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ダイジェスト

BIOCHEMISTRY

Biochemistry General

Efficient induction of proximity-dependent labelling by biotin feeding in BMAL1-BioID knock-in mice

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Proximity-dependent biotin identification (BioID) is a useful method to identify unknown protein–protein interactions. Few reports have described genetically engineered knock-in mouse models for *in vivo* BioID. Thus, little is known about the proper method for biotin administration and which tissues are applicable. Here, we established a BioID knock-in mouse model of Brain and Muscle ARNT-Like 1 (BMAL1) and the BirA biotin ligase with R118G mutation (BirA*). The BMAL1-BioID mouse model was used to investigate the effect of biotin diet feeding on protein biotinylation in several tissues. The BMAL1-BirA* fusion protein-retained proper intracellular localization of BMAL1 and binding to CLOCK protein in HEK293T cells. A biotin labelling assay in mouse embryonic fibroblasts revealed the protein biotinylation activity of BMAL1-BirA* expressed in knock-in mouse cells depending on biotin supplementation. Lastly, feeding a 0.5% biotin diet for 7 days induced protein biotinylation in the brain, heart, testis and liver of BMAL1-BioID mice without adverse effects on spermatogenesis. In the kidney, the biotin diet increased biotinylated protein levels in BMAL1-BioID and control mice, suggesting the existence of endogenous biotinylation activity. These results provide valuable information to optimize the *in vivo* BioID procedure.

Keywords; biotin feeding, BMAL1, circadian rhythm, in vivo BioID, knock-in mice

Protein Structure

A slight bending of an α-helix in FliM creates a counterclockwise-locked structure of the fl agellar motor in Vibrio

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Many bacteria swim by rotating flagella. The chemotaxis system controls the direction of flagellar rotation. *Vibrio alginolyticus*, which has a single polar flagellum, swims smoothly by rotating the flagellar motor counterclockwise (CCW) in response to attractants. In response to repellents, the motor frequently switches its rotational direction between CCW and clockwise (CW). We isolated a mutant strain that swims with a CW-locked rotation of the flagellum, which pulls rather than pushes the cell. This CW phenotype arises from a R49P substitution in FliM, which is the component in the C-ring of the motor that binds the chemotaxis signalling protein, phosphorylated CheY. However, this phenotype is independent of CheY, indicating that the mutation produces a CW conformation of the C-ring in the absence of CheY. The crystal structure of FliM with the R49P substitution showed a conformational change in the N-terminal α-helix of the middle domain of FliM ($FliM_M$). This helix should mediates FliM–FliM interaction. The structural models of wild type and mutant C-ring showed that the relatively small conformational change in $FliM_M$ induces a drastic rearrangement of the conformation of the $FliM_M$ domain that generates a CW conformation of the C-ring.

Keywords; bacteria, chemotaxis, conformation, molecular motors, X-ray crystallography

Protein Interaction and Recognition

Ectopic BH3-only protein Bim acts as a cochaperone to positively regulate Hsp70 in yeast

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The chaperone heat shock protein 70 (Hsp70) is conserved from bacteria to humans and is crucial for avoiding protein misfolding under stress. Bim functions, mainly as one of the B-cell lymphoma 2 (Bcl-2) family proapoptotic members, were identified to be a cochaperone of Hsp70. Herein, we reported that ectopic Bim could constitute the interactions with intrinsic Hsp70 and translate its positive cochaperone activity *in vitro* to the yeast growth promotion and help Hsp70 to fold its client Ras-like protein. With the help of a specific Hsp70/Bim disruptor, we illustrated that Hsp70/Bim dimers rescue yeast from heat shock. In an organism lacks apoptotic Bcl-2 factors, the proapoptotic Bim in mammalian cells exhibits prosurvival functions.

