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ダイジェスト

JB COMMENTARY

Regulation of the SNARE protein Ykt6 function by diprenylation and phosphorylation

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For proper intracellular vesicle transport, it is essential for transport vesicle membranes to fuse with the appropriate target membranes. Ykt6 is a SNARE protein with functions in diverse vesicle transport pathways, including secretory, endocytotic and autophagic pathways. To exert these functions, the association of Ykt6 with vesicle membranes and the change of its conformation from closed to open play key roles. Recent studies have revealed regulatory mechanisms involved in Ykt6 membrane association and conformation change. When in the cytosol, the vicinal cysteine residues within the C-terminal CCAIM sequence of Ykt6 undergo diprenylation (farnesylation of the distal cysteine residues by farnesyltransferase; this is followed by geranylgeranylation of the proximal cysteine residue by geranylgeranyltransferase-III). Phosphorylation of a serine residue within the SNARE domain triggers the conversion of the Ykt6 conformation from closed to open, allowing Ykt6 membrane association. In this commentary, I briefly summarize and discuss the recently revealed regulatory mechanisms of Ykt6 function by diprenylation and phosphorylation.

Keywords: farnesylation, geranylgeranylation, GGTase-III, SNARE, Ykt6

JB REVIEW

The molecular mechanisms of mammalian sperm maturation regulated by NELL2-ROS1 lumicrine signaling

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In terrestrial vertebrates, spermatozoa generated in the testis are transported through the reproductive tract toward outside the body. In addition to as the pathway of sperm transport, the male reproductive tract also functions as the site of post-testicular sperm maturation and the epididymis, which constitutes the majority of male reproductive tract, and plays central roles in such a sperm maturation. Recent studies with gene-modified animals have been unveiling not only the molecular mechanisms of sperm maturation in the epididymis but also the regulatory system by which the epididymis acquires and executes sperm-maturing functions. In this review, the mechanisms of mammalian sperm maturation will be summarized, based on recent findings, including the lumicrine regulation of sperm maturation.

Keywords: ROS1, OVCH2, NELL2, lumicrine, epididymis

RAPID COMMUNICATION

The role of TREM2 *N*-glycans in trafficking to the cell surface and signal transduction of TREM2

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Variants of triggering receptor expressed on myeloid cells 2 (TREM2) are associated with an increased incidence of Alzheimer's disease, as well as other neurodegenerative disorders. TREM2 is glycosylated *in vitro* and *in vivo*, but the significance of the modification is unknown. We previously established a sensitive and specific reporter cell model involving cultured Jurkat cells stably expressing a luciferase reporter gene and a gene encoding a TREM2DAP12 fusion protein to monitor TREM2-dependent signalling. In the present study, we prepared modified reporter cells to investigate the role of the *N*-glycans at N20 and N79. We show that the *N*-glycans at N79 have a requisite role in translocation of TREM2 to the cell surface, while the *N*-glycans at both N20 and N79 have a critical role in intracellular signal transduction. Our results indicate that structural changes to the TREM2 *N*-glycans may cause microglial dysfunction that contributes to the pathogenesis of neurodegenerative disorders and that maintaining the integrity of TREM2 *N*-glycosylation and the responsible glycosyltransferases may be a novel therapeutic strategy to treat these disorders.

Keywords: Alzheimer's disease, *N*-glycan, signal transduction, TREM2, trafficking

BIOCHEMISTRY

Biochemistry General

Compounds in cigarette smoke induce EGR1 expression via the AHR, resulting in apoptosis and COPD

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Chronic obstructive pulmonary disease (COPD) is a major cause of mortality worldwide, and pulmonary epithelial cell apoptosis is regarded as one of the most important factors in its pathogenesis. Here, we examined the molecular mechanisms of apoptosis caused by cigarette smoke (CS). In the normal bronchial epithelium cell line BEAS-2B, a CS extract markedly induced apoptosis together with transient early growth response 1 (EGR1) protein expression, which is activated over time via the aryl hydrocarbon receptor (AHR). The CS extract–induced apoptosis decreased cell count of BEAS-2B cells and was significantly reversed by knockdown of either *EGR1* or *AHR*. *In vivo*, the CS extract caused alveolar wall destruction, mimicking COPD, 1 week after intrathoracic injection. Bronchoalveolar lavage fluid (BALF) from the CS extract–treated mice contained massive numbers of apoptotic epithelial cells. Furthermore, it was found that aminoanthracene induced EGR1 expression and cell apoptosis. By contrast, the AHR antagonist stemregenin 1 (SR1) restored apoptosis upon CS treatment. These results suggest that aryl hydrocarbons, such as aminoanthracene, induce EGR1 expression via the AHR, resulting in cell apoptosis and that this can be prevented by administration of an antagonist of AHR.

