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ダイジェスト

JB Commentary

Regulation of HP1 protein by phosphorylation during transcriptional repression and cell cycle

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HP1 (heterochromatin protein 1), a key factor for the formation of heterochromatin, binds to the methylated lysine 9 of histone H3 (H3K9me) and represses transcription. While the H3K9me mark and HP1 binding are thought to be faithfully propagated to daughter cells, the heterochromatin structure could be dynamically regulated during cell cycle. As evidenced by the well-known phenomenon called position effect variegation (PEV), heterochromatin structure is dynamically and stochastically altered during developmental processes, and thus the expression of genes within or in the vicinity of heterochromatin could be affected by mutations in factors regulating DNA replication as well as by other epigenetic factors. Recent reports show that HP1 also plays an important role in the maintenance and transmission of chromosomes. Like many other factors ensuring faithful chromosome segregation, HP1 family proteins are subjected to posttranslational modifications, most notably phosphorylation, in a cell cycle-dependent manner. Recent studies identified a conserved phosphorylation site that profoundly affects the functions of HP1 during mitotic phase. In this commentary, we discuss dynamic regulation of HP1 protein by phosphorylation during transcriptional repression and cell cycle.

Keywords: heterochromatin, HP1, mitosis, position effect variegation, protein phosphorylation

JB Reviews

Post-translational modifi cation enzymes as key regulators of ciliary protein trafficking

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Primary cilia are evolutionarily conserved microtubule-based organelles that protrude from the surface of almost all cell types and decode a variety of extracellular stimuli. Ciliary dysfunction causes human diseases named ciliopathies, which span a wide range of symptoms, such as developmental and sensory abnormalities. The assembly, disassembly, maintenance and function of cilia rely on protein transport systems including intraflagellar transport (IFT) and lipidated protein intraflagellar targeting (LIFT). IFT is coordinated by three multisubunit protein complexes with molecular motors along the ciliary axoneme, while LIFT is mediated by specific chaperones that directly recognize lipid chains. Recently, it has become clear that several post-translational modification enzymes play crucial roles in the regulation of IFT and LIFT. Here, we review our current understanding of the roles of these post-translational modification enzymes in the regulation of ciliary protein trafficking as well as their regulatory mechanisms, physiological significance and involvement in human diseases.

Keywords: ICK/CILK1, kinase, MAK, retina, ubiquitin

Sweet modifi cation and regulation of death receptor signalling pathway

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Death receptors, members of the tumour necrosis factor receptor (TNFR) superfamily, are characterized by the presence of a death domain in the cytosolic region. TNFR1, Fas and TNF-related apoptosis-inducing ligand receptors, which are prototypical death receptors, exert pleiotropic functions in cell death, inflammation and immune surveillance. Hence, they are involved in several human diseases. The activation of death receptors and downstream intracellular signalling is regulated by various post-translational modifications, such as phosphorylation, ubiquitination and glycosylation. Glycosylation is one of the most abundant and versatile modifications to proteins and lipids, and it plays a critical role in the development and physiology of organisms, as well as the pathology of many human diseases. Glycans control a number of cellular events, such as receptor activation, signal transduction, endocytosis, cell recognition and cell adhesion. It has been demonstrated that oligo- and monosaccharides modify death receptors and intracellular signalling proteins and regulate their functions. Here, we review the current understanding of glycan modifications of death receptor signalling and their impact on signalling activity.

Keywords: apoptosis, death receptors, glycosylation, necroptosis

BIOCHEMISTRY

Biochemistry General

Fast autooxidation of a bis-histidyl-ligated globin from the anhydrobiotic tardigrade, Ramazzottius varieornatus, by molecular oxygen

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Tardigrades, a phylum of meiofaunal organisms, exhibit extraordinary tolerance to various environmental conditions, including extreme temperatures (−273 to 151°C) and exposure to ionizing radiation. Proteins from anhydrobiotic tardigrades with homology to known proteins from other organisms are new potential targets for structural genomics. Recently, we reported spectroscopic and structural characterization of a hexacoordinated haemoglobin (Kumaglobin [Kgb]) found in an anhydrobiotic tardigrade. In the absence of its exogenous ligand, Kgb displays hexacoordination with distal and proximal histidines. In this work, we analysed binding of the molecular oxygen ligand following reduction of haem in Kgb using a pulse radiolysis technique. Radiolytically generated hydrated electrons (e^-_{aq}) reduced the haem iron of Kgb within 20 µs. Subsequently, ferrous haem reacted with O_2 to form a ferrous-dioxygen intermediate with a second-order rate constant of $3.0 \times 10^6\,M^{-1}s^{-1}$. The intermediate was rapidly (within 0.1 s) autooxidized to the ferric form. Redox potential measurements revealed an E'_0 of −400 mV (vs. standard hydrogen electrode) in the ferric/ferrous couple. Our results suggest that Kgb may serve as a physiological generator of O_2^- via redox signalling and/or electron transfer.

