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ダイジェスト

JB Special Reviews
Skeletal muscle cell biology and disease

The roles of muscle stem cells in muscle injury, atrophy and hypertrophy
So-ichiro Fukada
Skeletal muscle is composed of multinuclear cells called myofibers. Muscular dystrophy (a genetic muscle disorder) induces instability in the cell membrane of myofibers and eventually causes myofibre damage. Non-genetic muscle disorders, including sarcopenia, diabetes, bedridden immobility and cancer cachexia, lead to atrophy of myofibres. In contrast, resistance training induces myofibre hypertrophy. Thus, myofibres exhibit a plasticity that is strongly affected by both intrinsic and extrinsic factors. There is no doubt that muscle stem cells (MuSCs, also known as muscle satellite cells) are indispensable for muscle repair/regeneration, but their contributions to atrophy and hypertrophy are still controversial. The present review focuses on the relevance of MuSCs to (i) muscle diseases and (ii) hypertrophy. Further, this review addresses fundamental questions about MuSCs to clarify the onset or progression of these diseases and which might lead to development of a MuSC-based therapy.

Ribitol-phosphate—a newly identified posttranslational glycosylation unit in mammals: structure, modification enzymes and relationship to human diseases
Motoi Kanagawa; Tatsushi Toda
Glycosylation is a crucial posttranslational modification that is involved in numerous biological events. Therefore, abnormal glycosylation can impair the functions of glycoproteins or glycolipids and is occasionally associated with cell dysfunction and human diseases. For example, aberrant glycosylation of dystroglycan (DG), a cellular receptor for matrix and synaptic proteins, is associated with muscular dystrophy and lissencephaly. DG sugar chains are required for high-affinity binding to ligand proteins, and thus disruption of DG-ligand linkages underlies disease conditions. Although their biological significance is well recognized, the sugar-chain structure of DG and its modification enzymes have long remained incompletely elucidated. However, recent seminal studies have finally revealed a highly regulated mechanism for DG glycosylation and have discovered a posttranslational unit, ribitol-phosphate, that was not previously known to be used in mammals. This review article introduces the structure, modification enzymes and functions of the sugar chains of DG, and then discusses their relationship to human diseases and therapeutic strategies.

Regular Papers

Biochemistry
Protein Structure

The assembly mechanism of coiled-coil domains of the yeast cargo receptors Emp46p/47p and the mutational alteration of pH-dependency of complex formation
Koichi Kato; Takahisa Furuhashi; Akifumi Oda; Eiji Kurimoto
The coiled-coil domains of the putative yeast cargo receptors Emp46p and Emp47p are responsible for their complex-formation in the Endoplasmic Reticulum. In vitro experiments using coiled-coil domains (Emp46pcc/47pcc) have indicated that formation of the hetero-complex is pH-dependent and that amino acid Glu303 of Emp46pcc is a key residue in this process. In this study, we investigated the effects of various mutations on complex formation and discovered the mechanism for its pH-dependency, which is that dissociation of the complex at low pH arises mainly from stabilization of Emp46pcc itself. Moreover, destabilization by the introduction of a histidine residue in Emp46pcc to repel a lysine residue in Emp47pcc, caused an upward shift in the pH profile of complex formation. Another mutation in Emp46pcc, a proline to an alanine (P291A), increased the stability of the helical structure, especially at low pH and shifted the transition pH upward. Combination of these pH-shifting mutations had an additive effect on the pH profile of complex formation. Thus, we successfully constructed coiled-coils that can react to a wide range of pH, encompassing more appropriate values for use in sensing physiological pH changes in the cell.

