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ダイジェスト

JB COMMENTARY

CDP-DAG synthesis by peripheral membrane-bound Tam41-type enzymes

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Cytidine diphosphate diacylglycerol (CDP-DAG) is a critical intermediate that is converted to multiple phospholipids in prokaryotes and eukaryotes. In budding yeast, CDP-DAG synthesis from cytidine triphosphate (CTP) and phosphatidic acid (PA) is catalyzed by the membrane-integrated protein Cds1 in the endoplasmic reticulum and the peripheral membrane-bound protein Tam41 in mitochondria. Although a recent study revealed that the fission yeast SpTam41 consists of a nucleotidyltransferase domain and a winged helix domain, forming an active-site pocket for CTP binding between the two domains together with a C-terminal amphipathic helix for membrane association, how CTP and Mg^{2+}, a most-favoured divalent cation, are accommodated with PA remains obscure. A more recent report by Kimura et al. (*J. Biochem.* 2022; 171: 429–441) solved the crystal structure of FbTam41, a functional ortholog from a Firmicutes bacterium, with CTP-Mg^{2+}, successfully providing a detailed molecular view of CDP-DAG synthesis. In this commentary, our current understanding of Tam41-mediated reaction is discussed.

Keywords: cardiolipin synthesis, cytidine diphosphate diacylglycerol, mitochondria, phosphatidic acid

JB REVIEW

Neurodegenerative diseases associated with the disruption of proteostasis and their therapeutic strategies using chemical chaperones

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Aberrant proteostasis is thought to be involved in the pathogenesis of neurodegenerative diseases. Some proteostasis abnormalities are ameliorated by chaperones. Chaperones are divided into three groups: molecular, pharmacological and chemical. Chemical chaperones intended to alleviate stress in organelles, such as the endoplasmic reticulum (ER), are now being administered clinically. Of the chemical chaperones, 4-phenylbutyrate (4-PBA) has been used as a research reagent, and its mechanism of action includes chaperone effects and the inhibition of histone deacetylase. Moreover, it also binds to the B-site of SEC24 and regulates COPII-mediated transport from the ER. Although its therapeutic effect may not be strong, elucidating the mechanism of action of 4-PBA may contribute to the identification of novel therapeutic targets for neurodegenerative diseases.

Keywords: 4-PBA, chaperone, COPII vesicles, endoplasmic reticulum, neurodegenerative diseases

RAPID COMMUNICATION

Neutral selection and clonal expansion during the development of colon cancer metastasis

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Intratumour heterogeneity has been shown to play a role in the malignant progression of cancer. The clonal evolution in primary cancer has been well studied, however, that in metastatic tumorigenesis is not fully understood. In this study, we established human colon cancer-derived organoids and investigated clonal dynamics during liver metastasis development by tracking barcode-labelled subclones. Long-term subclone co-cultures showed clonal drift, with a single subclone becoming dominant in the cell population. Interestingly, the selected subclones were not always the same, suggesting that clonal selection was not based on cell intrinsic properties. Furthermore, liver tumours developed by co-transplantation of organoid subclones into the immunodeficient mouse spleen showed a progressive drastic reduction in clonal diversity, and only one or two subclones predominated

in the majority of large metastatic tumours. Importantly, selections were not limited to particular subclones but appeared to be random. A trend towards a reduction in clonal diversity was also found in liver metastases of multiple colour-labelled organoids of mouse intestinal tumours. Based on these results, we propose a novel mechanism of metastasis development, i.e. a subclone population of the disseminated tumour cells in the liver is selected by neutral selection during colonization and constitutes large metastatic tumours.

