Journal of Biochemistry

Vol. 165, No. 5（2019 年 5 月 発 行）

ダイジェスト

JB Reviews

Featured article of the month: Multiple functions of the ER-resident VAP and its extracellular role in neural development and disease

[1]Kosuke Kamemura and [1]Takahiro Chihara

[1]Department of Biological Science, Graduate School of Science, Hiroshima University, Hiroshima, Japan

VAP (VAMP-associated protein) is a type II integral membrane protein of the endoplasmic reticulum (ER), and its N-terminal major sperm protein (MSP) domain faces the cytoplasmic side. VAP functions as a tethering molecule at the membrane contact sites between the ER and intracellular organelles and regulates a wide variety of cellular functions, including lipid transport, membrane trafficking, microtubule reorganization and unfolded protein response. VAP-point mutations in human *vapb* are strongly associated with amyotrophic lateral sclerosis. Importantly, the MSP domain of VAP is cleaved, secreted and interacts with the axon growth cone guidance receptors (Eph, Robo, Lar), suggesting that VAP could function as a circulating hormone similar to the *Caenorhabditis elegans* MSP protein. In this review, we discuss not only the intracellular functions of VAP but also the recently discovered extracellular functions and their implications for neurodegenerative disease.

Keywords: amyotrophic lateral sclerosis, endoplasmic reticulum, MSP domain, organelle tethering, VAP

Featured article of the month: Calcium signalling: a key regulator of neuronal migration

[1,2]Shin-ichiro Horigane, [1,2,3]Yukihiro Ozawa, [1,2]Hirokazu Yamada and [1,2,4]Sayaka Takemoto-Kimura

[1]Department of Neuroscience I, Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan, [2]Molecular/Cellular Neuroscience, Nagoya University Graduate School of Medicine, Nagoya, Japan, [3]Department of Pathology and Laboratory Medicine, Nagoya University Hospital, Nagoya, Japan, [4]Precursory Research for Embryonic Science and Technology (PRESTO), Japan Science and Technology Agency, Saitama, Japan

Neuronal migration is a crucial event in neuronal development for the construction of brain architecture and neuronal networks. Newborn neurons proliferate in the germinal zone and start migration toward their final destination. Migrating neurons adopt different routes, cell shapes and migratory modes depending on extracellular factors and outer physical substrates. Intracellular Ca^{2+} is an essential second messenger that regulates diverse cellular functions by activating Ca^{2+}-dependent signalling molecules that underlie Ca^{2+}-responsive cellular functions. Neuronal migration during brain architecture construction is no exception. Spontaneous Ca^{2+} transients are observed in several types of migrating neurons, and a series of Ca^{2+}-dependent signalling molecules governing neuronal migration has been identified. In this review, we first summarize the molecular mechanisms that trigger intracellular Ca^{2+} elevation in migrating neurons. In the latter half of this review, we provide an overview of the literature on Ca^{2+}-dependent signalling molecules underlying neuronal migration.

Keywords: Ca^{2+}, influx, Ca^{2+}, release, Ca^{2+}, signalling, neuronal migration, radial migration

BIOCHEMISTRY

Protein Structure

Structural insights into nucleotide and protein sequence of Ageritin: a novel prototype of fungal ribotoxin

[1]Nicola Landi, [1]Sara Ragucci, [1]Rosita Russo, [1]Paolo V Pedone, [1]Angela Chambery and [1]Antimo Di Maro

[1]Department of Environmental, Biological and Pharmaceutical Sciences and Technologies (DISTABIF), University of Campania 'Luigi Vanvitelli', Via Vivaldi 43, I Caserta, Italy

Here, we report the amino acid sequence of Ageritin, the first ribotoxin-like protein from basidiomycetes (*Agrocybe aegerita*). This protein consists of 135 amino acid residues with a theoretical molecular mass of 14,801.80 Da (experimental mass 14,802.84 Da, $[M+H^+]^+$). Unlike both the classic ribotoxins and homologous RNases T1 family from ascomycetes, Ageritin has a single free cysteinyl residue and does not show homology with known RNases endowed with the specific enzymatic activity on the universally conserved Sarcin Ricin Loop. On the other hand, our 3D homology study shows that Ageritin has a structural core consisting of an antiparallel β-sheet and an adjacent long α-helix, typical of ribotoxins and RNase T1 family, although the sheet has an orthogonal arrangement with respect to them. Thus, Ageritin is the first prototype of novel ribotoxin-like protein family from fungi.

