Journal of Biochemistry

Vol. 175, No. 2（2024 年 2 月 発 行）

ダイジェスト

JB SPECIAL ISSUE:

DYNAMIC ZONING OF CYTOPLASM BY MEMBRANES AND PHASE SEPARATION

GUEST EDITORS: NOBUO N. NODA AND MASAAKI KOMATSU

JB Special Issue-Reviews

Shaping transverse-tubules: central mechanisms that play a role in the cytosol zoning for muscle contraction

Kohei Kawaguchi[1] and Naonobu Fujita[1,2]
[1]Cell Biology Center, Institute of Innovative Research, Tokyo Institute of Technology, 4259 S2–11 Nagatsuta-cho, Midori-ku, Yokohama 226–8503, Japan; and [2]Graduate School of Life Science and Technology, Tokyo Institute of Technology, 4259 S2–11 Nagatsuta-cho, Midori-ku, Yokohama 226–8503, Japan

A transverse-tubule (T-tubule) is an invagination of the plasma membrane penetrating deep into muscle cells. An extensive membrane network of T-tubules is crucial for rapid and synchronized signal transmission from the cell surface to the entire sarcoplasmic reticulum for Ca^{2+} release, leading to muscle contraction. T-tubules are also in dispensable for the formation and positioning of other muscle organelles. Their structure and physiological roles are relatively well established; however, the mechanisms shaping T-tubules require further elucidation. Centronuclear myopathy (CNM), an inherited muscular disorder, accompanies structural defects in T-tubules. Membrane traffic-related genes, including MTM1 (Myotubularin 1), DNM2 (Dynamin 2), and BIN1 (Bridging Integrator-1), were identified as causative genes of CNM. In addition, causative genes for other muscle diseases are also reported to be involved in the formation and maintenance of T-tubules. This review summarizes current knowledge on the mechanisms of how T-tubule formation and maintenance is regulated.

Keywords: BIN1/Amph, dynamin, MTM, T-tubule, tubulation

Cytoplasmic zoning in membrane blebs

Yuki Fujii and Junichi Ikenouchi
Department of Biology, Faculty of Sciences, Kyushu University, Nishi-ku, Fukuoka 819–0395, Japan

Blebs are membrane structures formed by the detachment of the plasma membrane from the underlying actin cytoskeleton. It is now clear that a wide variety of cells, including cancer cells, actively form blebs for cell migration and cell survival. The expansion of blebs has been regarded as the passive ballooning of the plasma membrane by an abrupt increase in intracellular pressure. However, recent studies revealed the importance of 'cytoplasmic zoning', i.e. local changes in the hydro dynamic properties and the ionic and protein content of the cytoplasm. In this review, we summarize the current understanding of the molecular mechanisms behind cytoplasmic zoning and its role in bleb expansion.

Keywords: actin cortex, amoeboid migration, bleb, cytoplasmic zoning

p62 bodies: cytosolic zoning by phase separation

Reo Kurusu[1], Hideaki Morishita[1,2] and Masaaki Komatsu[1]
[1]Department of Physiology, Juntendo University Graduate School of Medicine, Bunkyo-ku, Tokyo 113–8421, Japan; and [2]Graduate School of Medical Sciences, Kyushu University, Higashi-ku, Fukuoka 812–8582, Japan

Cellular zoning or partitioning is critical in preventing macromolecules from random diffusion and orchestrating the spatiotemporal dynamics of biochemical reactions. Along with membranous organelles, membrane less organelles contribute to the precise regulation of biochemical reactions inside cells. In response to environmental cues, membrane less organelles rapidly form through liquid–liquid phase separation, sequester certain proteins and RNAs, mediate specific reactions and dissociate. Among membrane less organelles, ubiquitin-positive condensates, namely, p62 bodies, maintain cellular homeostasis through selective autophagy of themselves to contribute to intracellular quality control. p62 bodies also activate the antioxidative stress response regulated by the KEAP1NRF2system. In this review, we present an overview of recent advancements in cellular and molecular biology related to p62 bodies, high lighting their dynamic nature and functions.

Keywords: autophagy, intracellular quality control, KEAP1-NRF2 system, liquid–liquid phase separation, p62 body

Cytoplasmic zoning by protein phase transition after membrane permeabilization

Shinju Sugiyama, Kojiro Suda and Keiko Kono
Okinawa Institute of Science and Technology Graduate University, 1919–1 Tancha, Onna, Okinawa 904–0495, Japan