Keywords; B-cell lymphoma 2 family, Bim, cochaperone, 70 kilodalton heat shock protein, yeast

Enzymology

A mutant equipped with a regenerated disulphide for the missing His loop of a serine protease zymogen in the horseshoe crab coagulation cascade

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The lipopolysaccharide (LPS)-triggered coagulation cascade in horseshoe crabs is composed of three zymogens belonging to the trypsinogen family: prochelicerase C, prochelicerase B (proB) and the proclotting enzyme (proCE). Trypsinogen-family members contain three conserved disulphides located around the active site. While it is known that proB evolutionarily lost one of the disulphides, the His-loop disulphide, the roles of the missing His-loop disulphide in proB remain unknown. Here, we prepared a proB mutant, named proB-murasame, equipped with a regenerated His-loop disulphide. The activation rate by upstream α-chelicerase C for proB-murasame was indistinguishable from that for wild-type (WT) proB. The resulting protease chelicerase B-murasame exhibited an 8-fold higher k_{cat} value for downstream proCE than WT chelicerase B, whereas the K_m value of chelicerase B-murasame was equivalent to that of WT chelicerase B. WT serpins-1, -2 and -3, identified as scavengers for the cascade, had no reactivity against WT chelicerase B, whereas chelicerase B-murasame was inhibited by WT serpin-2, suggesting that WT chelicerae B may trigger as-yet-unsolved phenomena after performing its duty in the cascade. The reconstituted LPS-triggered cascade containing proB-murasame exhibited ~5-fold higher CE production than that containing WT proB. ProB-murasame might be used as a high value-adding reagent for LPS detection.

Keywords; haemolymph coagulation, lipopolysaccharide, recombinant protein, serine protease, serpin

Overexpression and characterization of Escherichia coli dihydropyrimidine dehydrogenase: a four iron-sulphur cluster containing fl avoprotein

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Escherichia coli dihydropyrimidine dehydrogenase (EcDPD) catalyses the NADH-dependent reduction of uracil and thymine to the corresponding 5,6-dihydropyrimidines to control their metabolite pools. EcDPD consists of two subunits, PreT and PreA, and requires FAD, FMN and Fe-S clusters for activity. Recombinant EcDPD with a C-terminal His_6-tagged-PreA subunit was overproduced in a DPD-lacking *E. coli* cells with augmented Fe-S cluster synthesis. Anaerobic purification resulted in purified enzyme with a specific activity of $13\,\mu mol\,min^{-1}mg^{-1}$. The purified EcDPD was a heterotetramer and contained 0.81 FAD, 0.99 FMN, 14 acid-labile sulphur and 15 iron per PreT-PreA dimer. The enzyme exhibited Michaelis–Menten kinetics for both the forward and reverse reactions, which is distinct from mammalian DPDs showing substrate inhibition kinetics. For uracil reduction, the k_{cat}, k_{cat}/K_{NADH} and k_{cat}/K_{uracil} values were constant over the pH range of 5.5–10. For dihydrouracil (DHU) dehydrogenation, the pH-dependence of the k_{cat} and k_{cat}/K_{NAD+} values indicated that a residue with a pK_a of 6.6 must be deprotonated for activity. Biochemical and kinetic comparisons with pig DPD revealed that protonation sates of the catalytically competent forms of EcDPD are distinct from those of pig enzyme.

Keywords; dihydropyrimidine dehydrogenase, flavin, iron-sulphur cluster, kinetics, uracil

Biochemistry of Proteolysis

Elastolytic activity is associated with infl ammation in bladder cancer

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Cancer development and progression is often associated with

inflammation. Late diagnosis of inflammation that directly leads to the development of neoplasm—cancer is associated with a reduction in the chance of successful treatment or is associated with therapeutic difficulties. A panel of chromogenic substrates was used for the qualitative determination of the specific activity of enzymes in urine of patients with confirmed inflammatory reaction and/or epithelial neoplasms in particular tumours at various stages of development. Urine of people with excluded inflammation was used as a control group. Proteolytic activity was determined in urine samples collected from patients with epithelial neoplasms and/or inflammation. What is more, we determine human neutrophil elastase activity-related inflammation based on the examination of urine samples. We suspect that the proteolytical activity of urine samples is due to neutrophil response to inflammation, which is directly related to cancer. This is the first study to determine elastolytic activity in bladder cancer urine samples. It supports wider use of urine for inflammation screening.

