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ダイジェスト

JB SPECIAL ISSUE—EVER-EXPANDING NGLY1 BIOLOGY

JB Special Issue—Commentary

Ever-expanding NGLY1 biology

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The cytosolic peptide:*N*-glycanase (PNGase; NGLY1 in humans) is a deglycosylating enzyme that is widely conserved in eukaryotes. This enzyme is involved in the degradation of misfolded *N*-glycoproteins that are destined for proteasomal degradation in the cytosol, a process that is called endoplasmic reticulum-associated degradation. Although the physiological significance of NGLY1 remained unknown until recently, the discovery of NGLY1 deficiency, a human genetic disorder bearing mutations in the *NGLY1* gene, has led to explosive research progress regarding the functional characterization of this enzyme. For example, it is now known that NGLY1 can also act as an 'editing enzyme' to convert *N*-glycosylated asparagine residues to aspartate residues, thus introducing negative charges into a core peptide and modulating the function of the target molecule. Diverse biological processes have also been found to be affected by compromised NGLY1 activity. In this special issue, recent research progress on the functional characterization of NGLY1 and its orthologues in worm/fly/rodents, assay methods/biomarkers useful for the development of therapeutics and the comprehensive transcriptome/proteome of *NGLY1*-KO cells as well as patient-derived cells are discussed.

Keywords: NGLY1, NGLY1 deficiency, model organisms, biomarker, trans-omics analysis

JB Special Issue—Reviews

NGLY1: insights from Caenorhabditis elegans

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Peptide:N-glycanase is an evolutionarily conserved deglycosylating enzyme that catalyses the removal of N-linked glycans from cytosolic glycoproteins. Recessive mutations that inactivate this enzyme cause NGLY1 deficiency, a multisystemic disorder with symptoms including developmental delay and defects in cognition and motor control. Developing treatments for NGLY1 deficiency will require an understanding of how failure to deglycosylate NGLY1 substrates perturbs cellular and organismal function. In this review, I highlight insights into peptide:N-glycanase biology gained by studies in the highly tractable genetic model animal *Caenorhabditis elegans*. I focus on the recent discovery of SKN-1A/Nrf1, an N-glycosylated transcription factor, as a peptide:N-glycanase substrate critical for regulation of the proteasome. I describe the elaborate post-translational mechanism that culminates in activation of SKN-1A/Nrf1 via NGLY1-dependent 'sequence editing' and discuss the implications of these findings for our understanding of NGLY1 deficiency.

Keywords: rare disease, proteasome, Nrf1, NGLY1, NFE2L1

Tracing the NGLY1 footprints: insights from Drosophila

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Recessive mutations in human *N*-glycanase 1 (*NGLY1*) cause a multisystem disorder with various phenotypes including global developmental delay. One of the models utilized to understand the biology of NGLY1 and the pathophysiology of NGLY1 deficiency is *Drosophila melanogaster*, a well-established, genetically tractable organism broadly used to study various biological processes and human diseases. Loss of the *Drosophila NGLY1* homolog (*Pngl*) causes a host of phenotypes including developmental delay and lethality. Phenotypic, transcriptomic and genome-wide association analyses on *Drosophila* have revealed links between NGLY1 and several critical developmental and cellular pathways/processes. Further, repurposing screens of Food and Drug Administration (FDA)-approved drugs have identified potential candidates to ameliorate some of the *Pngl*-mutant phenotypes. Here, we will summarize the insights gained into the functions of NGLY1 from *Drosophila* studies. We hope that the current review article will encourage additional studies in *Drosophila* and other model systems towards establishing a therapeutic strategy for NGLY1 deficiency patients.

Keywords: AMPKα, BMP signalling, deglycosylation, rare disease

Physiological importance of NGLY1, as revealed by rodent model analyses

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Cytosolic peptide: *N*-glycanase (NGLY1) is an enzyme that cleaves *N*-glycans from glycoproteins that has been retrotranslocated from the endoplasmic reticulum (ER) lumen into the cytosol. It is known that NGLY1 is involved in the degradation of cytosolic glycans (non-lysosomal glycan degradation) as well as ER-associated degradation, a quality control system for newly synthesized glycoproteins. The discovery of NGLY1 deficiency, which is caused by mutations in the human *NGLY1* gene and results in multisystemic symptoms, has attracted interest in the physiological functions of NGLY1 in mammals. Studies using various animal models led to the identification of possible factors that contribute to the pathogenesis of NGLY1 deficiency. In this review, we summarize phenotypic consequences that have been reported for various *Ngly1*-deficient rodent models and discuss future perspectives to provide more insights into the physiological functions of NGLY1.

Keywords: rodent model, NGLY1 deficiency, NGLY1, FBS2, ENGASE

Assay for the peptide: N-glycanase/NGLY1 and disease-specific biomarkers for diagnosing NGLY1 defi ciency

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Cytosolic peptide: *N*-glycanase (NGLY1 in mammals), a highly conserved enzyme in eukaryotes, catalyses the deglycosylation of *N*-glycans that are attached to glycopeptide/glycoproteins. In 2012, an autosomal recessive disorder related to the *NGLY1* gene, which was referred to as NGLY1 deficiency, was reported. Since then, more than 100 patients have been identified. Patients with this disease exhibit various symptoms, including various motor deficits and other neurological problems. Effective therapeutic treatments for this disease, however, have not been established. Most recently, it was demonstrated that the intracerebroventricular administration of an adeno-associated virus 9 vector expressing human NGLY1 during the weaning period allowed some motor functions to be recovered in *Ngly1*$^{-/-}$ rats. This observation led us to hypothesize that a therapeutic intervention for improving these motor deficits or other neurological symptoms found in the patients might be possible. To achieve this, it is critical to establish robust and facile methods for assaying NGLY1 activity in biological samples, for the early diagnosis and evaluation of the therapeutic efficacy for the treatment of NGLY1 deficiency. In this mini review, we summarize progress made in the development of various assay methods for NGLY1 activity, as well as a recent progress in the identification of NGLY1 deficiency-specific biomarkers.

JB Special Issue—Regular Papers

GlcNAc-Asn is a biomarker for NGLY1 deficiency

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Substrate-derived biomarkers are necessary in slowly progressing monogenetic diseases caused by single-enzyme deficiencies to identify affected patients and serve as surrogate markers for therapy response. *N*-glycanase 1 (NGLY1) deficiency is an ultra-rare autosomal recessive disorder characterized by developmental delay, peripheral neuropathy, elevated liver transaminases, hyperkinetic movement disorder and (hypo)-alacrima. We demonstrate that *N*-acetylglucosamine-asparagine (GlcNAc-Asn; GNA), is the analyte most closely associated with NGLY1 deficiency, showing consistent separation in levels between patients and controls. GNA accumulation is directly linked to the absence of functional NGLY1, presenting strong potential for its use as a biomarker. In agreement, a quantitative liquid chromatography with tandem mass spectrometry assay, developed to assess GNA from 3 to 3000 ng/ml, showed that it is conserved as a marker for loss of NGLY1 function in NGLY1-deficient cell lines, rodents (urine, cerebrospinal fluid, plasma and tissues) and patients (plasma and urine). Elevated GNA levels differentiate patients from controls, are stable over time and correlate with changes in NGLY1 activity. GNA as a biomarker has the potential to identify and validate patients with NGLY1 deficiency, act as a direct pharmacodynamic marker and serve as a potential surrogate endpoint in clinical trials.

