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ダイジェスト

JB Commentary

Direct and indirect roles of GRWD1 in the inactivation of p53 in cancer

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Glutamate-rich WD40 repeat containing 1 (GRWD1), also known as WDR28, interacts with various proteins through its WD domain and is involved in transcription, translation, cell cycle progression, ubiquitin-mediated degradation and DNA replication and repair. Ribosomal protein L11 (RPL11), which directly interacts with MDM2, inhibits MDM2 ubiquitin ligase activity, thus promoting p53 stabilization. Binding of GRWD1 to RPL11 disrupts the interaction between RPL11 and MDM2andpromotesp53ubiquitinationbyMDM2.In addition, a recent report by Fujiyama et al. found that GRWD1also directly interacts with wild-type p53 and suppresses its transcriptional activity. They propose that GRWD1isanoveltumor-promotingmoleculethat negatively regulates wild-type p53 via both indirect and direct mechanisms.

Keywords: p53, GRWD1, cancer

Structural insight into replicative helicase loading in Escherichia coli

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DNA replication is an essential, precisely regulated process that occurs once in a cell cycle. In the Gramnegative bacterium Escherichia coli, the replicative helicase EcDnaB and the helicase loader EcDnaC play key roles in the initiation step at the replication origin, oriC. EcDnaB and EcDnaC form a heterododecamer, in which hexameric EcDnaB is bound to hexameric EcDnaC. Using genetic, biochemical and structural biology approaches, many groups have probed the mechanism of replicative helicase loading, using helicases and helicase loaders from various species. Recent X-ray crystallography and cryogenic electron microscopy (cryo-EM) structural studies of the EcDnaB–EcDnaC complex revealed that the interaction of DnaC with DnaB triggers distortion accumulation on the closed ring of hexameric DnaB, inducing DnaB subunits to adopt the open helical form for replication progression. The high-resolution crystal structure of the DnaB–DnaC complex solved by Nagata et al. contributed to a better understanding of the conformational rearrangement of the DnaB ring. In addition to the structural alterations in DnaB subunits by DnaC, the binding of single-stranded DNA (ssDNA) substrates alters the ATP- and ADP-bound forms of Dna Band Dna C.Thesestudies have proposed mechanisms by which DnaC regulates helicase loading onto ssDNA.

Keywords: X-ray crystallography, replicative helicase, helicase loader, DNA replication, cryo-EM

JB Review

Advances in understanding the mechanisms of repairing damaged nuclear envelop

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The nuclear envelope (NE) separates genomic DNA from the cytoplasm in eukaryotes. The structure of the NE is dynamically altered not only in mitotic disassembly and reassembly but also during interphase. Recent studies have shown that the NE is frequently damaged by various cellular stresses that degenerate NE components and/or disrupt their functional interactions. These stresses are referred to as 'NE stress'. Accumulating evidence has demonstrated that NE stress potentially causes severe cellular dysfunctions, such as cell death and genome in stability. In this review, the concept of NE stress, the processes repairing damage of the NE caused by NE stress, and the molecular mechanisms by which NE stress contributes to disease pathogenesis are introduced.

Keywords: OASIS, nuclear envelope stress, nuclear envelop athies, LEM domain proteins, Lamins

BIOCHEMISTRY

Enzymology

Identification and biochemical characterization of a heteromeric cis-prenyltransferase from the thermophilic archaeon Archaeoglobus fulgidus

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cis-Prenyl transferases (cPTs) form linear polyprenyl pyrophosphates, the precursors of polyprenyl or dolichyl phosphates that

are essential for cell function in all living organisms. Polyprenyl phosphate serves as a sugar carrier for peptidoglycan cell wall synthesis in bacteria, a role that dolichyl phosphate performs analogously for protein glycosylation in eukaryotes and archaea. Bacterial cP Ts are characterized by their homodimeric structure, while cPTs from eukaryotes usually require two distantly homologous subunits for enzymatic activity. This study identifies the subunits Graphical Abstract of heteromeric cPT, Af1219 and Af0707, from a thermophilic sulphur-reducing archaeon, Archaeoglobus fulgidus. Both subunits are indispensable for cPT activity, and their protein–protein interactions were demonstrated by a pulldown assay. Gel filtration chromatography and chemical cross-linking experiments suggest that Af1219 and Af0707 likely form a hetero tetramer complex. Although this expected subunit composition agrees with a reported hetero tetrameric structure of human hCIT/NgBR cPT complex, the similarity of the quaternary structures is likely a result of convergent evolution.