Keywords: autooxidation, bis-histidyl-ligated globin, pulse radiolysis

Nanog-mediated stem cell properties are critical for MBNL3-associated paclitaxel resistance of ovarian cancer

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Paclitaxel (PTX) is the standard first-line treatment of ovarian cancer, but its efficacy is limited by multidrug resistance. Therefore, it is crucial to identify effective drug targets to facilitate PTX sensitivity for ovarian cancer treatment. Seventy PTX-administrated ovarian cancer patients were recruited in this study for gene expression and survival rate analyses. Muscleblind-like-3 (MBNL3) gain-of-function and loss-of-function experiments were carried out in ovarian cancer cells (parental and PTX-resistant) and xenograft model. Cancer cell viability, apoptosis, spheroids formation, Nanog gene silencing were examined and conducted to dissect the underlying mechanism of MBNL3-mediated PTX resistance. High expression of MBNL3 was positively correlated with PTX resistance and poor prognosis of ovarian cancer. MBNL3 increased cell viability and decreased apoptosis in ovarian stem-like cells, through upregulating Nanog. This study suggests the MBNL3-Nanog axis is a therapeutic target for the treatment of PTX resistance in ovarian cancer management.

Keywords: MBNL3, Nanog, ovarian cancer, paclitaxel resistance, stem-like cells

Protein Interaction and Recognition

Expression and affinity purifi cation of recombinant mammalian mitochondrial ribosomal small subunit (MRPS) proteins and protein–protein interaction analysis indicate putative role in tumourigenic cellular processes

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Mitochondrial ribosomal small subunit (MRPS) group of proteins is structural constituents of the small subunit of mitoribosomes involved in translation. Recent studies indicate role in tumourigenic process, however, unlike cytosolic ribosomal proteins, knowledge on the role of MRPS proteins in alternate cellular processes is very limited. Mapping protein–protein interactions (PPIs) onto known cellular processes can be a valuable tool to identify novel protein functions. In this study, to identify PPIs of MRPS proteins, we have constructed 31 glutathione-S-transferase (GST)/MRPS fusion clones. GST/MRPS fusion proteins were confirmed by MALDI-TOF analysis. GST pull-downs were performed using eight GST/MRPS proteins (MRPS9, MRPS10, MRPS11, MRPS18B, MRPS31, MRPS33, MRPS38 and MRPS39), GST alone as pull-down control and HEK293 cell lysate as the source for anchor proteins followed by nLC/MS/MS analysis and probable PPIs of eight MRPS proteins were identified. Three PPIs from GST pull-downs and interaction between six MRPS proteins and p53 previously reported in PPI database were validated. The PPI network analysis revealed putative role in cellular processes with implications for tumourigenesis. Gene expression screening of a cancer cell line panel indicated overexpression of MRPS10 and MRPS31 in breast cancer. Co-expression module identification tool analysis of breast cancer gene expression and MRPS10 and MRPS31 PPIs revealed putative role for PPI with acyl-CoA dehydrogenase in fatty acid

oxidation process regulated by brain-derived neurotrophic factor signalling pathway.

Keywords: extraribosomal functions, GST pull-down assay, LC/MS analysis, MRPS proteins, protein–protein interactions

A feasibility study of inverse contrast-matching small-angle neutron scattering method combined with size exclusion chromatography using antibody interactions as model systems

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Small-angle neutron scattering (SANS) and small- angle X-ray scattering (SAXS) are powerful techniques for the structural characterization of biomolecular complexes. In particular, SANS enables a selective observation of specific components in complexes by selective deuteration with contrast-matching techniques. In most cases, however, biomolecular interaction systems with heterogeneous oligomers often contain unfavorable aggregates and unbound species, hampering data interpretation. To overcome these problems, SAXS has been recently combined with size exclusion chromatography (SEC), which enables the isolation of the target complex in a multi-component system. By contrast, SEC–SANS is only at a preliminary stage. Hence, we herein perform a feasibility study of this method based on our newly developed inverse contrast-matching (iCM) SANS technique using antibody interactions as model systems. Immunoglobulin G (IgG) or its Fc fragment was mixed with 75% deuterated Fc-binding proteins, *i.e.* a mutated form of IgG-degrading enzyme of *Streptococcus pyogenes* and a soluble form of Fcγ receptor IIIb, and subjected to SEC–SANS as well as SEC–SAXS as reference. We successfully observe SANS from the non-deuterated IgG or Fc formed in complex with these binding partners, which were unobservable in terms of SANS in D_2O, hence demonstrating the potential utility of the SEC–iCM–SANS approach.

