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ダイジェスト

JB COMMENTARY

Maintenance of the Golgi ribbon structure by the KASH protein Jaw1

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The Golgi apparatus is an organelle responsible for modification, secretion and transport of biomolecules. Its structure and morphology are crucial for the efficient processing and proper transport of proteins. The maintenance of this Golgi ribbon structure involves multiple proteins including GRASP and golgin proteins and the microtubule network. Particularly, the microtubule network extending from the Golgi is important for the Golgi ribbon formation and positioning. A recent report by Okumura et al. (*J. Biochem.* 2023; 173: 383–392) demonstrated that Jaw1, one of the Klarsicht/ANC-1/Syne/homology (KASH) proteins that are components of the linker of nucleoskeleton and cytoskeleton (LINC) complex, is essential for maintaining the Golgi ribbon structure. Knockdown of Jaw1 disrupted the Golgi ribbon structure leading to the fragmentation, whilst the Golgi ministacks were preserved. Acetylated tubulin, a marker of the Golgi-derived microtubule network, became more dispersed, losing its local compactness in the Jaw-depleted cells. These phenomena suggest that Jaw1 is required to maintain the proper organization of the Golgi-derived microtubule network.

Keywords: biosynthesis, endoplasmic reticulum, lipid rafts, secretion, trafficking

Commentary for: a lipid scramblase TMEM41B is involved in the processing and transport of GPI-anchored proteins

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Glycosylphosphatidylinositol (GPI) anchoring is a conserved post-translational modification in eukaryotes. This modification allows acceptor proteins to be expressed at the cell surface as GPI-anchored proteins (GPI-APs), which play critical roles in various biological processes. It has been proposed that remodelling of GPI after transferring acceptor proteins, including the PGAP1-dependent deacylation of GPI-inositol, functions as a checkpoint for transporting mature GPI-APs from the endoplasmic reticulum (ER) to the Golgi. A previous study identified several factors involved in regulating PGAP1-dependent GPI-inositol deacylation, including proteins associated with the calnexin cycles, SELT and CLPTM1. A recent report by Cao *et al*., revealed that the loss of TMEM41B, an ER-resident lipid scramblase, rescues the defect in GPI-inositol deacylation in SELT-KO cells. Further investigation demonstrated that TMEM41B is essential for the efficient transport of both GPI-APs and transmembrane proteins from the ER to the Golgi. The study also found that PGAP1 proteins accumulate in the ER of TMEM41B-KO cells, suggesting that perturbations in the ER-membrane lipid integrity stabilize PGAP1 proteins, thereby enhancing the PGAP1 activity within the ER. These findings highlight that defects in TMEM41B impact two distinct processes: (i) the transport of GPI-APs from the ER to the Golgi, and (ii) the deacylation of GPI-APs.

Keywords: GPI-anchored protein, TMEM41B, lipid scramblase, protein trafficking, GPI-inositol deacylase

JB REVIEW

RMST: a long noncoding RNA involved in cancer and disease

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Long non-coding RNA rhabdomyosarcoma 2-associated transcript (RMST) is a crucial regulator in various biological processes, particularly in neurogenesis and cancer progression. This review summarizes current knowledge on structure, expression patterns and functional roles across different organs and diseases of RMST. RMST exhibits tissue-specific expression, notably in brain tissues and vascular endothelial cells, and plays a significant role in neuronal differentiation through interaction with SRY-box 2. In cancer, RMST predominantly functions as a tumour suppressor, with context-dependent roles observed across different cancer types. RMST is also implicated in neurological disorders, cardiovascular diseases and Hirschsprung's disease. Mechanistically, RMST acts as a competing endogenous RNA and a transcriptional regulator, interacting with various microRNAs and proteins to modulate gene expression. The potential of RMST as a biomarker and therapeutic target is increasingly recognized, particularly in atherosclerosis and cancer. While current findings are promising, further research is needed to fully elucidate the functions and translate these insights into clinical applications of RMST. This review underscores the significance of RMST in cellular processes and disease pathogenesis, highlighting its potential as a novel target for diagnostic and therapeutic interventions.