Protein Interaction and Recognition

Cell surface chondroitin sulphate proteoglycan 4 (CSPG4) binds to the basement membrane heparan sulphate proteoglycan, perlecan, and is involved in cell adhesion
Fengying Tang; Megan S Lord; William B Stallcup; John M Whitelock
Chondroitin sulphate proteoglycan 4 (CSPG4) is a cell surface proteoglycan highly expressed by tumour, perivascular and oligodendrocyte cells and known to be involved cell adhesion and migration. This study showed that CSPG4 was present as a proteoglycan on the cell surface of two melanoma cell lines, MM200 and Me1007, as well as shed into the conditioned medium. CSPG4 from the two melanoma cell lines differed in the amount of chondroitin sulphate (CS) decoration, as well as the way the protein core was fragmented. In contrast, the CSPG4 expressed by a colon carcinoma cell line, WiDr, was predominantly as a protein core on the cell surface lacking glycosaminoglycan

(GAG) chains. This study demonstrated that CSPG4 immunopurified from the melanoma cell lines formed a complex with perlecan synthesized by the same cultured cells. Mechanistic studies showed that CSPG4 bound to perlecan via hydrophobic protein–protein interactions involving multiple sites on perlecan including the C-terminal region. Furthermore, this study revealed that CSPG4 interacted with perlecan to support cell adhesion and actin polymerization. Together these data suggest a novel mechanism by which CSPG4 expressing cells might attach to perlecan-rich matrices so as those found in connective tissues and basement membranes.

Enzymology

Comparative studies on the activities of collagenases from Grimontia hollisae and Clostridium hystoliticum in the hydrolysis of synthetic substrates

Teisuke Takita; Jun Qian; Hongmin Geng; Zejian He; Sho Nemoto; Mariko Mori; Keisuke Tanaka; Shunji Hattori; Kenji Kojima; Kiyoshi Yasukawa

The collagenase produced by a gram-negative bacterium Grimontia hollisae strain 1706B (Ghcol) degrades collagen more efficiently than that produced by a gram-positive bacterium Clostridium histolyticum (Chcol), which is currently the most widely used collagenase in industry [Teramura et al. (Cloning of a novel collagenase gene from the gram-negative bacterium Grimotia (Vibrio) hollisae 1706B and its efficient expression in Brevibacillus choshinensis. J Bacteriol 2011; 193: 3049–3056)]. Here, we compared the Ghcol and Chcol activities using two synthetic substrates. In the hydrolysis of (7-methoxycoumarin-4-yl)acetyl-L-Lys-L-Pro-L-Leu-Gly-L-Leu-[N3-(2, 4-dinitrophenyl)-L-2, 3-diaminopropioyl]-L-Ala-L-Arg-NH2, Ghcol exhibited 350-fold higher activity than Chcol in the absence of CaCl2 and NaCl. The Ghcol activity markedly decreased with increasing concentrations of buffer, CaCl2 or NaCl, while the Chcol activity did not, suggesting that the Ghcol activity was sensitive to solvent components. In the hydrolysis of N-[3-(2-furyl)acryloyl]-L-Leu-Gly-L-Pro-Ala, Ghcol exhibited 16-fold higher activity than Chcol in the absence of CaCl2 and NaCl, and both enzyme activities did not decrease with increasing concentrations of buffer, CaCl2 or NaCl. pH dependences of activity revealed that the ionizable group responsible for acidic pKe may be Glu for Ghcol and Chcol, while that for alkaline pKe may be His for Ghcol and Tyr for Chcol. These striking differences suggest that the catalytic mechanism of Ghcol might be considerably different from that of clostridial collagenases.

Biochemistry in Diseases and Aging

Proteolytic inactivation of ADAMTS13 by plasmin in human plasma: risk of thrombotic thrombocytopenic purpura

Yongchol Shin; Haruki Miyake; Kenshi Togashi; Ryuichi Hiratsuka; Kana Endou-Ohnishi; Yasutada Imamura

