Journal of Biochemistry

Vol. 175, No. 6（2024 年 6 月 発 行）

ダイジェスト

JB REVIEW

Structure of cytotoxic amyloid oligomers generated during disaggregation

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Amyloidosis is characterized by the abnormal accumulation of amyloid proteins. The causative proteins aggregate from monomers to oligomers and fibrils, among which some intermediate oligomers are considered as major toxins. Cytotoxic oligomers are generated not only by aggregation but also via fibril disaggregation. However, little is known about the structural characteristics and generation conditions of cytotoxic oligomers produced during disaggregation. Herein, we summarized the structural commonalities of cytotoxic oligomers formed under various disaggregation conditions, including the addition of heat shock proteins or small compounds. *In vitro* experimental data demonstrated the presence of high-molecular-weight oligomers (protofibrils or protofilaments) that exhibited a fibrous morphology and *β*-sheet structure. Molecular dynamics simulations indicated that the distorted *β*-sheet structure contributed to their metastability. The tendency of these cytotoxic oligomers to appear under mild disaggregation conditions, implied formation during the early stages of disaggregation. This review will aid researchers in exploring the characteristics of highly cytotoxic oligomers and developing drugs that target amyloid aggregates.

Keywords: amyloid, cytotoxic oligomers, disaggregation, metastability, *β*-sheet structure

REGULAR PAPERS
BIOCHEMISTRY
Protein Structure

Homo-trimeric structure of the ribonuclease for rRNA processing, FAU-1, from Pyro coccus furiosus

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Crystal structure of a ribonuclease for ribosomal RNA processing, FAU-1, from *Pyrococcus furiosus* was determined with the resolution of 2.57 Å in a homo-trimeric form. The monomer structure consists of two domains: N-terminal and C-terminal domains. C-terminal domain forms trimer and each N-terminal domain locates outside of the trimer core. In the obtained crystal, a dinucleotide, pApUp, was bound to the N-terminal domain, indicating that N-terminal domain has the RNA-binding ability. The affinities to RNA of FAU-1 and a fragment corresponding to the N-terminal domain, FAU-ΔC, were confirmed by polyacrylamide gel electrophoresis and nuclear magnetic resonance (NMR). Interestingly, well-dispersed NMR signals were observed at 318K, indicating that the FAU-ΔC–F18 complex form an ordered structure at higher temperature. As predicted in our previous works, FAU-1 and ribonuclease (RNase) E show a structural similarity in their RNA-binding regions. However, structural similarity between RNase E and FAU-1 could be found in the limited regions of the N-terminal domain. On the other hand, structural similarity between C-terminal domain and some proteins including a phosphatase was found. Thus, it is possible that the catalytic site is located in C-terminal domain.

Keywords: Alphafold2, Pyrococcus furiosus, RNaseE, rRNA processing, X-ray crystallography

Protein Interaction and Recognition

Carbohydrate-binding ability of a recombinant protein containing the DM9 motif from Drosophila melanogaster

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Proteins containing DM9 motifs, which were originally identified in the *Drosophila melanogaster* genome, are widely distributed in various organisms and are assumed to be involved in their innate immune response. In this study, we produced a recombinant protein of CG13321 (rCG13321) from *D. melanogaster*, which consists of four DM9 motifs, in *Escherichia coli* cells. In affinity chromatography using a mannose-immobilized column, rCG13321 exhibited mannose-binding ability and was separated into high-affinity and low-affinity fractions, named HA and LA, respectively, based on its binding ability to the column. In addition to having a higher affinity for the column, HA exhibited self-oligomerization ability, suggesting slight differences in tertiary structure. Both LA and HA showed hemagglutinating activity and were able to agglutinate an oligomannose-containing

dendrimer, indicating that they have multiple carbohydrate-binding sites. Glycan array analysis suggested that rCG13321 primarily recognizes d-mannose and d-rhamnose through hydrogen bonding with the 2-, 3- and 4-hydroxy groups. Isothermal titration calorimetry demonstrated that rCG13321 has a comparable affinity to typical lectins. These findings suggest that CG13321 functions as a carbohydrate-binding protein or lectin that recognizes mannose and related carbohydrate-containing molecules on the surface of foreign organisms as a pattern recognition molecule.