Keywords: Agrocybe aegerita, homology modelling, mass spectrometry, mushrooms, ribotoxins

Glycobiology and Carbohydrate Biochemistry

Implications of altered *O*-glycosylation in tumour immune evasion

[1]Naoki Itano

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

Aberrant glycosylation on tumour cells has been implicated in tumour immune modulation. A recent article published in *The*

Journal of Biochemistry (Sutoh Yoneyama *et al.*, A mechanism for evasion of CTL immunity by altered *O*-glycosylation of HLA class I, *J. Biochem.* 2017; 161: 479–492) showed that bladder cancer cells evaded cytotoxic T lymphocyte-mediated antitumour immunity by a novel mechanism involving the loss of Core 2 structures on human leukocyte antigen Class I *O*-glycans and subsequent impairment of galectin–glycan lattice formation. The immunosuppressive action of *O*-glycans on natural killer cell-mediated tumour immunity is also considered an immune evasion system. Furthermore, sialylated *O*-glycans have been proposed to play a central role in tumour immune escape by modulating the production of immunoregulatory cytokines and growth factors through interactions with sialic acid-binding immunoglobulin-like lectins. Therefore, a better understanding of how alterations in *O*-glycosylation influence tumour immune evasion will enable the development of novel and more effective therapeutic options for cancer treatment.

Keywords: bladder cancer, C2GnT, HLA Class I, immune evasion, *O*-glycosylation

Biochemistry in Cell Membranes

Hepatitis B virus x protein accelerated the proliferation of hepatocellular carcinoma cell through lncRNA SNHG20/PTEN pathway

[1]Wenhui Tu, [1]Yonghong Yang, [1]Yulong Song and [1]Weijun Zhu

[1]Department of Infectious Disease, Taizhou Municipal Hospital, 381–1 Zhongshandong Rd, Jiaojiang District, Taizhou, Zhejiang Province, China

This study investigated the underlying mechanism of long noncoding RNA (lncRNA) small nucleolar RNA host gene 20 (SNHG20) in hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC). LncRNA SNHG20 and PTEN expression levels were detected by quantitative real-time polymerase chain reaction and western blot. The proliferation of HCC cells was measured by MTT assay, and the apoptosis of HCC cells was measured by flow cytometry analysis. SNHG20 expression level and HBx protein level were upregulated in HBV(+) group than that of HBV(−) group, whereas PTEN protein level was downregulated in HBV(+) group. Besides, SNHG20 was highly expressed in HBV(+) HCC cells than in HBV(−) HCC cells. SNHG20 expression level was positively associated with HBV x protein (HBx) in HCC cells, and HBx–SNHG20 involved in regulating the proliferation and apoptosis of HCC cells. Moreover, SNHG20 was confirmed to interact with PTEN, which negatively regulated PTEN protein level. Finally, we proved HBx–SNHG20–PTEN signalling pathway involved in the regulation of HCC cell proliferation and apoptosis. *In vivo* experiments showed SNHG20 knockdown inhibited tumour growth of HBV(+) HCC. HBx promoted the proliferation of HCC cell and reduced the apoptosis of HCC cells through the SNHG20/PTEN signalling pathway.

Keywords: HBx, hepatitis B virus, hepatocellular carcinoma, PTEN, SNHG20

MOLECULAR BIOLOGY

Molecular Biology General

Mitotic phosphorylation of HP1*α* regulates its cell cycle-dependent chromatin binding

[1]Gohei Nishibuchi, [2]Shinichi Machida, [3]Reiko Nakagawa, [4]Yuriko Yoshimura, [4,5]Kyoko Hiragami-Hamada, [6]Yusuke Abe, [2,7]Hitoshi Kurumizaka, [1]Hideaki Tagami and [1,4,5]Jun-ichi Nakayama