Keywords: N-glycanase 1, NGLY1 deficiency, GNA, GlcNAc-Asn, biomarker

Patient-derived gene and protein expression signatures of NGLY1 deficiency

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N-Glycanase 1 (NGLY1) deficiency is a rare and complex genetic disorder. Although recent studies have shed light on the molecular underpinnings of NGLY1 deficiency, a systematic characterization of gene and protein expression changes in patient-derived cells has been lacking. Here, we performed RNA-sequencing and mass spectrometry to determine the transcriptomes and proteomes of 66 cell lines representing four different cell types derived from 14 NGLY1 deficient patients and 17 controls. Although NGLY1 protein levels were up to 9.5-fold downregulated in patients compared with parents, residual and likely non-functional NGLY1 protein was detectable in all patient-derived lymphoblastoid cell lines. Consistent with the role of NGLY1 as a regulator of the transcription factor Nrf1, we observed a cell type-independent downregulation of proteasomal genes in NGLY1 deficient cells. In contrast, genes involved in ribosome biogenesis and mRNA processing were upregulated in multiple cell types. In addition, we observed cell type-specific effects. For example, genes and proteins involved in glutathione synthesis, such as the glutamate-cysteine ligase subunits GCLC and GCLM, were downregulated specifically in lymphoblastoid cells. We provide a web application that enables access to all results generated in this study at https://apps.embl.de/ngly1browser. This resource will guide future studies of NGLY1 deficiency in directions that are most relevant to patients.

Keywords: NGLY1 deficiency, patient-derived cell lines, multiomics profiling, RNA sequencing, N-glycanase 1

BIOCHEMISTRY

Biochemistry General

Multimerization of small G-protein H-Ras induced by chemical modifi cation at hyper variable region with caged compound

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The lipid-anchored small G protein Ras is a central regulator of cellular signal transduction processes, thereby functioning as a molecular switch. Ras forms a nanocluster on the plasma membrane by modifying lipids in the hypervariable region (HVR) at the C-terminus to exhibit physiological functions. In this study, we demonstrated that chemical modification of cysteine residues in HVR with caged compounds (instead of lipidation) induces multimerization of H-Ras. The sulfhydryl-reactive caged compound, 2-nitrobenzyl bromide, was stoichiometrically incorporated into the cysteine residue of HVR and induced the formation of the Ras multimer. Light irradiation induced the elimination of the 2-nitrobenzyl group, resulting in the conversion of the multimer to a monomer. Size-exclusion chromatography coupled with high-performance liquid chromatography and small-angle x-ray scattering analysis revealed that H-Ras forms a pentamer. Electron microscopic observation of the multimer showed a circular ring shape, which is consistent with the structure estimated from x-ray scattering. The shape of the multimer may reflect the physiological state of Ras. It was suggested that the multimerization and monomerization of H-Ras were controlled by modification with a caged compound in HVR under light irradiation.

Keywords: small angle x-ray scattering, multimer, H-Ras, chemical modification, caged compound

In vitro reconstitution of the Escherichia coli 70S ribosome with a full set of recombinant ribosomal proteins R

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Many studies of the reconstitution of the *Escherichia coli* small ribosomal subunit from its individual molecular parts have been reported, but contrastingly, similar studies of the large ribosomal subunit have not been well performed to date. Here, we describe protocols for preparing the 33 ribosomal proteins of the *E. coli* 50S subunit and demonstrate successful reconstitution of a functionally active 50S particle that can perform protein synthesis *in vitro*. We also successfully reconstituted both ribosomal subunits (30S and 50S) and 70S ribosomes using a full set of recombinant ribosomal proteins by integrating our developed method with the previously developed fully recombinant-based integrated synthesis, assembly and translation. The approach described here makes a major contribution to the field of ribosome engineering and could be fundamental to the future studies of ribosome assembly processes.

Keywords: cell-free protein synthesis, protein translation, PURE system, ribosomal protein, ribosome assembly

Nucleic Acid and Peptide Biochemistry

Interaction between a fluoroquinolone derivative and RNAs with a single bulge

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Interaction analysis between small molecules and RNA as well as structure determination of RNA–small molecule complexes will be the clues to search for compounds that bind to specific mRNA or non-coding RNA in drug discovery. In this study, the RNA-binding ability of a fluoroquinolone derivative, KG022, was examined against single-residue bulge-containing hairpin RNAs as RNA models. Nuclear magnetic resonance analysis indicated that KG022 interacts with the RNAs in the vicinity of the bulge residue, with preferring C and G as the bulge residues. The solution structures of the RNA–KG022 complexes showed that the KG022 binds to the RNAs at the bulge-out regions. Each substituent in KG022 interacts with specific position of RNAs around the bulge-out region probably contributing the specificity of the binding. This work provides a novel member for the RNA-targeted small molecules.

Keywords: small molecule, RNA, NMR, interaction, fluoroquinolone

Biochemistry in Diseases and Aging

Ubiquitin-dependent rapid degradation conceals a cell-protective function of cytoplasmic SIRT3 against oxidative stress

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SIRT3 is an NAD^+-dependent protein deacetylase localized in mitochondria. Several studies reported localization of SIRT3 in the cytoplasm or nucleus, but data of these studies were not consistent. We detected expression of mitochondrial (SIRT3mt) and cytoplasmic (SIRT3ct) *Sirt3 mRNAs* in the mouse brain, and we also found SIRT3 immunostaining of mitochondria and cytoplasm in the brain and cultured neural cells. However, expression levels of SIRT3ct in COS cells transfected with SIRT3ct cDNA were much lower than those of SIRT3mt. We found that SIRT3ct but not SIRT3mt was promptly degraded by ubiquitin-dependent degradation, in which SIRT3ct degradation was mediated mainly by ubiquitination of NH_2-terminal methionine and partly by that of lysine residues of SIRT3ct. SIRT3ct expression level was significantly enhanced by the treatment of cells with staurosporine or H_2O_2. H_2O_2 treatment promoted nuclear translocation of SIRT3ct and induced histone H3 deacetylation and superoxide dismutase 2 expression. Overexpression of SIRT3ct decreased cell death caused by H_2O_2 at levels similar to those achieved by overexpression of SIRT3mt. Knockdown of *Sirt3 mRNA* increased cell death caused by amyloid-β (Aβ), and overexpression of SIRT3ct suppressed the toxic function of Aβ in PC12 cells. These results indicate that SIRT3ct promotes cell survival under physiological and pathological conditions.

Keywords: neuronoxidative stress*SIRT3*ubiquitination

MOLECULAR BIOLOGY

Molecular Biology General

CircRPPH1 promotes cell proliferation, migration and invasion of non-small cell lung cancer via the PI3K/AKT and JAK2/STAT3 signalling axes

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Non-small cell lung cancer (NSCLC) has markedly increased morbidity and mortality rates worldwide. Circular RNAs were shown to regulate NSCLC progression. But the underlying pathways of the circRPPH1-mediated regulation of NSCLC still need further exploration. We evaluated circRPPH1 levels in NSCLC tissues and cell lines via qRT-PCR. Moreover, using ectopic plasmid incorporation and siRNA assays, we analysed the circRPPH1-mediated regulation of cell proliferation (CP), cell migration (CM) and cell invasion (CI) in NSCLC cell lines (H1975 and A549 cells), using CCK-8, colony forming, scratch wound and transwell assays, respectively. CircRPPH1 levels were remarkably high in the NSCLC tissues and cell lines. The transfection experiments showed that circRPPH1 overexpression was able to promote CP, CM and CI of NSCLC cells, while CP, CM and CI were significantly restrained by the knockdown of circRPPH1. We also displayed that circRPPH1 knockdown suppressed the cell progression via inactivating the PI3K/AKT and JAK2/STAT3 signalling axes. Subsequently, *in vivo* experiment in nude mice was demonstrated that the inhibition of circRPPH1 could reduce the tumour growth of NSCLC. circRPPH1 may accelerate the growth and metastasis of NSCLC, in culture conditions and in animal models, by stimulating the PI3K/AKT and JAK2/STAT3 signalling axes, thus promoting the development

of NSCLC.
Keywords: circRPPH1, JAK2/STAT3, NSCLC, PI3K/AKT

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JB Commentary

γ-Secretase structure and activity are modifi ed by alterations in its membrane localization and ambient environment