Keywords: cancer, competing endogenous RNA, long noncoding RNA, RMST, tumour suppressor

2-Oxoglutarate-dependent dioxygenases as oxygen sensors: their importance in health and disease

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Since low oxygen conditions below physiological levels, hypoxia, are associated with various diseases, it is crucial to understand the molecular basis behind cellular response to hypoxia. Hypoxia-inducible factors (HIFs) have been revealed to primarily orchestrate the hypoxic response at the transcription level and have continuously attracted great attention over the past three decades. In addition to these hypoxia-responsive effector proteins, 2-oxoglutarate-dependent dioxygenase (2-OGDD) superfamily including prolyl-4-hydroxylase domain-containing proteins (PHDs) and factor inhibiting HIF-1 (FIH-1) has attracted even greater attention in recent years as factors that act as direct oxygen sensors due to their necessity of oxygen for the regulation of the expression and activity of the regulatory subunit of HIFs. Herein, we present a detailed classification of 2-OGDD superfamily proteins, such as Jumonji C-domain-containing histone demethylases, ten-eleven translocation enzymes, AlkB family of DNA/RNA demethylases and lysyl hydroxylases, and discuss their specific functions and associations with various diseases. By introducing the multifaceted roles of 2-OGDD superfamily proteins in the hypoxic response, this review aims to summarize the accumulated knowledge about the complex mechanisms governing cellular adaptation to hypoxia in various physiological and pathophysiological contexts.

Keywords: 2-oxoglutarate-dependent dioxygenase (2-OGDD), epigenetics, gene regulation, hypoxia, metabolism

RAPID COMMUNICATION

GPLD1 + cancer stem cells contribute to chemotherapy resistance and tumour relapse in intestinal cancer

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Cancer stem cells (CSCs) play a central role in cancer progression, therapy resistance, and disease recurrence. With the use of a quadruple-mutant mouse intestinal cancer organoid model and single-cell RNA-sequencing analysis, we have now identified glycosylphosphatidylinositol-specific phospholipase D1 (GPLD1), an enzyme that catalyzes the cleavage of glycosylphosphatidylinositol (GPI) anchors of membrane proteins, as a marker of slowly cycling CSCs. Ablation of *Gpld1*+ cells in combination with 5-fluorouracil treatment greatly attenuated cell viability in and regrowth of the intestinal cancer organoids. In addition, we identified serine protease 8 (PRSS8) as a key substrate of GPLD1 in human colorectal cancer cells. GPLD1 cleaves the GPI anchor of PRSS8 and thereby mediates release of the protease from the plasma membrane, resulting in the activation of Wnt signalling and promotion of the epithelial-mesenchymal transition (EMT) in the cancer cells. Pharmacological inhibition of GPLD1 suppressed Wnt signalling activity and EMT in association with upregulation of the amount of functional PRSS8 at the plasma membrane. Our findings suggest that targeting of GPLD1 in colorectal cancer might contribute to a new therapeutic strategy that is based on suppression of Wnt signalling and EMT-related cancer progression driven by CSCs.

Keywords: cancer stem cell, chemotherapy resistance, *Gpld1*, intestinal cancer, Prss8

REGULAR PAPERS

BIOCHEMISTRY

Biochemistry General

Ionic control of small GTPase HRas using calmodulin

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HRas is a small GTPase that plays physiologically important roles in various intracellular signal transduction processes, such as cell growth and proliferation. The structure and action mechanisms of HRas have been well characterized, leading to its widespread use as a molecular switch in bionanomachines. Calmodulin (CaM), a calcium ion-binding protein, acts as an ion-binding molecular switch and activates the target enzymes. We previously demonstrated that the fusion protein of HRas (M13-HRas) with the CaM target peptide M13 at the N-terminus of HRas exhibits reversible regulation of GTPase activity and the interaction between M13-HRas and the downstream signalling factor Raf by calcium ions with CaM. In this study, we prepared two new HRas fusion proteins with the M13 peptide at the C-terminus (HRas-M13) and both termini (M13-HRas-M13) of HRas and analysed the calcium-dependent regulation of HRas function. M13-HRas-M13 more efficiently controlled GTPase, interaction with Raf and the HRas regulator GEF by calcium ions with CaM.