[1]Graduate School of Natural Sciences, Nagoya City University, Yamanohata 1, Mizuho-cho, Mizuho-ku, Nagoya, Japan, [2]Laboratory of Structural Biology, Graduate School of Advanced Science and Engineering, Waseda University, Shinjuku-ku, Tokyo, Japan, [3]Laboratory for Phyloinformatics, RIKEN Center for Biosystems Dynamics Research, 2–2–3 Minatojima-minamimachi, Chuo-ku, Kobe, Japan, [4]Division of Chromatin Regulation, National Institute for Basic Biology, Nishigonaka 38, Myodaiji, Okazaki, Japan, [5]Department of Basic Biology, School of Life Science, The Graduate University for Advanced Studies (SOKENDAI), Nishigonaka 38, Myodaiji, Okazaki, Japan, [6]Division of Experimental Pathology, Cancer Institute of the Japanese Foundation for Cancer Research, Koto-ku, Tokyo, Japan, [7]Institute for Quantitative Biosciences, The University of Tokyo, Bunkyo-ku, Tokyo, Japan

Heterochromatin protein 1 (HP1) is an evolutionarily conserved chromosomal protein that plays a crucial role in heterochromatin-mediated gene silencing. We previously showed that mammalian HP1*α* is constitutively phosphorylated at its N-terminal serine residues by casein kinase II (CK2), and that this phosphorylation enhances HP1*α*'s binding specificity for nucleosomes containing lysine 9-methylated histone H3 (H3K9me). Although the presence of additional HP1*α* phosphorylation during mitosis was reported more than a decade ago, its biological significance remains largely elusive. Here we found that mitosis-specific HP1*α* phosphorylation affected HP1*α*'s ability to bind chromatin. Using biochemical and mutational analyses, we showed that HP1*α*'s mitotic phosphorylation was located in its hinge region and was reversibly regulated by Aurora B kinase and serine/threonine phosphatases. In addition, chromatin fractionation and electrophoretic mobility shift assays revealed that hinge region-phosphorylated HP1*α* was preferentially dissociated from mitotic chromatin and exhibited a reduced DNA-binding activity. Although HP1's mitotic behaviour was previously linked to H3 serine 10 phosphorylation, which blocks the binding of HP1's chromodomain to H3K9me3, our findings suggest that mitotic phosphorylation in HP1*α*'s hinge region also contributes to changes in HP1*α*'s association with mitotic chromatin.

Keywords: Aurora B kinase, heterochromatin, HP1, phosphorylation, PP2 protein phosphatases

Genes and Other Genetic Materials

A novel splicing variant of small nucleolar RNA host gene 4 is a podocyte-selective non-coding RNA upregulated in response to puromycin aminonucleoside-induced podocyte injury

[1]Aya Horikawa, [1]Tomomi Yoneda, [2]Eishin Yaoita, [3]Katsushi Yamaguchi, [3]Shuji Shigenobu, [4]Mizuki Kuramochi, [4]Jyoji Yamate, [1]Takashi Inui and [1]Osamu Ishibashi

[1]Department of Applied Life Sciences, Osaka Prefecture University, Graduate School of Life and Environmental Sciences, 1–1 Gakuen-cho, Naka-ku, Sakai, Osaka, Japan, [2]Department of Structural Pathology, Kidney Research Center, Niigata University Graduate School of Medical and Dental Sciences, 1–757 Asahimachi-dori, Chuo-ku, Niigata, Japan, [3]NIBB Core Research Facilities, National Institute for Basic Biology, 38 Nishigonaka, Myodaiji, Okazaki, Japan, [4]Laboratory of Veterinary Pathology, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, 1–58 Rinku-Ourai-Kita, Izumisano, Osaka, Japan

Podocytes are terminally differentiated cells that function as the glomerular filtration barrier in the kidney, and podocyte injury leads to serious proteinuria and podocyte leakage into urine. Recent studies have demonstrated that the number of urinary podocytes is correlated with the progression of glomerular diseases. Therefore, urinary podocytes may serve as an indicator of podocyte injury. In this study, to explore podocyte injury-related genes, we performed comprehensive transcriptome analysis of primary rat podocytes cultured in the presence or absence of puromycin aminonucleoside (PAN), an agent commonly used to induce podocyte injury. RNA-seq revealed that a transcript containing the intronic sequence of small nucleolar RNA host gene 4 (*Snhg4*) was expressed in podocytes and upregulated by PAN. RT-qPCR analysis demonstrated that this transcript, but not *Snhg4*, was selectively expressed in podocytes. Therefore, we designated the novel transcript *Snhg4-pod.* 5′- and 3′-RACE experiments revealed that *Snhg4-pod* is a novel splice variant of *Snhg4* lacking a poly(A) tail. PAN induced *Snhg4-pod* expression in podocytes in a dose-dependent manner along with their mitochondria-mediated apoptotic cell death. Further, *Snhg4-pod* was detected in urinary sediments from PAN-induced nephrotic rats. Our findings suggest that *Snhg4-pod* may serve as a novel marker for the diagnosis of glomerular injury.