Keywords: AHR, apoptosis, cigarette smoke, COPD

Protein Interaction and Recognition

Molecular mechanism of negative cooperativity of ferredoxin-NADP$^+$ reductase by ferredoxin and NADP(H): role of the ion pair of ferredoxin Arg40 of and FNR Glu154

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Ferredoxin-NADP$^+$ reductase (FNR) in plants receives electrons from ferredoxin (Fd) and converts NADP$^+$ to NADPH at the end of the photosynthetic electron transfer chain. We previously showed that the interaction between FNR and Fd was weakened by the allosteric binding of NADP(H) on FNR, which was considered as a part of negative cooperativity. In this study, we investigated the molecular mechanism of this phenomenon using maize (*Zea mays* L.) FNR and Fd, as the 3D structure of this Fd:FNR complex is available. Site-specific mutants of several amino acid residues on the Fd:FNR interface were analysed for the effect on the negative cooperativity, by kinetic analysis of Fd:FNR electron transfer activity and by Fd-affinity chromatography. Mutations of Fd Arg40Gln and FNR Glu154Gln that disrupt one of the salt bridges in the Fd:FNR complex suppressed the negative cooperativity, indicating the involvement of the ion pair of Fd Arg40 and FNR Glu154 in the mechanism of the negative cooperativity. Unexpectedly, either mutation of Fd Arg40Gln or FNR Glu154Gln tends to increase the affinity between Fd and FNR, suggesting the role of this ion pair in the regulation of the Fd:FNR affinity by NADPH, rather than the stabilization of the Fd:FNR complex.

Keywords: ferredoxin, ferredoxin-NADP$^+$ reductase, negative cooperativity, protein–protein interaction, allosteric regulation

MOLECULAR BIOLOGY
Molecular Biology General

(Pro)renin receptor and insulin signaling regulate cell proliferation in MCF-7 breast cancer cells

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(Pro)renin receptor [(P)RR] is related to both the renin-angiotensin system and V-ATPase with various functions including stimulation of cell proliferation. (P)RR is implicated in the pathophysiology of diabetes mellitus and cancer. Hyperinsulinemia is observed in obesity-related breast cancer. However, the relationship between (P)RR and insulin has not been clarified. We have therefore studied the effect of insulin on (P)RR expression, cell viability and AKT phosphorylation under the conditions with and without (P)RR knockdown. Effects of insulin were studied in a human breast cancer cell line, MCF-7. Cell proliferation assay was performed by WST-8 assay. (P)RR expression was suppressed by (P)RR-specific siRNAs. The treated cells were analysed by western blotting and reverse transcriptase-quantitative polymerase chain reaction analysis. Insulin stimulated proliferation of MCF-7 cells and increased (P)RR protein expression, but not (P)RR mRNA levels. Moreover, autophagy flux was suppressed by insulin. Suppression of (P)RR expression reduced cell number of MCF-7 cells and AKT phosphorylation significantly in both the presence and the absence of insulin, indicating that (P)RR is important for cell viability and AKT phosphorylation. In conclusion, insulin upregulates the level of (P)RR protein, which is important for cell viability, proliferation, AKT phosphoryla-

tion and autophagy in breast cancer cells.

Keywords: (P)RR., insulin, cell proliferation, breast cancer, autophagy

Efficient DNA/RNA extraction from tarsal plates by SK mill, a freeze-crush apparatus

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The tarsal plate is an eyelid tissue that maintains lid structure from inside the upper/lower eyelids, and it surrounds the meibomian glands and supports their unique secretion mechanism. Sebaceous carcinoma, a malignant eyelid tumour, can sometimes develop from the meibomian glands and is usually excised together with the tarsal plate during surgery, so the tarsal plate serves as a control research tissue. However, since the plate is thick, hard and heterogeneous with few cells, obtaining enough genomic DNA and/or total RNA is often difficult. Therefore, we attempted to establish an efficient protocol to obtain DNA and RNA simultaneously by comparing the combinations of homogenization (mortar/pestle, pellet pestle or SK mill) and purification (organic solvent or spin column) methods using rabbit tarsal plates. Based on the yield, quality and hands-on time, the SK mill and spin column was found to be the most efficient combination. We then applied the established protocol to extract DNA/RNA from six human tarsal-plate samples and succeeded in generating high-quality exome and transcriptome datasets via a next-generation sequencer with sufficient coverage and meibomian gland-specific expression of representative genes, respectively. Our new findings will provide ideal reference data for future genetic and gene-expression studies of sebaceous carcinoma.