Keywords: DM9 motif, *Drosophila melanogaster*, innate immunity, lectin, mannose

Biochemistry in Cell Membranes

The ATPase activity of ABCA1 is increased by cholesterol in the presence of anionic lipids

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High-density lipoprotein (HDL) transports excess cholesterol from peripheral tissues back to the liver, and plasma HDL levels are inversely related to cardiovascular disease incidence. ATP-binding cassette A1 (ABCA1) is a member of the ABC protein superfamily, and generates nascent HDL, which consists of several hundreds of phospholipids and cholesterol wrapped by apolipoprotein A-I (apoA-I). However, it remains unclear whether cholesterol is a transport substrate of ABCA1. Since ATP hydrolysis of ABC proteins is typically increased by their transport substrates, we characterized the effects of cholesterol on the ATPase activity of purified ABCA1 using liposomes of various lipid compositions. ABCA1 showed substantial ATPase activity (20–30 $nmol \cdot min^{-1} \cdot mg^{-1}$) only in liposomes containing anionic lipids, including phosphatidylserine. Cholesterol increased the ATPase activity by 1.6- to 3-fold in the presence of anionic lipids. Moreover, phosphatidylserine addition to BHK/ABCA1 cells increased phosphatidylcholine and cholesterol efflux to apoA-I. Next, we investigated the sterol specificity of ABCA1. The ATPase activity of ABCA1 was strongly enhanced by desmosterol and zymosterol, similar to cholesterol. In contrast, 7-dehydrocholesterol and lathosterol weakly increased the ATPase activity, and no increase was observed with stigmasterol or brassicasterol. These findings suggest that ABCA1 transports cholesterol and prefers cholesterol over plant sterols as a transport substrate.

Keywords: ABCA1, cholesterol, liposomes, phosphatidylserine, sterol specificity

Neurochemistry

C-terminal truncation is a prominent post-translational modifi cation of human erythrocyte *α*-synuclein

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α-Synuclein (*α*-Syn) is a protein related to synucleinopathies with high expression in the central nervous system and erythrocytes which are a major source of peripheral *α*-Syn. Recent reports have suggested the presence of *α*-Syn within extracellular vesicles (EVs) derived from erythrocytes, potentially contributing to the pathogenesis of synucleinopathies. While Lewy bodies, intracellular inclusions containing aggregated *α*-Syn, are prominently observed within the brain, their occurrence in peripheral neurons implies the dissemination of synucleinopathy pathology throughout the body via the propagation of *α*-Syn. In this study, we found erythrocytes and circulating EVs obtained from plasma contained *α*-Syn, which was separated into four major forms using high-resolution clear native-PAGE and isoelectric focusing. Notably, erythrocyte *α*-Syn was classified into full-length and C-terminal truncated forms, with truncation observed between Y133 and Q134 as determined by LC–MS/MS analysis. Our finding revealed that C-terminally truncated *α*-Syn, which was previously reported to exist solely within the brain, was also present in erythrocytes and circulating EVs obtained from plasma.

Keywords: extracellular vesicles, neurodegenerative disease, Parkinson's disease, post-translational modification, *α*-synuclein

MOLECULAR BIOLOGY

Gene Expression

Whole blood transcriptome analysis for age- and gender-specific gene expression profiling in Japanese individuals

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Whole blood transcriptome analysis is a valuable approachin medical research, primarily due to the ease of sample collection and the richness of the information obtained. Since the expression profile of individual genes in the analysis is influenced by medical traits and demographic attributes such as age and gender, there has been a growing demand for a comprehensive database for blood transcriptome analysis. Here, we performed whole blood RNA sequencing (RNA-seq) analysis on 576 participants stratified by age (20–30s and 60–70s) and gender from cohorts of the Tohoku Medical Megabank (TMM). A part of female segment included pregnant women. We did not exclude the globin gene family in our RNA-seq study, which enabled us to identify instances of hereditary persistence of fetal hemoglobin based on the *HBG1* and *HBG2* expression information. Comparing stratified populations allowed us to identify groups of genes associated with age-related changes and gender differences. We also found that the immune response status, particularly measured by neutrophil-to-lymphocyte ratio (NLR), strongly influences the diversity of individual gene expression profiles in whole blood transcriptome analysis. This stratification has resulted in a data set that will be highly beneficial for future whole blood transcriptome analysis in the Japanese population.