Keywords; chromogenic substrates, inflammation, neoplasm

Biochemistry in Diseases and Aging

Functional analyses of plasmodium ferredoxin Asp97Tyr mutant related to artemisinin resistance of human malaria parasites

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Mutation of Asp97Tyr in the C-terminal region of ferredoxin (PfFd) in the apicoplast of malaria parasites was recently reported to be strongly related to the parasite's resistance to the frontline antimalarial drug, artemisinin. We previously showed that the aromatic amino acid in the C-terminal region of PfFd is important for the interaction with its electron transfer partner, Fd-$NADP^+$ reductase (PfFNR). Here, the importance of the aromatic–aromatic interaction between PfFd and PfFNR was shown using the kinetic analysis of the electron transfer reaction of site-directed mutants of PfFNR with PfFd. Mutation of Asp97Tyr of PfFd was further shown to increase the affinity with PfFNR by the measurements of the dissociation constant (K_d) using tryptophan fluorescence titration and the Michaelis constant (K_m) in the kinetic analysis with PfFNRs. Diaphorase activity of PfFNR was inhibited by D97Y PfFd at lower concentration as compared to wild-type PfFd. Ascorbate radical scavenging activity of PfFd and electron transfer activity to a heterogeneous Fd-dependent enzyme was lower with D97Y PfFd than that of wild-type PfFd. These results showed that D97Y mutant of PfFd binds to PfFNR tighter than wild-type PfFd, and thus may suppress the function of PfFNR which could be associated with the action of artemisinin.

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

Biochemical Pharmacology

Salivary neopterin and related pterins: their comparison to those in plasma and changes in individuals

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Neopterin (NP), biopterin (BP) and monapterin (MP) exist in saliva. The physiological role of salivary NP as well as the pathophysiological role of increased NP in the immune-activated state has been unclear. Saliva is a characteristic specimen different from other body fluids. In this study, we analysed salivary NP and related pterin compounds, BP and MP and revealed some of its feature. High-performance liquid chromatography (HPLC) analysis of saliva and plasma obtained from 26 volunteers revealed that salivary NP existed mostly in its fully oxidized form. The results suggested that salivary NP as well as BP would mostly originate from the oral cavity, perhaps the salivary glands, and that salivary NP levels might not reflect those in the plasma. We also found that a gender difference existed in correlations between concentrations of salivary total concentrations of NP (tNP) and BP (tBP). HPLC analysis of saliva obtained from 5 volunteers revealed that the concentrations of salivary tNP as well as tBP fluctuated in an irregular fashion in various individuals. MP, a diastereomer of NP, might have come from oral cavity NP itself or its precursor. These results indicated that the nature of salivary NP might be different from that of NP in the blood or urine.

Keywords; neopterin, biopterin, monapterin, salive, plasma

CELL

Biomembranes, Organelles, and Protein Sorting

Toxicity and membrane perturbation properties of the ribotoxin-like protein Ageritin

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Ageritin is the prototype of a new ribotoxin-like protein family, which has been recently identified also in basidiomycetes. The protein exhibits specific RNase activity through the cleavage of a single phosphodiester bond located at sarcin/ricin loop of the large rRNA, thus inhibiting protein biosynthesis at early stages. Conversely to other ribotoxins, its activity requires the presence of divalent cations. In the present study, we report the activity of Ageritin on both prokaryotic and eukaryotic cells showing that the protein has a prominent effect on cancer cells viability and no effects on eukaryotic and bacterial cells. In order to rationalize these findings, the ability of the protein to interact with various liposomes mimicking normal, cancer and bacterial cell membranes was explored. The collected results indicate that Ageritin can interact with DPPC/DPPS/Chol vesicles, used as a model of cancer cell membranes, and with DPPC/DPPG vesicles, used as a model of bacterial cell membranes, suggesting a selective interaction with anionic lipids. However, a different perturbation of the two model membranes, mediated by cholesterol redistribution, was observed and this might be at the basis of Ageritin selective toxicity towards cancer cells.
Keywords; Agrocybe aegerita, calorimetry, liposomes, membrane interactions, ribotoxin-like proteins

Receptors and Signal Transduction

ER membrane protein complex 1 interacts with STIM1 and regulates store-operated Ca^{2+} entry