Keywords: DNA/RNA extraction, eyelid sebaceous carcinoma, freeze crush, next-generation sequencer, tarsal plate

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ダイジェスト

JB REVIEW

Ubiquitin-mediated mitochondrial regulation by MITOL/MARCHF5 at a glance

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Mitochondria are involved in various cellular processes, such as energy production, inflammatory responses and cell death. Mitochondrial dysfunction is associated with many age-related diseases, including neurological disorders and heart failure. Mitochondrial quality is strictly maintained by mitochondrial dynamics linked to an adequate supply of phospholipids and other substances from the endoplasmic reticulum (ER). The outer mitochondrial membrane-localized E3 ubiquitin ligase MITOL/MARCHF5 is responsible for mitochondrial quality control through the regulation of mitochondrial dynamics, formation of mitochondria-ER contacts and mitophagy. MITOL deficiency has been shown to impair mitochondrial function, cause an excessive inflammatory response and increase vulnerability to stress, resulting in the exacerbation of the disease. In this study, we overview the ubiquitin-mediated regulation of mitochondrial function by MITOL and the relationship between MITOL and diseases.

Keywords: ubiquitin, MITOL/MARCHF5, mitochondrial dynamics, mitochondria-ER contacts, mitochondria

BIOCHEMISTRY

Protein Structure

Crystal structure of the sliding DNA clamp from the Gram-positive anaerobic bacterium Clostridioides difficile

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The sliding DNA clamp is a ring-shaped protein that encircles DNA within its central channel. It binds to multiple proteins, such as DNA polymerases and DNA repair enzymes, and stimulates their enzymatic activities, thereby playing a crucial role

in cell survival and proliferation. Accordingly, the bacterial clamp DnaN is considered to be a promising target for bacterial infection therapy. In this regard, 3D structures of DnaN from pathogenic bacteria are essential for the development of chemical compounds with antimicrobial activity. Here, the crystal structure of DnaN from a Gram-positive bacterium *Clostridioides difficile*, a human pathogen causing infectious diarrhoea, has been determined at 2.13 Å resolution. A comparison of the structures of DnaN from other bacteria indicates that the structural features of DnaN in terms of overall organization are essentially conserved within Gram-positive and Gram-negative bacteria. However, DnaN from *C. difficile* has structural differences in the potential binding pocket for partner proteins, implying a non-conventional interaction with its binding partners. Our findings will provide insight into the development of new therapies for *C. difficile* infection.

Keywords: Clostridioides difficile, crystal structure, DNA replication, molecular interaction, sliding DNA clamp

Structural insights into the substrate specificity of IMP-6 and IMP-1 metallo-*β*-lactamases

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IMP-type metallo-*β*-lactamases confer resistance to carbapenems and a broad spectrum of *β*-lactam antibiotics. IMP-6 and IMP-1 differ by only a point mutation: Ser262 in IMP-1 and Gly262 in IMP-6. The k_{cat}/K_m values of IMP-1 for imipenem and meropenem are nearly identical; however, for IMP-6, the k_{cat}/K_m for meropenem is 7-fold that for imipenem. In clinical practice, this may result in an ineffective therapeutic regimen and, consequently, in treatment failure. Here, we report the crystal structures of IMP-6 and IMP-1 with the same space group and similar cell constants at resolutions of 1.70 and 1.94 Å, respectively. The overall structures of IMP-6 and IMP-1 are similar. However, the loop region (residues 60–66), which participates in substrate binding, is more flexible in IMP-6 than in IMP-1. This difference in flexibility determines the substrate specificity of IMP-type metallo-*β*-lactamases for imipenem and meropenem. The amino acid at position 262 alters the mobility of His263; this affects the flexibility of the loop via a hydrogen bond with Pro68, which plays the role of a hinge in IMP-type metallo-*β*-lactamases. The substitution of Pro68 with a glycine elicited an increase in the K_m of IMP-6 for imipenem, whereas the affinity for meropenem remained unchanged.