Keywords: age and gender difference, globin, HPFH, RNA-seq, whole blood transcriptome

Protein Synthesis

Direct visualization of ribosomes in the cell-free system revealed the functional evolution of aminoglycoside

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The rapid emergence of multi-drug-resistant bacteria has raised a serious public health concern. Therefore, new antibiotic developments have been highly desired. Here, we propose a new method to visualize antibiotic actions on translating ribosomes in the cell-free system under macromolecular crowding conditions by cryo-electron microscopy, designated as the DARC method: the Direct visualization of Antibiotic binding on Ribosomes in the Cell-free translation system. This new method allows for acquiring a more comprehensive understanding of the mode of action of antibiotics on the translation inhibition without ribosome purification. Furthermore, with the direct link to biochemical analysis at the same condition as cryo-EM observation, we revealed the evolution of 2-DOS aminoglycosides from dibekacin (DBK) to arbekacin (ABK) by acquiring the synthetic tailored anchoring motif to lead to stronger binding affinity to ribosomes. Our cryo-EM structures of DBK and ABK bound ribosomes in the cell-free environment clearly depicted a synthetic tailored γ-amino-α-hydroxybutyryl (HABA) motif formed additional interactions with the ribosome enhancing antibiotic bindings. This new approach would be valuable for understanding the function of antibiotics for more efficient drug development.

Keywords: Antibiotics, cryo-electron microscopy, Ribosome structure < RNA, structural biology, Translation < RNA

CELL

Cell General

Extracellular histones promote calcium phosphate-dependent calcification in mouse vascular smooth muscle cells

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Vascular calcification, a major risk factor for cardiovascular events, is associated with a poor prognosis in chronic kidney disease (CKD) patients. This process is often associated with the transformation of vascular smooth muscle cells (VSMCs) into cells with osteoblast-like characteristics. Damage-associated molecular patterns (DAMPs), such as extracellular histones released from damaged or dying cells, are suspected to accumulate at calcification sites. To investigate the potential involvement of DAMPs in vascular calcification, we assessed the impact of externally added histones (extracellular histones) on calcium and inorganic phosphate-induced calcification in mouse VSMCs. Our study found that extracellular histones intensified calcification. We also observed that the histones decreased the expression of VSMC marker genes while simultaneously increasing the

expression of osteoblast marker genes. Additionally, histones treated with DNase I, which degrades dsDNA, attenuated this calcification, compared with the non-treated histones, suggesting a potential involvement of dsDNA in this process. Elevated levels of dsDNA were also detected in the serum of CKD model mice, underlining its potential role in vascular calcification in CKD. Our findings suggest that extracellular histones could play a pivotal role in the vascular calcification observed in CKD.
Keywords: chronic kidney disease, damage-associated molecular patterns, extracellular histones, vascular calcification, vascular smooth muscle cells

Biomembranes, Organelles and Protein Sorting

Formation of the NLRP3 inflammasome inhibits stress granule assembly by multiple mechanisms

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Proper regulation of cellular response to environmental stress is crucial for maintaining biological homeostasis and is achieved by the balance between cell death processes, such as the formation of the pyroptosis-inducing NLRP3 inflammasome, and pro-survival processes, such as stress granule (SG) assembly. However, the functional interplay between these two stress-responsive organelles remains elusive. Here, we identified DHX33, a viral RNA sensor for the NLRP3 inflammasome, as a SG component, and the SG-nucleating protein G3BP as an NLRP3 inflammasome component. We also found that a decrease in intracellular potassium (K^+) concentration, a key 'common' step in NLRP3 inflammasome activation, markedly inhibited SG assembly. Therefore, when macrophages are exposed to stress stimuli with the potential to induce both SGs and the NLRP3 inflammasome, such as cytoplasmic poly(I:C) stimulation, they preferentially form the NLRP3 inflammasome but avoid SG assembly by sequestering G3BP into the inflammasome and by inducing a reduction in intracellular K^+ levels. Thus, under such conditions, DHX33 is primarily utilized as a viral RNA sensor for the inflammasome. Our data reveal the functional crosstalk between NLRP3 inflammasome-mediated pyroptosis and SG-mediated cell survival pathways and delineate a molecular mechanism that regulates cell-fate decisions and anti-viral innate immunity under stress.
Keywords: DHX33, NLRP3 inflammasome, poly(I:C), potassium efflux, stress granules