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Store-operated calcium entry (SOCE) is the process by which the emptying of endoplasmic reticulum (ER) Ca^{2+} stores causes an influx of Ca^{2+} across the plasma membrane (PM). It is the major Ca^{2+} influx pathway in nonexcitable cells and has a wide array of physiological functions. Upon store depletion, stromal interaction molecule 1 (STIM1), an ER calcium sensor relocates into discrete puncta at the ER–PM junction region, which results in the coupling of Ca^{2+} channels to initiate SOCE. However, the mechanism regulating STIM1 activity remains poorly understood. Here, we performed affinity purification of STIM1 and uncovered ER membrane protein complex 1 (EMC1) as an STIM1 binding partner. We showed that this interaction occurred in the ER through the intraluminal region of STIM1. After store depletion, EMC1 does not cluster adjacent to the PM, which suggests that it is distributed differently from STIM1. EMC1 knockdown with small interfering RNA resulted in a marked decrease in SOCE. Thus, these findings suggest that EMC1 functions as a positive regulator of SOCE.
Keywords; calcium, endoplasmic reticulum, signal transduction, store-operated calcium entry

Stress Proteins and Molecular Chaperones

pH-regulated chaperone function of cyanobacterial Hsp90 and Hsp70: implications for light/dark regulation

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We have shown that cyanobacterial chaperonins have pH-dependent anti-aggregation activity. The pH in cyanobacterial cytosol increases by one pH unit following a shift from darkness to light. In this study, we examined whether other major chaperones such as Hsp90 (HtpG) and Hsp70 (DnaK2) from the cyanobacterium *Synechococcus elongatus* PCC 7942 also display pH-dependent activity. Suppressing aggregation of various heat-denatured proteins, especially lactate dehydrogenase, at an equimolar ratio of cyanobacterial Hsp90 to protein substrate was found to be pH-dependent. Hsp90 showed the highest activity at pH 8.5 over the examined pH range of 7.0 to 8.5. pH affected the anti-aggregation activity of DnaK2 in a similar manner to that of Hsp90 in the presence of half equimolar DnaK2 to the protein substrate. The ATPase activity of cyanobacterial Hsp90 was pH-dependent, with a fourfold increase in activity when the pH was raised from 7.0 to 8.5. The ATPase activity of DnaK2 was also regulated by pH in a similar manner. Finally, an increase in pH from 7.0 to 8.5 enhanced activities of both Hsp90 and Hsp70 in protein-folding assistance by two- to threefold. These results suggest that changes in pH may regulate chaperone function during a light–dark cycle in cyanobacterial cells.
Keywords; cyanobacteria, Hsp70, Hsp90, molecular chaperone, pH, photosynthesis

Heme oxygenase-1 induction by heat shock in rat hepatoma cell line is regulated by the coordinated function of HSF1, NRF2 and BACH1

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The mechanism of heme oxygenase-1 (HO-1) induction by heat shock (HS) loading remains unclear. Here, we investigated the contribution of transcription factors to HS-induced HO-1 expression, using a rat hepatoma cell line (H-4-II-E). Our results demonstrated that HS treatment resulted in a marked induction of HO-1. Immunohistochemical analysis showed a slight mismatch in the expression levels of HO-1 and HSP70 by HS among cells, suggesting a conflict between multiple induction mechanisms. We observed HS-induced nuclear localization of, not only phosphorylated HSF1 but also NRF2, which is a typical transcription factor activated by oxidative stress. HSF1 knockdown in H-4-II-E markedly reduced HO-1 induction by HS, while NRF2 knockdown resulted in a partial effect. The chromatin immunoprecipitation assay demonstrated that HS loading resulted in significant binding of HSF1 to the HSE in the promoter proximal region of *HO-1* gene and another HSE located close to the Maf recognition element (MARE) in the −4 kb upstream enhancer region 1, where NRF2 also bound, together with basic leucine zipper transcription factor 1, a negative transcription factor of HO-1. These observations indicate that HO-1 induction by HS is mainly mediated by HSF1 binding to the proximal HSE. NRF2 binding to MARE by HS is predominantly suppressed by an increased binding of BACH1.