Keywords: cancer evolution, liver metastasis, neutral selection, organoids

REGULAR PAPERS

BIOCHEMISTRY

Protein Interaction and Recognition

Amino acid residues responsible for the different pH dependency of cell-specific ferredoxins in the electron transfer reaction with ferredoxin-NADP+reductase from maize leaves

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In the chloroplast stroma, dynamic pH changes occur from acidic to alkaline in response to fluctuating light conditions. We investigated the pH dependency of the electron transfer reaction of ferredoxin-$NADP^+$ reductase (FNR) with ferredoxin (Fd) isoproteins, Fd1 and Fd2, which are localized in mesophyll cells and bundle sheath cells, respectively, in the leaves of C4 plant maize. The pH-dependent profile of the electron transfer activity with FNR was quite different between Fd1 and Fd2, which was mainly explained by the opposite pH dependency of the K_m value of these Fds for FNR. Replacement of the amino acid residue at position of 65 (D65N) and 78 (H78A) between the two Fds conferred different effect on their pH dependency of the K_m value. Double mutations of the two residues between Fd1 and Fd2 (Fd1D65N/H78A and Fd2N65D/A78H) led to the mutual exchange of the pH dependency of the electron transfer activity. This exchange was mainly explained by the changes in the pH-dependent profile of the K_m values. Therefore, the differences in Asp/Asn at position 65 and His/Ala at position 78 between Fd1 and Fd2 were shown to be the major determinants for their different pH dependency in the electron transfer reaction with FNR.

Keywords: electron transfer complex, ferredoxin, ferredoxin-NADP+ reductase, pH dependency, protein–protein interaction

Enzymology

The action of coenzyme B12 - dependent diol dehydratase on 3,3,3-trifluoro-1,2-propanediol results in elimination of all the fluorides with formation of acetaldehyde

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3,3,3-Trifluoro-1,2-propanediol undergoes complete defluorination in two distinct steps: first, the conversion into 3,3,3-trifluoropropionaldehyde catalyzed by adenosylcobalamin (coenzyme B**12**)-dependent diol dehydratase; second, non-enzymatic elimination of all three fluorides from this aldehyde to afford malonic semialdehyde (3-oxopropanoic acid), which is decarboxylated to acetaldehyde. Diol dehydratase accepts 3,3,3-trifluoro-1,2-propanediol as a relatively poor substrate, albeit without significant mechanism-based inactivation of the enzyme during catalysis. Optical and electron paramagnetic resonance (EPR) spectra revealed the steady-state formation of cob (II) alamin and a substrate-derived intermediate organic radical (3,3,3-trifluoro-1,2-dihydroxyprop-1-yl). The coenzyme undergoes Co–C bond homolysis initiating a sequence of reaction by the generally accepted pathway via intermediate radicals. However, the greater steric size of trifluoromethyl and especially its negative impact on the stability of an adjacent radical centre compared to a methyl group has implications for the mechanism of the diol dehydratase reaction. Nevertheless, 3,3,3-trifluoropropionaldehyde is formed by the normal diol dehydratase pathway, but then undergoes non-enzymatic conversion into acetaldehyde, probably via 3,3-difluoropropenal and malonic semialdehyde.

Keywords: adenosylcobalamin, coenzyme B12, defluorination, diol dehydratase, radical enzyme

Biochemical Pharmacology

Onnamide A suppresses the severe acute respiratory syndrome-coronavirus 2 infection without inhibiting 3-chymotrypsin-like cysteine protease

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Given the continuous emergence of new variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the

development of new inhibitors is necessary to enhance clinical efficacy and increase the options for combination therapy for the coronavirus disease 2019. Because marine organisms have been a resource for the discovery of numerous bioactive molecules, we constructed an extract library of marine invertebrates collected from the Okinawa Islands. In this study, the extracts were used to identify antiviral molecules against SARS-CoV-2. Using a cytopathic effect (CPE) assay in VeroE6/TMPRSS2 cells, an extract from the marine sponge *Theonella swinhoei* was found to reduce virus-induced CPE. Eventually, onnamide A was identified as an antiviral compound in the extract using column chromatography and NMR analysis. Onnamide A inhibited several SARS-CoV-2 variant-induced CPEs in VeroE6/TMPRSS2 cells as well as virus production in the supernatant of infected cells. Moreover, this compound blocked the entry of SARS-CoV-2 pseudo-virions. Taken together, these results demonstrate that onnamide A suppresses SARS-CoV-2 infection, which may be partially related to entry inhibition, and is expected to be a candidate lead compound for the development of anti-SARS-CoV-2 drugs.