Thrombotic thrombocytopenic purpura (TTP) is caused by inactivation of a von Willebrand factor (VWF)-cleaving enzyme, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13), which leads to platelet-rich thrombi comprising unusually large VWF multimers. We have found that ADAMTS13 can bind to the inactivated form of plasmin. In addition, plasmin cleaves purified ADAMTS13 into several fragments and inactivates it. Hence, we hypothesized that activation of plasminogen to plasmin becomes a new-onset factor for TTP due to ADAMTS13 inactivation. Plasmin was added exogenously or activated from plasminogen by streprokinase addition in human plasma (HP). ADAMTS13 digestion and effects of the digestion on ADAMTS13 activity were evaluated. Exogenous plasmin cleaved ADAMTS13 into several fragments, but a portion of ADAMTS13 remained in full-length form. Digestion profile of ADAMTS13 with streprokinase added exogenously in HP was similar to that of ADAMTS13 with exogenous plasmin. ADAMTS13 activity measured using FRETS-VWF73 decreased to ～40% compared with that for normal plasma. Endogenous VWF multimer-cleaving activity was attenuated more severely (～10%). These data suggest that endogenous plasmin cleaves ADAMTS13 into fragments and reduces its activity to ～10%. We suggest that endogenous plasmin activation alone is not sufficient to cause TTP, but plasmin activation with ADAMTS13 deficiency might increase the risk of TTP onset.

The GATA transcription factor ELT-2 modulates both the expression and methyltransferase activity of PRMT-1 in Caenorhabditis elegans

Sho Araoi; Hiroaki Daitoku; Atsuko Yokoyama; Koichiro Kako; Keiko Hirota; Akiyoshi Fukamizu

Protein arginine methyltransferase 1 (PRMT1) catalyzes asymmetric arginine dimethylation of cellular proteins and thus modulates various biological processes, including gene regulation, RNA metabolism, cell signaling and DNA repair. Since prmt-1 null mutant completely abolishes asymmetric dimethylarginine in C. elegans, PRMT-1 is thought to play a crucial role in determining levels of asymmetric arginine dimethylation. However, the mechanism underlying the regulation of PRMT-1 activity remains largely unknown. Here, we explored for transcription factors that induce the expression of PRMT-1 by an RNAi screen using transgenic C. elegans harbouring prmt-1 promoter upstream of gfp. Of 529 clones, we identify a GATA transcription factor elt-2 as a positive regulator of Pprmt-1::gfp expression and show that elt-2 RNAi decreases endogenous PRMT-1 expression at mRNA and protein levels. Nevertheless, surprisingly arginine methylation levels are increased when elt-2 is silenced, implying that erythroid-like transcription factor (ELT)-2 may also have ability to inhibit methyltransferase activ-

ity of PRMT-1. Supporting this idea, GST pull-down and co-immunoprecipitation assays demonstrate the interaction between ELT-2 and PRMT-1. Furthermore, we find that ELT-2 interferes with PRMT-1-induced arginine methylation in a dose-dependent manner. Collectively, our results illustrate the two modes of PRMT-1 regulation, which could determine the levels of asymmetric arginine dimethylation in C. elegans.

Molecular Biology
Replication and Recombination

Effect of daidzein and equol on DNA replication in MCF-7 cells

Mako Tsuji; Tomoki Tanaka; Ryosuke Nagashima; Yuka Sagisaka; Yuko Tousen; Yoriko Nishide; Yoshiko Ishimi; Yukio Ishimi

It has been reported that daidzein and equol stimulate DNA replication and proliferation of MCF-7 cells. However, their molecular mechanisms of action are still unclear. We examined the effects of daidzein and equol on DNA replication of MCF-7 cells, focusing on MCM2-7 proteins, which function as the replicative helicase. In the presence of either 1 μM of daidzein or equol, the number of cells in S-phase, which was determined by detecting bromodeoxyuridine incorporated into replicated DNA, almost doubled. The total amounts of MCM7 protein and chromatin-bound MCM7 protein increased in the presence of daidzein. The data suggest that phytoestrogens facilitate cell cycle progression in G1-phase by increasing the level of MCM proteins. In the presence of phytoestrogens, phosphorylation of Rb and levels of MCM2, 3 and 7 mRNA increased, suggesting that stimulation of MCM2-7 transcription is involved in the cell cycle progression. Under the same conditions, double-stranded DNA breakage in logarithmically growing MCF-7 cells, which was detected using anti-γ-H2AX antibodies, did not increase in the presence of equol.

