidonoylethanolamide (anandamide), palmitoylethanolamide and oleoylethanolamide. NAPEs are produced in mammals by *N*-acyltransferases, the enzymes which transfer an acyl chain of glycerophospholipids to the amino group of phosphatidylethanolamine. Recently, the ε isoform of cytosolic phospholipase A_2 ($cPLA_2\varepsilon$, also called PLA2G4E) was identified as Ca^{2+}-dependent *N*-acyltransferase. We showed that the activity is remarkably stimulated by phosphatidylserine (PS) *in vitro*. In the present study, we investigated whether or not endogenous PS regulates the function of $cPLA_2\varepsilon$ in living cells. When PS synthesis was suppressed by the knockdown of PS synthases in $cPLA_2\varepsilon$-expressing cells, the $cPLA_2\varepsilon$ level and its *N*-acyltransferase activity were significantly reduced. Mutagenesis studies revealed that all of C2, lipase and polybasic domains of $cPLA_2\varepsilon$ were required for its proper localization as well as the enzyme activity. Liposome-based assays showed that several anionic glycerophospholipids, including PS, phosphatidic acid and phosphatidylinositol 4,5-bisphosphate, enhance the Ca^{2+}-dependent binding of purified $cPLA_2\varepsilon$ to liposome membrane and stimulate its *N*-acyltransferase activity. Altogether, these results suggested that endogenous PS and other anionic phospholipids affect the localization and enzyme activity of $cPLA_2\varepsilon$.

Keywords: *N*-acylethanolamine, *N*-acyl-phosphatidylethanolamine, *N*-acyltransferase, lipid mediator, phosphatidylserine

Enzymology

Enzymatic characteristics of two adenylate kinases, AdkA and AdkB, from Myxococcus xanthus

[1]Yoshio Kimura, [1]Hiroyuki Yamamoto and [1]Shiori Kamatani

[1]Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, Ikenobe 2393, Miki-cho, Kagawa, Japan

Adenylate kinase (Adk) plays a critical role in energy metabolism and adaptation of bacteria to environmental stresses. We have previously shown that *Myxococcus xanthus* expresses polyphosphate kinase 1 (Ppk1) that also has Adk activity in the absence of polyphosphates. In this study, we investigated the Adk activity of the other two *M. xanthus* enzymes, AdkA and AdkB. The activity of AdkA was increased by dithiothreitol (DTT), which also enhanced enzyme stability. Site-directed mutagenesis of three cysteine residues (C130, C150, and C153) present in the LID domain of AdkA revealed that the Adk activity and stability of C150S and C153S mutants were not affected by DTT addition, suggesting formation of a disulfide bond between C150 and C153 in AdkA. The K_m of AdkA for AMP was 8 and 17 times lower than that for ADP and ATP, respectively. AdkB is a polyphosphate kinase 2 (Ppk2) homolog lacking the Ppk2 middle region and, consequently, Ppk activity. According to our analysis, AdkB also had Adk activity and its affinity for substrates was higher than that of AdkA. Thus, *M. xanthus* expresses three enzymes, AdkA, AdkB, and Ppk1, with Adk activity, which may function to support energy metabolism of the bacteria in different environmental conditions.

Keywords: adenylate kinase, disulfide bond, Myxococcus xanthus, polyphosphate kinase 2 homolog, substrate affinity

Metabolism and Bioenergetics

Lactate dehydrogenase C is required for the protein expression of a sperm-specific isoform of lactate dehydrogenase A

[1,2]Mina Dodo, [1]Hiroshi Kitamura, [3]Hiroki Shima, [4]Daisuke Saigusa, [1]Sisca Meida Wati, [1]Nao Ota [4]Fumiki Katsuoka, [5]Hatsune Chiba, [5]Hiroaki Okae, [5]Takahiro Arima, [3]Kazuhiko Igarashi, [2]Takeyoshi Koseki, [1]Hiroki Sekine and [1]Hozumi Motohashi

[1]Department of Gene Expression Regulation, Institute of Development, Aging and Cancer, Tohoku University, 4–1 Seiryo-machi, Aoba-ku, Sendai, Japan, [2]Department of Preventive Dentistry, Tohoku University Graduate School of Dentistry, 4–1 Seiryo-machi, Aoba-ku, Sendai, Japan, [3]Department of Biochemistry, Tohoku University Graduate School of Medicine, 2–1 Seiryo-machi, Aoba-ku, Sendai, Japan, [4]Department of Integrative Genomics, Tohoku Medical Megabank Organization, Tohoku University, 2–1 Seiryo-machi, Aoba-ku, Sendai, Japan, [5]Department of Informative Genetics, Tohoku University Graduate School of Medicine, 2–1 Seiryo-machi, Aoba-ku, Sendai, Japan

