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ダイジェスト

JB Reviews

Featured article of the month

How to rebuild the kidney: recent advances in kidney organoids

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It is difficult to restore kidney function once it has become severely impaired. Although kidney transplantation is a curative therapy, donor numbers remain limited. Thus, the generation of kidney organoids (mainly comprising glomeruli and renal tubules) from multipotent stem cells represents an important advance in regenerative medicine of the kidney. Recently, a protocol that can generate the higher-order structure of the mouse embryonic kidney was reported. Kidney organoids are now being used for disease modelling, and may eventually be applicable for clinical transplantation. In this review, we summarize the recent advances in kidney organoid research, and discuss the issues to be resolved toward kidney reconstruction.

Keywords: iPS cell, kidney, nephron progenitor, organoid, ureteric bud

Harnessing biomolecular condensates in living cells

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As part of the 'Central Dogma' of molecular biology, the function of proteins and nucleic acids within a cell is determined by their primary sequence. Recent work, however, has shown that within living cells the role of many proteins and RNA molecules can be influenced by the physical state in which the molecule is found. Within living cells, both protein and RNA molecules are observed to condense into non-membrane-bound yet distinct structures such as liquid droplets, hydrogels and insoluble aggregates. These unique intracellular organizations, collectively termed biomolecular condensates, have been found to be vital in both normal and pathological conditions. Here, we review the latest studies that have developed molecular tools attempting to recreate artificial biomolecular condensates in living cells. We will describe their design principles, implementation and unique characteristics, along with limitations. We will also introduce how these tools can be used to probe and perturb normal and pathological cell functions, which will then be complemented with discussions of remaining areas for technological advance under this exciting theme.

Keywords: biomolecular condensates, liquid droplets, hydrogels, chemically-induced dimerization, optogenetics

BIOCHEMISTRY

Biochemistry General

MSCs inhibits the angiogenesis of HUVECs through the miR-211/Prox1 pathway

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The aim of this study was to investigate the effect of mesenchymal stem cells (MSCs) on the angiogenesis of human umbilical vein endothelial cells (HUVECs). MSCs were subconjunctival injected into rat corneal alkali burn models. Their impacts on the degree of corneal neovascularization (CNV) and corneal opacity were evaluated at 3, 6, 9 and 12 days after injection. An *in vitro* experiment of MSCs affecting HUVECs angiogenesis was performed and evaluated using the tube formation assay. The results showed that both CNV and corneal opacity were decreased in rats after MSCs injection. In HUVECs, angiogenesis of cells was inhibited by miR-211 overexpression. miR-211 negatively regulated Prox1 expression. Knockdown of miR-211 blocked the decrease of Prox1 expression induced by MSCs and the inhibitory effect of MSCs on the angiogenesis of HUVECs. The critical role of miR-211 in MSCs inhibition of corneal angiogenesis was confirmed in rat experiments. We concluded that MSCs inhibited the angiogenesis of HUVEC through miR-211 mediating the down-regulation of Prox1.

Keywords: corneal angiogenesis, HUVECs, miR-211, MSCs, Prox1

Protein Structure

Distinct molecular assembly of homologous peroxiredoxins from Pyrococcus horikoshii and Thermococcus kodakaraensis

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Peroxiredoxins from *Pyrococcus horikoshii* (PhPrx) and *Thermococcus kodakaraensis* (TkPrx) are highly homologous proteins sharing 196 of the 216 residues. We previously reported a pentagonal ring-type decameric structure of PhPrx. Here, we present the crystal structure of TkPrx. Despite their homology, unlike PhPrx, the quaternary structure of TkPrx was found to be a dodecamer comprised of six homodimers arranged in a hexagonal ring-type assembly. The possibility of the redox-dependent conversion of the molecular assembly, which had been observed in PhPrx, was excluded for TkPrx based on the crystal structure of a mutant in which all of the cysteine residues were substituted with serine. The monomer structures of the dodecameric TkPrx and decameric PhPrx coincided well, but there was a slight difference in the relative orientation of the two domains. Molecular assembly of PhPrx and TkPrx in solution evaluated by gel-filtration chromatography was consistent with the crystallographic results. For both PhPrx and TkPrx, the gel-filtration elution volume slightly increased with a decrease in the protein concentration, suggesting the existence of an equilibrium state between the decameric/dodecameric ring and lower-order assembly. This structural assembly difference between highly homologous Prxs suggests a significant influence of quaternary structure on function, worthy of further exploration.