Keywords: podocyte, puromycin aminonucleoside, long noncoding RNA, splice variant, RNA-seq

CELL

Tumor and Immunology

PMEPA1/TMEPAI knockout impairs tumour growth and lung metastasis in MDA-MB-231 cells without changing monolayer culture cell growth

[1,2]Mohammed Abdelaziz, [1]Yukihide Watanabe and [1]Mitsuyasu Kato

[1]Department of Experimental Pathology, Graduate School of Comprehensive Human Sciences and Faculty of Medicine, University of Tsukuba, 1–1–1 Tennodai, Tsukuba, Ibaraki, Japan, [2]Department of Pathology, Faculty of Medicine, Sohag University, Nasr City, Eastern Avenue, Sohag, Egypt

Prostate transmembrane protein androgen-induced 1 (PMEPA1)/transmembrane prostate androgen-induced protein (TMEPAI), a direct target and a negative regulator of transforming growth factor beta signalling, has an oncogenic role in many cancers. We observed that knockout (KO) of *PMEPA1* in human breast cancer cell line MDA-MB-231 using a CRISPR-Cas9 system resulted in reduction of *in vivo* tumour growth and lung metastasis but not of *in vitro* monolayer growth capacity of these KO cell lines. This phenomenon was associated with *PMEPA1 KO*-mediated downregulation of the key proangiogenic factors vascular endothelial growth factor alpha (VEGFA) and interleukin-8 (IL8) that are essential for *in vivo* but not *in vitro* growing cells and are also substantial for initiation of lung metastasis.

Keywords: angiogenesis, IL8, metastasis, PMEPA1/TMEPAI, VEGFA

Vol. 165, No. 6（2019 年 6 月 発 行）

ダイジェスト

JB Reviews

Featured article of the month. Cellular and molecular mechanisms of sterile infl ammation in ischaemic stroke

[1,2]Koutarou Nakamura and [1,3]Takashi Shichita

[1]Stroke Renaissance Project, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan, [2]Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Chiba, Japan, [3]Japan Agency for Medical Research and Development (AMED), Tokyo, Japan

Cerebral inflammation is a promising therapeutic target for ischaemic stroke. After ischaemic stroke, inflammatogenic self-molecules, which originate from damaged brain tissue due to ischaemia, activate infiltrating immune cells (neutrophils, macrophages and lymphocytes) and thereby trigger sterile inflammation. Innate immunity plays the central role in sterile inflammation at the acute phase of brain ischaemia, although immune response by T lymphocytes (innate or acquired immunity) is also

implicated in inflammation at the subacute phase, which sustains ischaemic brain damage. In the recovery phase, infiltrating macrophages remove the damage-associated molecular patterns (DAMPs) from the ischaemic brain. These pro-resolving myeloid cells also produce neurotrophic factors involved in neural repair. Through a series of inflammatory mechanisms activated by ischaemic stroke, various immune cells change their functions from inflammation to repair in a precise process. In order to establish therapeutic strategies for the improvement of neurological deficits after ischaemic stroke, it is necessary to clarify the detailed molecular and cellular mechanisms of sterile inflammation after ischaemic brain injury.

Keywords: DAMPs, ischaemic stroke, lymphocytes, macrophage, sterile inflammation

Featured article of the month. Regulation of R-loops and genome instability in Fanconi anemia

[1,2]Yusuke Okamoto, [3]James Hejna and [1]Minoru Takata

[1]Laboratory of DNA Damage Signaling, Department of Late Effects Studies, Graduate School of Biostudies, Radiation Biology Center, Kyoto University, Yoshidakonoe-cho, Sakyo-ku, Kyoto, Japan, [2]Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, Yoshidakonoe-cho, Sakyo-ku, Kyoto, Japan, [3]Laboratory of Science Communication, Department of Biology Education and Heredity, Graduate School of Biostudies, Kyoto University, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto, Japan

Fanconi anemia (FA) is a devastating hereditary disorder with impaired genome stability resulting in physical abnormalities, gradual loss of hematopoietic stem cells and development of tumours and leukaemia. It has been suggested that functions of FA genes are required to maintain genome stability by counteracting endogenous metabolites, such as aldehydes, that damage DNA and stall replication forks. Recent studies have implicated co-transcriptional R-loops, consisting of a DNA : RNA hybrid and displaced single-stranded DNA, as one of the potential endogenous sources that induce genome instability and the FA phenotype. This review focuses on recent literature, including our own, regarding the interplay between FA proteins and R-loops, and will provide readers with a concise summary of this rapidly evolving field.