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γ-Secretase cleaves type I transmembrane proteins in a hydrophobic membrane environment following ectodomain shedding. Mutations in *PSEN* genes, encoding the catalytic subunits of *γ*-secretase, presenilins, are the most common cause of familial Alzheimer's disease (AD). Pathogenic mutations in *PSEN* genes increase production of longer and neurotoxic amyloid-*β* (A*β*) by intramembrane cleavage of membrane-associated amyloid-*β* protein precursor (APP) carboxyl-terminal fragment *β*, which is generated via primary cleavage of APP by *β*-site APP cleaving enzyme 1. The longer A*β* is prone to aggregate and accumulate in the brain; however, the accumulation of A*β* in brain is also a pathological feature of sporadic AD. Increased pathogenic A*β* generation, even in the absence of pathogenic *PSEN* gene mutations, is one of proposed mechanisms for sporadic AD pathogenesis. *γ*-Secretase digests substrates in the transmembrane region, generating A*β* peptide intermediates of various lengths. The end products, shorter A*β*40 and A*β*38 peptides, are less neurotoxic, whereas *PSEN* gene mutations increase the production ratio of longer, neurotoxic A*β* species such as A*β*42, an intermediate in A*β*38 production. *γ*-Secretase activity or structures is altered because of its aberrant membrane localization or changes in the ambient environment such as luminal acidification. Interestingly, *γ*-secretase has a pH sensor in presenilins.
Keywords: *γ*-secretase, intramembrane proteolysis, APP, amyloid-*β*, Alzheimer's disease, alcadein

Multiple interfaces to recognize nucleosomal targets

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In eukaryotic cells, DNA is tightly compacted as chromatin. Chromatin states must be dynamically changed to increase the accessibility of transcription factors (TFs) to chromatin or to stably silence genes by higher-order chromatin structures known as heterochromatin. The regulation of chromatin needs cooperative action performed by a variety of proteins. Specific binding of TFs to target DNA is the initial step of chromatin regulation and promotes changes in the post-translational modifications of histone tails, which themselves are recognized by a set of histone reader proteins. Recent biochemical studies have revealed that some TFs that recognize specific DNA sequences can also interact with histones. Furthermore, histone reader proteins that recognize specific histone tail modifications have been shown to have the ability to directly bind to DNA. In this commentary, we introduce recent advances in the elucidation of how chromatin regulating factors recognize nucleosomal targets.
Keywords: pioneer transcription factor, nucleosome, histone reader, histone methylation, heterochromatin

JB Reviews

Structural and biochemical elements of effi ciently degradable proteasome substrates

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Most regulated proteolysis in cells is conducted by the ubiquitin-proteasome system (UPS), in which proteins to be eliminated are selected through multiple steps to achieve high specificity. The large protease complex proteasome binds to ubiquitin molecules that are attached to the substrate and further interacts with a disordered region in the target to initiate unfolding for degradation. Recent studies have expanded our view of the complexity of ubiquitination as well as the details of substrate engagement by the proteasome and at the same time have suggested the characteristics of substrates that are susceptible to proteasomal degradation. Here, I review some destabilizing elements of proteasome substrates with particular attention to ubiquitination, initiation region and stability against unfolding and discuss their interplay to determine the substrate stability. A spatial perspective is important to understand the mechanism of action of proteasomal degradation, which may be critical for drug development targeting the UPS including targeted protein degradation.
Keywords: ubiquitin, protein unfolding, protein degradation, proteasome, initiation region

Long noncoding RNA and phase separation in cellular stress response

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Stress response is important for sensing and adapting to environmental changes. Recently, RNA-protein (RNP) condensates, which are a type of membrane-less organelle formed by liquid–liquid phase separation, have been proposed to regulate the stress response. Because RNP condensates are formed through interactions between positively charged proteins and negatively charged RNAs, the ratio of proteins to RNAs is critical for phase-separated condensate formation. In particular, long noncoding RNAs (lncRNAs) can efficiently nucleate phase-separated RNP condensates because of their secondary structure and long length. Therefore, increased attention has been paid to lncRNAs because of their potential role as a regulator of biological condensates by phase separation under stress response. In this review, we summarize the current research on the involvement of lncRNAs in the formation of RNP condensates under stress response. We also demonstrate that lncRNA-driven phase separation provides a useful basis to understanding the response to several kinds of cellular stresses.

Keywords: long noncoding RNA, phase separation, RNA-binding protein, RNA-protein condensate, stress response

BIOCHEMISTRY

Biochemistry General

Long noncoding RNAs transcribed downstream of the human *β*-globin locus regulate *β*-globin gene expression

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The five *β*-like globin genes (*ε*, G*γ*, A*γ*, *δ* and *β*) at the human *β*-globin gene locus are known to be expressed at specific developmental stages, although details of the underlying mechanism remain to be uncovered. Here we used an *in vitro* transcription assay to clarify the mechanisms that control this gene expression. We first tested nuclear RNA from HeLa cells using RT-qPCR and discovered a long noncoding RNAs (lncRNAs) within a 5.2-kb region beginning 4.4 kb downstream of the *β*-globin gene coding region. We investigated nuclear RNA from K562 cells using a primer-extension assay and determined the transcription start sites (TSSs) of these lncRNAs. To clarify their functional role, we performed knockdown (KD) of these lncRNAs in K562 cells. Hydroxyurea (HU), which induces differentiation of K562 cells, increased haemoglobin peptide production, and the effect was enhanced by KD of these lncRNAs, which also enhanced upregulation of the *γ*-globin expression induced by HU. To confirm these results, we performed an *in vitro* transcription assay. Noncoding single-stranded RNAs inhibited *β*-globin expression, which was upregulated by GATA1. Furthermore, lncRNAs interacted with GATA1 without sequence specificity and inhibited its binding to its target DNA response element *in vitro*. Our results suggest that lncRNAs downstream of the *β*-globin gene locus are key factors regulating globin gene expression.

Keywords: *γ*-globin, noncoding transcripts, in vitro transcription assay, human *β*-globin gene locus

Mitochondria metabolomics reveals a role of *β*-nicotinamide mononucleotide metabolism in mitochondrial DNA replication

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Mitochondrial DNA (mtDNA) replication is tightly regulated and necessary for cellular homeostasis; however, its relationship with mitochondrial metabolism remains unclear. Advances in metabolomics integrated with the rapid isolation of mitochondria will allow for remarkable progress in analyzing mitochondrial metabolism. Here, we propose a novel methodology for mitochondria-targeted metabolomics, which employs a quick isolation procedure using a hemolytic toxin from *Streptococcus pyogenes* streptolysin O (SLO). SLO isolation of mitochondria from cultured HEK293 cells is time- and labor-saving for simultaneous multi-sample processing and has been applied to various other cell lines in this study. Furthermore, our method can detect the time-dependent reduction in mitochondrial ATP in response to a glycolytic inhibitor 2-deoxyglucose, indicating the suitability to prepare metabolite analysis–competent mitochondria. Using this methodology, we searched for specific mitochondrial metabolites associated with mtDNA replication activation, and nucleotides and NAD^+ were identified to be prominently altered. Most notably, treatment of *β*-nicotinamide mononucleotide (*β*-NMN), a precursor of NAD^+, to HEK293 cells activated and improved the rate of mtDNA replication by increasing nucleotides in mitochondria and decreasing their degradation products: nucleosides. Our results suggest that *β*-NMN metabolism plays a role in supporting mtDNA replication by maintaining the nucleotide pool balance in the mitochondria.