Keywords: archaea, decamer, dodecamer, peroxiredoxin, quaternary structure

Protein Interaction and Recognition

Phosphorylation of PDE4A5 by MAPKAPK2 attenuates fi brin degradation via p75 signalling

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Phosphodiesterases (PDEs) shape local cAMP gradients to underpin the specificity of receptor function. Key to this process is the highly defined nature of the intra-cellular location of PDEs in the cell. PDE4A5 is a PDE isoform that specifically degrades cAMP and is known to associate with the p75 neurotrophin receptor (p75NTR) where it modulates cAMP signalling cascades that regulate extracellular matrix remodelling in the lungs. Here we map and validate novel protein–protein interaction sites that are important for formation of the PDE4A5–p75NTR complex and show, for the first time, that phosphorylation of PDE4A5 by MAPKAPK2 enhances PDE4A5 interaction with p75NTR and that this, in turn, serves to attenuate fibrin degradation.

Keywords: cyclic-AMP, extracellular matrix, p75-NTR, PDE4A, phosphodiesterase

Enzymology

Identification and characterization of cytochrome P450 1232A24 and 1232F1 from Arthrobacter sp. and their role in the metabolic pathway of papaverine

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Cytochrome P450 monooxygenases (P450s) play crucial roles in the cell metabolism and provide an unsurpassed diversity of catalysed reactions. Here, we report the identification and biochemical characterization of two P450s from *Arthrobacter* sp., a Gram-positive organism known to degrade the opium alkaloid papaverine. Combining phylogenetic and genomic analysis suggested physiological roles for P450s in metabolism and revealed potential gene clusters with redox partners facilitating the reconstitution of the P450 activities *in vitro*. CYP1232F1 catalyses the *para* demethylation of 3,4-dimethoxyphenylacetic acid to homovanillic acid while CYP1232A24 continues demethylation to 3,4-dihydroxyphenylacetic acid. Interestingly, the latter enzyme is also able to perform both demethylation steps with preference for the *meta* position. The crystal structure of CYP1232A24, which shares only 29% identity to previous published structures of P450s helped to rationalize the preferred demethylation specificity for the *meta* position and also the broader substrate specificity profile. In addition to the detailed characterization of the two P450s using their physiological redox partners, we report the construction of a highly active whole-cell *Escherichia coli* biocatalyst expressing CYP1232A24, which formed up to 1.77 g l^{-1} 3,4-dihydroxyphenylacetic acid. Our results revealed the P450s' role in the metabolic pathway of papaverine enabling further investigation and application of these biocatalysts.

Keywords: Arthrobacter sp, biochemical characterization, crystal structure, cytochrome P450, metabolism

The microenvironment surrounding FAD mediates its conversion to 8-formyl-FAD in Aspergillus oryzae RIB40 formate oxidase

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Aspergillus oryzae RIB40 formate oxidase has Arg87 and Arg554 near the formyl group and O(4) atom of 8-formyl-flavin adenine dinucleotide (FAD), respectively, with Asp396 neighbouring Arg554. Herein, we probed the roles of these three residues in modification of FAD to 8-formyl-FAD. Replacement of Arg87 or Arg554 with Lys or Ala decreased and abolished the modification, respectively. Replacement of Asp396 with Ala or Asn lowered the modification rate. The observation of unusual effects of maintaining pH 7.0 on the modification in R87K, R554K and D396 variants indicates initial and subsequent processes with different pH dependencies. Comparison of the initial process at pH 4.5 and 7.0 suggests that the microenvironment around Arg87 and the protonation state of Asp396 affect the initial process in the native enzyme. Comparison of the crystal structures of native and R554 variants showed that the replacements had minimal effect on catalytic site structure. The positively charged Arg87 might contribute to the formation of an anionic quinone-methide tautomer intermediate, while the positively charged Arg554, in collaboration with the negatively charged Asp396, might stabilize this intermediate and form a hydrogen bonding network with the N(5)/O(4) region, thereby facilitating efficient FAD modification.