Keywords: Size exclusion chromatography, small-angle neutron scattering, contrast matching, immunoglobulin G, Fc

Antiviral activity and mechanism of ESC-1GN from skin secretion of Hylarana guentheri against influenza A virus

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Development of new and effective antiinfluenza drugs is critical for prophylaxis and treatment of influenza A virus (IAV) infection. A wide range of amphibian skin secretions have been identified to show antiviral activity. Our previously reported ESC-1GN, a peptide from the skin secretion of *Hylarana guentheri*, displayed good antimicrobial and antiinflammatory effects. Here, we found that ESC-1GN possessed significant antiviral effects against IAVs. Moreover, ESC-1GN could inhibit the entry of divergent H5N1 and H1N1 virus strains with the IC_{50} values from 1.29 to 4.59 μM. Mechanism studies demonstrated that ESC-1GN disrupted membrane fusion activity of IAVs by interaction with HA2 subunit. The results of site-directed mutant assay and molecular docking revealed that E105, N50 and the residues around them on HA2 subunit could form hydrogen bonds with amino acid on ESC-1GN, which were critical for ESC-1GN binding to HA2 and inhibiting the entry of IAVs. Altogether, these not only suggest that ESC-1GN maybe represent a new type of excellent template designing drugs against IAVs, but also it may shed light on the immune mechanism and survival strategy of *H.guentheri* against viral pathogens.

Keywords: amphibian, antimicrobial peptide, ESC-1GN, haemagglutinin, influenza A virus

Metabolism and Bioenergetics

Thioredoxin pathway in Anabaena sp. PCC 7120: activity of NADPH-thioredoxin reductase C

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To understand the physiological role of NADPH-thioredoxin reductase C (NTRC) in cyanobacteria, we investigated an NTRC-deficient mutant strain of *Anabaena* sp., PCC 7120, cultivated under different regimes of nitrogen supplementation and light exposure. The deletion of *ntrC* did not induce a change in the cell structure and metabolic pathways. However, time-dependent changes in the abundance of specific proteins and metabolites were observed. A decrease in chlorophyll *a* was correlated with a decrease in chlorophyll *a* biosynthesis enzymes and photosystem I subunits. The deletion of *ntrC* led to a deregulation of nitrogen metabolism, including the NtcA accumulation and heterocyst-specific proteins while nitrate ions were available in the culture medium. Interestingly, this deletion resulted in a redox imbalance, indicated by higher peroxide levels, higher catalase activity and the induction of chaperones such as MsrA. Surprisingly, the antioxidant protein 2-CysPrx was downregulated. The deficiency in *ntrC* also resulted in the accumulation of metabolites such as 6-phosphogluconate, ADP and ATP. Higher levels of $NADP^+$ and NADPH partly correlated with higher G6PDH activity. Rather than impacting protein expression levels, NTRC appears to be involved in the direct regulation of enzymes, especially during the dark-to-light transition period.

Keywords: Anabaena, NADPH-thioredoxin reductase C, redox regulation, thioredoxin

Biochemistry in Cell Membranes

Inhibition of flippase-like activity by tubulin regulates phosphatidylserine exposure in erythrocytes from hypertensive and diabetic patients

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Plasma membrane tubulin is an endogenous regulator of P-ATPases and the unusual accumulation of tubulin in the erythrocyte membrane results in a partial inhibition of some their activities, causing hemorheological disorders like reduced cell deformability and osmotic resistance. These disorders are of particular interest in hypertension and diabetes, where the abnormal increase in membrane tubulin may be related to the disease development. Phosphatidylserine (PS) is more exposed on the membrane of diabetic erythrocytes than in healthy cells. In most cells, PS is transported from the exoplasmic to the cytoplasmic leaflet of the membrane by lipid flippases. Here, we report that PS is more exposed in erythrocytes from both hypertensive and diabetic patients than in healthy erythrocytes, which could be attributed to the inhibition of flippase activity by tubulin. This is supported by: (i) the translocation rate of a fluorescent PS analog in hypertensive and diabetic erythrocytes was slower than in healthy cells, (ii) the pharmacological variation of membrane tubulin in erythrocytes and K562 cells was linked to changes in PS translocation and (iii) the P-ATPase-dependent PS translocation in inside-out vesicles (IOVs) from human erythrocytes was inhibited by tubulin. These results suggest that tubulin regulates flippase activity and hence, the membrane phospholipid asymmetry.

Keywords: diabetes, erythrocytes, hypertension, phosphatidylserine, tubulin

MOLECULAR BIOLOGY

Molecular Biology General

Circular RNA circNINL promotes breast cancer progression through activating *β*-catenin signaling via miR-921/ADAM9 axis

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We investigated the expression and functions of circular RNA (circRNA) circNINL and miR-921 in breast cancer (BC) in this study. We found that the expression of circNINL increased while the expression of miR-921 decreased in BC tissues and cell lines, and their anomalous expressions were associated with malignant features and poor prognostic of BC. Then, we demonstrated that circNINL could interact with miR-921 and facilitate BC cells malignant process including proliferation acceleration, migration enhancement and apoptosis evasion via sponging miR-921 *in vitro*. Further investigations revealed that circNINL/miR-921 axis could mediate the expression of ADAM9 which was a direct target of miR-921. In addition, we exhibited that ADAM9 may activate *β*-catenin signaling by interacting with E-cadherin. We presented the vital roles of circNINL/miR-921/ADAM9/

β-catenin signaling in the progression of BC.