Keywords: streptolysin O, nucleotide metabolism, mitochon-

drial DNA, metabolomics, beta-nicotinamide mononucleotide (β-NMN)

Enzymology

Identifi cation of the active site and characterization of a novel sporulation-specific cysteine protease YabG from Bacillus subtilis

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In order to characterize the probable protease gene *yabG* found in the genomes of spore-forming bacteria, *Bacillus subtilis yabG* was expressed as a 35 kDa His-tagged protein (BsYabG) in *Escherichia coli* cells. During purification using Ni-affinity chromatography, the 35 kDa protein was degraded via several intermediates to form a 24 kDa protein. Furthermore, it was degraded after an extended incubation period. The effect of protease inhibitors, including certain chemical modification reagents, on the conversion of the 35 kDa protein to the 24 kDa protein was investigated. Reagents reacting with sulphhydryl groups exerted significant effects strongly suggesting that the *yabG* gene product is a cysteine protease with autolytic activity. Site-directed mutagenesis of the conserved Cys and His residues indicated that Cys218 and His172 are active site residues. No degradation was observed in the C218A/S and H172A mutants. In addition to the chemical modification reagents, benzamidine inhibitedGraphical Abstract the degradation of the 24 kDa protein. Determination of the N-terminal amino acid sequences of the intermediates revealed trypsin-like specificity for YabG protease. Based on the relative positions of His172 and Cys218 and their surrounding sequences, we propose the classification of YabG as a new family of clan CD in the MEROPS peptidase database.

Keywords: autolysis, catalytic dyad, cysteine protease, sporulation, YabG

MOLECULAR BIOLOGY

Molecular Biology General

Generation of endoplasmic reticulum stress-dependent reactive oxygen species mediates TGF-β1-induced podocyte migration

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Podocyte migration results in proteinuria and glomerulonephropathy. Transforming growth factor-β1 (TGF-β1), endoplasmic reticulum (ER) stress and reactive oxygen species (ROS) can mediate podocyte migration; however, the crosstalk between them is unclear. This study determined the relationships between these factors. ER stress biomarkers (GRP78, p-eIF2α or CHOP), intracellular ROS generation, integrin-β3 and cell adhesion and migration were studied in a treatment of experiment using TGF-β1 with and without the ER stress inhibitors: 4-phenylbutyric acid (4-PBA, a chemical chaperone), salubrinal (an eIF2α dephosphorylation inhibitor) and N-acetylcysteine (NAC, an antioxidant). ER stress biomarkers (p-eIF2α/eIF2α and GRP78), ROS generation and intergrin-β3 expression increased after TGF-β1 treatment. NAC down-regulated the expression of GRP78 after TGF-β1 treatment. 4-PBA attenuated TGF-β1-induced p-eIF2α/eIF2α, CHOP, ROS generation and intergrin-β3 expression. However, salubrinal did not inhibit TGF-β1-induced p-eIF2α/eIF2α, CHOP, ROS generation or integrin-β3 expression. NAC abrogated TGF-β1-induced integrin-β3 expression. At 24 h after treatment with TGF-β1, podocyte adhesion and migration increased. Furthermore, NAC, 4-PBA and an anti-interin-β3 antibody attenuated TGF-β1-induced podocyte adhesion and migration. This study demonstrated that TGF-β1-induced ER stress potentiates the generation of intracellular ROS to a high degree through the PERK/eIF2α/CHOP pathway. This intracellular ROS then mediates integrin-β3 expression, which regulates podocyte migration.

Keywords: eIF2α, ER stress, migration, podocyte, ROS, TGF-β1

Basic structure and cytocompatibility of giant membrane vesicles derived from paraformaldehyde-exposed human cells

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Exposure of cultured mammalian cells to paraformaldehyde

(PFA) is an effective approach to induce membrane blebs, which is followed by their detachment from the cellular cortex to yield giant membrane vesicles in extracellular spaces. Although PFA-induced giant vesicles have attracted significant interest in the field of cell membrane dynamics, their biochemical components and cytocompatibility remain largely unknown. In this report, we exposed human cervical cancer HeLa cells to PFA under metal-free buffer conditions to produce giant vesicles. We analyzed the components and structure of the purified PFA-induced giant vesicles. Co-culturing PFA-induced giant vesicles with exponentially growing HeLa cells resulted in docking of a significant number of the giant vesicles to the cell surface with seemingly no cytotoxicity. Intriguingly, we found that pre-treatment of HeLa cells with peptide-N-glycosidase and neuraminidase was effective in facilitating cellular uptake of constituents residing inside the vesicles. The results revealed further details about the effect of PFA on cell membranes and provide insights for studying the interaction between PFA-induced giant vesicles and cultured cells.

Keywords: membrane bleb., glycan, extracellular vesicle, cytocompatible vector

Gene Expression

PhoP induces RyjB expression under acid stress in Escherichia coli

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Bacterial small RNAs (sRNAs) play a pivotal role in post-transcriptional regulation of gene expression and participate in many physiological circuits. An ~80-nt-long RyjB was earlier identified as a novel sRNA, which appeared to be accumulated in all phases of growth in *Escherichia coli*. We have taken a comprehensive approach in the current study to understand the regulation of *ryjB* expression under normal and pH stress conditions. RpoS was not necessary for *ryjB* expression neither at normal condition nor under acid stress. Hfq also emerged to be unnecessary for RyjB accumulation. Interestingly, RyjB was detected as a novel acid stress induced sRNA. A DNA binding protein PhoP, a component of PhoP/Q regulon, was found to regulate *ryjB* expression at low pH, as the elimination of *phoP* allele in the chromosome exhibited a basal level of RyjB expression under acid stress. Ectopic expression of PhoP in Δ*phoP* cells restored the overabundance of RyjB in the cell. Overexpression of RyjB increased the abundance of *sgcA* transcripts, with which RyjB shares a 4-nt overlap. The current study increases our knowledge substantially regarding the regulation of *ryjB* expression in *E. coli* cell.

Keywords: acid stress, Hfq, small RNAs, sRNA-mediated gene regulation

CELL

Neurobiology

Upregulated mGluR5 induces ER stress and DNA damage by regulating the NMDA receptor subunit NR2B

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Dysfunction caused by mGluR5 expression or activation is an important mechanism in the development of Parkinson's disease (PD). Early clinical studies on mGluR5 negative allosteric modulators have shown some limitations. It is therefore necessary to find a more specific approach to block mGluR5-mediated neurotoxicity. Here, we determined the role of N-methyl-D-aspartate (NMDA) receptor subunit NR2B in mGluR5-mediated ER stress and DNA damage. *In vitro* study, rotenone-induced ER stress and DNA damage were accompanied by an increase in mGluR5 expression and overexpressed or activated mGluR5 with agonist (RS)-2-chloro-5-hydroxyphenylglycine (CHPG) induced ER stress and DNA damage, while blocking mGluR5 with antagonist 2-methyl-6-(phenylethynyl) pyridine hydrochloride (MPEP) alleviated the effect. Furthermore, the damage caused by CHPG was blocked by NMDA receptor antagonist MK-801. Additionally, rotenone or CHPG increased the p-Src and p-NR2B, which was inhibited by MPEP. Blocking p-Src or NR2B with PP2 or CP101,606 alleviated CHPG-induced ER stress and DNA damage. Overactivation of mGluR5 accompanied with the increase of p-Src and p-NR2B in the ER stress and DNA damage was found in rotenone-induced PD rat model. These findings suggest a new mechanism wherein mGluR5 induces ER stress and DNA damage through the NMDA receptor and propose NR2B as the molecular target for therapeutic strategy for PD.

