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ダイジェスト

JB Review

Featured article of the month.

An overview of mammalian mitochondrial DNA replication mechanisms

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While the majority of DNA is enclosed within the nucleus, the mitochondria also contain their own, separate DNA, the mitochondrial DNA (mtDNA). Mutations in mtDNA are associated with various human diseases, demonstrating the importance of mtDNA. Intensive studies over the last 18 years have demonstrated the presence of two distinct classes of mtDNA replication intermediates in mammals. One involves leading-strand DNA synthesis in the absence of synchronous lagging-strand DNA synthesis. Currently there are competing models in which the lagging-strand template is either systematically hybridized to processed mitochondrial transcripts, or coated with protein, until the lagging-strand DNA synthesis takes place. The other class of mtDNA replication intermediates has many properties of conventional, coupled leading- and lagging-strand DNA synthesis. Additionally, the highly unusual arrangement of DNA in human heart mitochondria suggests a third mechanism of replication. These findings indicate that the mtDNA replication systems of humans and other mammals are far more complex than previously thought, and thereby will require further research to understand the full picture of mtDNA replication.

Keywords: bootlace model, coupled leading- and lagging-strand DNA synthesis, mitochondrial DNA replication, RITOLS, strand-displacement mechanism model

Featured article of the month.

TMEPAI family: involvement in regulation of multiple signalling pathways

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The TMEPAI family, composed of TMEPAI and C18ORF1, is known to inhibit transforming growth factor-β (TGF-β) signalling via its competition for binding of receptor-regulated Smad with Smad anchor for receptor activation. However, TMEPAI has also been reported to be involved in androgen receptor signalling, phosphatase and tensin homologue deleted on chromosome 10 signalling, and formation of autophagosomes in addition to degradation of TβRI (TGF-β type I receptor) through lysosomes. Thus, TMEPAI seems to act as a regulator of multiple signalling pathways. A great deal of attention has already been paid to the relationship between the TMEPAI family and tumourigenicity. In this paper, therefore, we describe recent progresses in the understanding of how the TMEPAI family physiologically contributes to cellular functions and diseases.

Keywords; AR, C18ORF1, NEDD4, Smad, TGF-β: TMEPAI

BIOCHEMISTRY

Protein Structure

Slow luminescence kinetics of semi-synthetic aequorin: expression, purifi cation and structure determination of cf3-aequorin

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cf3-Aequorin is one of the semi-synthetic aequorins that was produced by replacing 2-peroxycoelenterazine (CTZ-OOH) in native aequorin with a 2-peroxycoelenterazine analog, and it was prepared using the C2-modified trifluoromethyl analog of coelenterazine (*cf3*-CTZ) and the histidine-tagged apoaequorin expressed in *Escherichia coli* cells. The purified *cf3*-aequorin showed a slow luminescence pattern with half-decay time of maximum intensities of luminescence of 5.0 s. This is much longer than that of 0.9 s for native aequorin, and its luminescence capacity was estimated to be 72.8% of that of native aequorin. The crystal structure of *cf3*-aequorin was determined at 2.15 Å resolution. The light source of 2-peroxytrifluoromethylcoelenterazine (*cf3*-CTZ-OOH) was stabilized by the hydrogen-bonding interactions at the C2-peroxy moiety and the *p*-hydroxy moiety at the C6-phenyl group. In native aequorin, three water molecules contribute to stabilizing CTZ-OOH through hydrogen bonds. However, *cf3*-aequorin only contained one water molecule, and the trifluoromethyl moiety at the C2-benzyl group of *cf3*-CTZ-OOH interacted with the protein by van der Waals interactions. The slow luminescence kinetics of *cf3*-aequorin could be explained by slow conformational changes due to the bulkiness of the trifluoromethyl group, which might hinder the

smooth cleavage of hydrogen bonds at the C2-peroxy moiety after the binding of Ca^{2+} to *cf3*-aequorin.