Keywords: archaea, cis-prenyl transferase, dolichol, enzyme structure, isoprenoid

Inhibitory effect of ascorbate on tryptophan 2,3-dioxygenase

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Tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO) catalyse the same reaction, oxidative cleavage of L-tryptophan (L-Trp) to Nformyl-kynurenine. In both enzymes, the ferric form is inactive and ascorbate (Asc) is frequently used as a reductant in in vitro assays to activate the enzymes by reducing the heme iron. Recently, it has been reported that Asc activates IDO2 by acting as a reductant; however, it is also a competitive inhibitor of the enzyme. Here, the effect of Asc on human TDO (hTDO) is investigated. Similar to its interaction with IDO2, Asc acts as both a reductant and a competitive inhibitor of hTDO in the absence of catalase, and its inhibitory effect was enhanced by the addition of H2O2. Interestingly, however, no inhibitory effect of Asc was observed in the presence of catalase. TDO is known to be activated by H2O2 and a ferryl-oxo (FeIV＝O) intermediate (Compound II) is generated during the activation process. The observation that Asc acts as a competitive inhibitor of hTDO only in the absence of catalase can be explained byassumingthatthetargetof Asc is Compound II. Asc seems to compete with L-Trp in an unusual manner.

Keywords: ascorbate, catalase, competitive inhibition, hydrogen peroxide, tryptophan 2,3-dioxygenase

Enzyme Inhibitors

Fragment-based drug discovery for Trypanosoma brucei Glycosylphosphatidylinositol-Specific Phospholipase C through biochemical and WaterLOGSY-NMR Methods

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Glycosylphosphatidylinositol-specific phospholipase C (GPI-PLC) of Trypanosoma brucei, the causative protozoan parasite of African trypanosomiasis, is a membrane-bound enzyme essential for antigenic variation, because it catalyses the release of the membrane bound form of variable surface glycoproteins. Here, we performed a fragment-based drug discovery of Tb GPIPLC inhibitors using a combination of enzymatic inhibition assay and water ligand observed viagradient spectroscopy (Water LOGSY) NMR experiment. The Tb GPI-PLC was cloned and overexpressed using an Escherichia coli expression system followed by purification using three-phase partitioning and gel filtration. Subsequently, the inhibitory activity of 873 fragment compound sagainst there combinant TbGPI- PLCledto the identification of 66 primary hits.These primary hits were subjected to the Water LOGSY NMR experiment where 10 fragment hits were confirmed to directly bind to the Tb GPI-PLC. These included benzothiazole, chlorobenzene, imidazole, indole, pyrazol and quinolinone derivatives. Molecular docking simulation indicated that six of them share a common binding site, which corresponds to the catalytic pocket. The present study identified chemically diverse fragment hits that could directly bind and inhibit the Tb GPI-PLC activity, which constructed a framework for fragment optimization or linking towards the design of novel drugs for African trypanosomiasis.

Keywords: antigenic variation, catalytic pocket, docking simulation, enzyme inhibition, GPI anchor

Biochemical Pharmacology

Hybrid molecule between platanic acid and LCL-161 as a yes-associated protein degrader

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Dysregulated yes-associated protein (YAP) is involved in several malignant cancers. However, discovering a druggable YAP inhibitor(s) is difficult because YAP itself does not have any enzymatic activity. In such cases, targeted protein degradation strategies based on hybrid molecules that bind to the target protein and an E3 ubiquitin ligase are useful for suppressing proteins that exhibit aberrant activation and/or excessive expression. Upon screening YAP-interacting small compounds, we identified HK13, a platanic acid, as a novel compound that interacts with YAP. Next, we synthesized hybrid compounds of platanic acid and LCL161, which reportedly shows a high affinity for cIAP, one of E3 ubiquitin ligases. Among these compounds, HK24possessedtheabilitytoinhibit the growth of YAP overexpressing NCI-H290 cells. This inhibitory activity may be mediated by YAP degradation, although HK24 exhibited weak YAP degradation. Furthermore, we confirmed involvement of proteasome pathway in HK24-dependent YAP degradation by culturing NCIH290 cells in the presence of a proteasome inhibitor. Therefore, it is possible that platanic acid is a potential candidate for molecular medicine targeting YAP.