Keywords: ADAM9, β-catenin signaling, breast cancer, circular RNA, miR-921

Gene Expression

Genome-wide analysis of chromatin structure changes upon MyoD binding in proliferative myoblasts during the cell cycle

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MyoD, a myogenic differentiation protein, has been studied for its critical role in skeletal muscle differentiation. MyoD-expressing myoblasts have a potency to be differentiated with proliferation of ectopic cells. However, little is known about the effect on chromatin structure of MyoD binding in proliferative myoblasts. In this study, we evaluated the chromatin structure around MyoD-bound genome regions during the cell cycle by chromatin immunoprecipitation sequencing. Genome-wide analysis of histone modifications was performed in proliferative mouse C2C12 myoblasts during three phases (G1, S, G2/M) of the cell cycle. We found that MyoD-bound genome regions had elevated levels of active histone modifications, such as H3K4me1/2/3 and H3K27ac, compared with MyoD-unbound genome regions during the cell cycle. We also demonstrated that the elevated H3K4me2/3 modification level was maintained during the cell cycle, whereas the H3K27ac and H3K4me1 modification levels decreased to the same level as MyoD-unbound genome regions during the later phases. Immunoblot analysis revealed that MyoD abundance was high in the G1 phase then decreased in the S and G2/M phases. Our results suggest that MyoD binding formed selective epigenetic memories with H3K4me2/3 during the cell cycle in addition to myogenic gene induction via active chromatin formation coupled with transcription.

Keywords: cell cycle, chromatin, epigenome, histone modification, myogenesis

CELL

Receptors and Signal Transduction

LncRNA PART1 promotes cell proliferation and inhibits apoptosis of oral squamous cell carcinoma by blocking EZH2 degradation

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Long non-coding RNAs (lncRNAs) have been considered as novel regulators in oral squamous cell carcinoma (OSCC). Enhancer of zeste homolog 2 (EZH2) can act as an oncogene in OSCC. This study intended to investigate whether lncRNA prostatic androgen-regulated transcription 1 (PART1) can exert its role in OSCC by regulating EZH2. The expression of PART1 in OSCC samples, tumour tissues or OSCC cell lines was detected by qRT-PCR. The proliferation and apoptosis of OSCC cells were detected by CCK-8 and flow cytometry assays, respectively. The expression of PART1 and EZH2 was highly expressed in clinical OSCC tumours and cell lines. The expression level of PART1 was positively correlated to the size, clinical stage and node metastasis of OSCC patients. Functionally, PART1 knockdown inhibited proliferation and facilitated apoptosis of OSCC cells. Mechanically, fused in sarcoma/translocated in liposarcoma (FUS) interacted with PART1 and EZH2. In addition, PART1 knockdown reduced the mRNA expression of EZH2, which was offset by FUS overexpression. The overexpression of FUS abrogated the effects of PART1 silence on proliferation and apoptosis of OSCC cells. The *in vivo* experiment revealed that PART1 knockdown inhibited tumour growth of OSCC cells in nude mice. This study indicated that PART1 exerts a carcinogenic role in OSCC by enhancing the stability of EZH2 protein.

Keywords: enhancer of zeste homolog 2, fused in sarcoma/translocated in liposarcoma, lncRNA PART1, oral squamous cell carcinoma

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JB Commentary

Targeting EZH2 as cancer therapy

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Enhancer of zeste homolog 2 (EZH2) is the catalytic subunit of polycomb repressive complex 2 (PRC2) that mediate repression of target genes by trimethylation of Lys27 in histone 3 (H3K27me3). Given the reported roles of EZH2 in cancer, it is perhaps not surprising that targeting EZH2 in cancer therapy has become a hot research topic. Indeed, different types of EZH2 inhibitors are currently under development and are being evaluated by clinical trials. Recently, Murashima *et al.* identified NPD13668, a novel EZH2 inhibitor, by using a cell-based high-

throughput screening assay. NPD13668 inhibited EZH2 methyltransferase activity, and repressed cell growth in multiple cancer cell lines, indicating a potential role for this compound in cancer treatment. In this review, we will focus on the current knowledge regarding the biological significance of PRC2 and H3K27me, and the recent advances in developing and testing drugs that target PRC2.

Keywords; EZH2, histones, PRC2, repressor, transcription

JB Reviews

D-Amino acid metabolism in bacteria

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Bacteria produce diverse D-amino acids, which are essential components of cell wall peptidoglycan. Incorporation of these D-amino acids into peptidoglycan contributes to bacterial adaptation to environmental changes and threats. D-Amino acids have been associated with bacterial growth, biofilm formation and dispersal and regulation of peptidoglycan metabolism. The diversity of D-amino acids in bacteria is primarily due to the activities of amino acid racemases that catalyse the interconversion of the D- and L-enantiomers of amino acids. Recent studies have revealed that bacteria possess multiple enzymes with amino acid racemase activities. Therefore, elucidating D-amino acid metabolism by these enzymes is critical to understand the biological significance and behaviour of D-amino acids in bacteria. In this review, we focus on the metabolic pathways of D-amino acids in six types of bacteria.