Keywords: amino acid substitution, crystal structure, formate oxidase, 8-formyl-FAD, positively charged amino acid

MOLECULAR BIOLOGY

Gene Expression

Phosphorylation of repressive histone code readers by casein kinase 2 plays diverse roles in heterochromatin regulation

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Heterochromatin is a condensed and transcriptionally silent chromatin structure and that plays important roles in epigenetic regulation of the genome. Two types of heterochromatin exist: constitutive heterochromatin is primarily associated with trimethylation of histone H3 at lysine 9 (H3K9me3), and facultative heterochromatin with trimethylation of H3 at lysine 27 (H3K27me3). The methylated histones are bound by the chromodomain of histone code 'reader' proteins: HP1 family proteins for H3K9me3 and Polycomb family proteins for H3K27me3. Each repressive reader associates with various 'effector' proteins that provide the functional basis of heterochromatin. Heterochromatin regulation is primarily achieved by controlling histone modifications. However, recent studies have revealed that the repressive readers are phosphorylated, like other regulatory proteins, suggesting that phosphorylation also participates in heterochromatin regulation. Detailed studies have shown that phosphorylation of readers affects the binding specificities of chromodomains for methylated histone H3, as well as the binding of effector proteins. Thus, phosphorylation adds another layer to heterochromatin regulation. Interestingly, casein kinase 2, a strong and predominant kinase within the cell, is responsible for phosphorylation of repressive readers. In this commentary, I summarize the regulation of repressive readers by casein kinase 2-dependent phosphorylation and discuss the functional meaning of this modification.

Keywords: casein kinase 2, chromodomain, heterochromatin, histone code reader, phosphorylation

Domains two and three of Escherichia coli ribosomal S1 protein confers 30S subunits a high affi nity for downstream A/U-rich mRNAs

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S1, a multi-domain ribosomal protein associated with the 30S subunit, is essential for translation initiation. S1 binds with high affinity to single-stranded mRNA containing A/U-rich patches upstream of the start codon. It was previously reported that domains 1–3 of S1 protein play a role in the docking and unfolding of structured mRNAs to the ribosome. Moreover, S1-deficient 30S subunits are still able to bind to low structured mRNAs. However, mRNAs containing A/U-rich patches in the early base positions after start codon enhance protein synthesis and mRNA binding to the ribosome, which suggests that S1 is also able to interact with these A/U-rich regions. To evaluate the essentiality of S1 domains in the binding to low structured mRNAs containing A/U/G nucleotides after the start codon as well as their role in translation and cell viability, S1 protein deletion variants were generated. We show that S1 domain 3 is necessary to discriminate these mRNAs according to the nucleotide nature since its absence abrogated S1 binding to A/U-rich mRNAs and allowed binding to G-rich mRNAs. Interestingly, domains 2 and 3 were required for the binding of mRNAs containing A/U-rich sequences after the start codon to 30S, *in vitro* translation and cell viability.

Keywords: A/U/G-rich mRNAs, ribosomal protein S1, S1 domains

CELL

Cytoskeleton/Cell Motility and Cell Shape

Effect of PlzD, a YcgR homologue of c-di-GMP-binding protein, on polar fl agellar motility in Vibrio alginolyticus