Biological membranes, including plasma membrane (PM) and organelle membranes, restrict the flux of ions, molecules and organelles. However, the barrier function of biological membranes is frequently compromised by various perturbations, including physical membrane damage and protein- or chemical-induced pore formation. Recent evidence suggests that, upon PM damage, protein gelation and solid condensation are utilized to restrict ion/molecule/organelle flux across the damaged mem-

branes by zoning the cytoplasm. In addition, membrane permeabilization dramatically alters intramembrane and extramembrane ion/molecule concentrations via the flux across the permeabilized membrane. The changes in ion/molecule concentration and their downstream pathways induce protein phase transition to form zones for biological processes or protein sequestration. Here, we review the mechanisms and functions of protein phase transition after biological membrane permeabilization.
Keywords: cellular wound healing, cytoplasmic zoning, liquid–liquid phase separation, membrane permeabilization, phase transition

Molecular mechanism of autophagy, cytoplasmic zoning by lipid membranes

Tetsuya Kotani[1], Yuri Yasuda[2] and Hitoshi Nakatogawa[1,2]
[1]Cell Biology Center, Institute of Innovative Research, Tokyo Institute of Technology, S2–14 4259 Nagatsuta-cho, Midori-ku, Yokohama, Kanagawa 226–8501, Japan; and [2]School of Life Science and Technology, Tokyo Institute of Technology, S2–14 4259 Nagatsuta-cho, Midori-ku, Yokohama, Kanagawa 226–8501, Japan
Autophagy is a highly conserved intracellular degradation mechanism. The most distinctive feature of autophagy is the formation of double-membrane structures called autophagosomes, which compartmentalize portions of the cytoplasm. The outer membrane of the autophagosome fuses with the vacuolar/lysosomal membrane, leading to the degradation of the contents of the autophagosome. Approximately 30 years have passed since the identification of autophagy-related (ATG) genes and Atg proteins essential for autophagosome formation, and the primary functions of these Atg proteins have been elucidated. These achievements have significantly advanced our understanding of the mechanism of autophagosome formation. This article summarizes our current knowledge on how the autophagosome precursor is generated, and how the membrane expands and seals to complete the autophagosome.
Keywords: autophagosome, autophagy, intracellular degradation, membrane dynamics, vacuole/lysosome

Mechanisms of mitochondrial reorganization

Tatsuro Maruyama[1], Yutaro Hama[1,2] and Nobuo N. Noda[1,2]
[1]Institute of Microbial Chemistry (BIKAKEN), 3–14–23 Kamiosaki, Shinagawa-ku, Tokyo 141–0021, Japan; and [2]Institute for Genetic Medicine, Hokkaido University, Kita 15, Nishi 7, Kita-ku, Sapporo 060–0815, Japan
The cytoplasm of eukaryotes is dynamically zoned by membrane-bound and membrane less organelles. Cytoplasmic zoning allows various biochemical reactions to take place at the right time and place. Mitochondrion is a membrane-bound organelle that provides a zone for intracellular energy production and metabolism of lipids and iron. A key feature of mitochondria is their high dynamics: mitochondria constantly undergo fusion and fission, and excess or damaged mitochondria are selectively eliminated by mitophagy. Therefore, mitochondria are appropriate model systems to understand dynamic cytoplasmic zoning by membrane organelles. In this review, we summarize the molecular mechanisms of mitochondrial fusion and fission as well as mitophagy unveiled through studies using yeast and mammalian models.
Keywords: cytoplasmic zoning, mitochondria, mitochondrial fission, mitochondrial fusion, mitophagy

Multi-dimensional condensation of intracellular biomolecules

Masataka Yanagawa[1,2] and Shunsuke F. Shimobayashi[3]
[1]Molecular and Cellular Biochemistry, Graduate School of Pharmaceutical Sciences, Tohoku University, 6–3 Aoba, Aramaki, Aoba-ku, Sendai, Miyagi 980–8578, Japan; [2]Cellular Informatics Laboratory, RIKEN Cluster for Pioneering Research, Wako, Saitama 351–0198, Japan; and [3]Department of Life Science Frontiers, Center for iPS Cell Research and Application, Kyoto University, 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606–8507, Japan
Liquid–liquid phase separation has been recognized as universal mechanisms in living cells for the formation of RNA-protein condensates and ordered lipid domains. These biomolecular condensates or domains nucleate, diffuse and interact with each other across physical dimensions to perform their biological functions. Here we summarize key features of biophysical principles underlying the multi-dimensional condensation of RNA-protein condensates and ordered lipid domains, which are related to nuclear transcription, and signaling on cell membranes. Uncovering physicochemical factors that govern the spatiotemporal coupling of those condensates presents a new avenue in their functions and associated human diseases.
Keywords: biomolecular condensates, diffusion, liquid–liquid phase separation, nucleation and growth, signaling

In situcryo-electron tomography: a new method to elucidate cytoplasmic zoning at the molecular level