Keywords: Parkinson's disease, NR2B subunit, metabotropic glutamate receptor 5, endoplasmic reticulum stress, DNA damage

Tumor and Immunology

The protein level of the tumour-promoting factor SET is regulated by cell density

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SET/I2PP2A is a multifunctional protein that acts as an intrinsic inhibitor of the tumour suppressor protein phosphatase 2A and as a histone chaperone. Increased SET levels have been observed in various cancers; however, the underlying molecular mechanisms remain unclear. In this study, we found that SET protein accumulates with the increasing density of cultured cells. This phenomenon was observed not only in cancer cell lines but also in non-cancer cell lines. The mRNA levels of SET were not affected by the cell density. Proteasome inhibition decreased SET levels, whereas autophagy inhibition led to SET accumulation, indicating the involvement of autophagy. The mRNA and protein expression of SETBP1, which stabilizes the SET protein, increased with cell density. The decrease in SET level due to the loss of SETBP1 was more pronounced in wild-type cells than that in autophagy-deficient cells. These results have revealed a mechanism underlying the regulation of SET level, wherein increased cell density induces SETBP1 expression and protects SET from autophagy.

Keywords: SETBP1, SET/I2PP2A, PP2A, cell density

Abbreviations: PP2A, Protein phosphatase 2A, SETBP1, SET binding protein 1

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ダイジェスト

JB REVIEWS

Branched ubiquitin code: from basic biology to targeted protein degradation

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Protein ubiquitylation regulates numerous pathways, and the diverse information encoded by various forms of ubiquitylation is known as the ubiquitin code. Recent studies revealed that branched ubiquitin chains are abundant in mammalian cells and regulate important pathways. They include proteasomal degradation of misfolded and disease-causing proteins, regulation of NF-κB signalling and apoptotic cell fate decisions. Targeted protein degradation through chemical degraders emerged as a transformative therapeutic paradigm aimed at inducing the disappearance of unwanted cellular proteins. To further improve the efficacy of target degradation and expand its applications, understanding the molecular mechanism of degraders' action from the view of ubiquitin code biology is required. In this review, I discuss the roles of the ubiquitin code in biological pathways and in chemically induced targeted protein degradation by focusing on the branched ubiquitin codes that we have characterized.

Key words: ubiquitin, targeted protein degradation, proteasome, PROTAC, mass spectrometry

New insights into the regulatory roles of glutathione in NLRP3-inflammasome-mediated immune and inflammatory responses

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Glutathione (GSH) is the most abundant non-protein thiol (–SH) in mammalian cells. Its synthesis and metabolism serve to maintain cellular reduction–oxidation (redox) homeostasis, which is important for multiple cellular processes including proliferation, differentiation and death. An accumulating body of evidence suggests that the essential roles of GSH extended far beyond its oxidant and electrophile scavenger activities and regulatory role in the lifespan of cells. Recent findings revealed that altered GSH levels are closely associated with a wide range of pathologies including bacterial and viral infections, neurodegenerative diseases and autoimmune disorders, all of which are also characterized by aberrant activation of the NLR family pyrin domain containing 3 (NLRP3) inflammasome. As a result of these findings, GSH was assigned a central role in influencing the activation of the NLRP3 inflammasome. To expand on our recent advances in understanding this process, we discuss here the emerging roles of GSH in activation of the NLRP3 inflammasome, and the therapeutic potential of GSH in its associated pathologies.

Keywords: redox regulation, NLRP3 inflammasome-related disorders, NLRP3 inflammasome, immune and inflammatory responses, glutathione

Into the matrix: current methods for mitochondrial translation studies

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In addition to the cytoplasmic translation system, eukaryotic cells house additional protein synthesis machinery in mitochondria. The importance of this *in organello* translation is exemplified by clinical pathologies associated with mutations in mitochondrial translation factors. Although a detailed understanding of mitochondrial translation has long been awaited, quantitative, comprehensive and spatiotemporal measurements have posed analytic challenges. The recent development of novel approaches for studying mitochondrial protein synthesis has overcome these issues and expands our understanding of the unique translation system. Here, we review the current technologies for the investigation of mitochondrial translation and the insights provided by their application.

Key words: translation, ribosome profiling, mitoribosome, mitochondria, FUNCAT

BIOCHEMISTRY

Biochemistry General

Crystal structure of Tam41 cytidine diphosphate diacylglycerol synthase from a irmicutes bacterium

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Translocator assembly and maintenance 41 (Tam41) catalyses the synthesis of cytidine diphosphate diacylglycerol (CDP-DAG), which is a high-energy intermediate phospholipid critical for generating cardiolipin in mitochondria. Although Tam41 is present almost exclusively in eukaryotic cells, a Firmicutes bacterium contains the gene encoding Tam41-type CDP-DAG synthase (FbTam41). FbTam41 converted phosphatidic acid (PA) to CDP-DAG using a ternary complex mechanism *in vitro*. Additionally, FbTam41 functionally substituted yeast Tam41 *in vivo*. These results demonstrate that Tam41-type CDP-DAG synthase functions in some prokaryotic cells. We determined the crystal structure of FbTam41 lacking the C-terminal 18 residues in the cytidine triphosphate (CTP)-Mg^{2+} bound form at a resolution of 2.6 Å. The crystal structure showed that FbTam41 contained a positively charged pocket that specifically accommodated CTP-Mg^{2+} and PA in close proximity. By using this structure, we constructed a model for the full-length structure of FbTam41 containing the last a-helix, which was missing in the crystal structure. Based on this model, we propose a molecular mechanism for CDP-DAG synthesis in bacterial cells and mitochondria.

Keywords: Tam41, phospholipid, mitochondria, Firmicutes bacterium, cardiolipin

Analytical Biochemistry

Evolution and properties of alanine racemase from Synechocystis sp. PCC6803

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Alanine racemase (EC 5.1.1.1) depends on pyridoxal 5′-phosphate and catalyses the interconversion between L- and D-Ala. The enzyme is responsible for the biosynthesis of D-Ala, which is an essential component of the peptidoglycan layer of bacterial cell walls. Phylogenetic analysis of alanine racemases demonstrated that the cyanobacterial enzyme diverged before the separation of gram-positive and gram-negative enzymes. This result is interesting considering that the peptidoglycans observed in cyanobacteria seem to combine the properties of those in both gram-negative and gram-positive bacteria. We cloned the putative alanine racemase gene (*slr0823*) of *Synechocystis* sp. PCC6803 in *Escherichia coli* cells, expressed and purified the enzyme protein and studied its enzymological properties. The enzymatic properties of the *Synechocystis* enzyme were similar to those of other gram-positive and gram-negative bacterial enzymes. Alignment of the amino acid sequences of alanine racemase enzymes revealed that the conserved tyrosine residue in the active centre of most of the gram-positive and gram-negative

bacterial enzymes has been replaced with tryptophan in most of the cyanobacterial enzymes. We carried out the site-directed mutagenesis involving the corresponding residue of *Synechocystis* enzyme (W385) and revealed that the residue is involved in the substrate recognition by the enzyme.

Keywords: alanine racemase, cyanobacteria, peptidoglycan, phylogenetic analysis, substrate recognition

MOLECULAR BIOLOGY

Molecular Biology General

Hoop-like role of the cytosolic interface helix in Vibrio PomA, an ion-conducting membrane protein, in the bacterial flagellar motor

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Vibrio has a polar flagellum driven by sodium ions for swimming. The force-generating stator unit consists of PomA and PomB. PomA contains four transmembrane regions and a cytoplasmic domain of approximately 100 residues, which interacts with the rotor protein, FliG, to be important for the force generation of rotation. The 3D structure of the stator shows that the cytosolic interface (CI) helix of PomA is located parallel to the inner membrane. In this study, we investigated the function of CI helix and its role as stator. Systematic proline mutagenesis showed that residues K64, F66 and M67 were important for this function. The mutant stators did not assemble around the rotor. Moreover, the growth defect caused by PomB plug deletion was suppressed by these mutations. We speculate that the mutations affect the structure of the helices extending from TM3 and TM4 and reduce the structural stability of the stator complex. This study suggests that the helices parallel to the inner membrane play important roles in various processes, such as the hoop-like function in securing the stability of the stator complex and the ion conduction pathway, which may lead to the elucidation of the ion permeation and assembly mechanism of the stator.