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YcgR, a cyclic diguanylate (c-di-GMP)-binding protein expressed in *Escherichia coli*, brakes flagellar rotation by binding to the motor in a c-di-GMP dependent manner and has been implicated in triggering biofilm formation. *Vibrio alginolyticus* has a single polar flagellum and encodes YcgR homologue, PlzD. When PlzD or PlzD-GFP was highly over-produced in nutrient-poor condition, the polar flagellar motility of *V. alginolyticus* was reduced. This inhibitory effect is c-di-GMP independent as mutants substituting putative c-di-GMP-binding residues retain the effect. Moderate over-expression of PlzD-GFP allowed its localization at the flagellated cell pole. Truncation of the N-terminal 12 or 35 residues of PlzD abolished the inhibitory effect and polar localization, and no inhibitory effect was observed by deleting *plzD* or expressing an endogenous level of PlzD-GFP. Subcellular fractionation showed that PlzD, but not its N-terminally truncated variants, was precipitated when over-produced. Moreover, immunoblotting and N-terminal sequencing revealed that endogenous PlzD is synthesized from Met33. These results suggest that an N-terminal extension allows PlzD to localize at the cell pole but causes aggregation and leads to inhibition of motility. In *V. alginolyticus*, PlzD has a potential property to associate with the polar flagellar motor but this interaction is too weak to inhibit rotation.

Keywords: c-di-GMP, flagellar motor, motility inhibition, polar flagellum, YcgR

Tumor and Immunology

Identifi cation of a chemical modulator of EZH2-mediated silencing by cell-based high-throughput screening assay

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Dysregulation of enhancer of zeste homologue 2 (EZH2), a methyltransferase component of polycomb repressive complex 2, is found in many types of cancers especially those that are highly progressive and aggressive. Specific catalytic inhibitors of EZH2 have high anti-tumour activity, particularly in lymphomas with EZH2 activating mutations. However, the clinical benefits of EZH2 catalytic inhibitors in tumours overexpressing EZH2 are still limited. Here, we identified NPD13668, a novel modulator of EZH2-mediated gene silencing, from 329,049 small chemical compounds using a cell-based high-throughput screening assay. NPD13668 reactivated the expression of silenced H3K27me3 target genes together with depletion of the H3K27me3 modification. In addition, NPD13668 repressed the cell growth of prostate cancer cell lines (PC3 and LNCaP) and ovarian cancer cell lines (SKOV3 and NIH-OVCAR3). NPD13668 partially inhibited the methyltransferase activity of EZH2 *in vitro*. Genome-wide expression analysis revealed that after NPD13668 treatment, about half of the upregulated genes overlapped with genes upregulated after treatment with GSK126, well-known EZH2 catalytic inhibitor, indicating that NPD13668 is a potential modulator of EZH2 methyltransferase activity. Our data demonstrated that targeting the pharmacological inhibition of EZH2 activity by NPD13668 might be a novel cancer treatment.

Keywords: anti-tumour agent, drug discovery, EZH2 inhibitor, high-throughput screening, histone H3 lysine 27 trimethylation

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ダイジェスト

JB Reviews

Featured article of the month

Not just a cargo receptor for large cargoes; an emerging role of TANGO1 as an organizer of ER exit sites

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Proteins synthesized within the endoplasmic reticulum (ER) are exported from ER exit sites via coat protein complex II (COPII)-coated vesicles. Although the mechanisms of COPII-vesicle for-

mation at the ER exit sites are highly conserved among species, vertebrate cells secrete a wide range of materials, including collagens and chylomicrons, which form bulky structures within the ER that are too large to fit into conventional carriers. Transport ANd Golgi Organization 1 (TANGO1) was initially identified as a cargo receptor for collagens but has been recently rediscovered as an organizer of ER exit sites. We would like to review recent advances in the mechanism of large cargo secretion and organization of ER exit sites through the function of TANGO1.