Lin Chen[1,2,3], Yuko Fukata[2,5] and Kazuyoshi Murata[1,2,4]
[1]Exploratory Research Center on Life and Living Systems (ExCELLS), National Institutes of Natural Sciences, 38 Nishigonaka, Myodaiji, Okazaki 444–8585, Japan; [2]National Institute for Physiological Sciences, National Institutes of Natural Sciences, 38 Nishigonaka, Myodaiji, Okazaki 444–8585, Japan; [3]School of life sciences, Zhejiang Chinese Medical University, No. 548 Binwen Road, Binjiang District, Hangzhou 310053, China; [4]Department of Physiological Sciences, School of Life Science, The Graduate University for Advanced Studies (SOKENDAI),

38 Nishigonaka, Myodaiji, Okazaki 444–8585, Japan; and 5Molecular and Cellular Pharmacology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466–8550, Japan

Cryo-electron microscopy was developed as a powerful tool for imaging biological specimens in near native conditions. Nowadays, advances in technology, equipment and computations make it possible to obtain structures of biomolecules with near-atomic resolution. Furthermore, cryo-electron tomography combined with continuous specimen tilting allows structural analysis of heterogeneous biological specimens. In particular, when combined with a cryo-focused ion beam scanning electron micro scope, it becomes possible to directly analyse the structure of the biomolecules within cells, a process known as in situ cryo-electron tomography. This technique has the potential to visualize cytoplasmic zoning, involving liquid–liquid phase separation, caused by biomolecular networks in aqueous solutions, which has been the subject of recent debate. Here, we review advances in structural studies of biomolecules to study cytoplasmic zoning by in situ cryo-electron tomography.

Keywords: cryo-electron microscopy, cryo-electron tomography, cryo-focused ion beam scanning electron microscope, cytoplasmic zoning, liquid–liquid phase separation

RAPID COMMUNICATION

Identification of effective CCR2 inhibitors for cancer therapy using humanized mice

Shigeaki Sugiyama[1], Kanae Yumimoto[1], Shun Fujinuma[1] and Keiichi I. Nakayama[1,2]

[1]Department of Molecular and Cellular Biology, Medical Institute of Bioregulation, Kyushu University, 3–1–1 Maidashi, Higashi-ku, Fukuoka, Fukuoka 812–8582, Japan; and [2]Anticancer Strategies Laboratory, TMDU Advanced Research Institute, Tokyo Medical and Dental University, 1–5–45 Yushima, Bunkyo-ku, Tokyo 113–8510, Japan

C-C chemokine receptor type 2 (CCR2) is the receptor for C-C motif chemokine 2 (CCL2) and is associated with various inflammatory diseases and cancer metastasis. Although many inhibitors for CCR2 have been developed, it remains unresolved which inhibitors are the most effective in the clinical setting. In the present study, we compared 10 existing human CCR2 antagonists in a calcium influx assay using human monocytic leukemia cells. Among them, MK0812 was found to be the most potent inhibitor of human CCR2. Furthermore, we generated a human CCR2B knock-in mouse model to test the efficacy of MK0812 against a lung metastasis model of breast cancer. Oral administration of MK0812 to humanized mice did indeed reduce the number of monocytic myeloid-derived suppressor cells and the rate of lung metastasis. These results suggest that MK0812 is the most promising candidate among the commercially available CCR2 inhibitors. We propose that combining these two screening methods may provide an excellent experimental method for identifying effective drugs that inhibit human CCR2.

Keywords: cancer metastasis, C-C chemokine receptor type 2 (CCR2), knock-in mice, knockout mice, monocytic myeloid-derived suppressor cells (M-MDSCs)

The largest subunit of human TFIIIC complex, TFIIIC220, a lysine acetyltransferase targets histone H3K18

Moumita Basu, Rohini Bhatt, Anjali Sharma, Ramachandran Boopathi

Sadhan Das*,‡ and Tapas K Kundu† Transcription and Disease Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore-560064, India

TFIIIC is a multi-subunit complex required for tRNA transcription by RNA polymerase III. Human TFIIIC holo-complex possesses lysine acetyltransferase activity that aids in relieving chromatin-mediated repression for RNA polymerase III-mediated transcription and chromatin assembly. Here we have characterized the acetyltransferase activity of the largest and DNA binding subunit of TFIIIC complex, TFIIIC220. Purified recombinant human TFIIIC220 acetylated core histones H3, H4 and H2A in vitro. Moreover, we have identified the putative catalytic domain of TFIIIC220 that efficiently acetylates core histones in vitro. Mutating critical residues of the putative acetyl-CoA binding ‘P loop’ drastically reduced the catalytic activity of the acetyltransferase domain. Further analysis showed that the knockdown of TFIIIC220 in mammalian cell lines dramatically reduces global H3K18 acetylation level, which was rescued by overexpression of the putative acetyltransferase domain of human TFIIIC220. Our findings indicated a possibility of a crucial role for TFIIIC220 in maintaining acetylation homeostasis in the cell.