Keywords; calcium-binding protein, EF-hand motif, photoprotein, van der Waals interaction, water molecules

Protein Interaction and Recognition

Plasmodium-specifi c basic amino acid residues important for the interaction with ferredoxin on the surface of ferredoxin-NADP+ reductase

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The malaria parasite (*Plasmodium falciparum*) possesses a plastid-derived, essential organelle called the apicoplast, which contains a redox system comprising plant-type ferredoxin (Fd) and Fd-$NADP^+$ reductase (FNR). This system supplies reducing power for the crucial metabolic pathways in this organelle. Electron transfer between *P. falciparum* Fd (PfFd) and FNR (PfFNR) is performed with higher affinity and specificity than that of plant Fd and FNR. To investigate the mechanism for such superior protein–protein interaction, we searched for the Fd interaction sites on the surface of PfFNR. Basic amino acid residues on the FAD binding side of PfFNR were comprehensively substituted to acidic amino acids by site-directed mutagenesis. Kinetic analysis of electron transfer to PfFd and plant Fds, physical binding to immobilized PfFd and thermodynamics of the PfFd binding using these PfFNR mutants revealed that several basic amino acid residues including those in *Plasmodium*-specific insertion region are important for the interaction with PfFd. Majority of these basic residues are *Plasmodium*-specific and not conserved among plant and cyanobacteria FNRs. These results suggest that the interaction mode of Fd and FNR is diverged during evolution so that PfFd: PfFNR interaction meets the physiological requirement in the cells of *Plasmodium* species.

Keywords; ferredoxin, ferredoxin-NADP+, reductase, malaria parasite, plastid evolution, protein–protein interaction

Metabolism and Bioenergetics

On the role of genetic polymorphisms in the sulfation of cholesterol by human cytosolic sulphotransferase SULT2B1b

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Sulphated cholesterol, like its unsulphated counterpart, is known to be biologically active and serves a myriad of biochemical/physiological functions. Of the 13 human cytosolic sulphotransferases (SULTs), SULT2B1b has been reported as the main enzyme responsible for the sulphation of cholesterol. As such, SULT2B1b may play the role as a key regulator of cholesterol metabolism. Variations in the sulphating activity of SULT2B1b may affect the sulphation of cholesterol and, consequently, the related physiological events. This study was designed to evaluate the impact of the genetic polymorphisms on the sulphation of cholesterol by SULT2B1b. Ten recombinant SULT2B1b allozymes were generated, expressed, and purified. Purified SULT2B1b allozymes were shown to display differential cholesterol-sulphating activities, compared with the wild-type enzyme. Kinetic studies revealed further their distinct substrate affinity and catalytic efficiency toward cholesterol. These findings showed clearly the impact of genetic polymorphisms on the cholesterol-sulphating activity of SULT2B1b allozymes, which may underscore the differential metabolism of cholesterol in individuals with different *SULT2B1b* genotypes.

Keywords; cytosolic sulphotransferase, cholesterol, SULT, SULT2B1b, sulphation

Biochemistry in Diseases and Aging

Rapid increase of 'brain-type' transferrin in cerebrospinal fluid after shunt surgery for idiopathic normal pressure hydrocephalus: a prognosis marker for cognitive recovery

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Idiopathic normal pressure hydrocephalus (iNPH) is a dementia-inducing disorder. Primary cause of iNPH is speculated to be a reduction of cerebrospinal fluid (CSF) absorption, which secondarily induces hydrocephalus, compression of brain, and reduction of CSF production. Patients are treated by surgically inserting a shunt to deliver excess CSF to the abdominal cavity. The prognosis for cognitive improvement after shunt surgery has been difficult to predict. We therefore investigated various CSF proteins, hoping to find a biomarker predictive of cognitive performance one to two years after shunt surgery. CSF proteins of 34 iNPH and 15 non-iNPH patients were analysed by Western blotting, revealing two glycan isoforms of transferrin (Tf); 'brain-type' Tf with *N*-acetylglucosaminylated glycans and 'serum-type' Tf with α2, 6-sialylated glycans. Brain-type Tf levels decreased in iNPH but rapidly returned to normal levels within 1–3 months after shunt surgery. This change was positively correlated with recovery from dementia, per Mini-Mental State Examination and Frontal Assessment Battery scores at 11.8 ± 7.7 months post-operation, suggesting that brain-type Tf is a prognostic marker for recovery from dementia after shunt surgery for iNPH. Histochemical staining with anti-Tf antibody and an *N*-acetylglucosamine-binding lectin suggests that brain-type Tf is secreted from choroid plexus, CSF-producing tissue.