Keywords: collagen, COPII, ER, secretion, TANGO1

Photoactive yellow protein and its chemical probes: an approach to protein labelling in living cells

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Labelling technologies developed over the past few years have changed the way of looking at biomolecules and have made a considerable contribution to our understanding of the functions and regulation of dynamic biological processes. One of the robust technologies employed to image proteins in a cellular environment is based on the use of chemical tags and their fluorescent probes, which provides flexibility in developing probes with a wide range of synthetic fluorophores. A variety of chemical tags, ranging from short amino acid sequences to small proteins, have been employed to generate protein-labelling systems. One such chemical tag is the photoactive yellow protein (PYP)-tag, which is a small bacterial protein, developed for the selective labelling and imaging of proteins. Herein, we briefly discuss the protein-labelling system developed based on PYP-tag technology, with a focus on the design strategy for PYP-tag labelling probes and their applications in protein imaging.

Keywords: chemical tag, live-cell imaging, protein labelling, PYP-tag

BIOCHEMISTRY

Biochemistry General

Developments of human adrenomedullin-IgG1 Fc fusion proteins

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Human adrenomedullin (hAM) is a hypotensive peptide hormone that exerts powerful anti-inflammatory effects. However, treatment required continuous administration of hAM, as the half-life of native hAM is quite short in blood. To resolve this problem, we designed two kinds of human IgG1 Fc fusion proteins containing either full-length hAM (IgG1-AM) or hAM residues 6–52 [IgG1-AM (6–52)]. A DNA construct was constructed by connecting DNA sequences encoding hAM and the IgG1 Fc region with a DNA sequence encoding a $(GGGGS)_3$ linker. The molecular weights of IgG1-AM and IgG1-AM (6–52) were determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and gel filtration chromatography. By protein sequencing, the N-terminal sequence of both recombinant AM-Fc fusions showed the expected human IgG1 sequence. Sufficient concentrations of both AM-Fc fusions were observed in blood 2 days after a single subcutaneous administration. IgG1-AM and IgG1-AM (6–52) stimulated cAMP production in human embryonic kidney-293 cells stably expressing the AM1 receptor. The activity of IgG1-AM (6–52) was higher than that of IgG1-AM. Treatment with IgG1-AM (6–52) inhibited blood pressure increase in spontaneously hypertensive rats. In addition, IgG1-AM (6–52) reduced total inflammation scores in the dextran sulfate sodium colitis model. Therefore, AM-IgG1 Fc fusions represent potential novel therapeutic agents.

Keywords: adrenomedullin, hypertension, immunoglobulin G, inflammatory bowel disease, recombinant protein

Protein Structure

Crystal structure of bacterial cyclopropane-fatty-acyl-phospholipid synthase with phospholipid

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The lipids containing cyclopropane-fatty-acid (CFA) protect bacteria from adverse conditions such as acidity, freeze-drying desiccation and exposure to pollutants. CFA is synthesized when cyclopropane-fatty-acyl-phospholipid synthase (CFA synthase, CFAS) transfers a methylene group from S-adenosylmethionine (SAM) across the *cis* double bonds of unsaturated fatty acyl chains. Here, we reported a 2.7-Å crystal structure of CFAS from *Lactobacillus acidophilus*. The enzyme is composed of N- and C-terminal domain, which belong to the sterol carrier protein and methyltransferase superfamily, respectively. A phospholipid in the substrate binding site and a bicarbonate ion (BCI) acting as a general base in the active site were discovered. To elucidate the mechanism, a docking experiment using CFAS from *L. acidophilus* and SAM was carried out. The analysis of this structure demonstrated that three groups, the carbons from the substrate, the BCI and the methyl of $S(CH_n)_3$ group, were close enough to form a cyclopropane ring with the help of amino acids in the active site. Therefore, the structure supports the hypothesis that CFAS from *L. acidophilus* catalyzes methyl transfer via a carbocation mechanism. These findings provide a structural basis to more deeply understand enzymatic cyclopropanation.