Keywords: common fragile sites, FANCD2, Fanconi anemia, replication–transcription collision, R-loops

BIOCHEMISTRY

Biochemistry General

Inhibition of protein phosphatase PPM1D enhances retinoic acidinduced differentiation in human embryonic carcinoma cell line

[1]Sari Ogasawara, [1]Yoshiro Chuman, [1]Takahiro Michibam, [1]Rui Kamada, [1]Toshiaki Imagawa and [1]Kazuyasu Sakaguchi

[1]Laboratory of Biological Chemistry, Department of Chemistry, Faculty of Science, Hokkaido University, North 10, West 8, Kita-ku, Sapporo, Japan

The protein phosphatase PPM1D (Wip1) was originally identified as a p53 target product. Activation of PPM1D through various mechanism promotes the tumorigenic potential of various cancers by suppressing p53 and other DNA damage response proteins. New functions of PPM1D have recently been revealed in physiological processes such as cell differentiation. However, the regulatory mechanisms of signalling pathway to maintain stemness and induce cell differentiation are still unclear. Here we report that PPM1D modulates retinoic acid (RA) signalling. PPM1D knockdown resulted in decreased alkaline phosphatase activity of the human teratocarcinoma cell line NT2/D1. Inhibition of PPM1D-induced cell differentiation and decreased gene expression of the stem cell marker Oct-4 (*POU5F1*). RA-induced cell differentiation was promoted by reducing PPM1D activity. RA treatment elicited activation of the MEK-ERK pathway and induced rapid and transient activation of the extracellular signal-regulated kinase 1/2 (ERK-1/2). PPM1D dephosphorylated a phosphopeptide with the TEY motif in ERK-1/2 *in vitro*. Moreover, phosphorylation of ERK-1/2 was facilitated by PPM1D inhibition. Our study shows that PPM1D plays an important role in maintaining the undifferentiation state and a new function in RA-induced ERK regulation and cell differentiation.

Keywords: differentiation, ERK, NT2/D1, PPM1D, retinoic acid signalling

Fine epitope mapping of a human disulphide-stabilized diabody against fi broblast growth factor-2

[1]Jiangchuan Zhong, [1]Simin Zhang, [1]Ligang Zhang, [1]Yaxiong Cai, [1]Yanrui Deng, [2]Qubo Zheng and [1]Ning Deng

[1]Department of Biology, Guangdong Province Engineering Research Center for Antibody Drug and Immunoassay, Jinan University, Huangpu Avenue, 601, Guangzhou, Guangdong, China, [2]The 458 Hospital of PLA, Dongfeng East Road, 801, Guangzhou, Guangdong, China

The human fibroblast growth factor-2 (FGF-2) highly expressed in tumours is an important factor to promote tumour angiogenesis and lymphangiogenesis. A disulphide-stabilized diabody (ds-Diabody) could specifically target FGF-2 and show its advantages in inhibition of tumour angiogenesis and growth. It is very important for antibody drugs to confirm the fine epitope. Here, theoretical structure models of FGF-2 and antibody were built by homology modelling. The amino acid residues in the interaction interface of antigen and antibody were analysed by molecular docking. The potential epitope was predicted by homology modelling and molecular docking of antigen–antibody and site-directed mutation assays of alanine scanning. The pre-

dicted epitope was verified by antigen mutagenesis and enzyme-linked immunosorbent assay (ELISA). The epitope mapping assay showed that the epitope of ds-Diabody against FGF-2 was defined by the discontinuous sites including six amino acid residues (P23, Q65, R69, G70, Y82 and R118). The results showed that the epitope was localized in the interaction interface of FGF-2 and ds-Diabody. The fine epitope mapping provided the important information for understanding the inhibition activity of ds-Diabody against FGF-2 and helping in the further development of ds-Diabody against FGF-2 as a potentially promising antibody drug for future cancer therapy.