Keywords; amino acid racemase, bacteria, D-amino acid, Thermotoga maritima, Thermus thermophilus

Karyopherin-*β*s play a key role as a phase separation regulator

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Recent studies have revealed that cells utilize liquid–liquid phase separation (LLPS) as a mechanism in assembly of membraneless organelles, such as RNP granules. The nucleus is a well-known membrane-bound organelle surrounded by the nuclear envelope; the nuclear pore complex on the nuclear envelope likely applies LLPS in the central channel to facilitate selective biological macromolecule exchange. Karyopherin-*β* family proteins exclusively pass through the central channel with cargos by dissolving the phase separated hydrogel formed by the phenylalanine-glycine (FG) repeats-containing nucleoporins. Karyopherin-*β*s also exhibit dissolution activity for the phase separation of cargo proteins. Many cargos, including RNA-binding proteins containing intrinsically disordered regions (IDRs), undergo phase separation; however, aberrant phase separation is linked to fatal neurodegenerative diseases. Multiple weak interactions between karyopherin-*β*s and phase separation-prone proteins, such as FG repeats-containing nucleoporins or IDR-containing karyopherin-*β* cargos, are likely to be important for passing through the nuclear pore complex and maintaining the soluble state of cargo, respectively. In this review, we discuss how karyopherin-*β*s regulate phase separation to function.

Keywords; karyopherin-*β*s, liquid–liquid phase separation, low-complexity domain, neurodegenerative disease, RNA binding proteins

BIOCHEMISTRY

Biochemistry General

Components from spider venom activate macrophages against glioblastoma cells: new potential adjuvants for anticancer immunotherapy

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Immunomodulation has been considered an important approach in the treatment of malignant tumours. However, the modulation of innate immune cells remains an underexplored tool. Studies from our group demonstrated that the *Phoneutria nigriventer* spider venom (PnV) administration increased the infiltration

of macrophage in glioblastoma, in addition to decreasing the tumour size in a preclinical model. The hypothesis that PnV would be modulating the innate immune system led us to the main objective of the present study: to elucidate the effects of PnV and its purified fractions on cultured macrophages. Results showed that PnV and the three fractions activated macrophages differentiated from bone marrow precursors. Further purification generated 23 subfractions named low weight (LW-1 to LW-12) and high weight (HW-1 to HW-11). LW-9 presented the best immunomodulatory effect. Treated cells were more phagocytic, migrated more, showed an activated morphological profile and induced an increased cytotoxic effect of macrophages on tumour cells. However, while M1-controls (LPS) increased IL-10, TNF-alpha and IL-6 release, PnV, fractions and subfractions did not alter any cytokine, with the exception of LW-9 that stimulated IL-10 production. These findings suggest that molecules present in LW-9 have the potential to be used as immunoadjuvants in the treatment of cancer.

Keywords; glioma, immunomodulation, innate immune system, phagocytosis, Phoneutria nigriventer

Protein Structure

Structural analysis and reaction mechanism of malate dehydrogenase from Geobacillus stearothermophilus

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Malate dehydrogenase (MDH) catalyzes the reversible reduction of oxaloacetate (OAA) to L-malate using nicotinamide adenine dinucleotide hydrogen. MDH has two characteristic loops, the mobile loop and the catalytic loop, in the active site. On binding to the substrate, the enzyme undergoes a structural change from the open-form, with an open conformation of the mobile loop, to the closed-form, with the loop in a closed conformation. In this study, three crystals of MDH from a moderate thermophile, *Geobacillus stearothermophilus* (gs-MDH) were used to determine four different enzyme structures (resolutions, 1.95–2.20 Å), each of which was correspondingly assigned to its four catalytic states. Two OAA-unbound structures exhibited the open-form, while the other two OAA-bound structures exhibited both the open- and closed-form. The structural analysis suggested that the binding of OAA to the open-form gs-MDH promotes conformational change in the mobile loop and simultaneously activates the catalytic loop. The mutations on the key amino acid residues involving the proposed catalytic mechanism significantly affected the gs-MDH activity, supporting our hypothesis. These findings contribute to the elucidation of the detailed molecular mechanism underlying the substrate recognition and structural switching during the MDH catalytic cycle.

