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ダイジェスト

SPECIAL ISSUE
—THE JOURNAL OF BIOCHEMISTRY 100TH ANNIVERSARY ISSUE

Editorial Towards the future of the Journal of Biochemistry
Makoto Nakanishi
The *Journal of Biochemistry* (*JB*) has just celebrated its 100th anniversary and I would like to express my sincere gratitude to the efforts of the successive Editors-in-Chief who have made it possible for *JB* to develop as a leading scientific journal in biochemistry and life sciences over such a long period of time. This special issue marks the 100th anniversary of the journal and we would like to thank Dr Mark A. Lemmon, Chair of the Editorial Board of the *Biochemical Journal*, and Dr Seamus J. Martin, Editor-in-Chief of the *FEBS Journal*, for their kind words of congratulations. Both journals, like *JB*, share a mission to serve the scientific community and are responding to major changes in the current scientific journal publishing industry, including the rise of open access and commercial journals, with a common awareness of the issues involved. The role of academic journals is not only to disseminate scientific results to the world, but also to contribute to the development of professional societies, the training of young researchers and the promotion of international exchange. Indeed, I am very proud to have published my first paper, which was also my dissertation, in *JB*. When I was a graduate student, I presented the results of my research every year at the annual meeting of the Japanese Biochemical Society and received comments from various professors, sometimes harsh, sometimes encouraging, as I advanced my own research. The journal must keep pace with such society activities and contribute to the scientific community. Continued

JB Special Issue—Commentary

A Prosperous Future
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The Journal of Biochemistry: 100 years of excellence in scientific publishing
Seamus J Martin
The FEBS Journal Editorial Office, Cambridge, CB2 3BZ, UK
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JB: A cradle for young Japanese biochemists
Tairo Oshima
Institute of Environmental Microbiology, Kyowa-kako Co., Professor Emeritus of Tokyo Institute of Technology, and Professor Emeritus of Tokyo University of Pharmacy and Life Sciences

Land-breaking publications and the impact of these publications in several research areas: commentary for the 100th anniversary of Journal of Biochemistry
Naoyuki Taniguchi
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Cherish JB, a unique journal that originated from Japan
Kohei Miyazono
Department of Molecular Pathology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Reflection
Akira Kikuchi
Department of Molecular Biology and Biochemistry, Graduate School of Medicine, Osaka University, Center for the Infectious Disease Education and Research, Osaka University

Reform of Journal of Biochemistry
Kenji Kadomatsu
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Fond Memories of Professor Sen-itiroh Hakomori
Jin-ichi Inokuchi
Division of Glycopathology, Institute of Molecular Biomembrane and Glycobiology (IMBG), Tohoku Medical and Pharmaceutical University, 4–4–1 Komatsushima, Aoba-ku, Sendai, Miyagi, 981–8558, Japan, Forefront Research Center, Graduate School of Sciences, Osaka University, Machikaneyama, Toyonaka, Osaka 560–0043, Japan

Obituary
In Memory of a Humble Scientifi c Pioneer, Tsuneo Omura
Yuzuru Ishimura
Professor Emeritus Department of Biochemistry, School of Medicine, Keio University and a past President of the Japanese Biochemical Society
(Ken-ichirou Morohashi and Bettie Sue S. Masters contributed to this article)

CELL

Extracellular Matrices and Cell Adhesion Molecules

Non-triple helical form of type IV collagen alpha1 chain suppresses vascular endothelial-cadherin mediated cell-to-cell junctions

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The CC chemokine receptor 5 (CCR5) antagonism represents a promising pharmacological strategy for therapeutic intervention as it plays a significant role in reducing the severity and progression of a wide range of pathological conditions. Here we designed and generated peptide ligands targeting the chemokine receptor, CCR5, that were derived from the critical interaction sites of the V3 crown domain of envelope protein glycoprotein gp120 (TRKSIHIGPGRAFYTTGEI) of HIV-1 using computational biology approach and the peptide sequence corresponding to this region was taken as the template peptide, designated as TMP-1. The peptide variants were synthesized by employing Fmoc chemistry using polymer support and were labelled with rhodamine B to study their interaction with the CCR5 receptor expressed on various cells. TMP-1 and TMP-2 were selected as the high-affinity ligands from *in vitro* receptor-binding assays. Specific receptor-binding experiments in activated peripheral blood mononuclear cells and HOS.CCR5 cells indicated that TMP-1 and TMP-2 had significant CCR5 specificity. Further, the functional analysis of TMP peptides using chemotactic migration assay showed that both peptides did not mediate the migration of responsive cells. Thus, template TMP-1 and TMP-2 represent promising CCR5 targeting peptide candidates.