Keywords: ds-Diabody against FGF-2, epitope mapping, homology modelling, molecular docking, site-directed mutagenesis

Glycobiology and Carbohydrate Biochemistry

A novel method for chemo-enzymatic synthesis of chitin oligosaccharide catalyzed by the mutant of inverting family GH19 chitinase using 4,6-dimethoxy-1,3,5-triazin-2-yl -chitobioside as a glycosyl donor

[1]Takayuki Ohnuma, [2]Tomonari Tanaka, [1]Atsushi Urasaki, [1]Satoshi Dozen and [1]Tamo Fukamizo

[1]Department of Advanced Bioscience, Kindai University, 3327–204 Nakamachi, Nara, Japan, [2]Department of Biobased Materials Science, Graduate School of Science and Technology, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto, Japan

A novel method for the chemo-enzymatic synthesis of chitin oligosaccharide catalyzed by mutants of BcChi-A, an inverting family GH19 chitinase from *Bryum coronatum*, has been developed using 4,6-dimethoxy-1,3,5-triazin-2-yl α-chitobioside [DMT-α-$(GlcNAc)_2$)] as a donor substrate. Based on the glycosynthase derived from BcChi-A, Glu70, which acts as a catalytic base, and Ser102, which fixes a nucleophilic water molecule, were changed to generate several single and double mutants of BcChi-A, which were employed in synthetic reactions. Among the double mutants tested, E70G/S102G, E70G/S102C and E70G/S102A were found to successfully synthesize chitotetraose [$(GlcNAc)_4$] from DMT-α-$(GlcNAc)_2$ and $(GlcNAc)_2$; however, the single mutants, E70G, S102G, S102C and S102A, did not. Among the mutants, E70G/S102A showed the highest synthetic activity. This is the first report of a glycosynthase that employs a dimethoxytriazine-type glycoside as a donor substrate.

Keywords: chitin oligosaccharide, DMT-glycoside, GH19 chitinase, glycosynthase

Enzyme Inhibitors

Screening of subtype-specifi c activators and inhibitors for diacylglycerol kinase

[1]Daiki Hayashi, [1]Ryosuke Tsumagari, [2]Ke Liu, [1]Shuji Ueda, [1]Minoru Yamanoue, [2]Fumio Sakane and [1]Yasuhito Shirai

[1]Department of Applied Chemistry in Bioscience, Graduate School of Agricultural Science, Faculty of Agriculture, Kobe University, 1–1 Rokkodai-cho, Nada-ku, Kobe, Japan, [2]Department of Chemistry, Graduate School of Science, Chiba University, 1–33 Yayoi-cho, Inage-ku, Chiba, Japan

Diacylglycerol kinase (DGK) is a lipid kinase that converts diacylglycerol (DG) into phosphatidic acid (PA). DG and PA function as lipid messengers contributing to various signalling pathways. Thus, DGK plays a pivotal role in the signalling pathways by maintaining DG and PA levels. For example, DGKδ is involved in diabetes and DGKβ is important for higher brain function including memory and emotion. Recently, we also revealed that the activation of DGKα ameliorated diabetic nephropathy (DN) in mice, suggesting that DGK can be therapeutic target. However, there is no commercially available DGK subtype-specific inhibitors or activators. Therefore, in a series of experiment to find DGK subtype-specific inhibitors or activators, we tried to screen novel DGKα activators from 9,600 randomly selected compounds by using high-throughput screening we had recently developed. Finally, we obtained two lead compounds for DGK α activators, KU-8 and KU-10. Focusing KU-8, we assessed the effect of KU-8 on all mammalian DGKs activities. Thus, KU-8 activates not only DGKα but also DGKθ by approximately 20%, and strongly inhibited DGKκ. In conclusion, KU-8 would be a good lead compound for DGKα and DGKθ activators, and useful as a DGKκ inhibitor.