Metabolites are sensitive indicators of moment-to-moment cellular status and activity. Expecting that tissue-specific metabolic signatures unveil a unique function of the tissue, we examined metabolomes of mouse liver and testis and found that an unusual metabolite, 2-hydroxyglutarate (2-HG), was abundantly accumulated in the testis. 2-HG can exist as D- or L-enantiomer, and both enantiomers interfere with the activities of 2-oxoglutarate (2-OG)-dependent dioxygenases, such as the Jumonji family of histone demethylases. Whereas D-2-HG is produced by onco-

genic mutants of isocitrate dehydrogenases (IDH) and known as an oncometabolite, L-2-HG was the major enantiomer detected in the testis, suggesting that a distinct mechanism underlies the testicular production of this metabolite. We clarified that lactate dehydrogenase C (LDHC), a testis-specific lactate dehydrogenase, is responsible for L-2-HG accumulation by generating and analysing *Ldhc*-deficient mice. Although the inhibitory effects of 2-HG on 2-OG-dependent dioxygenases were barely observed in the testis, the LDHA protein level was remarkably decreased in *Ldhc*-deficient sperm, indicating that LDHC is required for LDHA expression in the sperm. This unique functional interaction between LDH family members supports lactate dehydrogenase activity in the sperm. The severely impaired motility of *Ldhc*-deficient sperm suggests a substantial contribution of glycolysis to energy production for sperm motility.

Keywords: 2-hydroxyglutarate (2-HG), lactate dehydrogenase A (LDHA), lactate dehydrogenase C (LDHC), metabolism, sperm motility

Biochemistry in Cell Membranes

Repression of liver cirrhosis achieved by inhibitory effect of miR-454 on hepatic stellate cells activation and proliferation via Wnt10a

[1]Yong-Zhen Wang, [2]Wei Zhang, [3]Yan-Hua Wang, [4]Xi-Lin Fu and [1]Chen-Qi Xue

[1]Department of Interventional Radiology and Vascular Surgery, Nanjing Second Hospital, Nanjing University of Chinese Medicine, Nanjing, China, [2]College of Animal Science and Veterinary Medicine, Henan Institute of Science and Technology, Xinxiang, Henan, [3]Department of Ultrasound, Nanjing Second Hospital, Nanjing University of Chinese Medicine, Nanjing, China, [4]Department of Hepatopathy, Nanjing Second Hospital, Nanjing University of Chinese Medicine, Nanjing, China

As is known, hepatic stellate cells (HSCs) activation contributes to liver cirrhosis. This study aims to find out the acting mechanisms of miR-454 inhibiting the activation and proliferation of hepatic stellate cells. The expression of Col1A1, α-smooth muscle actin (α-SMA) and Wnt10a were determined by western blot, and the miR-454 level was determined by quantitative real-time PCR in this study. We took two objects as experiment subjects, one was liver cirrhosis rats, and the other was transforming growth factor (TGF)-β1-stimulated HSC-T6 cells. After activated with TGF-β1 and transfected with microRNA-454 mimic, separately or successively, the changes on the Col1A1 and α-SMA expression, HSC proliferation, miR-454 level and Wnt10a expression were examined in HSC-T6 cells, respectively. Interaction between miR-454 and Wnt10a was evaluated with dual luciferase reporter assay. MiR-454 expression was down-regulated in tissues of liver cirrhosis rats. TGF-β1 caused the down-regulation of the miR-454 in HSC-T6 cells. MiR-454 inhibited the activation and proliferation of HSC-T6 cells. Wnt10a had a targeting relationship with miR-454. TGF-β1 promoted HSC-T6 activation and proliferation via down-regulating miR-454 expression, which further up-regulated Wnt10a expression. MiR-454 mimic inhibited cirrhosis progression in liver cirrhosis rats. MiR-454 can inhibit the activation and proliferation of HSCs via suppressing the expression of Wnt10a, to restrain liver cirrhosis.