Keywords; basic leucine zipper transcription factor 1 (BACH1), chromatin immunoprecipitation (ChIP), heat shock factor protein 1 (HSF1), heme oxygenase, nuclear factor 2 (erythroid-derived 2-like factor) (NFE2 L2) (NRF2)

BIOTECHNOLOGY

Biotechnology General

Development of a novel human adrenomedullin derivative: human serum albumin-conjugated adrenomedullin

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Adrenomedullin is a biologically active peptide with multiple functions. Here, we have developed a novel human serum albumin-adrenomedullin (HSA-AM) conjugate, which was synthesized by the covalent attachment of a maleimide derivative of adrenomedullin to the 34th cysteine residue of HSA via a linker. Denaturing gel electrophoresis and western blotting for HSA-AM yielded a single band with adrenomedullin immunoreactivity at the position corresponding to a molecular weight (MW) of 73 kDa. Following gel-filtration chromatography, the purified HSA-AM showed a single main peak corresponding with an MW of 73 kDa, indicating that HSA-AM is a monomer. Both adrenomedullin and HSA-AM stimulated the intracellular accumulation of cyclic AMP (cAMP) in HEK-293 cells stably expressing the adrenomedullin 1 receptor. The pEC_{50} values for adrenomedullin and HSA-AM were 8.660 and 7.208 (equivalent to 2.19 and 61.9 nM as EC_{50}), respectively. The bioavailability of HSA-AM compared with that of adrenomedullin was much improved after subcutaneous administration in the rat, which was probably due to the superior resistance of HSA-AM towards endogenous proteases and its reduced clearance from the blood. HSA-AM may be a promising drug candidate for clinical application.

Keywords; adrenomedullin, cAMP, human serum albumin, maleimide, pharmacokinetics

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ダイジェスト

JB Reviews

Biotinylation-based proximity labelling proteomics: basics, applications and technical considerations

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Recent advances in biotinylation-based proximity labelling (PL) have opened up new avenues for mapping the protein composition of cellular compartments and protein complexes in living cells at high spatiotemporal resolution. In particular, PL combined with mass spectrometry-based proteomics has been successfully applied to defining protein–protein interactions, protein–nucleic acid interactions, (membraneless) organelle proteomes and secretomes in various systems ranging from cultured cells to whole animals. In this review, we first summarize the basics and recent biological applications of PL proteomics and then highlight recent developments in enrichment techniques for biotinylated proteins and peptides, focusing on the advantages of PL and technical considerations.

Keywords; affinity purification, biotinylation protein, interaction, proteomics, proximity labelling

BIOCHEMISTRY

Biochemistry General

Preparation of the ubiquitination-triggered active form of SETDB1 in Escherichia coli for biochemical and structural analyses

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Trimethylation of histone H3 at K9 by the lysine methyltransferase, SET domain bifurcated histone lysine methyltransferase 1 (SETDB1) plays a pivotal role in silencing tissue-specific genes and retrotransposable elements. In mammalian cells, SETDB1 undergoes monoubiquitination in the insertion region of the SET domain in an E3 ubiquitin ligase-independent manner. This ubiquitination has been shown to enhance the histone H3-K9 methyltransferase activity of SETDB1; however, the molecular mechanism underlying SETDB1 activation by ubiquitination is unknown. In this study, we developed an *Escherichia coli* ubiquitination plasmid for the preparation of ubiquitinated SETDB1. Western blotting and mutational analyses showed that co-expression of the SET domain of SETDB1 with the proteins encoded by the ubiquitination plasmid led to site-specific monoubiquitination of the SET domain at K867. An *in vitro* histone H3 methylation assay demonstrated that the ubiquitinated SET domain of SETDB1 acquired enzymatic activity. Taken together, these findings demonstrate successful preparation of the active form of SETDB1 with the *E.coli* ubiquitination system, which will aid biochemical and structural studies of ubiquitinated SETDB1.

Keywords; histone modifications, SETDB1/ESET, SET domain, ubiquitination

Protein Interaction and Recognition

Epitope-dependent thermodynamic signature of single-domain antibodies against hen egg lysozyme

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A substantial body of work has been carried out describing the structural features of the complex between single-domain antibodies (VHHs) and antigens, and the preeminence for epitopes located at concave surfaces of the antigen. However, the thermodynamic basis of binding is far less clear. Here, we have analysed the energetic profiles of five VHHs binding to the catalytic cleft or to a noncleft epitope of hen egg lysozyme. Various binding energetic profiles with distinctive enthalpic/entropic contributions and structural distribution of critical residues were found in the five antibodies analysed. Collectively, we suggest that from an energetic point of view the binding mechanism is influenced by the shape of the epitope. This information may be beneficial for the design of tailored epitopes for VHHs and their practical use.