Keywords: acetyltransferase, C646, embelin, H3K18ac, TFIIIC220

Journal of Biochemistry

Vol. 175, No. 3（2024 年 3 月 発 行）

ダイジェスト

JB COMMENTARY

Mitochondrial lipid dynamics regulated by MITOL-mediated ubiquitination

Koji Yamano[1], Hiroki Kinefuchi[1,2] and Waka Kojima[1]

[1]Department of Biomolecular Pathogenesis, Medical Research Institute, Tokyo Medical and Dental University, 1–5–45 Yushima, Bunkyo-ku, Tokyo 113–8510, Japan; and [2]Department of

Biosciences, School of Science, Kitasato University, 1–15–1 Kitasato, Minami-ku, Sagamihara, Kanagawa 252–0373, Japan

Mitochondria-endoplasmic reticulum (ER) contact sites in mammals provide platforms for various reactions, such as calcium signaling, lipid metabolism, organelle dynamics and autophagy. To fulfill these tasks, a number of proteins assemble at the contact sites including MITOL/MARCHF5, a critic all mitochondrial ubiquitin ligase. How MITOL regulates mitochondrial function from the contact site, however, has been largely unresolved. Recently, a new role for MITOL in the active transport of phosphatidic acid from the ER to mitochondria was reported. In this commentary, we briefly summarize our current understanding of mitochondria–ER contact sites and discuss the recently elucidated mechanism of MITOL fine-tuning phospholipid transfer activity through ubiquitination.

Keywords: contact site, mitochondria, phospholipid, ubiquitin, ubiquitin ligase

A commentary on 'Patient-derived gene and protein expression signatures of NGLY1 deficiency'

Tadashi Suzuki
Glycometabolic Biochemistry Laboratory, RIKEN Cluster for Pioneering Research, RIKEN, 2–1 Hirosawa, Wako, Saitama 351–0198, Japan

The cytosolic peptide: N-glycanase (PNGase; NGLY1 in human and PNG1 in budding yeast) is a deglyco-sylating enzyme widely conserved in eukaryotes. Initially, functional importance of this enzyme remained unknown as the png1Δ mutant in yeast did not exhibit any significant phenotypes. However, the discovery of NGLY1deficiency, a rare genetic disorder with biallelic mutations in NGLY1 gene, prompted an intensification of research that has resulted in uncovering the significance of NGLY1 as well as the proteins under its influence that are involved in numerous cellular processes. A recent report by Rauscher et al. (Patient-derived gene andproteinexpressionsignaturesofNGLY1deficiency. J. Biochem. 2022; 171: 187–199) presented a comprehensive summary of transcriptome/proteome analyses of various cell types derived from NGLY1-deficient patients. The authors also provide a web application called 'NGLY1 browser', which will allow researchers to have access to a wealth of information on gene and protein expression signature for patients with NGLY1 deficiency.

Keywords: NGLY1, NGLY1 browser, NGLY1 deficiency, proteome, transcriptome

JB REVIEW

Exploring protein lipidation by mass spectrometry-based proteomics

Kazuya Tsumagari[1,2,3], Yosuke Isobe[2,4,5], Koshi Imami[1,2,3] and Makoto Arita[2,4,5,6]
[1]Proteome Homeostasis Research Unit, RIKEN Center for Integrative Medical Sciences, 1–7–22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230–0045, Japan; [2]Laboratory for Metabolomics, RIKEN Center for Integrative Medical Sciences, 1–7–22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230–0045, Japan; [3]Laboratory for Integrative Genomics, RIKEN Center for Integrative Medical Sciences, 1–7–22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230–0045, Japan; [4]Division of Physiological Chemistry and Metabolism, Graduate School of Pharmaceutical Sciences, Keio University, 1–5–30 Shibakoen, Minato-ku, Tokyo 105–8512, Japan; [5]Cellular and Molecular Epigenetics Laboratory, Graduate School of Medical Life Science, Yokohama City University, 1–7–29 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230–0045, Japan; and [6]Human Biology-Microbiome-Quantum Research Center (WPI-Bio2Q), Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160–8582, Japan

Protein lipidation is a commonco-orpost-translational modification that plays a crucial role in regulating the localization, interaction and function of cellular proteins. Dysregulation of lipid modifications can lead to various diseases, including cancer, neurodegenerative diseases and infectious diseases. Therefore, the identification of proteins undergoing lipidation and their lipidation sites should provide insights into many aspects of lipid biology, as well as providing potential targets for therapeutic strategies. Bottom-up proteomics using liquid chromatography/tandem mass spectrometry is a powerful technique for the global analysis of protein lipidation. Here, we review proteomic methods for profiling protein lipidation, focusing on the two major approaches: the use of chemical probes, such as lipid alkyne probes, and the use of enrichment techniques for endogenous lipid-modified peptides. The challenges facing these methods and the prospects for developing them further to achieve a comprehensive analysis of lipid modifications are discussed.