Keywords: COVID-19, marine invertebrates, onnamide A, SARS-CoV-2, *Theonella swinhoei*

CELL

Cell General

Comprehensive analysis of non-selective and selective autophagy in yeast atg mutants and characterization of autophagic activity in the absence of the Atg8 conjugation system

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Most autophagy-related genes, or *ATG* genes, have been identified through studies using budding yeast. Although the functions of the *ATG* genes are well understood, the contributions of individual genes to non-selective and various types of selective autophagy remain to be fully elucidated. In this study, we quantified the activity of non-selective autophagy, the cytoplasm-to-vacuole targeting (Cvt) pathway, mitophagy, endoplasmic reticulum (ER)-phagy and pexophagy in all *Saccharomyces cerevisiae atg* mutants. Among the mutants of the core autophagy genes considered essential for autophagy, the *atg13* mutant and mutants of the genes involved in the two ubiquitin-like conjugation systems retained residual autophagic functionality. In particular, mutants of the Atg8 ubiquitin-like conjugation system (the Atg8 system) exhibited substantial levels of non-selective autophagy, the Cvt pathway and pexophagy, although mitophagy and ER-phagy were undetectable. Atg8-system mutants also displayed intravacuolar vesicles resembling autophagic bodies, albeit at significantly reduced size and frequency. Thus, our data suggest that membranous sequestration and vacuolar delivery of autophagic cargo can occur in the absence of the Atg8 system. Alongside these findings, the comprehensive analysis conducted here provides valuable datasets for future autophagy research.

Keywords: *Atg8* conjugation system, autophagy, core autophagy genes, Cvt pathway, mitophagy

BIOTECHNOLOGY

RNA Technology

Selection and characterization of aptamers targeting the Vif-CBFβ-ELOB-ELOC-CUL5 complex

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The viral infectivity factor (Vif) of human immunodeficiency virus 1 forms a complex with host proteins, designated as Vif-CBFβ-ELOB-ELOC-CUL5 (VβBCC), initiating the ubiquitination and subsequent proteasomal degradation of the human antiviral protein APOBEC3G (A3G), thereby negating its antiviral function. Whilst recent cryo-electron microscopy (cryo-EM) studies have implicated RNA molecules in the Vif-A3G interaction that leads to A3G ubiquitination, our findings indicated that the VβBCC complex can also directly impede A3G-mediated DNA deamination, bypassing the proteasomal degradation pathway. Employing the Systematic Evolution of Ligands by EXponential enrichment (SELEX) method, we have identified RNA aptamers with high affinity for the VβBCC complex. These aptamers not only bind to the VβBCC complex but also reinstate A3G's DNA deamination activity by inhibiting the complex's function. Moreover, we delineated the sequences and secondary

structures of these aptamers, providing insights into the mechanistic aspects of A3G inhibition by the VβBCC complex. Analysis using selected aptamers will enhance our understanding of the inhibition of A3G by the VβBCC complex, offering potential avenues for therapeutic intervention.

Keywords: aptamer, human immunodeficiency virus, SELEX, viral infectivity factor

Drug Delivery Systems

Chondroitin sulfate liposome: clustering toward high functional efficiency

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Chondroitin sulfate (CS) is a linear polysaccharide chain of alternating residues of glucuronic acid (GlcA) and *N*-acetylgalactosamine (GalNAc), modified with sulfate groups. Based on the structure, CS chains bind to bioactive molecules specifically and regulate their functions. For example, CS whose GalNAc is sulfated at the C4 position, termed CSA, and CS whose GalNAc is sulfated at both C4 and C6 positions, termed CSE, bind to a malaria protein VAR2CSA and receptor type of protein tyrosine phosphatase sigma (RPTPσ), respectively, in a specific manner. Here, we modified CSA and CSE chains with phosphatidylethanolamine (PE) at a reducing end, attached them to liposomes containing phospholipids and generated CSA and CSE liposomes. The CS-PE was incorporated into the liposome particles efficiently. Inhibition ELISA revealed specific interaction of CSA and CSE with recombinant VAR2CSA and RPTPσ, respectively, more efficiently than CS chains alone. Furthermore, CSE liposome was specifically incorporated into RPTPσ-expressing HEK293T cells. These results indicate CS liposome as a novel and efficient drug delivery system, especially for CS-binding molecules.

Keywords: chondroitin sulfate, drug delivery system, liposome, RPTPσ, VAR2CSA