Keywords: hepatic stellate cell, liver cirrhosis, miR-454, TGF-β1, Wnt10a

Biochemistry in Diseases and Aging

Epigenetic interplays between DNA demethylation and histone methylation for protecting oncogenesis

[1]Kanji Furuya, [2]Masae Ikura and [2]Tsuyoshi Ikura

[1]Laboratory of Genome Maintenance, [2]Laboratory of Chromatin Regulatory Network, Department of Genome Biology, Radiation Biology Center, Graduate School of Biostudies, Kyoto University, Yoshida Konoe-cho, Sakyo-ku, Kyoto, Japan

Epigenetic systems are organized by different types of modifications on histones and DNA. To determine how epigenetic systems can produce variable, yet stable cellular outcomes, understanding the collaboration between these modifications is the key. A recent study by Yamagata and Kobayashi revealed the direct interplay between the regulation of two epigenetic modifications: DNA de-methylation by TET2 and histone H3-K36 methylation. Mechanistically, this finding could explain how cells are protected from oncogenesis by maintaining the integrity of active transcription. The recent identification of epigenetic modifier mutations in leukaemia suggested that it is not just the turning 'on' and 'off' of particular transcriptional events that causes disease occurrence, but rather it is the aberration in epigenetic regulation, i.e. the timing and duration of the activation/inactivation of these transcripts. Thus, a comprehensive understanding of how epigenetic interplays tune transcription will be the new perspective for disease research.

Keywords: DNA methylation, epigenetic, histone methylation, oncogenesis, TET

Neurochemistry

A DISC1 point mutation promotes oligomerization and impairs information processing in a mouse model of schizophrenia

[1]Kyosuke Kakuda, [2]Ayumi Niwa, [1]Ryo Honda, [3]Kei-ichi Yamaguchi, [2]Hiroyuki Tomita, [4]Md Nojebuzzaman, [2]Akira Hara, [3]Yuji Goto, [4]Masatake Osawa and [1]Kazuo Kuwata

[1]United Graduate School of Drug Discovery and Medical Information Sciences, Gifu University, 1–1 Yanagido, Gifu, [2]Department of Tumor Pathology, Gifu University Graduate School of Medicine, 1–1 Yanagido, Gifu, [3]Institute for Protein Research,

Osaka University, 3–2 Yamadaoka, Suita, Osaka, [4]Division of Regeneration Technology, Gifu University Graduate School of Medicine, Gifu, Japan

Disrupted-in-schizophrenia 1 (DISC1) is strongly associated with schizophrenia, but it remains elusive how the modification of the intermolecular interaction of DISC1 affects the information processing in brain. We show that a DISC1 point mutation alters intermolecular cohesiveness promoting the phase separation, and disrupts sensorimotor gating monitored by the prepulse inhibition in a mouse model of schizophrenia. Although the conformation of DISC1 partial peptide with the schizophrenia-related mutation L607F in human or the corresponding L604F in mouse was essentially indistinguishable from the wild type (WT) as long as monitored by fluorescence, circular dichroism, ultracentrifugation, dynamic light scattering and nuclear magnetic resonance, the atomic force microscopy was able to detect their morphological distinctions. The WT peptides were round and well dispersed, while mutants were inhomogeneous and disrupted to form dimer to trimer that aligned along one direction without apparent aggregate formation. Homozygous L604F mutant mice created by CRISPR exhibited the significant decrease in DISC1 level in the immunohistopathology at the hippocampal region compared to the WTs. The ratio of prepulse inhibition of the homozygous mutant mice was significantly impaired compared to WTs. Altered DISC1 distribution or function caused by aberrant intermolecular interactions may contribute to information processing characteristics in schizophrenia.

Keywords: atomic force microscopy, DISC1 L607F mutation, immunohistochemistry, oligomerization, prepulse inhibition

MOLECULAR BIOLOGY

Molecular Biology General

SMAD3 silencing enhances DNA damage in radiation therapy by interacting with MRE11-RAD50-NBS1 complex in glioma

[1]Zheng Jiang, [2]Yan Guo, [3]Lifeng Miao, [1]Lizhang Han, [4]Wei Zhang and [1]Yuquan Jiang

[1]Department of Neurosurgery, Qilu Hospital of Shandong University, No. 107 Wenhua West Road, Lixia District, Jinan, Shandong Province, P. R. China, [2]Department of Internal Medicine, People's Hospital of Qingzhou, No. 1726 Linglongshan Road, Qingzhou, Shandong Province, P. R. China, [3]Department of Neurosurgery, Dezhou People Hospital, No. 1751 Xinhu Street, Dezhou, Shandong Province, P. R. China, [4]Department of Neurosurgery, Weifang Yidu Central Hospital, No. 4138 Linglongshan Road, Qingzhou, Shandong Province, P. R. China