Keywords; cerebrospinal fluid, choroid plexus, dementia, shunt surgery, transferrin

CELL

Biomembranes, Organelles, and Protein Sorting

Determination of cytoplasmic optineurin foci sizes using image correlation spectroscopy

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Optineurin (OPTN) plays an important role in membrane trafficking processes such as exocytosis and autophagy. The sizes and rate of formation of accumulated structures comprising OPTN, such as foci or inclusion bodies (IBs), are often disrupted by amyotrophic lateral sclerosis (ALS) and glaucoma-associated mutants of OPTN. Therefore, methods for the quantitative measurement of the size of the accumulated structure are necessary. Here, we show that, using spatial image correlation spectroscopy (ICS), the average diameter of accumulated structures of the wild-type and disease-associated mutants in living cells may be easily determined. Although OPTN was found to frequently form foci in the cytoplasm, regardless of ALS- and glaucoma-associated mutation, the diameter of OPTN foci decreased in an ALS-associated mutant and increased in a glaucoma-associated mutant. However, a portion of cells carried IBs of the ALS-associated mutant that were larger than micrometre and ellipse-like shape, suggesting that this mutant accumulates non-uniformly in the IBs. The findings suggest that changes in their accumulation, determined via quantitative comparison of the OPTN foci and IBs in the cells, are involved in pathological features of ALS. In addition, this method enables rapid comparison of the average sizes of various other intracellular structures such as granules.

Keywords; foci, image correlation spectroscopy, inclusion body, optineurin, vesicular trafficking

BIOTECHNOLOGY

Biotechnology General

Photo-control of the mitotic kinesin Eg5 using a novel photochromic inhibitor composed of a spiropyran derivative

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In this study, we synthesized a novel photochromic inhibitor of the mitotic kinesin Eg5, which is composed of the photochromic compound spiropyran to photo-control the function of Eg5. The compound (S)-2, 3-dispiropyran propionic acid (DSPPA) exhibits reversible *spiropyran–merocyanine* photo-isomerization upon irradiation with visible or ultra-violet light. DSPPA induced reversible changes in the inhibitory effect on Eg5 ATPase and motor activities, which correlates with the *spiropyran–merocyanine* photo-isomerization. Microtubule-dependent ATPase activity was significantly more inhibited by the *spiropyran* isomer of DSPPA than by the *merocyanine* isomer. Additionally, an *in vitro* motility assay revealed that the microtubule gliding velocity was reduced more by the *spiropyran* isomer than by the *merocyanine* isomer. This indicates that the spiropyran derivative may be useful in regulating the function of the mitotic kinesin.

Keywords; Eg5, inhibitor, kinesin, photo-control, photochromic molecule

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ダイジェスト

JB Special Reviews—Cellular plasticity in epithelial homeostasis and diseases

Featured article of the month.

Involvement of partial EMT in cancer progression

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The epithelial-mesenchymal transition (EMT) provides an outstanding example of cellular plasticity during embryonic development and cancer progression. During EMT in embryonic development, epithelial cells lose all vestiges of their epithelial origin and acquire a fully mesenchymal phenotype, known as complete EMT, which is typically characterized by a so-called cadherin switch. Conversely, during EMT in cancer progression, cancer cells that originate from epithelial cells exhibit both mesenchymal and epithelial characteristics, that is the hybrid E/M phenotype in a process known as partial EMT. Partial EMT in cancer cells is thought to enhance their invasive properties, generate circulating tumour cells and cancer stem cells, and promote resistance to anti-cancer drugs. These phenotypic changes are regulated by extracellular matrix components, exosomes and soluble factors, which regulate several transcription factors known as EMT transcription factors. In this review, I summarize our current understanding of the EMT program during cancer progression.