Journal of Biochemistry

Vol. 176, No. 1（2024 年 7 月 発 行）

ダイジェスト

JB REVIEW

Cryo-EM advances in GPCR structure determination

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G-protein-coupled receptors (GPCRs) constitute a prominent superfamily in humans and are categorized into six classes (A–F) that play indispensable roles in cellular communication and therapeutics. Nonetheless, their structural comprehension has been limited by challenges in high-resolution data acquisition. This review highlights the transformative impact of cryogenic electron microscopy (cryo-EM) on the structural determinations of GPCR–G-protein complexes. Specific technologies, such as nanobodies and mini-G-proteins, stabilize complexes and facilitate structural determination. We discuss the structural alterations upon receptor activation in different GPCR classes, revealing their diverse mechanisms. This review highlights the robust foundation for comprehending GPCR function and pave the way for future breakthroughs in drug discovery and therapeutic targeting.
Keywords: cryo-EM, G-protein-coupled receptor, structural biology

REGULAR PAPERS
BIOCHEMISTRY
Biochemistry General

Photocontrol of small GTPase Ras fused with a photo responsive protein

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The small GTPase Ras plays an important role in intracellular signal transduction and functions as a molecular switch. In this study, we used a photoresponsive protein as the molecular regulatory device to photoregulate Ras GTPase activity. Photo zipper (PZ), a variant of the photoresponsive protein Aureochrome1 developed by Hisatomi et al. was incorporated into the C-terminus of Ras as a fusion protein. The three constructs of the Ras–PZ fusion protein had spacers of different lengths between Ras and PZ. They were designed using an *Escherichia coli* expression

system. The Ras–PZ fusion proteins exhibited photoisomerization upon blue light irradiation and in the dark. Ras–PZ dimerized upon light irradiation. Moreover, Ras GTPase activity, which is accelerated by the Ras regulators guanine nucleotide exchange factors and GTPase-activating proteins, is controlled by photoisomerization. It has been suggested that light-responsive proteins are applicable to the photoswitching of the enzymatic activity of small GTPases as photoregulatory molecular devices.

Keywords: Nanomachine, Photo zipper, Photoregulation, Ras, Small GTPase

Structure-specific DNA endonuclease T7 endonuclease Icleaves DNA containing UV-induced DNA lesions

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The T7 gene 3 product, T7 endonuclease I, acts on various substrates with DNA structures, including Holliday junctions, heteroduplex DNAs and single-mismatch DNAs. Genetic analyses have suggested the occurrence of DNA recombination, replication and repair in *Escherichia coli*. In this study, T7 endonuclease I digested UV-irradiated covalently closed circular plasmid DNA into linear and nicked plasmid DNA, suggesting that the enzyme generates single- and double-strand breaks (SSB and DSB). To further investigate the biochemical functions of T7 endonuclease I, we have analysed endonuclease activity in UV-induced DNA substrates containing a single lesion, cyclobutane pyrimidine dimers (CPD) and 6–4 photoproducts (6–4PP). Interestingly, the leading cleavage site for CPD by T7 endonuclease I is at the second and fifth phosphodiester bonds that are 5′ to the lesion of CPD on the lesion strand. However, in the case of 6–4PP, the cleavage pattern on the lesion strand resembled that of CPD, and T7 endonuclease I could also cleave the second phosphodiester bond that is 5′ to the adenine–adenine residues opposite the lesion, indicating that the enzyme produces DSB in DNA containing 6–4PP. These findings suggest that T7endonuclease I accomplished successful UV damage repair by SSB in CPD and DSB in 6–4PP.

Keywords: 6–4 photoproducts, cyclobutane pyrimidine dimers, DNA endonuclease, T7 endonuclease I, UV-induced DNA lesions

Production of CA125 with Tn antigens using a glycosylphosphatidylinositol anchoring system