Keywords: supramolecular complex; stator; MotB; MotA; bacterial flagellum

Gene Expression

MAB21L4 regulates the TGF-*β*-induced expression of target genes in epidermal keratinocytes

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Smad proteins transduce signals downstream of transforming growth factor-*β* (TGF-*β*) and are one of the factors that regulate the expression of genes related to diseases affecting the skin. In the present study, we identified MAB21L4, also known as male abnormal 21 like 4 or *C2orf54*, as the most up-regulated targets of TGF-*β* and Smad3 in differentiated human progenitor epidermal keratinocytes using chromatin immunoprecipitation sequencing (ChIP-seq) and RNA sequencing (RNA-seq). We found that TGF-*β* induced expression of the barrier protein involucrin (encoded by the *IVL* gene). Transcriptional activity of the *IVL* promoter induced by TGF-*β* was inhibited by MAB21L4 siRNAs. Further analysis revealed that MAB21L4 siRNAs also down-regulated the expression of several target genes of TGF-*β*. MAB21L4 protein was located mainly in the cytosol, where it was physically bound to Smad3 and a transcriptional corepressor c-Ski. siRNAs for MAB21L4 did not inhibit the binding of Smad3 to their target genomic regions but down-regulated the acetylation of histone H3 lys 27 (H3K27ac), an active histone mark, near the Smad3 binding regions. These findings suggest that TGF-*β*-induced MAB21L4 up-regulates the gene expression induced by TGF-*β*, possibly through the inhibition of c-Ski via physical interaction in the cytosol.

Keywords: TGF-*β*, epidermal keratinocytes, Smad3, c-Ski, ChIP-seq

A leaderless mRNA including tRNA-like sequence encodes a small peptide that regulates the expression of GcvB small RNA in Escherichia coli

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A tRNA-like sequence conserved in the genomes of all *Escherichia coli* strains was found. The sequence resembles arginine-tRNA, which is present in *E. coli* pathogenic islands and phages. Expression experiments revealed that this sequence is a part of a leaderless mRNA encoding a short peptide (60 amino acids: XtpA). A deletion mutant of this gene is more sensitive than wild-type cell to several aminoglycoside antibiotics at low concentrations. Further analyses indicated that XtpA positively

regulates the expression of GcvB small RNA, which is involved in the intrinsic resistance to aminoblycosides in *E. coli*.
Keywords: tRNA-like sequence, leaderless mRNA, GcvB small RNA, *E. coli*, aminoglycoside antibiotics

CELL
Cell Cycle

Long non-coding RNA DANCR accelerates colorectal cancer progression via regulating the miR-185-5p/HMGA2 axis

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Long non-coding RNAs (lncRNAs) are crucial players in tumour progression. Herein, this work was designated to decipher the clinical significance, function and molecular mechanism of a lncRNA, differentiation antagonizing non-coding RNA (DANCR) in colorectal cancer (CRC). Quantitative real-time polymerase chain reaction was adopted to examine DANCR, miR-185-5p and HMGA2 mRNA expressions in CRC tissues and cells. Both gain-of-function and loss-of-function cell models for DANCR were established, and then MTT, wound healing and Transwell, flow cytometry assays were carried out to detect the proliferation, migration, invasion, cell cycle and apoptosis of CRC cells. Dual-luciferase reporter gene assay and RIP assay were utilized to validate the targeting relationships between DANCR and miR-185-5p. Western blot was employed for detecting high mobility group A2 (HMGA2) expressions in CRC cells. In this study, we demonstrated that the expression of DANCR was elevated in CRC tissues and cell lines, and its high expression was significantly associated with increased TNM stage and positive lymph node metastasis. DANCR overexpression promoted CRC cell proliferation, migration, invasion and cell cycle progression, but inhibited apoptosis; while knocking down DANCR caused the opposite effects. DANCR was further identified as a molecular sponge for miR-185-5p, and DANCR could indirectly increase the expression of HMGA2 via repressing miR-185-5p. In conclusion, DANCR/miR-185-5p/HMGA2 axis participated in the progression of CRC.
Keywords: CRC, DANCR, high mobility group A2, miR-185-5p

Lidocaine relieves spinal cord ischemia–reperfusion injury via long non-coding RNA MIAT-mediated Notch1 downregulation

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Microglial activation and inflammatory response play a critical role in spinal cord ischemia reperfusion injury (SCIRI). This study aimed to investigate whether lidocaine relieves SCIRI via modulating myocardial infarction-associated transcript (MIAT)-mediated Notch1 downregulation. Mouse SCIRI was induced by the obstruction of the aortic arch. Lidocaine was injected after reperfusion. Microglial activation and inflammatory response were assessed by Iba1, interleukin 1 beta (IL-1β) and tumour necrosis factor alpha (TNF-α) levels. The interaction between MIAT and Notch1 was assessed by RNA pull-down and RNA immunoprecipitation assays. Lidocaine treatment relieved SCIRI by reducing Iba1 and serum TNF-α and IL-1β levels. After lidocaine treatment, MIAT expression was elevated in lipopolysaccharide-induced BV2 cells. The interference of MIAT and the overexpression of MIAT and Notch1 restored TNF-α and IL-1β levels in supernatants. Notch1 protein was existent in MIAT-pull-down compounds, and the expression of MIAT was markedly elevated in Notch1-immunoprecipitants. The overexpression of MIAT markedly promoted the degradation of Notch1 and increased the level of ubiquitin-bound Notch1 complex. The therapeutic effect of lidocaine on SCIRI mice could be reversed by adeno-associated virus-mediated MIAT knockdown. In conclusion, lidocaine treatment relieved SCIRI via inhibiting microglial activation and reducing the inflammatory response. The molecular mechanism was partly through MIAT-mediated Notch1 downregulation.
Keywords: spinal cord ischemia reperfusion injury, Notch1, microglial activation, MIAT, lidocaine, inflammatory response

MicroRNA-30c-2-3p targets STRIP2 to suppress malignant progression of gastric cancer cells

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MicroRNA plays a crucial part in genesis and development of gastric cancer (GC). We uncovered that microRNA-30c-2-3p was down-regulated in GC tissue and cell lines. Suppression of microRNA-30c-2-3p promoted progression of GC cells *in vitro*. STRIP2 was confirmed as a target for microRNA-30c-2-3p. MicroRNA-30c-2-3p overexpression remarkably suppressed cell malignant behaviours, while reintroduction of STRIP2 partially restored the anticancer effect of microRNA-30c-2-3p. Taken together, these findings suggested that microRNA-30c-2-3p acted as a candidate tumour suppressor in GC by directly targeting STRIP2. Therefore, microRNA-30c-2-3p can be used as a towardly GC therapeutic target.

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ダイジェスト

JB SPECIAL ISSUE: MOLECULAR CONNECTIONS BETWEEN CIRCADIAN CLOCK AND HEALTH/AGING GUEST EDITORS: YASUKAZU NAKAHATA, YOSHITAKA FUKADA AND SALVADOR AZNAR BENITAH

Special Issue—JB Commentary

Molecular connections between circadian clock and health/ageing

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For decades, considerable efforts have been expended for solving the molecular mechanisms of disease progression. An important clue to tackle this question is the circadian clock. Recent findings have uncovered previously unknown molecular connections between circadian clock and disease incidence, consequently causing the ageing process. Furthermore, 'chronotherapy' is emerging as a new concept of optimizing the time of the day for drug administration according to target gene expressions in order to maximize therapeutic efficacy and minimize the side effects. This concept will help cure patients and prevent them from suffering evitable pain and side effects. This JB special issue 'Molecular connections between circadian clock and health/ageing' discusses how the circadian clocks link to health and ageing from molecular to organismal levels.