Keywords: V3 crown, RANTES, gp120, CCR5 antagonism, CCR5

Receptors and Signal Transduction

Trehalose decreases mRNA and protein expressions of c-Jun and JunB in human cervical cancer HeLa cells

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Non-triple helical collagen polypeptide α1(IV) (NTH α1(IV)) is a gene product of COL4A1 and is secreted as a polypeptide chain without the triple helix structure under physiological conditions. Studies have shown that NTH α1(IV) is up-regulated in and around vascular endothelial cells during neovascularization and vascular-like networks of in vitro angiogenesis models, suggesting its involvement in angiogenesis. In the present study, we examined the effect of NTH α1(IV) on endothelial cell-to-cell junctions, and we found that NTH α1(IV) suppressed VE-cadherin (vascular endothelial cadherin) mediated junctions and promoted cellular migration in human umbilical vein endothelial cell cultures. NTH α1(IV) is potentially a factor that induces VE-cadherin endocytosis and promotes neovascular sprouting and elongation. The possible mechanism entails endocytosis of NTH α1(IV) by its cellular receptor(s), Endo180 and/or other proteins, which results in the clearance of the cellular receptor(s) from the cell surface, thus inducing the endocytosis of VE-cadherin. Because the NC1 domain of the α1 chain of type IV collagen, called arresten, is Graphical Abstract and one α2(IV) chain in a triple helix structure (3).It has been shown that polypeptides without the triple helix structure, called non-triple helical collagen polypeptides (NTHs), are secreted through cellular biosynthesis (4–8). The NTH encoded in the COL4A1 gene is known as NTH α1(IV). Thus far, NTHs have been found for type IV collagen and type VI collagen. considered an endogenous inhibitor of angiogenesis, it seems that the single polypeptide chain of NTH α1(IV) has conflicting functions.

Keywords: VE-cadherin, NTH α1(IV), endocytosis, Endo180, angiogenesis

BIOTECHNOLOGY

Synthetic Peptides and Oligonucleotides

Novel small synthetic HIV-1 V3 crown variants: CCR5 targeting ligands

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Increasing evidence suggests that trehalose, a non-reducing disaccharide, ameliorates disease phenotypes by activating autophagy in animal models of various human diseases, including neurodegenerative diseases. Multiple *in vitro* studies suggest that activation of transcription factor EB, a master regulator of lysosomal biogenesis and autophagy genes, is a major contributor to trehalose-induced autophagy at later stages of exposure. However, underlying causes of trehalose-induced autophagy possibly occur at the early stage of the exposure period. In this study, we investigated the effects of short-term exposure of HeLa cells to trehalose on several signal transduction pathways to elucidate the initial events involved in its beneficial effects. Phospho-

protein array analysis revealed that trehalose decreases levels of phosphorylated c-Jun, a component of the transcription factor activator protein-1, after 6 h. Trehalose also rapidly reduced mRNA expression levels of c-Jun and JunB, a member of the Jun family, within 1 h, resulting in a subsequent decrease in their protein levels. Future studies, exploring the interplay between decreased c-Jun and JunB protein levels and beneficial effects of trehalose, may provide novel insights into the mechanisms of trehalose action.

Keywords: autophagy, cell signaling, c-Jun, JunB, trehalose

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ダイジェスト

JB REVIEW

Structural basis for molecular interactions on the eukaryotic DNA sliding clamps PCNA and RAD9-RAD1-HUS1

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DNA sliding clamps are widely conserved in all living organisms and play crucial roles in DNA replication and repair. Each DNA sliding clamp is a doughnut-shaped protein with a quaternary structure that encircles the DNA strand and recruits various factors involved in DNA replication and repair, thereby stimulating their biological functions. Eukaryotes have two types of DNA sliding clamp, proliferating cell nuclear antigen (PCNA) and RAD9-RAD1-HUS1 (9-1-1). The homo-trimer PCNA physically interacts with multiple proteins containing a PCNA-interacting protein box and/or AlkB homologue 2 PCNA-interacting motif. The two motifs bind to PCNA by a similar mechanism; in addition, the bound PCNA structure is similar, implying a universality of PCNA interactions. In contrast to PCNA, 9-1-1 is a heterotrimer composed of RAD9, RAD1 and HUS1 subunits. Although 9-1-1 forms a trimeric ring structure similar to PCNA, the C-terminal extension of the RAD9 is intrinsically unstructured. Based on the structural similarity between PCNA and 9-1-1, the mechanism underlying the interaction of 9-1-1 with its partners was thought to be analogous to that of PCNA. Unexpectedly, however, the recent structure of the 9-1-1 ring bound to a partner has revealed a novel interaction distinct from that of PCNA, potentially providing a new principle for molecular interactions on DNA sliding clamps.