Keywords: anti-cancer drug, malignant mesothelioma, platanic acid, SNIPER, yes-associated protein

MOLECULAR BIOLOGY

Molecular Biology General

MiR-29a regulates cardiomyocyte apoptosis by targeting Bak1 in diabetic cardiomyopathy

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This studysoughttoinvestigate the association between microRNA-29a (miR-29a) and cardiomyocyte apoptosis in diabetic cardiomyopathy (DCM). DCMratmodel was established by treating rats with streptozotocin Graphical Abstract Control Streptozotocin (STZ) DCM DCM+NC (STZ), followed byinjection of NCormiR-29a-3pmimics into the myocardium of rats. High glucose (HG)treated H9c2 cells were transfected with NC and miR29a-3p mimics. DCM rats presented elevated levels of blood glucose, HbA1c, blood pressure, urine output, decreased bodyweightandcardiaccontractile function after modeling. MiR-29a was lowly expressed in STZtreated rats and HG-treated H9c2 cells. Upregulation of miR-29a improved cardiac structure and function and attenuated, alleviated myocardial histological abnormalities and fibrosis and lowered cardiomyocyte apoptosis in DCM rats. Meanwhile, HG promoted H9c2 cell apoptosis, while miR-29a overexpression attenuated the function of HG. Compared with control group, the protein expression of Bax, cleaved-caspase3 and Bak1 in DCM and HG groups were significantly upregulated, and the expression of Bcl-2 and Mcl-1 was downregulated, while miR-29a overexpression exerted opposite effect. Bioinformatics prediction methodandwesternblotrevealedthatmiR-29adirectly targeted Bak1 and downregulated Bak1 expression. Overall, miR-29a regulated STZ- and HG-induced cardiomyocyte apoptosis by targeting Bak1, providing a novel understanding of the pathogenesis of DCM.

Keywords: miR-29a, high glucose, diabetic cardiomyopathy, cardiomyocyte apoptosis, Bak1

Gene Expression

vp1524, a Vibrio parahaemolyticus NAD +-dependent deacetylase, regulates host response during infection by induction of host histone deacetylation

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Gram-negative intracellular pathogen Vibrio parahaemolyticus manifests its infection through a series of effector proteins released into the host via the type III secretion system. Most of these effector proteins alter sign alling pathways of the host to facilitate survival and proliferation of bacteria inside host cells. Here, we report V. parahaemolyticus (serotype O3:K6) infection induced histone deacetylation in host intestinal epithelial cells, particularly deacetylation of H3K9, H3K56, H3K18 and H4K16 residues. We found a putative NAD+-dependent deacetylase, vp1524 (vp Cob B) of V. parahaemolyticus, was overexpressed during infection. BiochemicalassaysrevealedthatVp1524isafunctional NAD+-dependent Sir2 family deacetylase in vitro, which was capable of deacetylating acetylated histones. Furthermore,

we observed that vp1524 is expressed and localized to the nuclear periphery of the host cells during infection. Consequently, Vp1524 translocated to nuclear compartments of transfected cells, deacetylated histones, specifically causing deacetylation of those residues (K56, K16, K18) associated with V. parahaemolyticus infection. This infection induced deacetylation resulted in transcriptional repression of several host genes involved in epigenetic regulation, immune response, autophagy etc. Thus, our study shows that a V. parahaemolyticus lysine deacetylase Vp1524issecretedinsidethehostcells during infection, modulating host gene expression through histone deacetylation.

Keywords: V. parahaemolyticus proteins, prokaryotic NAD+-dependent deacetylase, nuclear localization, lysine deacetylation, host–pathogen interaction

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ダイジェスト

JB Reviews

Structural basis for the linkage specificity of ubiquitin-binding domain and eubiquitinase

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Ubiquitination is a post-translational modification system essential for regulating a wide variety of biological processes in eukaryotes. Ubiquitin (Ub) itself under goes post-translational modifications, including ubiquitination. All seven lysine residues and one N-terminal amino group of Ub can act as acceptors for further ubiquitination, producing eight types of Ub chains. Ub chains of different linkage types have different cellular functions and are referred to as the ‘ubiquitin code’. Decoder molecules that contain linkage-specific Ubbinding domains (UBDs) recognize the Ub chains to regulate different cellular functions. On the other hand, deubiquitinases (DUBs) cleave Ub chains to reverse ubiquitin signals. This review discusses the molecular mechanisms of linkage-specific recognitions of Ub chains by UBDs and DUBs, which have been revealed by structural studies.