Keywords: chemical probe, lipidation, mass spectrometry, post-translational modification, proteomics

RAPID COMMUNICATION

Dephosphorylation of NFAT by Calcineurin inhibits Skp2-mediated degradation

Shunsuke Hanaki[1], Makoto Habara[1], Yuki Sato[1], Haruki Tomiyasu[1], Yosei Miki[1], Shusaku Shibutani[2] and Midori Shimada[1,3]
[1]Department of Veterinary Biochemistry, Yamaguchi University, 1677–1 Yoshida, Yamaguchi, Yamaguchi 753–8511, Japan; [2]Laboratory of Veterinary Hygiene, Yamaguchi University, 1677–1 Yoshida, Yamaguchi, Yamaguchi 753–8511, Japan; and [3]Department of Molecular Biology, Nagoya University, Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466–8550, Japan

The transcription factor NFAT plays key roles in multiple bio-

logical activities, such as immune responses, tissue development and malignant transformation. NFAT is dephosphorylated by calcineurin, which is activated by intracellular calcium levels, and translocated into the nucleus, resulting in transcriptional activation. Calcineurin dephosphorylates various target proteins and regulates their functions. However, the regulation of NFAT degradation is largely unknown, and it is unclear whether calcineurin contributes to the stability of NFAT. We investigated the effect of calcineurin inhibition on NFAT protein stability and found that the dephosphorylation of NFAT by calcineurin promotes the NFAT stabilization, whereas calcineurin mutant that is defective in phosphatase activity was unable to stabilize NFAT. Increased intracellular calcium ion concentration, which is essential for calcineurin activation, also induced NFAT stability. In addition, we identified S-phase kinase associated protein 2 (Skp2), an F-box protein of the SCF ubiquitin ligase complex, as a factor mediating degradation of NFAT when calcineurin was depleted. In summary, these findings revealed that the dephosphorylation of NFAT by calcineurin protects NFAT from degradation by Skp2 and promotes its protein stability. *Keywords: calcineurin, calcium, NFAT, phosphorylation, Skp2*

REGULAR PAPER
BIOCHEMISTRY
Protein Structure

An oscillating magnetic field suppresses ice-crystal growth during rapid freezing of muscle tissue of mice

Kana Okuda[1,2], Kunitani Kaori[1], Aiko Kawauchi[1,2], Ishii Miyu[1,2] and Kentaro Yomogida[1,3] [1]Department of Innovative Food Science, Mukogawa Women’s University, 6–46 Ikebiraki-cho, 663–8558 Nishinomiya, Japan; [2]Abi Inc., 1–12–1 Ohtakanomori-higashi, Nagareyama 270–0138, Japan; and 3Institute for Bioscience, Mukogawa Women’s University, 6–46 Ikebiraki-cho, 663–8558 Nishinomiya, Japan

Regenerative medicine would benefit from a safe and efficient cryopreservation method to prevent the structural disruption caused by ice-crystal formation in cells and tissue. Various attempts have been made to overcome this problem, one of which is the use of an oscillating magnetic field (OMF). However, the underlying mechanism is unclear. In this study, to evaluate the effect of an OMF on ice-crystal formation in the leg muscles of mice, we used to use the frozen section method with as lower freezing rate than is, usual which resulted in ice crystals forming in the tissue. We assessed the mean size and number per unit area of intracellular ice holes in sections of muscle tissue, with and without OMF. Ice-crystal growth was reduced in frozen tissue subjected to OMF. Furthermore, we evaluated the structure and function of proteins in frozen tissue subjected to OMF by immunostaining using an anti-dystrophin antibody and by enzymatic histochemistry for NADH-TR and myosin ATPase. The results imply that the ability of OMF to suppress ice crystal growth might be related to their stabilization of bound water in biomolecules during freezing.

Keywords: cryopreservation, frozen section, oscillating magnetic field, protein structure, skeletal muscle

Glycobiology and Carbohydrate Biochemistry

Novel synthetic biological study on intracellular distribution of human GlcNAc-1-phosphotransferase expressed in insect cells

Kei Kiriyama[1,2], Keisuke Fujioka[3], Kaito Kawai[2], Teru Mizuno[1,2], Yasuo Shinohara[1,2,3] and Kohji Itoh[2,3,4]

[1]Institute for Genome Research, Tokushima University, 3–18–15 Kuramotocho, Tokushima-shi, Tokushima 770–8503, Japan; [2]Department of Medicinal Biotechnology, Institute for Medicinal Research, Graduate School of Pharmaceutical Sciences, Tokushima University, 1–78–1 Shoumachi, Tokushima-shi, Tokushima 770–8505, Japan; [3]Department of Medicinal Biotechnology, Faculty of Pharmaceutical Sciences, Tokushima University, 1–78–1 Shoumachi, Tokushima-shi, Tokushima 770–8505, Japan; and [4]Department of Pediatrics, Jichi Medical University School of Medicine, 3311–1 Yakushiji, Shimotsuke-shi, Tochigi 329–0498, Japan