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Cancer antigen 125 (CA125) is a serum marker associated with ovarian cancer. Despite its widespread use, CA125 levels can also be elevated in benign conditions. Recent reports suggest that detecting serum CA125 that carries the Tn antigen, a truncated *O*-glycan containing only *N*-acetylgalactosamine on serine or threonine residues, can improve the specificity of ovarian cancer diagnosis. In this study, we engineered cells to express CA125 with a Tn antigen. To achieve this, we knocked out *C1GALT1* and *SLC35A1*, genes encoding Core1 synthase and a transporter for cytidine-5′-monophospho-sialic acid respectively, in human embryonic kidney 293 (HEK293) cells. In ClGALT1-SLC35A1-knockout (KO) cells, the expression of the Tn antigen showed a significant increase, whereas the expression of the T antigen (galactose-β1,3-*N*-acetylgalactosamine on serine or threonine residues) was decreased. Due to the inefficient secretion of soluble CA125, we employed a glycosylphosphatidylinositol (GPI) anchoring system. This allowed for the expression of GPI-anchored CA125 on the cell surface of ClGALT1-SLC35A1-KO cells. Cells expressing high levels of GPI-anchored CA125 were then enriched through cell sorting. By knocking out the *PGAP2* gene, the GPI-anchored form of CA125 was converted to a secretory form. Through the engineering of *O*-glycans and the use of a GPI-anchoring system, we successfully produced CA125 with Tn antigen modification.

Keywords: CA125, Glycoengineering, Glycosylphosphatidylinositol, Mucin-type *O*-glycan, Tn antigen

MOLECULAR BIOLOGY

Molecular Biology General

SdrR, a LysR-type regulator, responds to the mycobacterial antioxidant defense

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Protection against oxidative stress is a vital defense mechanism for *Mycobacterium tuberculosis* within the host. However, few transcription factors that control bacterial antioxidant defense are known. Here, we present evidence that SdrR, encoded by the *MSMEG_5712* (*Ms5712*) gene, functions as an oxidative stress response regulator in *Mycobacterium smegmatis*. SdrR

recognizes an 11-bp motif sequence in the operon's upstream regulatory region and negatively regulates the expression of short-chain dehydrogenases/reductases (SDR). Overexpressing *sdrR* inhibited SDR expression, which rendered the strain oxidative more stress-sensitive. Conversely, *sdrR* knockout alleviates SDR repression, which increases its oxidative stress tolerance. Thus, SdrR responds to oxidative stress by negatively regulating *sdr* expression. Therefore, this study elucidated an underlying regulatory mechanism behind mycobacterial oxidative stress adaptation.

Keywords: Mycobacterium smegmatis, antioxidant defense, LysR-type regulator, short-chain dehydrogenases, transcriptional regulation

Gene Expression

G protein-coupled receptor 84 gene expression is regulated by the ER stress response in the liver

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G protein-coupled receptor 84 (Gpr84) is reportedly activated by medium-chain fatty acids and is involved in the pathology of liver fibrosis. Inflammatory stimulants, such as lipopolysaccharide and tumor necrosis factor-*α*, upregulate Gpr84 expression. However, the detailed molecular mechanism by which Gpr84 is induced remains unknown. Inflammatory stimulation also evokes endoplasmic reticulum (ER) stress, but there has been no direct evidence to link Gpr84 expression and the ER stress response. Administration of tunicamycin (Tm) provokes ER stress and acute steatosis in the liver tissue of mice. Here, *in situ* hybridization analysis revealed that induction of *Gpr84* expression occurred in parenchymal cells in the liver tissue following Tm administration. Gene expression analysis using a reporter assay showed that the intron 1 region of *Gpr84* was involved in induction of the gene under ER stress conditions. Furthermore, Tm-dependent upregulation of *Gpr84* was blocked by the small chemical compound AEBSF, an inhibitor of ER stress transducers, *in vitro* and *in vivo*. In conclusion, the current study marks the discovery that the ER stress agent Tm induces the expression of *Gpr84*.

Keywords: Creb3 family, endoplasmic reticulum stress, G protein-coupled receptor 84, liver, tunicamycin

BIOTECHNOLOGY

Gene and Protein Engineering

Structure-based design, biophysical characterization, and biochemical application of the heterodimeric affinity purification tag based on the Schistosoma japonicum glutathione-S-transferase (SjGST) homodimer