Keywords: bicarbonate ion, cyclopropane-fatty-acyl-phospholipid synthase, enzymatic cyclopropanation, phospholipid

Metal binding to cutinase-like enzyme from Saccharomonospora viridis AHK190 and its effects on enzyme activity and stability

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A cutinase from *Saccharomonospora viridis* AHK190, Cut190, can hydrolyze polyethylene terephthalate and has a unique feature that the activity and stability are regulated by Ca^{2+} binding. Our recent structural and functional analyses showed three Ca^{2+} binding sites and their respective roles. Here, we analysed the binding thermodynamics of Mn^{2+}, Zn^{2+} and Mg^{2+} to Cut190 and their effects on the catalytic activity and thermal stability. The binding affinities of Mn^{2+} and Zn^{2+} were higher than that of Mg^{2+} and are all entropy driven with a binding stoichiometry of three, one and one for Zn^{2+}, Mn^{2+} and Mg^{2+}, respectively. The catalytic activity was measured in the presence of the respective metals, where the activity of 0.25 mM Mn^{2+} was comparable to that of 2.5 mM Ca^{2+}. Our 3D Reference Interaction Site Model calculations suggested that all the ions exhibited a high occupancy rate for Site 2. Thus, Mn^{2+} and Mg^{2+}would most likely bind to Site 2 (contributes to stability) with high affinity, while to Sites 1 and 3 (contributes to activity) with low affinity. We elucidate the metal-dependent structural and functional properties of Cut190 and show the subtle balance on structure stability and flexibility is controlled by specific metal ions.

Keywords: circular dichroism, enzyme activity, isothermal titration calorimetry, metal–protein interaction, 3D-RISM calculations

Structure of the periplasmic domain of Sfl A involved in spatial regulation of the fl agellar biogenesis of Vibrio reveals a TPR/SLR-like fold

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Bacteria have evolved various types of flagellum, an organella for bacterial motility, to adapt to their habitat environments. The number and the spatial arrangement of the flagellum are precisely controlled to optimize performance of each type of the flagellar system. *Vibrio alginolyticus* has a single sheathed flagellum at the cell pole for swimming. SflA is a regulator protein to prevent peritrichous formation of the sheathed flagellum, and consists of an N-terminal periplasmic region, a transmembrane helix, and a C-terminal cytoplasmic region. Whereas the cytoplasmic region has been characterized to be essential for inhibition of the peritrichous growth, the role of the N-terminal region is still unclear. We here determined the structure of the N-terminal periplasmic region of SflA ($SflA_N$) at 1.9-Å resolution. The core of $SflA_N$ forms a domain-swapped dimer with tetratricopeptide repeat (TPR)/Sel1-like repeat (SLR) motif, which is often found in the domains responsible for protein–protein interaction in various proteins. The structural similarity and the following mutational analysis based on the structure suggest that SflA binds to unknown partner protein by $SflA_N$ and the binding signal is important for the precise control of the SflA function.

Keywords: bacterial flagellum, crystal structure, flagellar biogenesis, Sel1-like repeat, tetratricopeptide repeat

Enzyme Inhibitors

Morellofl avone as a novel inhibitor of mitotic kinesin Eg5

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Among 40 plant-derived biflavonoids with inhibitory potential against Eg5, morelloflavone from *Garcinia dulcis* leaves was selected for further testing based on *in silico* analysis of binding modes, molecular interactions, binding energies and functional groups that interact with Eg5. Computational models predicted that morelloflavone binds the putative allosteric pocket of Eg5, within the cavity surrounded by amino acid residues of Ile-136, Glu-116, Glu-118, Trp-127, Gly-117, Ala-133, Glu-215, Leu-214 and Tyr-211. Binding energy was −8.4 kcal/mol, with a single hydrogen bond formed between morelloflavone and Tyr-211. The binding configuration was comparable to that of a reference inhibitor, S-trityl-L-cysteine. Subsequent biochemical analysis *in vitro*confirmed that morelloflavone inhibited both the basal and microtubule-activated ATPase activity of Eg5 in a manner that does not compete with ATP binding. Morelloflavone also suppressed Eg5 gliding along microtubules. These results suggest that morelloflavone binds the allosteric binding site in Eg5 and

thereby inhibits ATPase activity and motor function of Eg5.
Keywords: biflavonoids, in silico, kinesin Eg5, microtubule gliding, morelloflavone