Keywords: calcium ion, calmodulin, HRas, ionic control, small GTPase

Lipid Biochemistry

Characterization of UGT8 as a monogalactosyl diacylglycerol synthase in mammals

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Monogalactosyl diacylglycerol (MGDG) is a major membrane lipid component in plants and is crucial for proper thylakoid functioning. However, MGDG in mammals has not received much attention, partly because of its relative scarcity in mammalian tissues. In addition, the biosynthetic pathway of MGDG in mammals has not been thoroughly analysed, although some reports have suggested that UGT8, a ceramide galactosyltransferase, has the potential to catalyse MGDG biosynthesis. Here, we successfully captured the endogenous levels of MGDG in HeLa cells using liquid chromatography quadrupole time-of-flight mass spectrometry (LC–QTOF-MS)-based lipidomics. Cellular MGDG was completely depleted in CRISPR/Cas9-mediated UGT8 knockout (KO) HeLa cells. Transient overexpression of UGT8 enhanced MGDG production in HeLa cells, and the corresponding cell lysates displayed MGDG biosynthetic activity *in vitro*. Site-directed mutagenesis revealed that His358 within the UGT signature sequence was important for its activity. UGT8 was localized in the endoplasmic reticulum and activation of the unfolded protein response by membrane lipid saturation was impaired in UGT8 KO cells. These results demonstrate that UGT8 is an MGDG synthase in mammals and that UGT8 regulates membrane lipid saturation signals in cells.

Keywords: endoplasmic reticulum stress, glycolipid, mass spectrometry, membrane lipid, unfolded protein response

Biochemical Pharmacology

Species-specific differences in acetaminophen hepatotoxicity depend on HSP70 expression level

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Acetaminophen (N-Acetyl-p-aminophenol: APAP) is one of the most commonly used analgesic/antipyretic drugs with proven safety at therapeutic doses, however, over-dosage causes dose-dependent liver damage, leading to acute liver failure in severe cases. The level of APAP-induced liver injury has been known to vary amongst animal species, and APAP concentrations that induce cell death have been investigated using primary cultured cells. We constructed *in vitro* model of APAP-induced hepatotoxicity using mouse, rat and human hepatoma cell lines to investigate species differences in the APAP-induced cytotoxicity by monitoring cell death as a marker. The EC_{50} for each cell line was Hepa1–6 (mouse) < H-4-II-E (rat) < Hep3B (human), whilst the expression of heat shock protein 70 (HSP70), which was a typical molecular chaperone, positively correlated with the EC_{50} of each cell. Heat shock treatment, which caused activation of heat shock factor 1 (HSF1) followed by significant induction of HSP70, partially suppressed APAP-induced cell death in Hepa1–6 and H-4-II-E. Moreover, HSP70 or HSF1 siRNA treatment in Hep3B enhanced APAP-induced cell death. These results suggest that APAP-induced cell death in hepatoma cell lines may be partly mediated by protein denaturation and that the expression level of HSP70 has an inhibitory effect.

Keywords: Acetaminophen, heat shock protein, liver, species-specific differences, hepatotoxicity

MOLECULAR BIOLOGY

Gene Expression

Two-sided function of osteopontin during osteoblast differentiation

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Osteopontin (OPN) is expressed in various cell types including osteoblasts. OPN expression level is robustly increased during osteoblast differentiation. Although OPN was initially found as a secretory protein (sOPN), recent reports identified the intracellular isoform of OPN (iOPN). Distinct functions of each OPN isoform in osteoblasts, however, are not well established. Here, using the Tet-On inducible expression system, we examined the role of each OPN isoform during osteoblast differentiation. Induced overexpression of wild type OPN (wtOPN), which

includes both sOPN and iOPN, significantly increased matrix mineralization and osteogenic marker gene expression during osteogenic differentiation induced by either ascorbic acid or bone morphogenetic protein (BMP) 9. In contrast, these osteogenic differentiation processes were significantly inhibited by the specific overexpression of iOPN. Furthermore, the addition of recombinant OPN or neutralizing anti-OPN antibody to the culture medium exerted promotive or inhibitory effect on osteoblast differentiation, respectively. These data strongly indicate that iOPN exerts inhibitory effects on osteoblast differentiation, whereas sOPN exerts positive effects. We also found that the secretion process of OPN is positively regulated by c-Jun N-terminal kinase (JNK) activity in osteoblasts.