Many lysosomal enzymes contain N-glycans carrying mannose 6-phosphate (M6P) residues. Modifying lysosomal enzymes by M6P residues requires a twostep process in the Golgi apparatus. Then the lysosomal enzymes with M6P residues are transported from the trans-Golgi network to endosomes and lysosomes by M6P receptors. In insect cells, M6P residues are not added to N-glycans. Therefore, many in sect lysosomal enzymes are transported to lysosomes by the M6P-independent pathway. The expression and subcellular distribution of M6P-modifying enzymes were examined by amplifying DNA fragments of M6Pmodifying enzymes, generating the corresponding plasmid constructs, and transfection each construct into Sf9 cells, an insect cell line. The human GlcNac1-phosphotransferase α/β subunit, one of the M6Pmodifying enzymes, was found to differ in maturation and localization between mammalian and insect cells. In mammalian cells, newly biosynthesized α/β subunit localized in the cis-Golgi. In Sf9 cells, most of the α/β sub unit was localized in the endoplasmic reticulum, and few mature forms of α/β subunit were observed. However, by the co-expression of the human site-1 protease, the mature forms were observed significantly and colocalization with each protein. Our study indicates new insights into regulating the intracellular distribution of the human GlcNac-1-phosphotransferase α/β subunit in insect cells.

Keywords: GlcNac-1-phosphotransferase, GlcNac-1-phosphotransferase *α*/*β* subunit, M6P-modifying enzymes, mannose 6-phosphate, ste-1 protease

Enzyme Inhibitors

Involvement of cardiac glycosides targeting Na/K-ATPase in their inhibitory effects on c-Myc expression via its transcription, translation and proteasomal degradation

Muneshige Tokugawa[1], Yasumichi Inoue[1], Hiromasa Aoki[2], Chiharu Miyajima[1], Kan'ichiro Ishiuchi[3], Kento Tsurumi[1], Chisane Kujirai[1], Daisuke Morishita[1,4], Michiyo Matsuno[5], Hajime Mizukami[5], Masaki Ri[6], Shinsuke Iida[6], Toshiaki Makino[3], Mineyoshi Aoyama[2] and Hidetoshi Hayashi[1]
[1]Department of Cell Signaling, Graduate School of Pharmaceutical Sciences, Nagoya City University, 3–1 Tanabe-dori, Mizuho-ku, Nagoya 467–8603, Japan; [2]Department of Pathobiology, Graduate School of Pharmaceutical Sciences, Nagoya City University, 3–1 Tanabe-dori, Mizuho-ku, Nagoya 467–8603, Japan; [3]Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences, Nagoya City University, 3–1 Tanabe-dori, Mizuho-ku, Nagoya 467–8603, Japan; [4]Chordia Therapeutics Inc., 26–1 Muraoka-Higashi 2-chome, Fujisawa, Kanagawa 251–0012, Japan; [5]Plant research section, The Kochi Prefectural Makino Botanical Garden, 4200–6 Godaiyama, Kochi 781–8125, Japan; and [6]Department of Hematology and Oncology, Graduate School of Medical Sciences, Nagoya City University, 1 Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467–8601, Japan

Cardiac glycosides (CGs) have been used for decades to treat heart failure and arrhythmic diseases. Recent non-clinical and epidemiological findings have suggested that CGs exhibit antitumor activities. Therefore, CGs may be repositioned as drugs for the treatment of cancer. A detailed understanding of the anticancer mechanisms of CGs is essential for their application to the treatment of targetable cancer types. To elucidate the factors associated with the antitumor effects of CGs, we performed transcriptome profiling on human multiple myeloma AMO1 cells treated with periplocin, one of the CGs. Periplocin significantly down-regulated the transcription of MYC (c-Myc), a well-established oncogene. Periplocin also suppressed c-Myc expression at the protein levels. This repression of c-Myc was also observed in several cell lines. To identify target proteins for the inhibition of c-Myc, we generated CG-resistant (C9) cells using a sustained treatment with digoxin. We confirmed that C9 cells acquired resistance to the inhibition of c-Myc expression and cell proliferation by CGs. Moreover, the sequencing of genomic DNA in C9 cells revealed the mutation of D128N in α1-Na/K-ATPase, indicating the target protein. These results suggest that CGs suppress c-Myc expression in cancer cells via α1-Na/K-ATPase, which provides further support for the anti-tumor activities of CGs.