Cell
Differentiation, Development, and Aging

ヌクレオメチリン欠損による胎児造血障害の解析

村上昌平[1]；鈴木琢磨[1, 2]；横山　航[3]；八木理子[1]；松村啓汰[1]；仲島由佳[3]；張替秀郎[2]；深水昭吉[3]；本橋ほづみ[1]
([1]東北大学 加齢医学研究所 遺伝子発現制御分野；[2]東北大学 大学院医学系研究科 血液・免疫病学分野；[3]筑波大学 生命領域学際研究センター ゲノム情報生物学分野)

ヌクレオメチリン（NML）はリボソーム形成に重要であり，その機能抑制はp53を活性化させることが示されている．我々は，NML欠損マウスの大半が胎生中期で造血障害を呈し，致死となることを見いだした．NML欠損の胎児肝臓ではp53依存的なp21発現上昇がみられたが，p53を欠損させてもNML欠損マウスの造血障害・胎性致死は改善しなかった．よって，これらの障害はp53非依存的であると考えられる．

Biotecgnology

Biotechnology General

Efficient development of a stable cell pool for antibody production using a single plasmid

Yi Yang; Min You; Fentian Chen; Tianrong Jia; Yuanzhi Chen; Bing Zhou; Qingyu Mi; Zhiqiang An; Wenxin Luo; Ningshao Xia

Therapeutic antibodies are the fastest growing group of biopharmaceuticals. Evaluation of drug candidates requires a sufficient amount of antibodies. Production of antibodies with stable cell pools is an efficient strategy to produce grams of proteins for drug candidate selection. Many methods have been described for developing stable cell pools for antibody expression. However, most of the reported methods are laborious due to the low frequency of high producers. In this study, we determined optimal vectors and screening parameters to develop a strategy for efficient construction of stable antibody expressing cell pools. The cell pool constructed using the optimized strategy consistently yielded a higher expression titer, up to 10-fold improvement. Further, this method resulted in a higher ratio of the cell pools with the main product peak above 95% as assessed by size-exclusion chromatography. High producers could be obtained by means of screening five 96-well plates. This strategy will greatly reduce clone-screening size during Clinical Lead Selection. This study provides a platform with efficient design of plasmids and screening strategies for significant cost and labour savings in high expression of two-subunit proteins such as antibodies.

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JB Special Reviews
Neuronal functions and disorders

Postsynaptic density proteins and their involvement in neurodevelopmental disorders

T. Kaizuka; T. Takumi

Synaptic connections are essential for neural circuits in order to convey brain functions. The postsynaptic density (PSD) is a huge protein complex associated with postsynaptic membranes of excitatory synapses. In mammals, the PSD is composed of more than 1,000 proteins including receptors, scaffold proteins, signalling enzymes and cytoskeletal proteins. PSD proteins are crucial for synaptic transmission and plasticity. Proteomic stud-

ies have revealed the composition of PSD proteins in various species, brain regions and specific physiological conditions. Abnormalities with PSD proteins are linked to various neuropsychiatric diseases including neurodevelopmental disorders such as autism spectrum disorder and schizophrenia. Here, we review different kinds of proteomic studies of the PSD and the involvement of PSD proteins in physiological and pathological conditions.

Activity-dependent functions of non-electrical glial cells

D. Kato; K. Eto; J. Nabekura; H. Wake

Electrical activity is essential for brain function. However, neurons, the electrically active cells, are less numerous than the non-electrical glial cells in the central nervous system. The non-electrical components modify the function of neural circuits, depending on the electrical neuronal activity, by wrapping synapses, myelinating axons and phagocytozing the neuronal components. Moreover, recent evidence has suggested that they contribute to neurological and psychiatric disease by regulating neuronal circuits, ultimately affecting their behaviour. In this review, we highlight the physiological functions of glial cells, particularly the electrical activity-dependent processes, to provide further insight into their role in brain function.