Keywords: 3D structure, protein–protein interactions, PCNA, DNA sliding clamp, 9-1-1

Adipose tissue thermogenesis by calcium futile cycling

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Brown and beige adipocytes produce heat and control systemic energy via non-shivering thermogenesis. Historically, thermogenesis in brown and beige adipocytes was thought to be exclusively through a mitochondria-localized protein, uncoupling protein 1 (UCP1). However, recent studies identified UCP1-independent thermogenic mechanisms in adipocytes. Importantly, UCP1-independent pathways significantly contribute to systemic energy and glucose homeostasis. The finding of UCP1-independent mechanisms provided new opportunities to target the pathways *in vivo*. In this review, we discuss the current understandings of thermogenic mechanisms in adipocytes with a focus on Ca^{2+} futile cycling.

Keywords: uncoupling protein1, Thermogenic adipocytes, alcium cycling thermogenesis, brown

BIOCHEMISTRY

Biochemistry in Cell Membranes

Oligomeric state of the aspartate: alanine transporter from Tetragenococcus halophilus

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The aspartate: alanine exchanger family of membrane transporters includes industrially important transporters such as succinate exporter and glutamate exporter. No high-resolution structure is available from this family so far, and the transport mechanism of these transporters also remains unclear. In the present study, we focus on the oligomeric status of the aspartate:alanine antiporter (AspT) of *Tetragenococcus halophilus*, which is the prototype of this family. To investigate the oligomeric structure of AspT, we

established a system that produces high yields of highly purified AspT and determined the oligomeric structure of AspT by analysis with size exclusion chromatography coupled with multi-angle light scattering and blue native PAGE and by comparison of the wild-type AspT with a single-cysteine mutant that forms spontaneous inter-molecular thiol crosslinking. All the results consistently support the notion that AspT is a homodimer in solutions and in membranes.

Keywords: SEC-MALS, blue native PAGE, AspT, amino acid transporter

Lunapark ubiquitinates atlastin-2 for the tubular network formation of the endoplasmic reticulum

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Endoplasmic reticulum (ER) tubules are interconnected by three-way junctions, resulting in the formation of a tubular ER network. Lunapark (Lnp) localizes to and stabilizes the three-way junctions. The N-terminal cytoplasmic domain in Lnp has a ubiquitin ligase activity. However, the molecular mechanism of how the ubiquitin ligase activity of Lnp is involved in the formation of the tubular ER network remains unknown. In this study, we examined whether the ER membrane proteins responsible for the formation of the tubular ER network are ubiquitinated by Lnp. We found that atlastin-2 (ATL2), an isoform of the ATL family mediating the generation of the three-way junctions by connecting the ER tubules, is a novel substrate for ubiquitination by Lnp. The localization of Lnp at the three-way junctions is important for ubiquitination of ATL2. Lysine 56, 57, 282 and 302 are the potential ubiquitination sites by Lnp. Silencing ATL2 decreased the number of the three-way junctions, and the expression of the ATL2 mutant in which the lysine residues are substituted with arginine failed to rescue the decrease of the three-way junctions in the ATL2 knocked-down cells. These results suggest that Lnp ubiquitinates ATL2 at the three-way junctions for the proper tubular ER network formation.