Keywords: cardiac glycosides, c-Myc, multiple myeloma, periplocin, α1-Na/K-ATPase

Biochemistry in Diseases and Aging

Super complex formation of mitochondrial respiratory chain complexes in leukocytes from patients with neurodegenerative diseases

Tsukasa Hara[1], Ryosuke Amagai[1], Ryuji Sakakibara[2] and Ayako Okado-Matsumoto[1]
[1]Department of Biology, Faculty of Science, Toho University, Miyama 2–2–1, Funabashi, Chiba 274–8510, Japan; and [2]Division of Neurology, Department of Internal Medicine, Sakura Medical Center, Toho University, Shimoshizu 564–1, Sakura, Chiba 285–8741, Japan

With population aging, cognitive impairments and movement disorders due to neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD) and dementia with Lewy bodies (DLB), are increasingly considered as key social issues. Clinically, it has remained challenging to diagnose them before the onset of symptoms because of difficulty to observe the progressive loss of neurons in the brain. Therefore, with exploratory research into biomarkers, a number of candidates have previously been proposed, such as activities of mitochondrial respiratory chain complexes in blood in AD and PD. In this study, we focused on the formation of mitochondrial respiratory chain super complexes (SCs) because the formation of SC itself modulates the activity of each complex. Here we investigated the SC formation in leukocytes from patients with AD, PD and DLB. Our results showed that SCs were well formed in AD and PD compared with controls, while poorly formed in DLB. We highlighted that the disruption of the SC formation correlated with the progression of PD and DLB. Taking our findings together, we propose that pronounced SC formation would already have occurred before the onset of AD, PD and DLB and, with the progression of neurodegeneration, the SC formation would gradually be disrupted.

Keywords: Alzheimer's disease, dementia with Lewy bodies, high-resolution clear native polyacrylamide gel electrophoresis, in-gel activity assay, mitochondrial respiratory chain complexes, Parkinson's disease

MOLECULAR BIOLOGY

Gene Expression

NRIP1 regulates cell proliferation in lung adenocarcinoma cells

Fumihiko Watanabe[1,2], Shigemitsu Sato[1,3],Takuo Hirose[1,3,4], Moe Endo[1], Akari Endo[1,4], Hiroki Ito[1,4], Koji Ohba[1], Takefumi Mori[3,4] and Kazuhiro Takahashi[1]
[1]Department of Endocrinology and Applied Medical Science, Tohoku University Graduate School of Medicine, 2–1 Seiryo, Aoba, Sendai 980–8575, Japan; [2]Department of Blood Trans-

fusion and Transplantation Immunology, Fukushima Medical University School of Medicine, 1, Hikarigaoka, Fukushima, Fukushima 960–1295, Japan; [3]Division of Integrative Renal Replacement Therapy, Faculty of Medicine, Tohoku Medical and Pharmaceutical University, 1–15–1 Fukumuro, Miyagino, Sendai 983–8536, Japan; and [4]Division of Nephrology and Endocrinology, Faculty of Medicine, Tohoku Medical and Pharmaceutical University, 1–15–1, Fukumuro, Miyagino, Sendai 983–8536, Japan

Nuclear receptor interacting protein 1 (NRIP1) is a transcription cofactor that regulates the activity of nuclear receptors and transcription factors. Functional expression of NRIP1 has been identified in multiple cancers. However, the expression and function of NRIP1 in lung adenocarcinoma have remained unclear. Thus, we aimed to clarify the NRIP1 expression and its functions in lung adenocarcinoma cells. NRIP1 and Ki-67 were immunostained in the tissue microarray section consisting of 64 lung adenocarcinoma cases, and the association of NRIP1 immunoreactivity with clinical phenotypes was examined. Survival analysis was performed in lung adenocarcinoma data from The Cancer Genome Atlas (TCGA). Human A549 lung adenocarcinoma cell line with an NRIP1-silencing technique was used in vitro study. Forty-three of 64 cases were immunostained with NRIP1. Ki-67positive cases were more frequent in NRIP1-positive cases as opposed to NRIP1-negative cases. Higher NRIP1 mRNA expression was associated with poor prognosis in the TCGA lung adenocarcinoma data. NRIP1 was mainly located in the nucleus of A549 cells. NRIP1 silencing significantly reduced the number of living cells, suppressed cell proliferation, and induced apoptosis. These results suggest that NRIP1 participates in the progression and development of lung adenocarcinoma. Targeting NRIP1 may be a possible therapeutic strategy against lung adenocarcinoma.

Keywords: apoptosis, cancer, cell cycle, lung, transcriptional factor

CELL

Bio membranes, Organelles and Protein Sorting

Comparative study of the steady-state subcellular distribution of lysosome-associated membrane glycoprotein-2 (LAMP-2) isoforms with GYXXΦ-type tyrosine-based motifs that interact differently with four adaptor protein (AP) complexes

Fumiaki Yamaguchi, Hiroshi Sakane and Kenji Akasaki
Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Fukuyama, Hiroshima 729–0292, Japan