Keywords: carbapenemase, X-ray crystallography, substrate specificity, metallo-*β*-lactamase, loop flexibility

Protein Interaction and Recognition

Characterization of KDM5 lysine demethylase family substrate preference and identification of novel substrates

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The KDM5/JARID1 sub-family are 2-oxoglutarate and Fe(II)-dependent lysine-specific histone demethylases that are characterized by their Jumonji catalytic domains. The KDM5 family is known to remove tri-/di-methyl modifications from lysine-4 of histone H3 (*i.e.* H3-K4me2/3), a mark associated with active gene expression. As a result, studies to date have revolved around the influence of KDM5 on disease through their ability to regulate H3-K4me2/3. Recent evidence demonstrates that KDM5 may influence disease beyond H3-K4 demethylation, making it critical to further investigate KDM5-mediated demethylation of non-histone proteins. To help identify potential non-histone substrates for the KDM5 family, we developed a library of 180 permutated peptide substrates, with sequences that are systematically altered from the wild-type H3-K4me3 substrate. From this library, we characterized recombinant KDM5A/B/C/D substrate preference and developed recognition motifs for each KDM5 demethylase. The recognition motifs developed were used to predict potential substrates for KDM5A/B/C/D and profiled to generate a list of high-ranking and medium/low-ranking substrates for further *in vitro* validation. Through this approach, we identified 66 high-ranking substrates in which KDM5 demethylases displayed significant *in vitro* activity towards.

Keywords: Jumonji domain, lysine demethylase, non-histone substrates, permutated substrates, substrate preference

BIOTECHNOLOGY

Biotechnology General

Screening of *α*-amino acid ester acyl transferase variant with improved activity by combining rational and random mutagenesis

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Random and rational mutagenesis of an *α*-amino acid ester acyl transferase from *Sphingobacterium siyangensis* AJ2458 (SAET)

was conducted to examine the production of aspartame, an *α*-L-aspartyl-L-phenylalanine methyl ester. We previously reported aspartame production via combination of enzymatic and chemical methods. However, the productivity of the aspartame intermediate by SAET was approximately one-fifth that of L-alanyl-L-glutamine (Ala-Gln), whose production method has already been established. Here, to improve the enzymatic activity of SAET, we performed random mutagenesis in the gene encoding SAET and obtained 10 mutations that elevated the enzymatic activity (1.2- to 1.7-fold increase) relative to that of wild-type SAET. To further improve the activity, we performed mutagenesis to optimize the combination of the obtained mutations and finally selected one SAET variant with 10 amino acid substitutions (M35-4 SAET). An *Escherichia coli* strain overexpressing M35-4 SAET displayed a 5.7-fold higher activity than that of the wild-type SAET, which was almost equal to that of Ala-Gln by an *E. coli* strain overexpressing wild-type SAET. The V_{max} value of M35-4 SAET was 2.0-fold greater, and its thermostability was higher than those of wild-type SAET. These results suggest that the obtained SAET variants contribute to improvement in aspartame production.

Keywords: *α*-amino acid ester acyl transferase, *Sphingobacterium siyangensis*, rational mutagenesis, random mutagenesis, aspartame production

CELL

Differentiation/Development and Aging

nudt7 gene depletion causes transcriptomic change in early development of zebrafish

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The Nudt family has been identified as enzymes performing Coenzyme A to 35-ADP+4-phospho pantetheine catalysis. The members of this family have been shown to be particularly involved in lipid metabolism, while their involvement in gene regulation through regulating transcription or mRNA metabolism has also been suggested. Here, we focused on peroxisomal NUDT7, possessing enzymatic activity similar to that of its paralog, peroxisomal NUDT19, which is involved in mRNA degradation. No reports have been published about the Nudt family in zebrafish. Our transcriptomic data showed that the Nudt family members are highly expressed around zygotic gene activation (ZGA) in developing zebrafish embryos. Therefore, we confirmed the computational prediction that the products of the nudt7 gene in zebrafish were localized in the peroxisome and highly expressed in early embryogenesis. The depletion of nudt7 genes by the CRISPR/Cas9 system did not affect development; however, it decreased the rate of transcription in ZGA. In addition, H3K27ac ChIP-seq analysis demonstrated that this decrease in transcription was correlated with the genome-wide decrease of H3K27ac level. This study suggests that peroxisomal Nudt7 functions in regulating transcription in ZGA via formation of the H3K27ac domain in active chromatin.

Keywords: zygotic gene activation, zebrafish, RNA-seq, Nudt family, ChIP-seq