Keywords: ubiquitin ligase, three-way junction, endoplasmic reticulum, amphipathic helix

Biochemistry in Diseases and Aging

Tau-binding protein PRMT8 facilitates vacuole degeneration in the brain

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Amyloid-β and tau pathologies are important factors leading to neurodegeneration in Alzheimer's disease (AD); however, the molecular mechanisms that link these pathologies remain unclear. Assuming that important though as yet unidentified factors inhibit/accelerate tau pathology and neuronal cell death under amyloid pathology, we sought to isolate and identify tau-interacting proteins from mouse brains with or without amyloid pathology. Among the proteins that were identified, we focused on protein arginine methyltransferase 8 (PRMT8), which interacts with tau specifically in the absence of amyloid pathology. To investigate the role of PRMT8 in the pathogenesis of AD, we conducted *Prmt8* gene deletion and overexpression experiments in $App^{NL\text{-}G\text{-}F}$/*MAPT* double knock-in mice and analysed the resulting pathological alterations. PRMT8-knockout did not alter the AD pathology in double knock-in mice, whereas PRMT8-overexpression promoted tau phosphorylation, neuroinflammation and vacuole degeneration. To evaluate if such a PRMT8-induced vacuole degeneration depends on tau pathology, PRMT8 was overexpressed in tau-KO mice, which were consequently found to exhibit vacuole degeneration. In addition, proteomic analyses showed that PRMT8 overexpression facilitated the arginine methylation of vimentin. Abnormal protein methylation could be involved in PRMT8-induced brain pathologies. Taken together, PRMT8 may play an important role in the formation of tau pathology and vacuole degeneration.

Keywords: vacuole, Tau, PRMT8, App knockin, Alzheimer's disease

Protein Interaction and Recognition

Cloaking the ACE2 receptor with salivary cationic proteins inhibits SARS-CoV-2 entry

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Saliva contributes to the innate immune system, which suggests that it can prevent SARS-CoV-2 entry. We studied the ability of healthy salivary proteins to bind to angiotensin-converting enzyme 2 (ACE2) using biolayer interferometry and pull-down assays. Their effects on binding between the receptor-binding domain of the SARS-CoV-2 spike protein S1 (S1) and ACE2 were determined using an enzyme-linked immunosorbent assay. Saliva bound to ACE2 and disrupted the binding of S1 to ACE2 and four ACE2-binding salivary proteins were identified, including cationic histone H2A and neutrophil elastase, which inhibited the S1-ACE2 interaction. Calf thymus histone (ct-histone) also inhibited binding as effectively as histone H2A. The results of a cell-based infection assay indicated that ct-histone suppressed SARS-CoV-2 pseudoviral invasion into ACE2-expressing host cells. Manufactured polypeptides, such as ε-poly-L-lysine, also disrupted S1-ACE2 binding, indicating the importance of the cationic properties of salivary proteins in ACE2 binding. Overall, we demonstrated that positively charged salivary proteins are a barrier against SARS-CoV-2 entry by cloaking the negatively charged surface of ACE2 and provided a view that the cationic polypeptides represent a preventative and therapeutic treatment against COVID-19.

Keywords: SARS-CoV-2 spike protein, saliva, neutrophil elastase, histone H2A, angiotensin-converting enzyme 2

MOLECULAR BIOLOGY

RNA Processing

Analysis of ribonucleotide content in the genomic DNA of ribonuclease H2 A subunit (RH2A)-knockout NIH3T3 cells after transient expression of wild-type RH2A or RH2A variants with an Aicardi–Goutières syndrome-causing mutation

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Ribonuclease (RNase) H2 is involved in the removal of ribonucleotides embedded in genomic DNA. Eukaryotic RNase H2 is a heterotrimer consisting of the catalytic A subunit (RH2A) and the accessory B and C subunits. This study aimed to compare the cellular activities of wild-type ribonuclease (RNase) H2 and its variants with a mutation causing neuroinflammatory autoimmune disease, Aicardi–Goutières syndrome (AGS). We first analyzed cellular RNase H2 activity and ribonucleotide content in the genomic DNA of RH2A-knockout (KO) mouse fibroblast NIH3T3 cells after transfection with a transient expression plasmid encoding mouse wild-type RH2A. From 4 h after transfection, the RNase H2 activity increased and the amount of ribonucleotides decreased, as compared with the corresponding non-transfected RH2A-KO cells. This demonstrated the rapidness of ribonucleotide turnover in mammalian genomic DNA and the importance of continuous expression of RNase H2 to maintain the ribonucleotide amount low. Next, we expressed mouse RH2A variants with a mutation corresponding to a human AGS-causing mutation in RH2A-KO NIH3T3 cells. Neither increase in RNase H2 activity nor decrease in ribonucleotide amount was observed for G37S; however, both conditions were observed for N213I and R293H. This corresponded with our previous results on the activity of recombinant human RNase H2 variants.

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