Keywords; cancer, differentiation, E-cadherin, EMT, invasion

Epithelial-mesenchymal transition in haematopoietic stem cell development and homeostasis

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Epithelial-mesenchymal transition (EMT) is a morphogenetic process of cells that adopt an epithelial organization in their developmental ontogeny or homeostatic maintenance. Abnormalities in EMT regulation result in many malignant tumours in the human body. Tumours associated with the haematopoietic system, however, are traditionally not considered to involve EMT and haematopoietic stem cells (HSCs) are generally not associated with epithelial characteristics. In this review, we discuss the ontogeny and homeostasis of adult HSCs in the context of EMT intermediate states. We provide evidence that cell polarity regulation is critical for both HSC formation from embryonic dorsal aorta and HSC self-renewal and differentiation in adult bone marrow. HSC polarity is controlled by the same set of surface and transcriptional regulators as those described in canonical EMT processes. With an emphasis on partial EMT, we propose that the concept of EMT can be similarly applied in the study of HSC generation, maintenance and pathogenesis.

Keywords; partial EMT/MET, epithelial polarity, stem cell niche, metastable, malignancy

Mitotic spindle orientation in epithelial homeostasis and plasticity

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Polarized epithelia are a foundation of organ and appendage structures throughout Metazoa and serve as a physical barrier to preserve physiological functions. In proliferating epithelia, planar cell division occurs by orienting the mitotic spindle within the plane of the epithelium to ensure tissue organization. Conversely, loss of tissue architecture is a hallmark of carcinoma, and aberrant spindle orientation is hypothesized to contribute to tissue disorganization through dysplasia and cell dissemination. Recent *in vivo* studies have uncovered a role of planar spindle alignment in the robust maintenance of tissue architecture, which accompanies homeostatic mechanisms such as cell delamination and re-integration of misplaced cells following abnormal cell division. Furthermore, perpendicular spindle orientation shifts have been suggested as causes of cell fate change and epithelial plasticity manifested by epithelial-to-mesenchymal transition. This review describes the mechanism by which planar spindle orientation is tightly regulated and discusses the roles of mitotic spindle orientation in epithelial development and disease.

Keyword; scell junctions, cell polarity, EMT, epithelial homeostasis, mitotic spindle orientation

BIOCHEMISTRY

Biochemistry General

Functional characterization of the partially purifi ed Sac1p independent adenine nucleotide transport system (ANTS) from yeast endoplasmic reticulum

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Several ATP-depending reactions take place in the endoplas-

mic reticulum (ER). Although in *Saccharomyces cerevisiae* ER the existence of a Sac1p-dependent ATP transport system was already known, its direct involvement in ATP transport was excluded. Here we report an extensive biochemical characterization of a partially purified adenine nucleotide transport system (ANTS) not dependent on Sac1p. Highly purified ER membranes from the wild-type and Δ*sac1* yeast strains reconstituted into liposomes transported ATP with the same efficiency. A chromatography on hydroxyapatite was used to partially purify ANTS from Δ*sac1* ER extract. The two ANTS-enriched transport activity eluted fractions showed essentially the presence of four bands, one having an apparent MW of 56 kDa, similar to that observed for ANTS identified in rat liver ER. The two fractions reconstituted into liposomes efficiently transported, by a strict counter-exchange mechanism, ATP and ADP. ATP transport was saturable with a Km of 0.28 mM. The ATP/ADP exchange mechanism and the kinetic constants suggest that the main physiological role of ANTS is to catalyse the transport of ATP into ER, where it is used in several energy-requiring reactions and to export back to the cytosol the ADP produced.