Special Issue—JB Review

The circadian clock and cancer: links between circadian disruption and disease Pathology

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There is growing evidence that disruption of our 24-h clock increases our risk for acquiring several diseases and disorders. One of these diseases is cancer. While the mechanistic links between circadian clock disruption and cancer initiation or progression are an active area of study, significantly more work needs to be done to understand the molecular substrates involved. Of particular complexity remains the functions of the clock in individual cells during the process of transformation (cancer initiation) versus the functions of the clock in tumour-surrounding stroma in the process of tumour progression or metastasis. Indeed, the nexus of cellular circadian dynamics, metabolism and carcinogenesis is drawing more attention, and many new studies are now highlighting the critical role of circadian rhythms and clock proteins in cancer prevention. In this brief review, we cover some of the basic mechanisms reported to link circadian disruption and cancer at the level of gene expression and metabolism. We also review some of the human studies addressing circadian disruption and cancer incidence as well as some controlled laboratory studies connecting the two in pre-clinical models. Finally, we discuss the tremendous opportunity to use circadian approaches for future prevention and treatment in the context of cancer in specific organs.

Basis for diurnal exacerbation of neuropathic pain hypersensitivity and its application for drug development

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In addition to diurnal rhythms in physiology and behavior, a variety of pathological conditions also exhibit marked day–night changes in symptom intensity, exemplified by allergic rhinitis, arthritis, asthma, myocardial infarction, congestive heart failure, stroke and chronic pain disorders. Currently, novel therapeutic approaches are facilitated by the development of chemical compounds targeted to key proteins that cause diurnal exacerbation of pathological events. Neuropathic pain is a chronic condition that occurs by tumor-induced nerve compression, cancer cell infiltration into the nerve, diabetes and herpes virus infection. One troublesome hallmark symptom of neuropathic pain is hypersensitivity to normally innocuous stimuli known as 'mechanical allodynia' that is often refractory to common analgesic therapies. Millions of patients worldwide presently endure neuropathic pain. We summarize the recent insights gained into the mechanism of diurnal exacerbation of neuropathic pain hypersensitivity and introduce the strategy of circadian clock-based drug development.

Keywords: circadian clock, drug development, neuropathic pain, SGK-1, sulfasalazine

Cellular senescence and its impact on the circadian clock

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Ageing is one of the greatest risk factors for chronic non-communicable diseases, and cellular senescence is one of the major causes of ageing and age-related diseases. The persistent presence of senescent cells in late life seems to cause disarray in a tissue-specific manner. Ageing disrupts the circadian clock system, which results in the development of many age-related diseases such as metabolic syndrome, cancer, cardiac diseases and sleep disorders and an increased susceptibility to infections. In this review, we first discuss cellular senescence and some of its basic characteristics and detrimental roles. Then, we discuss a relatively unexplored topic on the link between cellular senescence and the circadian clock and attempt to determine whether cellular senescence could be the underlying factor for circadian clock disruption.

Keywords: NAD+, metabolites, circadian clock, cellular senescence, ageing

Special Issue—Regular Paper

Effects of cryptochrome-modulating compounds on circadian behavioural rhythms in zebrafish

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The circadian clock controls daily rhythms of various physiological processes, and impairment of its function causes many diseases including sleep disorders. Chemical compounds that regulate clock function are expected to be applied for treatment of circadian clock-related diseases. We previously identified small-molecule compounds KL001, KL101 and TH301 that lengthen the period of cellular circadian clock by directly targeting clock proteins cryptochromes (CRYs) in mammals. KL001 targets both CRY1 and CRY2 isoforms, while KL101 and TH301 are isoform-selective compounds and require CRY C-terminal region for their effects. For further application of these compounds, the effects on locomotor activity rhythms at the organismal level need to be investigated. Here we used zebrafish larvae as an *in vivo* model system and found that KL001 lengthened the period of locomotor activity rhythms in a dose-dependent manner. In contrast, KL101 and TH301 showed no effect on the period. The amino acid sequences of CRY C-terminal regions are diverged in zebrafish and mammals, supporting the importance of this region for the effects of KL101 and TH301. This study demonstrated efficacy of CRY modulation for controlling circadian behavioural rhythms in organisms and suggested species-dependent differences in the effects of isoform-selective CRY-modulating compounds.

Keywords: circadian clock, cryptochrome, locomotor activity rhythm, small-molecule compounds, zebrafish

Diurnal shift of mouse activity by the deficiency of an ageing-related gene Lmna

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Nuclear lamina is a fundamental structure of the cell nucleus and regulates a wide range of molecular pathways. Defects of components of the nuclear lamina cause ageing-like physiological disorders, called laminopathy. Generally, ageing and diseases are often associated with perturbation of various time-of-day–dependent regulations, but it remains elusive whether laminopathy induces any changes of the circadian clock and physiological rhythms. Here, we demonstrated that deficiency of *Lmna* gene in mice caused an obvious shift of locomotor activities to the daytime. The abnormal activity profile was accompanied by a remarkable change in phase angle between the central clock in the suprachiasmatic nucleus (SCN) and the lung peripheral clocks, leaving the phase of the SCN clock unaffected by the mutation. These observations suggest that *Lmna* deficiency causes a change of the habitat from nocturnal to diurnal behaviours. On the other hand, molecular oscillation and its phase resetting mechanism were intact in both the *Lmna*-deficient cells and progeria-mimicking cells. Intriguingly, high-fat diet feeding extended the short lifespan and ameliorated the abnormalities of the behaviours and the phase of the peripheral clock in the *Lmna*-deficient mice. The present study supports the important contribution of the energy conditions to a shift between the diurnal and nocturnal activities.

Keywords: circadian, diseases, diurnal and nocturnal, *Lmna*, metabolism

BIOCHEMISTRY

Protein Interaction and Recognition

MITOL regulates phosphatidic acid-binding activity of RMDN3/PTPIP51

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The transfer of phospholipids from the endoplasmic reticulum (ER) to mitochondria via the mitochondria-ER contact site (MERCS) is essential for maintaining mitochondrial function and integrity. Here, we identified RMDN3/PTPIP51, possessing phosphatidic acid (PA)-transfer activity, as a neighbouring protein of the mitochondrial E3 ubiquitin ligase MITOL/MARCH5 by proximity-dependent biotin labelling using APEX2. We found that MITOL interacts with and ubiquitinates RMDN3. Mutational analysis identified lysine residue 89 in RMDN3 as a site of ubiquitination by MITOL. Loss of MITOL or the substitution of lysine 89 to arginine in RMDN3 significantly reduced the PA-binding activity of RMDN3, suggesting that MITOL regulates the transport of PA to mitochondria by activating RMDN3. Our findings imply that ubiquitin signalling regulates phospholipid transport at the MERCS.

Keywords: E3 ubiquitin ligase, mitochondria-ER contact site, MITOL, phospholipid, RMDN3

Glycobiology and Carbohydrate Biochemistry

Sialyl-Tn antigen facilitates extracellular vesicle-mediated transfer of FAK and enhances motility of recipient cells

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Protein glycosylation plays a pivotal role in tumour development by modulating molecular interactions and cellular signals. Sialyl-Tn (sTn) antigen is a tumour-associating carbohydrate epitope whose expression correlates with metastasis and poor prognosis of various cancers; however, its pathophysiological function is poorly understood. Extracellular vesicles (EVs) derived from cancer cells act as a signal mediator amongst tumour microenvironments by transferring cargo molecules. sTn antigen has been found in the glycans of EVs, thereby the functional relevance of sTn antigen to the regulation of tumour microenvironments could be expected. In the present study, we showed that sTn antigen induced TP53 and tumour suppressor–activated pathway 6 (TSAP6) and consequently enhanced EV production. Besides, the genetic attenuation of TSAP6 resulted in the reduction of the EV production in the sTn antigen expressing cells. The enhanced EV production in the sTn antigen–expressing cells consequently augmented the delivery of EVs to recipient cells. The produced EVs selectively and abundantly encased focal adhesion kinase and transferred it to EV-recipient cells, and thus, their cellular motility was enhanced. These findings would contribute to facilitate the elucidation of the pathophysiological significance of the sTn antigen in the tumour microenvironments and tumour development.