Keywords; conformational change, malate dehydrogenase, oxaloacetate, substrate recognition, thermophile

Glycobiology and Carbohydrate Biochemistry

Cell engineering for the production of hybrid-type N-glycans in HEK293 cells

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Glycoprotein therapeutics are among the leading products in the biopharmaceutical industry. The heterogeneity of glycans in therapeutic proteins is an issue for maintaining quality, activity and safety during bioprocessing. In this study, we knocked out genes encoding Golgi α-mannosidase-II, *MAN2A1* and *MAN2A2* in human embryonic kidney 293 (HEK293) cells, establishing an M2D-KO cell line that can produce recombinant proteins mainly with hybrid-type N-glycans. Furthermore, *FUT8*, which encodes α1,6-fucosyltransferase, was knocked out in the M2D-KO cell line, establishing a DF-KO cell line that can express noncore fucosylated hybrid-type N-glycans. Two recombinant proteins, lysosomal acid lipase and constant fragment of human IgG_1, were expressed in the M2D-KO and DF-KO cell lines. Glycan structural analysis revealed that complex-type N-glycans were removed in both M2D-KO and DF-KO cells. Our results suggest that these cell lines are suitable for the production of therapeutic proteins with hybrid-type N-glycans. Moreover, KO cell lines would be useful as models for researching the mechanism of antimetastatic effects in human tumours by swainsonine treatment.

Keywords; core fucose modification, genome editing, HEK293, hybrid-type N-glycans, therapeutic protein

Enzymology

Dynamics of D-amino acid oxidase ain kidney epithelial cells under amino acid starvation

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D-amino acid oxidase (DAO) is a flavoenzyme catalyzing the oxidation of D-amino acid (AA)s. In the kidney, its expression is detected in proximal tubules, and DAO is considered to play a role in the conversion of D-form AAs to α-keto acids. LLC-PK_1 cells, a pig renal proximal tubule cell line, were used to elucidate the regulation of DAO protein synthesis and degradation. In this study, we showed that trypsinization of LLC-PK_1 cells in culture system rapidly reduced the intracellular DAO protein level to ~33.9% of that before treatment, even within 30 min. Furthermore, we observed that the DAO protein level was decreased when LLC-PK_1 cells were subjected to AA starvation. To determine the degradation pathway, we treated the cells with chloroquine and MG132. DAO degradation was found to be inhibited by chloroquine, but not by MG132 treatment. We next examined whether or not DAO was degraded by autophagy. We found that AA starvation led to an increased accumulation of LC3-II, suggesting that DAO protein is degraded by autophagy due to AA starvation conditions. Furthermore, treatment with cycloheximide inhibited DAO protein degradation. Taken together, DAO protein is degraded by autophagy under starvation. The present study revealed the potential dynamics of DAO correlated with renal pathophysiology.

Keywords; amino acid starvation, autophagy, D-amino acid oxidase, kidney epithelial cell, peroxisomes

Enzyme Inhibitors

Kinetic analysis of the inhibition mechanism of bovine mitochondrial F1-ATPase inhibitory protein using biochemical assay

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ATPase inhibitory factor 1 (IF_1) is a mitochondrial regulatory protein that blocks ATP hydrolysis of F_1-ATPase, by inserting its *N*-terminus into the rotor–stator interface of F_1-ATPase. Although previous studies have proposed a two-step model for IF_1-mediated inhibition, the underlying molecular mechanism remains unclear. Here, we analysed the kinetics of IF_1-mediated inhibition under a wide range of [ATP]s and [IF_1]s, using bovine mitochondrial IF_1 and F_1-ATPase. Typical hyperbolic curves of inhibition rates with [IF_1]s were observed at all [ATP]s tested, suggesting a two-step mechanism: the initial association of IF_1 to F_1-ATPase and the locking process, where IF_1 blocks rotation by inserting its *N*-terminus. The initial association was dependent on ATP. Considering two principal rotation dwells, binding dwell and catalytic dwell, in F_1-ATPase, this result means that IF_1 associates with F_1-ATPase in the catalytic-waiting state. In contrast, the isomerization process to the locking state was almost independent of ATP, suggesting that it is also independent of the F_1-ATPase state. Further, we investigated the role of Glu30 or Tyr33 of IF_1 in the two-step mechanism. Kinetic analysis showed that Glu30 is involved in the isomerization, whereas Tyr33 contributes to the initial association. Based on these findings, we propose an IF_1-mediated inhibition scheme.

Keywords; ATPase inhibitory factor 1 (IF1), ATP synthase, enzyme kinetics, F1-ATPase, rotary molecular motor

MOLECULAR BIOLOGY

Molecular Biology General

Lidocaine activates autophagy of astrocytes and ameliorates chronic constriction injury-induced neuropathic pain