Radiotherapy is the major treatment modality for malignant glioma. However, the treatment response of radiotherapy is suboptimal due to resistance. Here we aimed to explore the effect and mechanism of Mothers against decapentaplegic homologue (*SMAD3*) silencing in sensitizing malignant glioma to radiotherapy. Clonogenic assay was used to evaluate the sensitivity of glioma cells to increasing doses of radiation. Glioma cells were transfected with small-interfering RNAs (siRNAs) specific to *SMAD3*. Overexpression of *SMAD3* was achieved by transfecting expression plasmid encoding *SMAD3* cDNA. Changes in *MRE11-RAD50-NBS1* mRNA and protein levels were assessed through qPCR analysis and western blot analysis, respectively. Chromatin immunoprecipitation (ChIP) was used to confirm the interaction between *SMAD3* and *MRE11-RAD50-NBS1* (MRN) complex. Silencing of *SMAD3* increased sensitivity of glioma cells to radiotherapy. *MRE11, RAD50* and *NBS1* were overexpressed in response to radiotherapy, which was attenuated by *SMAD3*silencing while boosted by *SMAD3* overexpression. ChIP analysis confirmed the interaction of *SMAD3* with *MRE11, RAD50* and *NBS1* under radiotherapy, which was inhibited by *SMAD3* silencing. *SMAD3* silencing is an effective strategy for sensitizing glioma to radiotherapy, which is mediated by the interaction of *SMAD3* with the MRN complex.

Keywords: DNA damage, glioma, radiation therapy, RAD50, SMAD3

Possible cold-adaptation for the fungal kinesin in compensation for thermal stability acquired by single amino acid substitution

[1]Youské Shimizu, [1]Toru Togawa and [1]Shigeru Chaen

[1]Department of Biosciences, College of Humanities and Sciences, Nihon University, 3–25–40 Sakurajosui, Setagaya-ku, Tokyo, Japan

The amino acid sequence of the motor domain of AnKinA, kinesin-1 from *Aspergillus nidulans*, growing optimally at 37°C, was compared with that of SbKin1, kinesin-1 from the snow mold *Sclerotinia borealis*. For cold-adaptation, some enzymes are thought to exhibit augmented protein structure flexibility, acquired most effectively by substituting a glycine residue for another amino acid residue. By the comparison described above, two glycine residues proximal to tightly bound ADP were identified in the SbKin1 motor domain, of which the corresponding residues of AnKinA were non-glycine ones (P60 and S323). We made AnKinA recombinant kinesin (AnKinA-WT (WT)) along with P60G and S323G mutants. From the basal ATPase activity (without microtubules), these kinesins showed similar characteristics in activation energies, while deviation from the linearity of the ATPase activity time-course was detected at 34°C for WT and P60G but at 24°C for S323G. The microtubule translocation velocity of WT, P60G or S323G exhibited an activation energy of 60, 58 or 53 kJ/mol, respectively; for S323G, the activation energy was lower and the velocity at low temperatures was higher than those for the other two. These results suggest that the point mutation S323G would offer possible cold-adaptation in

compensation for thermal stability.

Keywords: activation energy, cold-adaptation, kinesin, temperature dependence, thermal stability

Gene Expression

KDM5D-mediated H3K4 demethylation is required for sexually dimorphic gene expression in mouse embryonic fibroblasts

[1,2]Hayase Mizukami, [1]Jun-Dal Kim, [3]Saori Tabara, [4]Weizhe Lu, [2]Chulwon Kwon, [2]Misaki Nakashima and [1,4]Akiyoshi Fukamizu

[1]Life Science Center for Survival Dynamics, Tsukuba Advanced Research Alliance (TARA), [2]Graduate School of Life and Environmental Sciences, [3]College of Agro-Biological Resource Sciences, [4]Ph.D. Program in Human Biology, School of Integrative Global Majors (SIGMA), [4]International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, 1–1–1 Tennodai, Tsukuba, Ibaraki, Japan

Males and females share the same genetic code, but gene expression profile often displays differences between two sexes. Mouse embryonic fibroblasts (MEFs) have been used to experiment as a useful tool to test gene function. They have also been characterized by gender-based differences in expressed genes such as Y-linked *Sry* or X-linked *Hprt*. However, there is no report on sex differences in global gene expression. Here, using the next-generation RNA sequencing, we compared the comprehensive transcriptome of MEFs derived from two sexes. In comparison with the female group, the male group up-regulated 27 differentially expressed genes (DEGs), in which a male-specific histone demethylase KDM5D gene is included, and 7 DEGs were down-regulated. Based on the results by searching the ENCODE analysis, it was shown that the expression of 15 genes identified is potentially regulated by the methylation of H3K4me1 or H3K4me3. Interestingly, we demonstrated that both of H3K4 methylation are induced by knocking down KDM5D, which causes changes in patterns of eight DEGs found in male MEFs. Collectively, these data not only suggest an importance of KDM5D-mediated demethylation of H3K4 involved in the sexually dimorphic gene expression in male MEFs, but also may provide information regarding sex-dependent changes in gene expression when MEFs are used for experiments.

Keywords: H3K4 demethylation, KDM5D, MEFs, RNA-Seq, sexually dimorphic gene expression