Lysosome-associated membrane protein-1 and-2 (LAMP-1 and LAMP-2, respectively) are type I transmembrane proteins. LAMP-2 comprises three splice isoforms (LAMP-2A, -B and-C) with different cytoplasmic tails (CTs). These three CTs possess different tyrosine-based motifs (GYXXΦ, where is a bulky hydrophobic amino acid) at their C-termini. Interactions between tyrosine-based motifs and μ-subunits of four tetrameric adaptor protein (AP) complexes are necessary for their vesicular transport to lysosomes. Little is known about how the interaction strengths of these tyrosine motifs with μ-subunits affect the localization of isoforms to lysosomes. The interactions were first investigated using a yeast two-hybrid system to address this question. LAMP-2A-CT interacted with all four μ-subunits (μ1, μ2, μ3A and μ4ofAP-1, AP2, AP-3 and AP-4, respectively). The interaction with μ3A was more robust than that with other μ-subunits. LAMP-2B-CT interacted exclusively and moderately with μ3A. LAMP-2C-CT did not detectably interact with any of the four μ-subunits. Immunofluorescence microscopy showed that all isoforms were localized in late endosomes and lysosomes. LAMP-2C was present in the plasma membrane and early endosomes; however, LAMP-2A and-2B were barely detectable in these organelles. In cell fractionation, LAMP2A was the most abundant in the dense lysosomes, whereasLAMP-2Cwassignificantlypresentinthelowdensity fraction containing the plasma membrane and early endosomes, in addition to the dense lysosomes. LAMP-2B considerably existed in the low-density late endosomal fraction. These data strongly suggest that the LAMP-2 isoforms are distributed differently in endocytic organelles depending on their interaction strengths with AP-3.

Keywords: adaptor protein complex, isoform, lysosome targeting, subcellular distribution, tyrosine-based motif

The incorporation of extracellular vesicle markers varies among vesicles with distinct surface charges

Koki Maeda[1,2], Simon Goto[1], Koya Miura[1], Koki Saito[1,2] and Eiji Morita[1,2]
[1]Department of Biochemistry and Molecular Biology, Faculty of Agriculture and Life Science, Hirosaki University, 3 Bunkyocho, Hirosakishi, Aomori 036–8561, Japan; and [2]Division of Biomolecular Function, Bioresources Science, United Graduate School of Agricultural Sciences, Iwate University, 3 Bunkyocho, Hirosakishi, Aomori 036–8561, Japan

Extracellular vesicles (EVs) are important mediators of intercellular communication. However, the methods available for distinguishing the heterogeneity of secreted EVs and isolating and purifying them are limited. This study introduced a HiBiT-tag to detect various EV markers, including CD63, CD9, Epidermal Growth Factor Receptor (EGFR), Flotilin1, and Syndecan-1, and investigated whether these marker-containing vesicles were capable of binding to differently charged column carriers. Four column carriers, Diethylaminoethyl (DEAE), Capto Adhere, Blue and Heparin, showed affinity for CD63containing EVs, but the irelution patterns varied. Furthermore, we observed that the elution patterns of the EV markers differed among vesicles with distinct surface charges when a DEAE column was used. This suggests that the incorporation of EV markers varied between these vesicles. The markers showed different subcellular

localizations, indicating that the site of vesicle formation may contribute to the production of vesicles with varying charges and marker incorporation. These findings may have implications for the development of methods to purify homogeneous EVs, which could be useful in EV-mediated drug delivery systems.

Keywords: column chromatography, DEAE, exosome, extracellular vesicle, HiBiT

Cell Death

Merlin/NF2 regulates SLC7A11/xCT expression and cell viability under glucose deprivation at high cell density in glioblastoma cells

Itsuki Yamaguchi[1,2] and Hironori Katoh[2]
[1]Laboratory of Molecular Neurobiology, Graduate School of Biostudies, Kyoto University, Yoshidakonoe-cho, Sakyo-ku, Kyoto 606–8501, Japan; and [2]Department of Biological Chemistry, Graduate School of Science, Osaka Metropolitan University, Gakuen-cho, Naka-ku, Sakai, Osaka 599–8531, Japan

The cystine/glutamate transporter SLC7A11/xCT is highly expressed in many cancer cells and plays an important role in antioxidant activity by supplying cysteine for glutathione synthesis. Under glucose-depleted conditions, however, SLC7A11-mediated cystine uptake causes oxidative stress and cell death called disulfidptosis, a new form of cell death. We previously reported that high cell density (HD) promotes lysosomal degradation of SLC7A11 in glioblastoma cells, allowing them to survive under glucose-depleted conditions. In this study, we found that the neurofibromatosis type 2 gene, Merlin/NF2 is a key regulator of SLC7A11in glioblastoma cells at HD. Deletion of Merlin increased SLC7A11protein level and cystine uptake at HD, leading to promotion of cell death under glucose deprivation. Furthermore, HD significantly decreased SLC7A11 mRNA level, which was restored by Merlin deletion. This study suggests that Merlin suppresses glucose deprivation-induced cell death by downregulating SLC7A11expressioninglioblastomacellsat HD.

Keywords: amino acid transport, cell death, cell density, glioblastoma, transcriptional regulation