Keywords; Biomolecular recognition, Nanobody, Protein-protein interactions, Epitope shape, Hot-spot residue

Plasmodium falciparum metacaspase-2 capture its natural substrate in a non-canonical way

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Programmed cell death (PCD) is a multi-step process initiated by a set of proteases, which interacts and cleaves diverse proteins, thus modulating their biochemical and cellular functions. In metazoans, PCD is mediated by proteolytic enzymes called caspases, which triggered cell death by proteolysis of human Tudor staphylococcus nuclease (TSN). Non-metazoans lack a close homologue of caspases but possess an ancestral family of cysteine proteases termed 'metacaspases'. Studies supported that metacaspases are involved in PCD, but their natural substrates remain unknown. In this study, we performed the *Plasmodium falciparum* TSN (*Pf*TSN) cleavage assay using wild and selected mutants of *P. falciparum* metacaspases-2 (*Pf*MCA-2) *in vitro and in vivo*. Interestingly, *Pf*MCA-2, cleaved a phylogenetically conserved protein, *Pf*TSN at multiple sites. Deletion or substitution mutation in key interacting residues at the active site, Cys^{157} and His^{205} of *Pf*MCA-2, impaired its enzymatic activity with the artificial substrate, z-GRR-AMC. However, the mutant $Tyr^{224}A$ did not affect the activity with z-GRR-AMC but abolished the cleavage of *Pf*TSN. These results indicated that the catalytic dyad, Cys^{157} and His^{205} of *Pf*MCA-2 was essential for its enzymatic activity with an artificial substrate, whereas Tyr^{224} and Cys^{157} residues were responsible for its interaction with the natural substrate and subsequent degradation of *Pf*TSN. Our results suggested that MCA-2 interacts with TSN substrate in a non-

canonical way using non-conserved or conformationally available residues for its binding and cleavage. In future, it would be interesting to explore how this interaction leads to the execution of PCD in the *Plasmodium*.

Keywords; cell death, malaria, metacaspase-2, protein–protein interaction, Tudor staphylococcal nuclease

Glycobiology and Carbohydrate Biochemistry

Accumulation of Ne-(carboxyethyl) lysine in Caenorhabditis elegans is correlated with the formation of ketone body

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Advanced glycation end-products (AGEs) are formed when proteins react with carbonyl compounds, and they gradually accumulate with age. However, AGE accumulation with ageing is not fully understood because longevity studies in mammals are time-consuming. Therefore, we used *Caenorhabditis elegans* to evaluate the correlation between ageing and AGE accumulation. Age-synchronized *C.elegans* nematodes were cultured for 3 and 12 days. The levels of N^{ε}-(carboxymethyl) lysine, N^{ω}-(carboxymethyl) arginine, N^{δ}-(5-hydro-5-methyl-4-imidazolone-2-yl) ornithine and N^{ε}-(carboxyethyl) lysine (CEL) were compared. Glucose, methylglyoxal and acetol were incubated with human serum albumin, and CEL formation was evaluated. The levels of methylglyoxal and ketone bodies in *C.elegans* were quantified. CEL accumulation increased significantly with culture duration. Methylglyoxal and ketone bodies—possible forerunners of AGE accumulation—were also quantified with respect to culture duration. The levels of ketone bodies increased significantly during culture, and correlated closely with CEL accumulation ($R^2 = 0.72$, $P = 0.0008$), whereas the levels of methylglyoxal did not increase over time. CEL was formed *in vitro* in a time-dependent manner from methylglyoxal and acetol when incubated with human serum albumin (HSA) at the same temperature as *C.elegans* culture, suggesting that increased levels of CEL in *C.elegans* are attributable to ketone bodies.