Keywords: diacylglycerol, diacylglycerol kinase, diebetic nephrophathy, hypospadias, phosphatyidic acid

Neurochemistry

Function of essential chloride and arginine residue in nucleotide binding to vesicular nucleotide transporter

[1]Yuma Iwai, [1]Setsuko Kamatani, [1]Sawako Moriyama and [1]Hiroshi Omote

[1]Department of Membrane Biochemistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 1–1–1 Tsushimanaka, Kita-ku, Okayama, Okayama, Japan

Vesicular nucleotide transporter (VNUT) plays a key role in purinergic signalling through its ability to transport nucleotides. VNUT belongs to the SLC17 family, which includes vesicular glutamate transporters (VGLUTs) and Type I Na^+/phosphate cotransporters. All of these transporters exhibit membrane potential and Cl^--dependent organic anion transport activity and have essential arginine in the transmembrane region. Previously, we reported that ketoacids inhibit these transporters through modulation of Cl^- activation. Although this regulation is important to control signal transmission, the mechanisms underlying Cl^--dependent regulation are unclear. Here, we examined the functional roles of Cl^- and essential arginine residue on ATP binding to VNUT using the fluorescent ATP analogue trinitrophenyl-

ATP (TNP-ATP). The fluorescence of TNP-ATP was enhanced by VNUT, whereas no enhancement was observed by VGLUT. Concentration-dependence curves showed that TNP-ATP was a high-affinity fluorescent probe for VNUT, with a K_d of 4.8 μM. TNP-ATP binding was competitive to ATP and showed similar specificity to transport activity. Addition of Cl^- and ketoacids did not affect the apparent affinity for TNP-ATP. The Arg119 to Ala mutant retained TNP-ATP binding ability with slightly reduced affinity. Overall, these results indicated that Cl^- and essential arginine were not important for ATP binding.

Keywords: chloride, ketone body, SLC17, trinitrophenyl-ATP, vesicular nucleotide transporter

MOLECULAR BIOLOGY

Molecular Biology General

A DNA-binding domain in the C-terminal region of Cdt2 enhances the DNA synthesis-coupled CRL4Cdt2 ubiquitin ligase activity for Cdt1

[1]Muadz Ahmad Mazian, [1]Naohiro Suenaga, [1]Takashi Ishii, [1]Akiyo Hayashi, [1]Yasushi Shiomi and [1]Hideo Nishitani

[1]Graduate School of Life Science, University of Hyogo, Kamigori, Akogun Hyogo, Japan

The Cullin-RING ubiquitin ligase $CRL4^{Cdt2}$ maintains genome integrity by mediating the cell cycle- and DNA damage-dependent degradation of proteins such as Cdt1, p21 and Set8. Human Cdt2 has two regions, a conserved N-terminal seven WD40 repeat region and a less conserved C-terminal region. Here, we showed that the N-terminal region is sufficient for complex formation with CRL4, but the C-terminal region is required for the full ubiquitin ligase activity. UV irradiation-induced polyubiquitination and degradation of Cdt1 were impaired in Cdt2 (N-terminus only)-expressing cells. Deletion and mutation analysis identified a domain in the C-terminal region that increased ubiquitination activity and displayed DNA-binding activity. The identified domain mediated binding to double-stranded DNA and showed higher affinity binding to single-stranded DNA. As the ligase activity of $CRL4^{Cdt2}$ depends on proliferating cell nuclear antigen (PCNA) loading onto DNA, the present results suggest that the DNA-binding domain facilitates the $CRL4^{Cdt2}$-mediated recognition and ubiquitination of substrates bound to PCNA on chromatin.

Keywords: cell cycle, DNA damage, DNA replication, proteolysis, ubiquitination

Gene Expression

Do the charges matter?—balancing the charges of the chromodomain proteins on the nucleosome

[1,2]Kyoko Hiragami-Hamada and [1,2]Jun-ichi Nakayama

[1]Division of Chromatin Regulation, National Institute for Basic Biology, Okazaki, Aichi, Japan, [2]Department of Basic Biology, School of Life Science, SOKENDAI (The Graduate University for Advanced Studies), Okazaki, Aichi, Japan

The chromodomain (CD) is a member of the Royal family of conserved chromatin-binding motifs with methylated substrate binding ability, and is often found in 'readers' or 'writers' of repressive histone marks. The regions upstream or downstream of the CD are generally highly charged. Several previous studies suggested that these charged regions modulate the CD's chromatin-binding activity. Considering the relatively weak interaction between the CD and a modified histone tail, it is puzzling how the highly charged CD-flanking regions are 'balanced' on the highly charged nucleosomes to mediate a modification-dependent interaction. Interestingly, the charge distributions along the CD and surrounding regions appear to be distinct among different types of readers and writers, indicating their functional relevance. Here, we describe and discuss the current understanding of the highly charged CD-flanking regions and the potential experimental concerns caused by the regions.

Keywords: chromodomain, heterochromatin, histones, histone methylation, nucleosome