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Schistosoma japonicum glutathione-S-transferase (SjGST), the so-called GST-tag, is one of the most widely used protein tags for the purification of recombinant proteins by affinity chromatography. Attachment of SjGST enables the purification of a protein of interest (POI) using commercially available glutathione-immobilizing resins. Here we produced an SjGST mutant pair that forms heterodimers by adjusting the salt bridge pairs in the homodimer interface of SjGST. An MD study confirmed that the SjGST mutant pair did not disrupt the heterodimer formation. The modified SjGST protein pair coexpressed in *Escherichia coli* was purified by glutathione-immobilized resin. The stability of the heterodimeric form of the SjGST mutant pair was further confirmed by size exclusion chromatography. Surface plasmon resonance measurements unveiled the selective formation of heterodimers within the pair, accompanied by a significant suppression of homodimerization. The heterodimeric SjGST exhibited enzymatic activity in assays employing a commercially available fluorescent substrate. By fusing one member of the heterodimeric SjGST pair with a fluorescent protein and the other with the POI, we were able to conveniently and sensitively detect protein–protein interactions using fluorescence spectroscopy in the pull-down assays. Thus, utilization of the heterodimeric SjGST would be a useful tag for protein science.

Keywords: domainswapping, heterodimer, homodimer, proteinengineering, saltbridge

Journal of Biochemistry

Vol. 176, No. 2（2024 年 8 月 発 行）

ダイジェスト

JB REVIEW

Sex chromosome cycle as a mechanism of stable sex determination

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Recent advances in DNA sequencing technology have enabled the precise decoding of genomes in non-model organisms, providing a basis for unraveling the patterns and mechanisms of sex chromosome evolution. Studies of different species have yielded conflicting results regarding the traditional theory that sex chromosomes evolve from autosomes via the accumulation of deleterious mutations and degeneration of the Y (or W) chromosome. The concept of the 'sex chromosome cycle,' emerging from this context, posits that at any stage of the cycle (i.e., differentiation, degeneration, or loss), sex chromosome turnover can occur while maintaining stable sex determination. Thus, understanding the mechanisms that drive both the persistence and turnover of sex chromosomes at each stage of the cycle is crucial. In this review, we integrate recent findings on the mechanisms underlying maintenance and turnover, with a special focus on several organisms having unique sex chromosomes. Our review suggests that the diversity of sex chromosomes in the maintenance of stable sex determination is underappreciated and emphasizes the need for more research on the sex chromosome cycle.

Keywords: recombination suppression, sex chromosome loss, sex chromosome turnover, sex determination, transposable elements

REGULAR PAPERS

BIOCHEMISTRY

Biochemistry General

Effects of eyestalk ablation and seawater temperature on D-glutamate levels in the reproductive tissues of male kuruma prawn Marsupenaeus japonicus

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D-Glutamate, a novel D-amino acid found in animal tissues, exclusively exists in the male reproductive tissues of the kuruma prawn, *Marsupenaeus japonicus*. Herein, changes in the D-glutamate content were determined in the male reproductive tissues of *M. japonicus* during acclimation to breeding seawater temperatures of 18–22°C and unilateral eyestalk ablation. The D-glutamate content in the testis increased with increasing seawater temperature and with unilateral eyestalk ablation. This suggests that both stimulations induced D-glutamate synthesis in the testis. Although the D-alanine content in the testis increased after unilateral eyestalk ablation, it did not change with elevated seawater temperature. Furthermore, we determined the D-glutamate distribution in the *M. japonicus* spermatophore. This indicates that D-glutamate is crucial in prawn fertilization.

Keywords: D-alanine, D-amino acid, D-glutamate, male reproductive organs, *Marsupenaeus japonicus*

Glycobiology and Carbohydrate Biochemistry

Essential dextrin structure as donor substrate for 4-α-glucano transferase in glycogen debranching enzyme