Keywords: ubiquitin code, ubiquitin chain, ubiquitin binding domain, linkage specificity, deubiquitinase

Epigenetic and environmental regulation of adipocyte function

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Adipocytes play an essential role in the maintenance of whole-body energy homeostasis. White adipocytes regulate energy storage, whereas brown and beige adipocytes regulate energy expenditure and heat production. De novo production of adipocytes (i.e. adipogenesis) and their functions are dynamically controlled by environmental cues. Environmental changes (e.g. temperature, nutrients, hormones, cytokines) are transmitted via intracellular signaling to facilitate short-term responses and long-term adaptation in adipocytes; however, the molecular mechanisms that link the environment and epigenome are poorly understood. Our recent studies have demonstrated that environmental cues dynamically regulate interactions between transcription factors and epigenomic chromatin regulators, which together trigger combinatorial changes in chromatin structure to influence gene expression in adipocytes. Thus, environmental sensing by the concerted action of multiple chromatin associated protein complexes is a key determinant of the epigenetic regulation of adipocyte functions.

Keywords: post-translational modifications, phosphorylation, histone methylation, histone acetylation, adipogenesis

BIOCHEMISTRY

Biochemistry General

Investigation of serum markers of esophageal squamous cell carcinoma based on machine learning methods

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Esophageal squamous cell carcinoma (ESCC) is one of the malignant tumors with high mortality in humans, and there is a lack of effective and convenient early diagnosis methods. By analyzing the serum miRNA expression data in ESCC tumor samples and normal samples, on the basis of the maximal relevance and minimal redundancy (mRMR) feature selection and the incremental feature selection method, a random forest classifier constructed by five-feature miRNAs was acquired in our study. The receiver operator characteristic curve showed that the model was able to distinguish samples. Principal component analysis (PCA) and sample hierarchical cluster analysis showed that five-feature miRNAs could well distinguish ESCC patients from healthy individuals. The expression levels of miR-663a, miR-

5100 and miR-221-3p all showed a higher expression level in ESCC patients than those in healthy individuals. On the contrary, miR-6763-5p and miR-7111-5p both showed lower expression levels in ESCC patients than those in healthy individuals. In addition, the collected clinical serum samples were used for qRT-PCR analysis. It was uncovered that the expression trend softhe five-feature miRNAs follow eda similar pattern with those in the training set. The above findings indicated that the five-feature miRNAs may be serum tumor markers of ESCC. This study offers new insights for the early diagnosis of ESCC.

Keywords: tumor serum markers, principal component analysis, mRMR feature selection, machine learning, incremental feature selection

Enzymology

Identification and characterization of a serine racemase in the silkworm Bombyx mori

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The pupae of lepidopterans contain high concentrations of endogenous d-serine. In the silkworm Bombyx mori, d-serine is negligible during the larval stage but increases markedly during the pupal stage, reaching 50% of the total free serine. However, the physiological function of d-serine and the enzyme responsible for its production is unknown. Herein, we identified a new type of pyridoxal 5-phosphate (PLP)-dependent serine racemase (SR) that catalyses the racemization of l-serine to d-serine in B. mori. This silkworm SR (BmSR) has an N-terminalPLP-binding domain that is homologous to mammalian SR and a C-terminal putative ligand-binding regulatory-like domain (ACT-like Graphical Abstract domain) that is absent in mammalian SR. Similar to mammalian SRs, BmSR catalyses the racemization and dehydration of both serine isomers. However, BmSR is different from mammalian SRs as evidenced by its insensitivity to Mg^{2+}/Ca^{2+} and Mg-ATP—which are required for activation of mammalian SRs—and high d-serine dehydration activity. At the pupal stage, the SR activity was predominantly detected in the fat body, which was consistent with the timing and localization of BmSR expression. The results are an important first step in elucidating the physiological significance of dserine in lepidopterans.