Keywords; AGEs, CEL, C.elegans, Glycation, Ketone body

Dermatan sulphate is an activating ligand of anaplastic lymphoma kinase

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Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase (RTK) that harbours a tyrosine kinase domain in its intracellular region and is expressed in both central and peripheral nervous systems. RTKs are activated upon ligand binding and receptor clustering; however, ALK remains an orphan receptor despite its pathological significance, especially in malignancy. Recent biochemical work showed that heparan sulphate (HS), an unbranched sulphated glycan, acts as a ligand for and activates ALK. Here, we show that dermatan sulphate (DS, chondroitin sulphate B) directly interacts with the extracellular N-terminal region of ALK as well as HS. The tetrasaccharide of DS was required and was sufficient for inducing autophosphorylation of ALK at tyrosine 1604, a marker for activated ALK. Interestingly, longer oligosaccharides caused enhanced activation of ALK, as was the case for HS. Our results provide a novel example of glycans as signalling molecules and shed light on the pathophysiological roles of ALK.

Keywords; anaplastic lymphoma kinase, dermatan sulphate, ligand, phosphorylation, receptor tyrosine kinase

Enzyme Inhibitors

Kolafl avanone, a bifl avonoid derived from medicinal plant Garcinia, is an inhibitor of mitotic kinesin Eg5

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Mitotic kinesin Eg5 remains a validated target in antimitotic therapy because of its essential role in the formation and maintenance of bipolar mitotic spindles. Although numerous Eg5 inhibitors of synthetic origin are known, only a few inhibitors derived from natural products have been reported. In our study, we focused on identifying novel Eg5 inhibitors from medicinal plants, particularly *Garcinia* species. Herein, we report the inhibitory effect of kolaflavanone (KLF), a *Garcinia* biflavonoid, on the ATPase and microtubule-gliding activities of mitotic kinesin Eg5. Additionally, we showed the interaction mechanism between Eg5 and KLF via *in vitro* and *in silico* analyses. The results revealed that KLF inhibited both the basal and microtubule-

activated ATPase activities of Eg5. The inhibitory mechanism is allosteric, without a direct competition with adenosine-5′-diphosphate for the nucleotide-binding site. KLF also suppressed the microtubule gliding of Eg5 *in vitro*. The Eg5–KLF model obtained from molecular docking showed that the biflavonoid exists within the α2/α3/L5 (α2: Lys111–Glu116 and Ile135–Asp149, α3: Asn206–Thr226; L5: Gly117–Gly134) pocket, with a binding pose comparable to known Eg5 inhibitors. Overall, our data suggest that KLF is a novel allosteric inhibitor of mitotic kinesin Eg5.

Keywords; ATPase activity, biflavonoid, kinesin Eg5, kolaflavanone, molecular interaction

Biochemistry in Diseases and Aging

Transmembrane protein 168 mutation reduces cardiomyocyte cell surface expression of Nav1.5 through B-crystallin intracellular dynamics

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Transmembrane protein 168 (TMEM168) was found to be localized on the nuclear membrane. A heterozygous mutation (c.1616G>A, p. R539Q) in TMEM168 was identified in patients with Brugada syndrome. This mutation reduced expression of cardiomyocyte sodium channel $Na_v1.5$ via Nedd4-2 E3 ubiquitin ligase-induced ubiquitination and degradation. However, the detailed molecular mechanism provoked by the TMEM168 mutant remains unclear. Here, we demonstrated that small heat shock protein αB-crystallin, which can bind to $Na_v1.5$ and Nedd4-2 and interfere with the association of both proteins, was strongly recruited from the cell surface to the perinuclear region because of the much higher affinity of αB-crystallin with the TMEM168 mutant than with wild-type TMEM168. Following knockdown of αB-crystallin in HL-1 cardiomyocytes, the interaction of $Na_v1.5$ with Nedd4-2 was increased, despite the reduced expression of $Na_v1.5$. Moreover, reduction of $Na_v1.5$ expression by αB-crystallin knockdown was rescued in the presence of a proteasome inhibitor MG-132, suggesting the importance of the αB-crystallin-modulated ubiquitin–proteasome system for the stability of $Na_v1.5$ expression. Collectively, the balance of molecular interactions among $Na_v1.5$, Nedd4-2 and αB-crystallin plays a role in the regulation of cardiomyocyte cell surface expression of $Na_v1.5$, and the TMEM168 mutant disturbs this balance, resulting in a decrease in $Na_v1.5$ expression.