Regular Papers

Biochemistry

Protein Interaction and Recognition

Acceleration of nucleation of prion protein during continuous ultrasonication

K.-i. Yamaguchi; R.P. Honda; A.E. Elhelaly; K. Kuwata

Although pulsatile irradiation of ultrasonication is frequently used for generating amyloid fibrils in vitro, the potential for inducing amyloid fibrillation of proteins during continuous ultrasonication is unknown. In this study, we implemented a continuous irradiation system and measured far-ultraviolet circular dichroism in a real-time manner. During the continuous ultrasonication, the conformation of full-length mouse prion protein (mPrP) was rapidly altered without a lag time and electron microscopy revealed that distorted fibrils, β-oligomers and amorphous aggregates were formed at pH 2.2, 4.0 and 9.1, respectively. Similarly, hen egg white lysozyme formed distorted fibrils and small and large amorphous aggregates at pH 2.2 and 7.1 and 11.9, respectively, without a lag time. The concentration dependencies of the initial rates were different between the two systems. The aggregate formation of mPrP followed a first-order reaction, whereas that of lysozyme followed the zeroth-order reaction. Importantly, the reactions were immediately stopped by switching off ultrasonication, and restarted instantaneously when ultrasonication was restarted. Thus, the continuous ultrasonication significantly accelerates the nucleations of mPrP and lysozyme aggregates by the interaction between monomer and cavitation bubble. These cavitation bubbles may act as catalysts that decrease the activation free energy for nucleation, which is low in mPrP and high in lysozyme.

Nucleic Acid and Peptide Biochemistry

rRNA adenine methylation requires T07A9.8 gene as rram-1 in Caenorhabditis elegans

W. Yokoyama; K. Hirota; H. Wan; N. Sumi; M. Miyata; S. Araoi; N. Nomura; K. Kako; A. Fukamizu

RNAs are post-transcriptionally modified in all kingdoms of life. Of these modifications, base methylations are highly conserved in eukaryote ribosomal RNA (rRNA). Recently, rRNA processing protein 8 (Rrp8) and nucleomethylin (NML) were identified as factors of N1-methyladenosine (m1A) modification in yeast 25 S and mammalian 28 S rRNA, respectively. However, m1A modification of rRNA is still poorly understood in Caenorhabditis elegans (C. elegans). Here, using the liquid chromatography/tandem mass spectrometry analysis and RNA immunoprecipitation assay, we have identified that the m1A modification is located around position 674 (A674) of 26 S rRNA in C. elegans. Furthermore, quantitative PCR-based analysis revealed that T07A9.8, a C. elegans homolog of yeast Rrp8 and human NML, is responsible for m1A modification at A674 of 26 S rRNA. This m1A modification site in C. elegans corresponds to those in yeast 25 S rRNA and human 28 S rRNA. Intriguingly, T07A9.8 is not associated with pre-rRNA transcription under normal nutrient conditions. Since the m1A modification of 26 S rRNA requires T07A9.8 in C. elegans, we designated the gene as rRNA adenine methyltransferase-1 (rram-1).

Enzymology

NADPH oxidase 4 function as a hydrogen peroxide sensor

Y. Nisimoto; H. Ogawa; Y. Kadokawa; S. Qiao

Nox4, a member of the NADPH- and oxygen-dependent oxidoreductases that generate reactive oxygen species (ROS), is widely expressed and constitutively active. To understand better its function and regulation, specific mutations in the Nox4 dehydrogenase (DH) domain were examined for effects on Nox4 oxidase activity. Transfection of His6-tagged Nox4 increased the amount of p22phox subunit in HEK293 cells, and a higher level of the heterodimer was observed in the nucleus-enriched fraction (NEF). NEF from Nox4-expressing HEK293 cells exhibited oxygen and H2O2 concentration-dependent NADPH oxidation rate. In Nox4-expressing cells, NEF and its partially purified form, the Nox4(P437H) mutant almost completely lost its oxidase activity, while Nox4(C546S), Nox4(C546L) or/and (C547L) had a significantly decreased rate of ROS production. The NADPH-dependent reduction of cytochrome c or cyto-

chrome b5 by purified Nox4 DH domain was found regulated by the H2O2 concentration, and C546L and C547L mutants showed lower rates of the hemeprotein reduction. These conserved Cys residues in the DH domain respond to the cytosolic H2O2 concentration to regulate Nox4 activity.