Keywords: silkworm, serine racemase, D-serine, D-amino acid, B. mori

Biochemical Pharmacology

Down-regulation of ABCB1 by collateral sensitivity drugs reverses multidrug resistance and up-regulates enolase I

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The emergence of drug resistance remains an obstacle in the clinical treatment of cancer. Recent developments in the studies of drug resistance have identified compounds such as verapamil and tamoxifen that specifically target ABCB1-expressing multi drug resistant (MDR) cells, through an ATP-dependent ROS-generating mechanism. In this report, we demonstrate that treatment of ABCB1-expressing MDR cells (CHORC5 or MDA-Doxo400) or individual clones of the latter with sublethal concentrations of tamoxifen or verapamil down-regulates ABCB1 protein and mRNA expression in surviving clones. Consequently, tamoxifen- and verapamil-treated cells show increased sensitivity to chemotherapeutic drugs (e.g., colchicine and doxorubicin) and decreased sensitivity to collateral sensitivity drugs (e.g., verapamil and tamoxifen). Importantly, we show for the first time that downregulation of ABCB1 expression resulting from tamoxifen treatment and CRISPR-knockout of ABCB1 expression up-regulate α-enolase (enolase I) protein levels and activity. These findings demonstrate a possible effect of ABCB1 expression on the metabolic homeostasis of MDR cells. Moreover, given the use of tamoxifen to prevent the recurrence of oestrogen receptor-positive breast cancer, the findings of this study may be clinically important in modulating activity of other drugs.

Keywords: tamoxifen, multidrug Resistance, enolase I, collateral sensitivity, ABCB1

BIOTECHNOLOGY

Gene and Protein Engineering

Effect of an intermolecular disulfide bond introduced into the first loop of CH1 domain of Adalimumab Fab on thermal stability and antigen-binding activity

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The introduction of intermolecular disulfide bonds by amino acid mutations is an effective method for stabilizing dimeric proteins. X-ray crystal structure of Fab of a therapeutic antibody, adalimumab, revealed the first loop of the CH1 domain to be partially unsolved at position 135–141. To find new sites

for the introduction of intermolecular disulfide bonds in adalimumab Fab, Fab mutants targeting the unsolved region were predicted using molecular simulation software. Four Fab mutants, H:K137C-L:I117C, H:K137C-L:F209C, H:S138C-L:F116C and H:S140CL:S114C, were expressed in the methylotrophic yeast Pichia pastoris. SDS-PAGE analysis of these mutants indicated that H:K137C-L:F209C, H:S138C-L:F116C and H:S140C-L:S114C mutants mostly formed intermolecular disulfide bonds, whereas some H:K137CL:I117C mutants formed intermolecular disulfide bonds and some did not. Differential scanning calorimetry measurements showed increased thermal stability in all Fabmutants with engineer eddi sulfide bonds. The bio-layer interferometry measurements, for binding of the antigen tumor necrotic factor α, indicated that Fab mutants had less antigen-binding activity than wildtype Fab. In particular, the KD value of H:K137CL:F209C was ～17 times higher than that of wild-type Fab. Thus, we successfully introduced intermolecular disulfide bonds between the first loop region of the CH1 and CL domains and observed that it increases the thermostability of Fab and affects the antigen-binding activity.

Keywords: thermostability, internal disulfide bond, Fab, antigen-binding activity, adalimumab

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ダイジェスト

JB Commentary

TT-pocket/HIRAN: binding to 3 -terminus of DNA for recognition and processing of stalled replication forks

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Stalled replication forks need to be swiftly detected and protected from collapse and the cause for fork stall be removed to restore the active replication fork. In bacteria, stalled forks are recognized and stabilized by PriA, aDEXH-type helicase, which also facilitatesre assembly of an active replication fork. A TT-pocket (three-prime terminus binding pocket) present in the N-terminal segment of PriA plays a crucial role in stabilization of the stalled forks by specifically binding to the 3′terminusof then ascent leading strand. Eukaryoticproteins, Rad5/HLTF, contain a TT-pocket related domain, HIRAN, that specifically binds to 3′-terminus of DNA and play a role installed fork processing. While the TT pocket of Pri A facilitates the formation of an apparently stable and immobile complex on a fork with a 3′ terminus at the fork junction, HIRAN of Rad5/HLTF facilitates fork regression by itself. A recent report shows that HIRAN can displace 3 nucleotides at the end of the duplex DNA, providing mechanistic insight into how stalled forks arer ever sedineukaryotes. Inthis article, I will compare the roles of 3′-terminus binding domains installed fork processing in prokaryotes and in eukaryotes.