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Lidocaine is a commonly used drug to alleviate neuropathic pain (NP). This work aims to investigate the mechanism of lidocaine in alleviating NP. Chronic constriction injury (CCI) rats were established by surgery to induce NP. We observed the mechanical withdrawal threshold (MWT) and thermal withdrawal latency (TWL) of rats. Immunofluorescence staining was performed to determine the LC3/glial fibrillary acidic protein (GFAP)-positive cells. Rat astrocytes were treated with lipopolysaccharide (LPS) to induce CCI, and then treated with lidocaine or 3-MA (autophagy inhibitor). CCK-8 was performed to detect cell proliferation. Western blot and enzyme-linked immunosorbent assay were performed to detect the level of protein and inflammatory factor. CCI rats exhibited a decrease of MWT and TWL, which was effectively abolished by lidocaine. Lidocaine enhanced the number of LC3/GFAP-positive cells in CCI rats. Moreover, lidocaine inhibited the expression of GFAP and p62, and enhanced LC3-II/LC3-I expression in the LPS-treated astrocytes. Lidocaine inhibited the level of TNF-α and IL-1β in the LPS-treated astrocytes. The influence conferred by lidocaine was effectively abolished by 3-MA. In conclusion, our work demonstrates that lidocaine activates autophagy of astrocytes and ameliorates CCI-induced NP. Thus, our study provides a further experimental basis for the mechanism of lidocaine to alleviate NP.

Keywords; astrocytes, autophagy, chronic constriction injury, lidocaine, neuropathic pain

RAD18 mediates DNA double-strand break-induced ubiquitination of chromatin protein

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The E3 ubiquitin ligase RAD18 mono-ubiquitinates PCNA to promote bypass of replication fork-stalling DNA lesions. On the other hand, RAD18 also contributes to DNA double-strand break (DSB) repair. RAD18 is recruited to ionizing radiation (IR)-induced DSB and colocalizes with ubiquitinated chromatin proteins. RAD18 interacts with the ubiquitinated chromatin proteins via its ubiquitin-binding Zinc finger (UBZ) domain and is proposed to propagate DNA DSB signalling and recruit DNA repair proteins. We found that purified human RAD18 protein complexed with RAD6B (RAD6B–RAD18) catalyzes mono- and poly-ubiquitination of histone H2A *in vitro* while UBZ domain-mutated RAD18 complexed with RAD6B protein catalyzes mono- but not poly-ubiquitination of histone H2A. Human RAD18$^{-/-}$cells synchronized at the G1 phase show a reduced signal of ubiquitinated protein in chromatin after IR when compared to that of wild-type control cells. The reduced signal of ubiquitinated protein in RAD18$^{-/-}$cells is rescued by the introduction of RAD18 cDNA but to a lesser extent by the introduction of cDNA coding RAD18 lacking UBZ domain. Taken together, these results indicate that RAD18 mediates DSB-induced ubiquitination of chromatin protein during the G1 phase.

Keywords; DNA double-strand break repair, histone H2A, RAD18, ubiquitination

Gene Expression

Long non-coding RNA ZEB1-AS1 promotes proliferation and metastasis of hepatocellular carcinoma cells by targeting miR-299-3p/E2F1 axis

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There is emerging evidence that dysregulation of long non-coding RNAs (lncRNAs) is associated with hepatocellular carcinoma (HCC). Zinc finger E-box binding homeobox 1 antisense 1 (ZEB1-AS1) functions as an oncogenic regulator in various malignancies. Nonetheless, the potential role of ZEB1-AS1 in HCC remains poorly elucidated. Herein, qRT-PCR was employed for examining ZEB1-AS1, miR-299-3p and E2F transcription factor 1 (E2F1) mRNA expressions in HCC cells and tissues. MTT assay was performed to evaluate cell proliferation. Transwell assay was utilized for evaluating cancer cell migration and invasion. Western blot was employed for measuring E2F1 protein expression. What's more, dual-luciferase reporter assay was utilized for verifying the targeting relationships between ZEB1-AS1 and miR-299-3p, as well as E2F1 and miR-299-3p. It was demonstrated that, in HCC tissues and cells, ZEB1-AS1 expression was markedly increased, and meanwhile, its high expression level is related to the unfavourable clinicopathologic indicators. ZEB1-AS1 overexpression facilitated HCC cell proliferation, migration and invasion, while its knockdown led to the opposite effects. In terms of mechanism, we discovered that ZEB1-AS1 could decoy miR-299-3p and upregulate E2F1 expression. This work reveals the functions and mechanism of ZEB1-AS1 in HCC tumourigenesis and progression, which provides novel biomarkers and therapeutic targets for HCC.

Keywords; E2F1, hepatocellular carcinoma, lncRNA, MiR-299-3p, ZEB1-AS1

RNA Processing

Characterization of the aberrant splicing of MAP3K7 induced by cancer-associated SF3B1 mutation

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SF3B1, an essential RNA splicing factor, is frequently mutated in various types of cancers, and the cancer-associated SF3B1 mutation causes aberrant RNA splicing. The aberrant splicing of several transcripts, including *MAP3K7*, promotes tumorigenesis. Here, we identify a premature termination codon in the aberrantly spliced transcript of *MAP3K7*. Treatment of HEK293T cells transfected with the K700E-mutated SF3B1 with cycloheximide leads to increased accumulation of the aberrant spliced transcript of *MAP3K7*, demonstrating that the aberrantly spliced transcript of *MAP3K7* is targeted by nonsense-mediated decay. The aberrantly spliced *MAP3K7* transcript uses an aberrant 3′ splice sites and an alternative branchpoint sequence. In addition, the aberrant splicing of *MAP3K7* requires not only the polypyrimidine tract associated with normal splicing but also an alternative polypyrimidine tract upstream of the aberrant 3′ splice site. Other cancer-associated *SF3B1* mutations also cause the aberrant splicing of *MAP3K7*, which depends on the same sequence features. Our data provide a further understanding of the mechanisms underlying aberrant splicing induced by cancer-associated *SF3B1* mutation, and reveal an important role of alternative polypyrimidine tract in diseases.