Biochemistry in Diseases and Aging

Age- and gender-dependent D-amino acid oxidase activity in mouse brain and peripheral tissues: implication for aging and neurodegeneration

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D-amino acid oxidase (DAO) is a flavoenzyme, catalysing oxidative deamination of D-amino acids to produce corresponding α-keto acids, ammonia and hydrogen peroxide. In our search for DAO activity among various tissues, we developed a sensitive assay based on hydrogen peroxide production involving enzyme-coupled colorimetric assay with peroxidase. We first optimized buffer components to extract DAO protein from mouse tissues. Here we show that DAO activity was detected in kidney, cerebellum, medulla oblongata, midbrain and spinal cord, but not in liver. In addition, we observed that DAO activity and expression were decreased in thoracic and lumbar regions of spinal cord in aged mice when compared with young mice, indicating that decreased DAO is involved in motoneuron degeneration during senescence. We also found gender difference in DAO activity in the kidney, suggesting that DAO activity is influenced by sexual dimorphism. We newly detected DAO activity in the epididymis, although undetected in testis. Furthermore, DAO activity was significantly higher in the caput region than corpus and cauda regions of epididymis, indicating that D-amino acids present in the testis are eliminated in epididymis. Taken together, age- and gender-dependent DAO activity in each organ may underlie the human pathophysiology regulated by D-amino acid metabolism.
Keywords: age- and gender-dependence, D-amino acid oxidase, epididymis, kidney, spinal cord

CELL

Biomembranes, Organelles, and Protein Sorting

Phosphorylation of TMEM55B by Erk/MAPK regulates lysosomal positioning Tumor and Immunology

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TMEM55B is first identified as phosphatidylinositol-4,5-$P_2$4-phosphatases (PtdIns-4,5-$P_2$4-phosphatases) that catalyse dephosphorylation of PtdIns-4,5-P_2 to PtdIns-5-P. We demonstrate for the first time that TMEM55B is phosphorylated by Erk/MAPK and that this mechanism participates in regulation of lysosomal clustering. Exposure of RAW264.7 macrophages to various stimuli induces phosphorylation of TMEM55B on Ser76 and Ser169, sites corresponding to consensus sequences (PX(S/T)P) for phosphorylation by MAPK. Of these stimuli, Toll-like receptor ligands most strongly induce TMEM55B phosphorylation, and this is blocked by the MEK1/2 inhibitor U0126. However, phosphorylation does not impact intrinsic phosphatase activity of TMEM55B. TMEM55B has recently been implicated in starvation induced lysosomal translocation. Amino acid starvation induces perinuclear lamp1 clustering in RAW264.7 macrophages, which was attenuated by shRNA-mediated knockdown or CRISPR/Cas9-mediated knock-out of TMEM55B. Cells exposed to U0126 also exhibit attenuated lamp1 clustering. Overexpression of TMEM55B but not TMEM55A notably enhances lamp1 clustering, with TMEM55B mutants (lacking phosphorylation sites or mimicking the phosphorylated state) exhibiting lower and higher efficacies (respectively) than wild-type TMEM55B. Collectively, results suggest that phosphorylation of TMEM55B by Erk/MAPK impacts lysosomal dynamics.
Keywords: Erk, lysosome, phagosome, phosphorylation, TMEM55B

Regulation of collagen type XVII expression by miR203a-3p in oral squamous cell carcinoma cells

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Collagen type XVII (COL17) is expressed in various tissues and its aberrant expression is associated with tumour progression. In this study, we investigated the regulation of COL17 expression in oral squamous cell carcinoma (OSCC) using the cell lines NA, SAS, Ca9-22, and Sa3. COL17 was induced upon p53 activation by cisplatin in SAS; however, this effect was more limited in NA and hardly in Ca9-22 and Sa3, with mutated p53. Moreover, COL17 was found to be regulated by miR203a-3p in all cell lines. Our data suggest that COL17 expression in OSCC cell lines is regulated by p53 and miR203a-3p.
Keywords: collagen 17, miR203a-3p, oral squamous cell carcinoma, p53, tumour progression