Keywords: HLTF, Pri A, stalled replication fork, 3′-terminus binding, fork reversal, TT-pocket/HIRAN

JB Review

Targeted protein degradation and drug discovery

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Targeted protein degradation attracts attention as a novel modality for drug discovery, as well as for basic research. Various types of degrader molecules have been developed so far, which include proteolysis targeting chimaeras (PROTACs) and specific and nongenetic IAP-dependent protein erasers (SNIPERs), E3 modulators, hydrophobic tagging molecules, IAP antagonists and deu biquity lase inhibitors. PROTACs and SNIPERs are chimeric degrader molecules consisting of a target ligand linked to another ligand that binds to an E3 ubiquitin ligase. In the cells, they recruit an E3 ligase to the target protein, thereby inducing ubiquitylation and proteasomal degradation of the target protein. Because of their modular structure, novel PROTACs and SNIPERs targeting proteins of your interest can be rationally developed by substituting target ligands. In this article, various compounds capable of inducing protein degradation were overviewed, including SNIPER compounds developed in our laboratory.

Keywords: PROTACs, proteasome, SNIPERs, targeted protein degradation, ubiquitin

Rapid Communication

2,2,6,6-Tetramethylpiperidine-1-oxyl acts as a volatile inhibitor of ferroptosis and neurological injury

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Ferroptosis, a type of oxidative stress cell death, has been implicated in cell injury in several diseases, and treatments with specific inhibitors have been shown to protect cells and tissues. Here we demonstrated that a treatment with the nitroxide radical, 2,2,6,6tetramethylpiperidine-N-oxyl(TEMPO),prevented the ferroptotic cell death in an airborne manner. Other TEMPO derivatives and lipophilic antioxidants, such as Trolox and ferrostatin-1, also prevented cell death induced by erastin and RSL3; however, only TEMPO exhibited inhibitory activity from a physically distant location. TEMPO vaporized without decomposing and then dissolved again into a nearby water solution. Volatilized TEMPO inhibited glutamate-induced cell death in mouse hippocampal cell lines and also reduced neuronal cell death in a mouse ischemia model. These results suggest that TEMPO is a unique cell protective agent that acts in a volatility-mediated manner.

Keywords: gas, ischemia, lipid peroxidation, oxytosis, radical scavenger

BIOCHEMISTRY

Biochemistry General

Structural and biochemical analyses of the nucleosome containing Komagataella pastoris histones

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Komagataella pastoris is a methylotrophic yeast that is commonly used as a host cell for protein production. In the present study, we reconstituted the nucleosome with *K. pastoris* histones and determined the structure of the nucleosome core particle by cryogenic electron microscopy. In the *K. pastoris* nucleosome, the histones form an octamer and the DNA is left-handedly wrapped around it. Micrococcal nuclease assays revealed that the DNA ends of the *K. pastoris* nucleosome are somewhat more accessible, as compared with those of the human nucleosome. *In vitro* transcription assays demonstrated that the *K. pastoris* nucleosome is transcribed by the *K. pastoris* RNA polymerase II (RNAPII) more efficiently than the human nucleosome, while the RNAPII pausing positions of the *K. pastoris* nucleosome are the same as those of the human nucleosome. These results suggested that the DNA end flexibility may enhance the transcription efficiency in the nucleosome but minimally affect the nucleosomal pausing positions of RNAPII.

Keywords: transcription, nucleosome, *K. pastoris*, histone, cryo-EM

Decreased EMILIN2 correlates to metabolism phenotype and poor prognosis of ovarian cancer

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This study aimed to explore the function and related mechanisms of elastin microfibril inter facer 2 (EMILIN2) in ovarian cancer. First, the expression level of EMILIN2 was detected in patient tissues and its correlation with overall survival rate was analysed. Then, EMILIN2 was overexpressed in ovarian cancer cell lines to observe its function and effect on Warburg effect. By detecting its promoter region methylation, the epigenetic regulatory role was explored. Finally, through the luciferase reporter assay and siRNA tools, the regulatory mechanism of p53 on EMILIN2 was investigated. It was detected in clinical samples that down-regulated EMILIN2 was associated with poor prognosis of ovarian cancer. It was further found that EMILIN2 regulated the metabolic phenotype of ovarian cancer cells. The expression of EMILIN2 was epigenetically regulated by its promoter methylation. Also, it was found that p53 regulated the expression of EMILIN2 and the p53/EMILIN2 axis regulated the Warburg effect in ovarian cancer cells. EMILIN2 was inhibited by methylation in ovarian cancer. In summary, p53 can promote and regulate its transcription bybindingtothepromoterregionofEMILIN2,t hereby affecting the Warburg effect and inhibiting tumours. Therefore, EMILIN2 might be a potential target for clinical diagnosis and treatment of ovarian cancer.