Keywords: bone morphogenetic protein, bone regeneration, osteoblast differentiation, osteopontin, Tet-On system

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ダイジェスト

JB SPECIAL ISSUE: BIOCHEMISTRY OF AGING: LATEST INSIGHTS INTO AGING AND CELLULAR SENESCENCE
GUEST EDITOR: AKIKO TAKAHASHI
***JB SPECIAL ISSUE*—REVIEW**

Cellular senescence: mechanisms and relevance to cancer and aging

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Cellular senescence is an irreversible cell cycle arrest induced by stresses such as telomere shortening and oncogene activation. It acts as a tumor suppressor mechanism that prevents the proliferation of potentially tumorigenic cells. Paradoxically, senescent stromal cells that arise in the tumor microenvironment have been shown to promote tumor progression. In addition, senescent cells that accumulate *in vivo* over time are thought to contribute to aging and age-related diseases. These deleterious effects of senescent cells involve the secretion of bioactive molecules such as inflammatory cytokines and chemokines, a phenomenon known as the senescence-associated secretory phenotype. While the role of cellular senescence *in vivo* is becoming increasingly clear, the intracellular signaling pathways that induce the expression of senescent phenotypes are not fully understood. In this review, we outline senescence-associated signaling pathways and their relevance to cancer and aging.

Keywords: aging, cancer, cellular senescence, SASP, stress signalling

Cellular senescence in the cancer microenvironment

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In this ageing society, the number of patients suffering from age-related diseases, including cancer, is increasing. Cellular senescence is a cell fate that involves permanent cell cycle arrest. Accumulated senescent cells in tissues over time present senescence-associated secretory phenotype (SASP) and make the inflammatory context, disturbing the tumour microenvironment. In particular, the effect of senescent cancer-associated fibroblasts on cancer progression has recently come under the spotlight. Although scientific evidence on the impact of cellular senescence on cancer is emerging, the association between cellular senescence and cancer is heterogeneous and the comprehensive mechanism is still not revealed. Recently, a therapy targeting senescent cells, senotherapeutics, has been reported to be effective against cancer in preclinical research and even clinical trials. With further research, the development of senotherapeutics as a novel cancer therapy is expected.

Keywords: cancer, cancer-associated fibroblast, cellular senescence, senescent cell, senotherapeutics

Targeting senescent cells for the treatment of age-associated diseases

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Cellular senescence, which entails cellular dysfunction and inflammatory factor release—the senescence-associated secretory phenotype (SASP)—is a key contributor to multiple disorders, diseases and the geriatric syndromes. Targeting senescent cells using senolytics has emerged as a promising therapeutic

strategy for these conditions. Among senolytics, the combination of dasatinib and quercetin (D + Q) was the earliest and one of the most successful so far. D + Q delays, prevents, alleviates or treats multiple senescence-associated diseases and disorders with improvements in healthspan across various pre-clinical models. While early senolytic therapies have demonstrated promise, ongoing research is crucial to refine them and address such challenges as off-target effects. Recent advances in senolytics include new drugs and therapies that target senescent cells more effectively. The identification of senescence-associated antigens—cell surface molecules on senescent cells—pointed to another promising means for developing novel therapies and identifying biomarkers of senescent cell abundance.