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Glycogen debranching enzyme is a single polypeptide with distinct catalytic sites for 4-α-glucanotransferase and amylo-α-1,6-glucosidase. To allow phosphorylase to degrade the inner tiers of highly branched glycogen, 4-α-glucanotransferase converts the phosphorylase-limit biantennary branch G-G-G-G-(G-G-G-G↔)G-G- (G: d-glucose, hyphens: α-1,4-linkages; double-headed arrow: α-1,6-linkage) into the G-G-G-G-(G↔)G-G- residue, which is then subjected to amylo-α-1,6-glucosidase to release the remaining G↔ residue. However, while the essential side-chain structure of the 4-α-glucanotransferase donor substrate has been determined to be the G-G-G-G↔ residue (Watanabe, Y., *et al.* (2008) *J. Biochem.*143, 435–440), its essential main-chain structure remains to be investigated. In this study, we probed the 4-α-glucanotransferase donor-binding region using novel fluorogenic dextrins G_m-(G_4↔) G-G_n-F (F: 1-deoxy-1-[(2-pyridyl)amino]-d-glucitol) and maltohexaose (G_6) as the donor and acceptor substrates, respectively. 4-α-Glucanotransferase exhibited maximum activity towards G_4-(G_4↔) G-F and G_4-(G_4↔) G-G-F, indicating that recognition of the G_4-(G_4↔) G-moiety was essential for full enzyme function. Notably, when the 4-α-glucanotransferase activity towards G_4-(G_4↔) G-G-F was taken as unity, those towards nonbranching dextrins were < 0.001. This indicated that the disproportionation activities towards maltooligosaccharides (G*m*) are abnormal behaviours of 4-α-glucanotransferase. Notably, however, these activities have been traditionally measured to identify the 4-α-glucanotransferase mutations causing glycogen storage disease type III. This study provides a basis for more accurate identification.

Keywords: 4-α-glucanotransferase, biantennary dextrin, donor substrate specificity, glycogen debranching enzyme, glycogen storage disease type III

Mtc6/Ehg2 is a novel endoplasmic reticulum-resident glycoprotein essential for high-pressure tolerance

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Hydrostatic pressure is a common mechanical stressor that modulates metabolism and reduces cell viability. Eukaryotic cells have genetic programs to cope with hydrostatic pressure stress and maintain intracellular homeostasis. However, the mechanism underlying hydrostatic pressure tolerance remains largely unknown. We have recently demonstrated that maintenance of telomere capping protein 6 (Mtc6) plays a protective role in the survival of the budding yeast *Saccharomyces cerevisiae* under hydrostatic pressure stress by supporting the integrity of nutrient permeases. The current study demonstrates that Mtc6 acts as an endoplasmic reticulum (ER) membrane protein. Mtc6 comprises two transmembrane domains, a C-terminal cytoplasmic domain and a luminal region with 12 Asn (N)-linked glycans attached to it. Serial mutational analyses showed that the cytoplasmic C-terminal amino acid residues GVPS Mtc6 activity. Multiple N-linked glycans in the luminal region are involved in the structural conformation of Mtc6. Moreover, deletion of *MTC6* led to increased degradation of the leucine permease Bap2 under hydrostatic pressure, suggesting that Mtc6 facilitates the proper folding of nutrient permeases in the ER under stress conditions. We propose a novel model of molecular function in which the glycosylated luminal domain and cytoplasmic GVPS sequences of Mtc6 cooperatively support the nutrient permease activity.

Keywords: endoplasmic reticulum protein, hydrostatic pressure, *Mtc6/Ehg2*, nutrient permeases, *Saccharomyces cerevisiae*

CELL

Receptors and Signal Transduction

Identification of two critical amino acid residues in short-chain aldehyde-responsive odorant receptors

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Mammalian odorant receptors (ORs) are crucial for detecting a broad spectrum of odorants, yet their functional expression poses a significant challenge, often requiring Receptor-transporting proteins (RTPs). This study examines mouse Olfr733 and Olfr732, which, despite high homology, show different functional expression profiles in heterologous cell systems. Our research aimed to identify key amino acids impacting Olfr733's functional expression. We discovered that G112F$^{BW3.40}$ and L148P$^{BW4.49}$ (Ballesteros–Weinstein numbering in superscript) substitutions in Olfr732 markedly enhance its RTP-independent expression and ligand responsiveness, mirroring Olfr733. These substitutions, particularly Phe112 and Leu148, are crucial for aldehyde recognition and membrane localization in Olfr733, respectively. While Olfr732-type ORs are conserved across species, Olfr733-types, unique to specific rodents, appear to have evolved from Olfr732, with Pro148 enhancing membrane expression and aldehyde sensitivity. Mouse ORs with Pro$^{BW4.49}$ tend to exhibit improved membrane expression compared to their paralogs, especially when co-expressed with RTP1S. This study concludes that the Pro residue in the fourth transmembrane domain significantly contributes to the structural stability of certain olfactory receptors, highlighting the intricate molecular mechanisms underlying OR functionality and evolution.