Keywords: Warburg effect, P53, methylation, metabolism, glycolysis

MOLECULAR BIOLOGY

Molecular Biology General

Functional analysis of the N-terminal region of Vibrio FlhG, a MinD-type ATPase in fl agellar number control

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GTPase FlhF and ATPase FlhG are two key factors involved in

regulating the flagellum number in Vibrio alginolyticus. FlhG is a paralogue of the Escherichia coli cell division regulator MinD and has a longer N-terminal region than MinD with a conserved DQAxxLR motif. The deletion of this N-terminal region or a Q9A mutation in the DQAxxLR motif prevents FlhG from activating the GTPase activity of FlhF in vitro and causes a multiflagellation phenotype. The mutant FlhG proteins, especially the N-terminally deleted variant, were remarkably reduced compared to that of the wild-type protein in vivo. When the mutant FlhG was expressed at the same level as the wild-type FlhG, the number of flagella was restored to the wild-type level. Once synthesized in Vibrio cells, the N-terminal region mutation in FlhG seems not to affect the protein stability. We speculated that the flhG translation efficiency is decreased by N-terminal mutation. Our results suggest that the N-terminal region of FlhG controls the number of flagella by adjusting the FlhF activity and the amount of FlhG in vivo. We speculate that there gelation by FlhG, achieved through transcription by the master regulator FlaK, is affected by the mutations, resulting in reduced flagellar formation by FlhF.

Keywords: Vibrio alginolyticus, MinD, FlhF, FleN, flagella, ATPase

Polyamines produced by an extreme thermophile are essential for cell growth at high temperature

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An extreme thermophile, Thermus thermophilus grows at an optimum temperature of around 70°C and produces 16different polyamines including long-chain and branched-chain polyamines. We found that the composition of polyamines in the thermophile cells changes with culture temperature. Long-chain and branch edchain polyamines (unusual polyamines) were increased in the cells grown at high temperature such as 80°C, but they were minor components in the cells grown at relatively lower temperature such as 60°C. The effects of polyamines on cell growth were studied using T. thermophilus HB8 speA deficient in arginine decarboxylase. Cell growth of this mutant strain was significantly decreased at 70°C. This mutant strain cannot produce polyamines and grows poorly at 75°C. It was also determined whether polyamines are directly involved in protecting DNA from DNA double-strand breaks (DSBs) induced by heat. Polyamines protected DNA against double-strand breaks. Therefore, polyamines play essential roles in cell growth at extremely high temperature through maintaining a functional conformation of DNA against DSBs and depurination.

Keywords: Thermus thermophilus, polyamine, high temperature, DNA double-strand breaks, cell growth

RNA Processing

Cancer-associated mutations in SF3B1 disrupt the interaction between SF3B1 and DDX42

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While cancer-associated SF3B1 mutations causes alternative RNA splicing, the molecular mechanism underlying the alternative RNA splicing is not fully elucidated. Here, we analysed the proteins that interacted with the wild-type and K700E-mutated SF3B1 and found that the interactions of two RNA helicases, DDX42 and DDX46, with the mutated SF3B1 were reduced. Overexpression of DDX42 restored the decreased interaction between DDX42 and the K700Emutated SF3B1, and suppressed some alternative RNA splicing associated with the SF3B1 mutation. Mutation that decreased the ATP hydrolysis activities of DDX42 abolished the suppressive effects of DDX42 on the alternative RNA splicing, suggesting that the ATP hydrolysis activity of DDX42 is involved in the mechanism of the altered RNA splicing associated with the SF3B1 mutation. Our study demonstrates an important function of the interaction between DDX42 and SF3B1 on regulating RNA splicing and revealed a potential role of DDX42 in the altered RNA splicing associated with the SF3B1 mutation.

Keywords: U2 snRNP, SF3B1, DDX42, ATP hydrolysis, alternative splicing