Keywords: cell surface proteins, cellular senescence, immunotherapy, seno-antigens, senolytics

Functional diversity of senescent cells in driving ageing phenotypes and facilitating tissue regeneration

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As the global population continues to age, understanding the complex role of cellular senescence and its implications in healthy lifespans has gained increasing prominence. Cellular senescence is defined as the irreversible cessation of cell proliferation, accompanied by the secretion of a range of pro-inflammatory factors, collectively termed the senescence-associated secretory phenotype (SASP), in response to various cellular stresses. While the accumulation of senescent cells has been strongly implicated in the ageing process and the pathogenesis of age-related diseases owing to their pro-inflammatory properties, recent research has also highlighted their essential roles in processes such as tumour suppression, tissue development and repair. This review provides a comprehensive examination of the dual nature of senescent cells, evaluating their deleterious contributions to chronic inflammation, tissue dysfunction and disease, as well as their beneficial roles in maintaining physiological homeostasis. Additionally, we explored the therapeutic potential of senolytic agents designed to selectively eliminate detrimental senescent cells while considering the delicate balance between transient and beneficial senescence and the persistence of pathological senescence. A deeper understanding of these dynamics is critical to develop novel interventions aimed at mitigating age-related dysfunctions and enhancing healthy life expectancies.

Keywords: age-related diseases, cellular senescence, healthspan, senolytics, tissue regeneration

JB COMMENTARY

Commentary on: *γ*-enolase (ENO2) is methylated at the N*τ* position of His-190 among enolase isozymes

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Post-translational modifications play crucial roles in regulating protein function. Protein methylation, occurring at lysine, arginine, and histidine, has gained attention, particularly for histone methylation. However, the mechanism and significance of protein methylation at histidine remain poorly understood. Kasai *et al.* developed a novel method to identify histidine-methylated proteins and discovered that *γ*-enolase in the mouse brain undergoes N*τ*-methylation at His190. This modification reduces dimerization and enzymatic activity, suggesting this reaction plays a physiological role. This work will accelerate research on histidine methylation and help elucidate unknown phenomena in the brain.

Commentary on 'Structural insights into a bacterial *β*-glucosidase capable of degrading sesaminol triglucoside to produce sesaminol: towards the understanding of the aglycone recognition mechanism by the C-terminal lid domain'

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Sesaminol is an organic compound that shows the strong antioxidant, anti-inflammatory and neuroprotective properties. Sesaminol triglucoside (STG) is a glycosylated form of sesaminol and abundantly exists in sesame seeds. However, typical *β*-glucosidases could not deglycosylate STG probably due to its bulky aglycone. PSTG1 and 2 are *β*-glucosidases lately isolated from *Paenibacillis* sp. KB0459 and have the capacity to deglycosylate STG. A recent report by Yanai *et al.* (*J. Biochem*. 2023; 174: 335–344) revealed the unique domain architecture of PSTG1. Apart from other *β*-glucosdasies in the GH3 family, PSTG1 has a novel accessary domain (domain 4) at the C-terminus. Domain 4 contributes to the dimer formation and is located close to the active site. Interestingly, several hydrophobic residues are exposed, suggesting that this domain may recognize the hydrophobic aglycone of STG. The physiological functions of the non-catalytic domains in glyco-enzymes are sometimes overlooked. This paper sheds light on the aglycone recognition by novel accessary domain.

Keywords: aglycone recognition, glycosidase, glycosyltransferase

RAPID COMMUNICATION

Etomoxir suppresses the expression of PPARγ2 and inhibits the thermogenic gene induction of brown adipocytes through pathways other than β-oxidation inhibition

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Brown adipocytes are characterized by a high abundance of mitochondria, allowing them to consume fatty acids for heat production. Increasing the number of brown adipocytes is considered a promising strategy for combating obesity. However, the molecular mechanisms underlying their differentiation remain poorly understood. In this study, we demonstrate that etomoxir, an inhibitor of Carnitine Palmitoyltransferase 1 (CPT1), inhibits their differentiation through mechanisms independent of β-oxidation inhibition. In the presence of etomoxir during brown adipocyte differentiation, reduced expression of the thermogenic gene UCP1 and decreased lipid droplets formation were observed. Furthermore, a transient reduction in the expression of PPARγ2, a critical factor in adipocyte differentiation, was also observed in the presence of etomoxir. These findings suggest the presence of a regulatory mechanism that specifically enhances PPARγ2 expression during brown adipocyte differentiation, thereby modulating thermogenic gene expression.