Keywords; adenine nucleotide transport system, endoplasmic reticulum, HTP purification, Sac1p, transport

CELL

Cell General

Jaw1/LRMP has a role in maintaining nuclear shape via interaction with SUN proteins

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Jaw1/LRMP is characterized as a Type II integral membrane protein that is localized to endoplasmic reticulum, however, its physiological functions have been poorly understood. An alignment of amino acid sequence of Jaw1 with Klarsicht/ANC-1/Syne/homology (KASH) proteins, outer nuclear membrane proteins, revealed that Jaw1 has a partial homology to the KASH domain. Here, we show that the function of Jaw1 is to maintain nuclear shape in mouse melanoma cell line. The siRNA-mediated knockdown of Jaw1 caused a severe defect in nuclear shape, and the defect was rescued by ectopic expression of siRNA-resistant Jaw1. Since co-immunoprecipitation assay indicates that Jaw1 interacts with Sad-1/UNC-84 (SUN) proteins that are inner nuclear proteins and microtubules, this study suggests that Jaw1 has a role in maintaining nuclear shape via interactions with SUN proteins and microtubules.

Keywords; Jaw1/LRMP, KASH proteins, LINC complex, nuclear envelope, SUN proteins

Cell Cycle

The effect of a gene associated with retinoid-interferon-induced mortality 19 (GRIM-19) on STAT3-induced gene expression in renal carcinoma

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This study aimed to investigate the exact regulatory mechanisms of retinoid-interferon-induced mortality 19 (GRIM-19) in renal carcinoma. Tumour tissue samples from patients with renal carcinoma ($n=30$, there were seven cases of Stage I, eight cases of Stage II, eight cases of Stage III, seven cases of Stage IV) and control subjects were selected from adjacent normal tissue ($n=10$). Real-time quantitative PCR and western blotting were used to assess the level of GRIM-19, signal transducer and activator of transcription-3 (STAT3) and its downstream molecules. CD31 was detected by immunohistochemistry. The MTT assay was used to measure cell proliferation. The amount of apoptosis cells was analysed by Flow cytometry. The results showed that expression of GRIM-19 was decreased in renal carcinoma. However, in tumour tissue, STAT3 and its downstream signalling molecules showed the higher expression compared with control. Overexpression of GRIM-19, inhibited tumour growth apoptosis by mediating activators of STAT3 signal. In addition, interferon-β and all-trans-retinoic acid inhibited the renal carcinoma cell growth and induced apoptosis, and effect of drug combinations was particularly evident. In conclusion, GRIM-19 expression is associated with hyperactivation of STAT3-induced gene expression in renal carcinoma.

Keywords; apoptosis, GRIM-19, proliferation, renal carcinoma STAT3

BIOTECHNOLOGY

Biotechnology General

Highly efficient photocontrol of mitotic kinesin Eg5 ATPase activity using a novel photochromic compound composed of two azobenzene derivatives

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Mitotic kinesin Eg5 plays an important physiological role in cell division. Several small-molecule inhibitors of Eg5 are the focus of cancer therapies. Azobenzene is a photochromic compound exhibiting *cis*–*trans* isomerization upon ultraviolet (UV) and visible (VIS) light irradiation. Photochromic compounds of azobenzene derivatives, mimicking Eg5-specific inhibitors of STLC, indicated photoreversible inhibitory effects on Eg5 ATPase activity; however, the photoreversible switching efficiency was not significant. This study presents a novel synthesized photochromic Eg5 inhibitor 2,3-bis[(2,5-dioxo-1-{4-[(*E*)-2-phenyldiazen-1-yl]phenyl}pyrrolidin-3-yl)sulfanyl] butanedioic acid (BDPSB), which is composed of two azobenzenes. BDPSB exhibited *cis*–*trans* isomerization with UV and VIS light irradiation. The *trans*form of BDPSB significantly inhibited microtubule-dependent ATPase activity of Eg5, with an IC_{50} of 74 μM. *Cis* BDPSB showed weak effects on the microtubule-dependent ATPase activity. The results suggest that the novel photochromic Eg5 inhibitor BDPSB, which exhibits highly efficient photoswitching, shows a switch 'ON' and 'OFF' behaviour with VIS and UV light irradiation.

Keywords; azobenzene, inhibitor, mitotic kinesin, photochromic compound