Keywords; 3′, splice site, branch point sequence, nonsense-mediated decay, polypyrimidine tract, RNA splicing

CELL

Differentiation/Development and Aging

Epigenetic effects induced by the ectopic expression of Pax7 in 3T3-L1

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Although skeletal muscle cells and adipocytes are derived from the same mesoderm, they do not transdifferentiate *in vivo* and are strictly distinct at the level of gene expression. To elucidate some of the regulatory mechanisms underlying this strict distinction, Pax7, a myogenic factor, was ectopically expressed in 3T3-L1 adipose progenitor cells to perturb their adipocyte differentiation potential. Transcriptome analysis showed that ectopic expression of Pax7 repressed the expression of some adipocyte genes and induced expression of some skeletal muscle cell genes. We next profiled the epigenomic state altered by Pax7 expression using H3K27ac, an activating histone mark, and H3K27me3, a repressive histone mark, as indicators. Our results show that ectopic expression of Pax7 did not result in the formation of H3K27ac at loci of skeletal muscle-related genes, but instead resulted in the formation of H3K27me3 at adipocyte-related gene loci. These findings suggest that the primary function of ectopic Pax7 expression is the formation of H3K27me3, and muscle gene expression results from secondary regulation.

Keywords; adipogenesis, epigenome, myogenesis, transcription factor, transcriptome

Neurobiology

Protein phosphatase 6 promotes neurite outgrowth by promoting mTORC2 activity in N2a cells

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Understanding the molecular mechanism of neuronal differentiation is important to overcome the incurable diseases caused by nervous system damage. Neurite outgrowth is prerequisite for neuronal differentiation and regeneration, and cAMP response element-binding protein (CREB) is one of the major transcriptional factors positively regulating this process. Neuronal differentiation stimuli activate mammalian target of rapamycin (mTOR) complex 2 (mTORC2)/Akt signalling to phosphorylate CREB; however, the precise molecular mechanism of this event has not been fully understood. In this manuscript, we show that neuronal differentiation stimuli increased a protein level of protein phosphatase 6 (PP6), a member of type 2A Ser/Thr protein phosphatases. PP6 knockdown suppressed mTORC2/Akt/CREB signalling and results in failure of neurite outgrowth. SIN1 is a unique component of mTORC2 that enhances mTORC2 activity towards Akt when it is in dephosphorylated form. We found PP6 knockdown increased SIN1 phosphorylation. These data suggest that PP6 may positively regulate neurite outgrowth by dephosphorylating SIN1 to activate mTORC2/Akt/CREB signalling.

Keywords; mTORC2, N2a cell, neurite outgrowth, PP6, SIN1

Tumor and Immunology

Targeting Nrf2-antioxidant signalling reverses acquired cabazitaxel resistance in prostate cancer cells

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Prostate cancer is known to have a relatively good prognosis, but long-term hormone therapy can lead to castration-resistant prostate cancer (CRPC). Cabazitaxel, a second-generation taxane, has been used for the CRPC treatment, but its tolerance is an urgent problem to be solved. In this study, to elucidate the acquisition mechanism of the cabazitaxel-resistance, we established cabazitaxel-resistant prostate cancer 22Rv1 (Cab-R) cells, which exhibited ~sevenfold higher LD_{50} against cabazitaxel than the parental 22Rv1 cells. Cab-R cells showed marked increases in nuclear accumulation of NF-E2 related factor 2 (Nrf2) and expression of Nrf2-inducible antioxidant enzymes compared to 22Rv1 cells, suggesting that Nrf2 signalling is homeostatically activated in Cab-R cells. The cabazitaxel sensitivity of Cab-R cells was enhanced by silencing of Nrf2, and that of 22Rv1 cells was reduced by activation of Nrf2. Halofuginone (HF) has been

recently identified as a potent Nrf2 synthetic inhibitor, and its treatment of Cab-R cells not only suppressed the Nrf2 signalling by decreasing both nuclear and cytosolic Nrf2 protein levels, but also significantly augmented the cabazitaxel sensitivity. Thus, inhibition of Nrf2 signalling may be effective in overcoming the cabazitaxel resistance in prostate cancer cells.
Keywords; antioxidant enzymes, cabazitaxel resistance, CRPC, halofuginone, Nrf2