Keywords; Brugada syndrome, heat shock protein, protein interaction, sodium channel, ubiquitination

Analytical Biochemistry

An inexpensive, simple and eff ective method of genome DNA fragmentation for NGS libraries

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Next-generation sequencing (NGS)-library preparation for whole-genome sequencing (WGS) starts with DNA fragmentation, and sonication is a physical approach used most often due to its simplicity and reproducibility. However, the commercially available Covaris instrument has a high price for both the device and consumables. Here, we describe our in-house method of DNA shearing by sonication with small (100–600 μm) glass beads and an ultrasonic bath. The fragmentation conditions were optimized for the bacterial WGS with ～550-bp fragment size (the ultrasonic bath water temperature 5–10°C, glass beads 0.06 g, the fragmentation time 50 s) and for human DNA with ～250 bp (fragmentation with the same parameters for 4 min). Fragmentation results were compared with the Covaris instrument for preparing several bacterial NGS libraries for Illumina NGS platforms by several characteristics. We obtained close mean fragment lengths (523–623 versus 480–646), similar mono- and dinucleotide specificity of shearing, and comparable indicators of read alignment and *de novo* assembly for both methods. Thus, the described method is a new fast, and effective DNA fragmentation approach that can be used in different WGS applications.

Keywords; DNA fragmentation, glass beads, shearing, sonication, whole-genome sequencing

MOLECULAR BIOLOGY

Molecular Biology General

Regulation of polyphosphate glucokinase gene expression through cotranscriptional processing in Mycobacterium tuberculosis H37Rv

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Transcription is a molecular process that involves the synthesis of RNA chain into the 5′–3′ direction, and simultaneously nascent RNA chain tends to form geometric structures, known as cotranscriptional folding. This folding determines the functional properties of RNA molecules and possibly has a critical role during the synthesis. This functioning includes the characterized properties of riboswitches and ribozymes, which are significant when the transcription rate is comparable to the cellular environment. This study reports a novel noncoding region important in the genetic expression of *polyphosphate glucokinase (ppgk) in Mycobacterium tuberculosis*. This noncoding element of *ppgk* gene undergoes cleavage activity during the transcriptional process in *M.tuberculosis*. We revealed that cleavage occurs within the nascent RNA, and the resultant cleaved 3′RNA fragment carries the Shine–Dalgarno (SD) sequence and expression platform. We concluded cotranscriptional processing at the noncoding region as the required mechanism for *ppgk* expression that remains constitutive within the bacterial environment. This study defines the molecular mechanism dependent on the transient but highly active structural features of the nascent RNA.

Keywords; cleavage activity, cotranscriptional processing, noncoding region, reporter gene expression, Shine–Dalgarno sequestration domain

CELL

Tumor and Immunology

ETV4 mediates the Wnt/b-catenin pathway through transcriptional activation of ANXA2 to promote hepatitis B virus-associated liver hepatocellular carcinoma progression

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ETS variant 4 (ETV4) has been implicated in the development of various cancers. However, the molecular events mediated by ETV4 in liver cancer are poorly understood, especially in hepatitis B virus (HBV)-associated liver hepatocellular carcinoma (LIHC). Here, we aimed to identify the target involved in ETV4-driven hepatocarcinogenesis. Bioinformatics analysis revealed that ETV4 was highly expressed in patients with HBV-associated LIHC, and HBV infection promoted the expression of ETV4 in LIHC cells. Inhibition of ETV4 repressed the proliferation, migration, invasion of LIHC cells and suppressed the secretion of HBV and the replication of HBV DNA. ANXA2 expression in LIHC patients was positively correlated with ETV4 expression. Chromatin immunoprecipitation and dual-luciferase reporter assays revealed that ETV4 elevated the ANXA2 expression at the transcriptional level by binding to the ANXA2 promoter. Overexpression of ANXA2 reversed the inhibitory effect of sh-ETV4 on the malignant biological behaviours of HBV-infected LIHC cells by activating the Wnt/*β*-catenin pathway. In conclusion, ETV4 mediates the activation of Wnt/*β*-catenin pathway through transcriptional activation of ANXA2 expression to promote HBV-associated LIHC progression.

Keywords; ANXA2, ETV4, hepatitis B virus, liver hepatocellular carcinoma, Wnt/*β*-catenin pathway