Keywords: brown adipocytes, differentiation, etomoxir, mitochondria, PPARγ2

Polymerase-usage sequencing identifies initiation zones with less bias across S phase in mouse embryonic stem cells

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Various methods have been developed to map replication initiation zones (IZs) genome-wide, often finding far fewer IZs than expected. In particular, IZs corresponding to later stages of S phase are under-represented. Here, we reanalysed IZs with respect to replication timing in mouse ES cells. These datasets identified over five times as many early IZs compared to late IZs. In addition, we have set up a polymerase-usage sequencing (Pu-seq) system in mouse ES cells to map IZs genome-wide. Pu-seq showed less bias towards early IZs, potentially indicating better sensitivity for identifying IZs in late S phase.

Keywords: DNA replication, mouse embryonic stem cells, polymerase-usage sequencing, replication initiation zones, replication timing

REGULAR PAPERS

BIOCHEMISTRY

Biochemistry in Diseases and Aging

Supplementation of essential amino acids suppresses age-associated sleep loss and sleep fragmentation but not loss of rhythm strength under yeast-restricted malnutrition in Drosophila

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Sleep quality and quantity decrease with age, and sleep disturbance increases the risk of many age-associated diseases. There is a significant relationship between nutritional status and sleep outcomes, with malnutrition inducing poor sleep quality in older adults. However, it remains elusive whether, and if so how, nutritional supplementation prevents age-associated sleep problems. Here, we utilized *Drosophila* to investigate the effects of a malnutrition diet with restricted yeast, a primary protein source, and supplementation of 10 essential amino acids (EAAs) on sleep profiles during ageing. Compared with the standard diet containing 2.7% yeast, the malnutrition diet containing 0.27% yeast significantly decreased target of rapamycin (TOR) signalling and shortened the lifespan of male Canton-S flies. By contrast, age-associated sleep loss, sleep fragmentation and loss of rhythm strength were similarly observed under both diets. Supplementation of the malnutrition diet with EAAs in restricted yeast significantly ameliorated age-associated sleep loss and sleep fragmentation without altering loss of rhythm strength. It also rescued decreased TOR signalling activity but not the shortened lifespan, suggesting that the effects of EAAs on sleep integrity are independent of TOR activity and lifespan regulation. These results may help to develop dietary interventions that improve age-related sleep problems in humans.

Keywords: *Drosophila*, sleep, malnutrition, ageing, amino acid

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ダイジェスト

JB REVIEW

Profiling translation in the nervous system

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Regulation at the level of translation is critical in the nervous system, such as for the formation of cell-type-specific proteomes or plastic changes in neural circuits. Whilst current knowledge of the translatome is relatively limited compared to transcriptome, a growing array of tools to analyse translation is becoming available. In this review, we discuss techniques for profiling translation on a genome-wide scale with a special emphasis on cell-type-specific analyses in the nervous system. This includes polysome-profiling-seq, Translating Ribosome Affinity Purification (TRAP)-seq and ribosome profiling (Ribo-seq). We review recent advances to achieve spatial resolution of translatome analysis, such as genetic labelling of the targeted cells and cell sorting, and discuss the biological implications of translational regulation in the brain and potential future extensions.

Keywords: nervous system, polysome-profiling-seq, Ribo-seq, translatome, TRAP-seq

RAPID COMMUNICATION

Replication across O6-methylguanine activates futile cycling of DNA mismatch repair attempts assisted by the chromatin-remodelling enzyme Smarcad1

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S_N1-type alkylating reagents generate O^6-methylguanine (^{me}G) lesions that activate the mismatch repair (MMR) response. Since post-replicative MMR specifically targets the nascent strand, ^{me}G on the template strand is refractory to rectification by MMR and, therefore, can induce non-productive MMR reactions. The cycling of futile MMR attempts is proposed to cause DNA double-strand breaks in the subsequent S phase, leading to ATR-checkpoint-mediated G2 arrest and apoptosis. However, the mechanistic details of futile MMR cycling, especially how this reaction is maintained in chromatin, remain unclear. Using replication-competent *Xenopus* egg extracts, we herein establish an *in vitro* system that recapitulates futile MMR cycling in the chromatin context. The ^{me}G–T mispair, but not the ^{me}G–C pair, is efficiently targeted by MMR in our system. MMR attempts on the ^{me}G-strand result in the ^{me}G-to-A correction, whilst those on the T-strand induce iterative cycles of strand excision and resynthesis. Likewise, replication across ^{me}G generates persistent single-strand breaks on the daughter DNA containing ^{me}G. Moreover, the depletion of Smarcad1, a chromatin remodeller previously reported to facilitate MMR, impairs the retention of single-strand breaks. Our study thus provides experimental evidence that chromatin replication across ^{me}G induces futile MMR cycling that is assisted by Smarcad1.