Keywords: cellular motility, focal adhesion kinase, TSAP6, extracellular vesicle, Sialyl-Tn antigen

MOLECULAR BIOLOGY

Gene Expression

CEBPB is required for NRF2-mediated drug resistance in NRF2-activated non-small cell lung cancer cells

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NRF2 is a transcription activator that plays a key role in cytoprotection against oxidative stress. Although increased NRF2 activity is principally beneficial for our health, NRF2 activation in cancer cells is detrimental, as it drives their malignant progression. We previously found that CCAAT/enhancer-binding protein B (CEBPB) cooperates with NRF2 in NRF2-activated lung cancer and enhances tumour-initiating activity by promoting *NOTCH3* expression. However, the general contribution of CEBPB in lung cancer is rather controversial, probably because the role of CEBPB depends on cooperating transcription factors in each cellular context. To understand how NRF2 shapes the function of CEBPB in NRF2-activated lung cancers and its biological consequence, we comprehensively explored NRF2-CEBPB–coregulated genes and found that genes involved in drug metabolism and detoxification were characteristically enriched. Indeed, CEBPB and NRF2 cooperatively contribute to the drug resistance. We also found that CEBPB is directly regulated by NRF2, which is likely to be advantageous for the coexpression and cooperative function of NRF2 and CEBPB. These results

suggest that drug resistance of NRF2-activated lung cancers is achieved by the cooperative function of NRF2 and CEBPB.

Keywords: transcriptional regulation, gene expression, enhancers, drug resistance, cancer

NRF2 pathway activation attenuates ageing-related renal phenotypes due to α-klotho deficiency

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Oxidative stress is one of the major causes of the age-related functional decline in cells and tissues. The KEAP1–NRF2 system plays a central role in the regulation of redox balance, and NRF2 activation exerts antiageing effects by controlling oxidative stress in aged tissues. α-Klotho was identified as an ageing suppressor protein based on the premature ageing phenotypes of its mutant mice, and its expression is known to gradually decrease during ageing. Because α-klotho has been shown to possess antioxidant function, ageing-related phenotypes of α-klotho mutant mice seem to be attributable to increased oxidative stress at least in part. To examine whether NRF2 activation antagonizes ageing-related phenotypes caused by α-klotho deficiency, we crossed α-klotho–deficient ($Kl^{-/-}$) mice with a *Keap1*-knockdown background, in which the NRF2 pathway is constitutively activated in the whole body. NRF2 pathway activation in $Kl^{-/-}$ mice extended the lifespan and dramatically improved ageing-related renal phenotypes. With elevated expression of antioxidant genes accompanied by an oxidative stress decrease, the antioxidant effects of NRF2 seem to make a major contribution to the attenuation of ageing-related renal phenotypes of $Kl^{-/-}$ mice. Thus, NRF2 is expected to exert an antiageing function by partly compensating for the functional decline of α-Klotho during physiological ageing.

Keywords: α-klotho, oxidative stress, kidney, KEAP1–NRF2 system, ageing

CELL

Neurobiology

Autophagy-independent cytoprotection by optineurin from toxicity of aggregates formed by mutant huntingtin and mutant ataxin-3

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An important feature of several neurodegenerative diseases is the formation of pathological structures containing aggregated proteins. The autophagy receptor optineurin/OPTN is frequently observed in these structures. The role played by optineurin in these aggregates is not clear. In this study, we explored whether optineurin has a cytoprotective role in the cells having mutant protein aggregates. We overexpressed mutant huntingtin having 97 glutamine repeats (mHtt) and mutant ataxin-3 having 130 glutamine repeats (mAtax-3) in wild-type and optineurin-deficient neuronal (N2A) and non-neuronal cells ($Optn^{-/-}$ mouse embryonic fibroblasts) and determined the percentage of dead cells with mutant protein aggregates. Optineurin-deficient cells having mHtt or mAtax-3 aggregates showed higher cell death as compared to wild-type cells having mutant protein aggregates. Confocal microscopy revealed that optineurin formed a shell around mHtt and mAtax-3 aggregates through its C-terminal domain. The C-terminal domain of optineurin, which lacks LC3-interacting region required for autophagy, was necessary and sufficient to reduce cytotoxicity of mHtt and mAtax-3 aggregates. Our results show that in the absence of optineurin, mutant protein aggregates are highly toxic, revealing an autophagy-independent cytoprotective function of optineurin, which is mediated by its C-terminal domain.

Keywords: optineurin, neurodegeneration, mutant protein aggregates, mutant huntingtin, mutant ataxin-3, autophagy

Visualization of Reelin Secretion from Primary Cultured Neurons by Bioluminescence Imaging

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Reelin is a secreted glycoprotein important for brain development and synaptic plasticity in the adult brain. Some reports suggest that Reelin is secreted from the nerve terminals and functions as a neurotransmitter. However, the mechanism of Reelin secretion is unknown. In this study, we visualized Reelin secretion by bioluminescence imaging using a fusion protein of Reelin and *Gaussia* luciferase (GLase-Reelin). GLase-Reelin expressed in HEK293T cells was correctly processed and secreted. Luminescence signals from the secreted GLase-Reelin of primary cultured neurons were visualized by bioluminescence microscopy. Reelin secretory events were observed at neurites and cell bodies. Bioluminescence imaging was also performed

before and after KCl depolarization to compare the secretory events of Reelin and brain-derived neurotrophic factor (BDNF). The secretion of BDNF increased markedly shortly after depolarization. In contrast, the frequency of Reelin secretion did not change significantly by depolarization. Thus, Reelin secretion from neurites might not be regulated in a neuronal activity-dependent manner.

Keywords: Bioluminescence imaging, Gaussia luciferase, neuron, Reelin, secretion

BIOTECHNOLOGY

Biomimetic Chemistry

A circular RNA derived from FAT atypical cadherin 3 promotes lung cancer progression via forming a regulatory loop with oncogenic ELAV like RNA binding protein 1

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Circular RNA (circRNA) is a covalently closed endogenous RNA that participates in disease progression. However, its role in lung cancer is largely undetermined. In the present study, we found an onctogenic circRNA in lung cancer, FAT atypical cadherin 3 (FAT3) circRNA (circ-FAT3) was remarkably up-regulated in lung cancer in comparison to paired normal tissues. High circ-FAT3 was closely linked to larger tumour size, lymph node metastasis, later clinical stage, as well as dismal outcome. Stable knockdown of circ-FAT3 inhibited cell proliferation and metastasis both *in vitro* and *in vivo*. RNA binding protein ELAV like RNA binding protein 1 (HuR) was found to bind to introns flanking circ-FAT3, promoting the cyclization and generation of circ-FAT3. Further, circ-FAT3 was able to sponge miR-136-5p by acting as a competing endogenous RNA (ceRNA), alleviating the repressive effect of miR-136-5p on HuR mRNA at the transcriptional and post-transcriptional levels. Moreover, circ-FAT3 expression in lung cancer tissues was strongly positively and negatively correlated with HuR and miR-136-5p expression, respectively. Overall, our data reveal the previously uncharacterized regulatory loop of circ-FAT3/miR-136-5p/HuR in lung cancer and provide novel evidence for the importance of circRNA as a ceRNA in tumorigenesis.

Keywords: RNA binding protein, microRNA, lung cancer, competing endogenous RNA, circular RNA