Biochemistry in Cell Membranes

Immune neutralization of the receptor for advanced glycation end products reduce liver oxidative damage induced by an acute systemic injection of lipopolysaccharide

C. Tiefensee Ribeiro; J. Gasparotto; A.A. Teixeira; L.V.C. Portela; V.N.L. Flores; J.C.F. Moreira; D.P. Gelain

The receptor for advanced glycation end products (RAGE) is a multi-ligand receptor, which activation amplifies and perpetuates inflammatory reactions. RAGE activation also strongly stimulates the production of reactive oxygen species, leading an imbalance of redox cellular state. The extent of liver damage caused by inflammation is crucial to the systemic response during proinflammatory episodes. To investigate the role of RAGE in liver damage caused by systemic inflammation, we evaluated the effect of RAGE blocking in oxidative stress parameters induced by systemic lipopolysaccharide (LPS) injection. Wistar rats received an intraperitoneal injection of RAGE antibody (50 mg/kg), 1 h prior intraperitoneal injection of LPS (5 mg/kg). Twenty-four hours later, the liver was isolated for analysis. The LPS-induced effect in protein oxidative damage, mitochondrial complex II activity, catalase activity, signal transducer and activator of transcription 3 phosphorylation and caspase 3 activation was prevented by prior treatment with RAGE antibody. However, RAGE blocking was not able to inhibit reactive oxygen species production and the impairment in non-enzymatic antioxidant capacity induced by LPS. The present results indicate that RAGE is an important mediator of liver oxidative damage induced by an acute systemic injection of LPS, although other mechanisms may also be responsible for liver function impairment during inflammation.

Cell

Receptors and Signal Transduction

Homodimer formation by the ATP/UTP receptor P2Y2 via disulfide bridges

M. Abe; K. Watanabe; Y. Kuroda; T. Nakagawa; H. Higashi

Many class C G-protein coupled receptors (GPCRs) function as homo- or heterodimers and several class A GPCRs have also been shown to form a homodimer. We expressed human P2Y2 receptor (P2Y2R) in cultured cells and compared SDS-PAGE patterns under reducing and non-reducing conditions. Under non-reducing conditions, approximately half of the P2Y2Rs were electrophoresed as a dimer. We then produced Cys to Ser mutants at four sites (Cys25, Cys106, Cys183 and Cys278) in the extracellular domains of P2Y2R and examined the effect on dimer formation and receptor activity. All single mutants formed dimers similarly to the wild-type protein, but C25S, C106S and C183S P2Y2R lost activity, while C278S P2Y2R maintained weak activity. Coexpression with wild-type P2Y2R recovered the activity of the C25S mutant. These results show that Cys106 and Cys183 are required for monomer or homodimer activity; Cys25 is required for monomer activity, but it is not needed in one protomer for homodimer activity; and Cys278 can be replaced in the monomer and homodimer. Approximately, half of C25S/C278S double mutants were electrophoresed as a dimer, similarly to the wild-type and single mutants, and dimers with the wild-type protein were active. These results suggest involvement of Cys106 and Cys183 in disulfide bonding between protomers in homodimer formation.

Biotechnology

Gene and Protein Engineering

Characterization of glycoengineered anti-HER2 monoclonal antibodies produced by using a silkworm–baculovirus expression system

Y. Egashira; S. Nagatoishi; M. Kiyoshi; A. Ishii-Watabe; K. Tsumoto

Silkworm–baculovirus expression systems are efficient means for the production of recombinant proteins that provide high expression levels and post-translational modifications. Here, we characterized the stability, glycosylation pattern and antibody-dependent cell-mediated cytotoxicity activity of anti-HER2 monoclonal antibodies containing native or glycoengineered mammalian-like N-glycans that were produced by using a silkworm–baculovirus expression system. Compared with a monoclonal antibody produced by using a Chinese hamster ovary cell expression system, the glycoengineered monoclonal antibody had comparable thermal stability and a higher antibody-dependent cell-mediated cytotoxicity activity. These results suggest that silkworm–baculovirus expression systems are next-generation expression systems potentially useful for the cost-effective production of therapeutic antibodies.