Keywords: DNA replication, mismatch repair, O6-methylguanine, Smarcad1, Xenopus egg extract

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Biochemistry General

SSB promotes DnaB helicase passage through DnaA complexes at the replication origin oriC for bidirectional replication

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For bidirectional replication in *Escherichia coli*, higher order complexes are formed at the replication origin *oriC* by the initiator protein DnaA, which locally unwinds the left edge of *oriC* to promote the loading of two molecules of DnaB helicases onto the unwound region via dynamic interactions with the helicase-loader DnaC and the *oriC*-bound DnaA complex. One of the two helicases must translocate rightwards through *oriC*-bound DnaA complex. Here, we used a synthetic forked *oriC* DNA, which mimics the unwound state of *oriC*, to examine DnaB translocation through the *oriC*-bound DnaA complex. We found that DnaB helicase alone cannot pass through the *oriC*-bound DnaA complex without the help of single-strand binding protein (SSB). In the presence of SSB, DnaB passed through this complex along with its helicase function, releasing DnaA molecules. In addition, DnaB helicase activity is known to be inhibited by oversupply of DnaC, but this inhibition was relieved by SSB. These results suggest a mechanism that when two DnaB helicases are loaded at *oriC*, one translocates leftwards to expand the DnaA-unwound region and allows SSB binding to the single-stranded DNA, and

such SSB molecules then stimulate translocation of the other helicase rightwards through the *oriC*-bound DnaA complex.
Keywords: bidirectional replication, helicase, reconstituted assays, replication origin, SSB

Protein Interaction and Recognition

Identification of a novel Eps 15 homology domain-containing protein 1 (EHD1) and EHD4-binding motif in phostensin

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Phostensin (PTS) encoded by *KIAA1949* binds to protein phosphatase 1, F-actin, Eps 15 homology domain-containing protein 1 (EHD1) and EHD4. Most EHD-binding proteins contain a consensus motif, Asn-Pro-Phe (NPF), which interacts with the C-terminal EH domain of EHD proteins. Nevertheless, the NPF motif is absent in PTS. The binding motif for PTS to interact with EHD1 (or EHD4) remains unknown. Here, we identified that PTS-α binds to EHD1 (or EHD4) through the region of residues 51–80, which contains a consensus motif, ^{64}ILV(X)$_4$(L/V)RL^{74}S. This novel consensus motif is also found in vacuolar protein sorting-35 (vps35). Replacement of ^{64}ILV(X)$_4$(L/V)RL^{74}S with ^{64}AAA(X)$_4$(L/V)RL^{74}S or with ^{64}ILV(X)$_4$AEA^{74}A significantly reduces the binding efficiency of PTS-α to either EHD1 or EHD4 in GST pull-down assay and far western blotting assay. In addition, replacement of ^{218}ILV(X)$_4$VRL^{228}S with ^{218}AAA(X)$_4$AEA^{228}A decreases the binding ability of vps35 to EHD4 in far western blotting assay. Overexpression of the PTS-β in 293 T cells attenuated the endocytic trafficking of transferrin. However, this attenuation of transferrin in endocytic trafficking was disrupted when 293 T cells overexpressed the mutant PTS-β with a defective EHD-binding motif, suggesting that PTS-β can regulate the endocytic recycling via associating with EHD1 or EHD4.