Keywords: odorant receptor, receptor-transporting protein, membrane traffic, aldehyde, heterologous expression

Differentiation / Development and Aging

Intracellular acidification and glycolysis modulate inflammatory pathway in senescent cells

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Senescent cells accumulate in various organs with ageing, and its accumulation induces chronic inflammation and age-related physiological dysfunctions. Several remodelling of intracellular environments have been identified in senescent cells, including enlargement of cell/nuclear size and intracellular acidification. Although these alterations of intracellular environments were reported to be involved in the unique characteristics of senescent cells, the contribution of intracellular acidification to senescence-associated cellular phenotypes is poorly understood. Here, we identified that the upregulation of *TXNIP* and its paralog *ARRDC4* as a hallmark of intracellular acidification in addition to KGA-type GLS1. These genes were also upregulated in response to senescence-associated intracellular

acidification. Neutralization of the intracellular acidic environment ameliorated not only senescence-related upregulation of *TXNIP*, *ARRDC4* and KGA but also inflammation-related genes, possibly through suppression of PDK-dependent anaerobic glycolysis. Furthermore, we found that expression of the intracellular acidification-induced genes, *TXNIP* and *ARRDC4*, correlated with inflammatory gene expression in heterogeneous senescent cell population *in vitro* and even *in vivo*, implying that the contribution of intracellular pH to senescence-associated cellular features, such as SASP.

Keywords: glycolysis, intracellular acidification, SASP, senescence

BIOTECHNOLOGY

Bioactive Substances

Cytotoxic stress caused by azalamellarin D (AzaD) interferes with cellular protein translation by targeting the nutrient-sensing kinase mTOR

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Analogs of pyrrole alkaloid lamellarins exhibit anticancer activity by modulating multiple cellular events. Lethal doses of several lamellarins were found to enhance autophagy flux in HeLa cells, suggesting that lamellarins may modulate protein homeostasis through the interference of proteins or kinases controlling energy and nutrient metabolism. To further delineate molecular mechanisms and their targets, our results herein show that azalamellarin D (AzaD) cytotoxicity could cause translational attenuation, as indicated by a change in eIF2α phosphorylation. Intriguingly, acute AzaD treatment promoted the phosphorylation of GCN2, a kinase that transduces the integrated stress response (ISR), and prolonged exposure to AzaD could increase the levels of the phosphorylated forms of eIF2α and the other ISR kinase protein kinase R (PKR). However, the effects of AzaD on ISR signalling were marginally abrogated in cells with genetic deletion of GCN2 and PKR, and evaluation of protein target engagement by cellular thermal shift assay (CETSA) revealed no significant interaction between AzaD and ISR kinases. Further investigation revealed that acute AzaD treatment negatively affected mechanistic target of rapamycin (mTOR) phosphorylation and signalling. The analyses by CETSA and computational modelling indicated that mTOR may be a possible protein target for AzaD. These findings indicate the potential for developing lamellarins as novel agents for cancer treatment.

Keywords: amino acid–sensing kinases, cancer, cytotoxicity, lamellarin pyrrole alkaloids, translational attenuation

Gene and Protein Engineering

Evaluation of the cyclic single-chain Fv antibody derived from nivolumab by biophysical analyses and in vitro cell-based bioassay

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Single-chain Fv (scFv) is a recombinant small antibody in which a polypeptide linker connects the variable regions of the light chain (VL) and the heavy chain (VH). The practical use of scFv, however, has been prevented by its tendency to aggregate due to interchain VL–VH interactions. We recently developed a cyclic scFv whose N-terminus and C-terminus were connected by protein ligation techniques. Biophysical comparisons between cyclic and linear scFv have been conducted, but cell biological evaluations remain unexplored. Here we studied the properties of cyclic and linear scFv derived from nivolumab. Biophysical studies revealed that the thermal stability was not changed but that the antigen-binding activity was approximately 3-fold higher as a result of circularization. A cell-based PD-1/PD-L1 interaction inhibitory assay revealed that the biological activity of scFv was markedly higher in the circularized form. In addition, biophysical analysis of scFv proteins incubated in the presence of serum revealed that circularization suppressed the decrease in antigen-binding activity. It could be assumed that circularization of scFv improved stability in the presence of serum, which in turn would suggest the applicability of cyclic scFv as a biopharmaceutical format.

Keywords: antibody engineering, cyclic scFv, nivolumab, PD-1, single-chain Fv