Keywords: EHD4, EHD-binding motif, Eps 15 homology domain-containing protein 1 (EHD1), phostensin, vacuolar protein sorting 35

Enzymology

Biochemical characterization and mutational analysis of lysophosphatidic acid acyltransferases of Escherichia coli highlighting their involvement in the generation of membrane phospholipid diversity

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Lysophosphatidic acid acyltransferase (LPAAT) is an enzyme responsible for the second acylation step of phospholipid biosynthesis and transforms lysophosphatidic acid to phosphatidic acid, a universal precursor of various phospholipids. In addition to the well-studied *plsC*-encoded LPAAT (EcPlsC), we previously found that *Escherichia coli* has another LPAAT that is encoded by *yihG* (EcYihG). EcPlsC and EcYihG are integral membrane proteins and have never been solubilized and purified in their active form. To better understand the difference in their enzymatic functions and how the two paralogs differently contribute to lipid diversity, we established a method to purify both enzymes in their active form and comparatively analysed their biochemical characteristics. Our findings illustrate that EcPlsC possesses the highest activity at pH 8.0 and 37°C with selectivity for unsaturated fatty acyl-CoAs (*e.g.* palmitoleoyl-CoA), whereas EcYihG works optimally at pH 7.5 and 30°C and prefers saturated fatty acyl-CoAs (*e.g.* myristoyl-CoA). In addition, we performed a mutational analysis based on AlphaFold2 models and revealed that one residue, which is located at the putative acyl-donor-selectivity tunnel entrance, plays a pivotal role in selecting acyl donor substrates. This provides new insights into how LPAATs recognize specific fatty acyl groups and incorporate them into membrane phospholipids.

Keywords: lysophosphatidic acid acyltransferase, phospholipid biosynthesis, *PlsC*, YihG

CELL

Biomembranes/Organelles and Protein Sorting

Interaction mapping between nucleoporins in the fission yeast Schizosaccharomyces pombe using mass-spectrometry

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Nuclear pore complexes (NPCs) act as gateways across the nuclear envelope for molecular transport between the nucleus and the cytoplasm in eukaryotes. NPCs consist of several subcomplexes formed by multiple copies of approximately 30 different proteins known as nucleoporins (Nups). In the fission yeast *Schizosaccharomyces pombe*, the NPC structure is unique, particularly in its outer ring subcomplexes, where the cytoplasmic and nucleoplasmic outer rings are composed of distinct sets of proteins. However, it remains unclear how this unique outer ring structure in *S. pombe* is supported by interactions between subcomplexes or individual Nups. In this study, we investigated protein–protein interactions between *S. pombe* Nups using mass spectrometry and identified Nups that interact with each subcomplex or a specific Nup. The cytoplasmic outer ring Nups bind to both the cytoplasmic filament Nups and the inner ring Nups, while the nucleoplasmic outer ring Nups bind to the nuclear basket Nups in addition to the inner ring Nups. Among the inner ring Nups, Nup155 interacts with most of the cytoplasmic and nucleoplasmic outer ring Nups, suggesting that Nup155 may serve as a hub supporting the uniquely asymmetric outer ring structure of the *S. pombe* NPC.

Keywords: inner ring, mass spectrometry, nuclear pore complex, Nup155, outer ring

Extracellular Matrices and Cell Adhesion Molecules

Production of non-triple-helical collagen polypeptides under hypoxia and the implication for tumour

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Non-triple-helical collagen polypeptides (NTHs) are alternative gene products lacking the typical collagen triple-helical structure. This study investigated NTH production in tumour cells and tissues. NTH α1(IV) was detected in various human tumour cell lines and extracted from human lung cancer tissues and tumours in mice. NTH production was significantly affected by serum concentration and occurred under hypoxic or hypoxia-mimetic conditions, even with sufficient ascorbic acid. This suggests NTHs are produced under physiological hypoxia, potentially contributing to tumour angiogenesis. NTH production generally coincided with hypoxia-inducible factor-1α (HIF-1α) accumulation, except with cobalt chloride, indicating HIF-1α is not directly involved in NTH α1(IV) production. NTH electrophoretic mobility on SDS-PAGE was higher under hypoxia or deferoxamine treatment, likely due to suppressed lysyl hydroxylase 3 activity. This study demonstrates NTH production in tumour cells and tissues under hypoxia, suggesting their association with tumour angiogenesis and potential as therapeutic targets.

Keywords: angiogenesis, HIF-1αhypoxia, non-triple-helical collagen polypeptide, tumour, Type IV collagen