Journal of Biochemistry

Vol.167, No.1（2020 年 1 月 発 行）

ダイジェスト

BIOCHEMISTRY

Biochemistry General

MiRNA-96-5p contributed to the proliferation of gastric cancer cells by targeting FOXO3

Xionghui He[1] and Kejian Zou[1]

[1]Department of General Surgery, Hainan General Hospital, Hainan Medical University, Haikou, Hainan, China

Various microRNAs (miRNAs, miRs) and the forkhead box O (FOXO) family proteins have been shown to influence gastric cancer progression and development. Here, we aimed to identify the gastric cancer related miRNAs and their relationship with the FOXO family. MiRNA profiles were generated by miRNA microarray screening from pre-operative plasma samples. Quantitative reverse transcription PCR and western bolt were used to determine the expression levels of miR-96 and FOXO family. 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide assay and colony formation assay were used to test the cell viability. The miR-96-5p and FOXO3 interaction were confirmed by luciferase reporter assay. Our results demonstrated the excessive expression of miR-96-5p in gastric cancer cell lines and plasma samples from gastric cancer patients. In addition, the protein levels of FOXO3 were decreased in tissue samples from gastric cancer patients. Moreover, miR-96-5p accelerated the gastric cancer cell proliferation by directly targeting FOXO3. Therefore, we conclude that iR-96-5p might promote the progression of gastric cancer by directly targeting FOXO3 mRNA and downregulating the expression of FOXO3 protein, which provides new insights for the molecular mechanism of gastric cancer.

Keywords: forkhead box O3 (FOXO3), gastric cancer, MiR-96-5p

RAPID COMMUNICATION

Protein Structure

Crystal structure of the complex of the interaction domains of *Escherichia coli* DnaB helicase and DnaC helicase loader: structural basis implying a distortion-accumulation mechanism for the DnaB ring opening caused by DnaC binding

Koji Nagata[1], Akitoshi Okada[1], Jun Ohtsuka[1], Takatoshi Ohkuri[2], Yusuke Akama[3], Yukari Sakiyama[3], Erika Miyazaki[3], Shoichiro Horita[1], Tsutomu Katayama[3], Tadashi Ueda[2] and Masaru Tanokura[1]

[1]Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo, Japan, [2]Department of Protein Structure, Function and Design, Graduate School of Pharmaceutical Sciences, Kyushu University, Higashi-ku, Fukuoka, Japan, [3]Department of Molecular Biology, Graduate School of Pharmaceutical Sciences, Kyushu University, Higashi-ku, Fukuoka, Japan

Loading the bacterial replicative helicase DnaB onto DNA requires a specific loader protein, DnaC/DnaI, which creates the loading-competent state by opening the DnaB hexameric ring. To understand the molecular mechanism by which DnaC/DnaI opens the DnaB ring, we solved 3.1-Å co-crystal structure of the interaction domains of *Escherichia coli* DnaB–DnaC. The structure reveals that one N-terminal domain (NTD) of DnaC interacts with both the linker helix of a DnaB molecule and the C-terminal domain (CTD) of the adjacent DnaB molecule by forming a three α-helix bundle, which fixes the relative orientation of the two adjacent DnaB CTDs. The importance of the intermolecular interface in the crystal structure was supported by the mutational data of DnaB and DnaC. Based on the crystal structure and other available information on DnaB–DnaC structures, we constructed a molecular model of the hexameric DnaB CTDs bound by six DnaC NTDs. This model suggested that the binding of a DnaC would cause a distortion in the hexameric ring of DnaB. This distortion of the DnaB ring might accumulate by the binding of up to six DnaC molecules, resulting in the DnaB ring to open.

Keywords: crystal structure, distortion-accumulation mechanism, DnaB helicase, DnaC helicase loader, helicase ring opening

Enzymology

Kinetic and solvent isotope effects in oxidation of halogen derivatives of tyramine catalyzed by monoamine oxidase A

Małgorzata Pająk[1]

[1]Department of Chemistry, Warsaw University, Pasteur 1 Str, Warsaw, Poland

The isotope effects approach was used to elucidate the mechanism of oxidative deamination of 3′-halotyramines, catalyzed by monoamine oxidase A (EC 1.4.3.4). The numerical values of kinetic isotope effect (KIE) and solvent isotope effect (SIE) were established using a non-competitive spectrophotometric technique. Based upon KIE and SIE values, some of the mechanistic details of investigated reaction were discussed.

Keywords: deuterium, enzyme mechanism, 3′-halotyramines, isotope effects, MAO A

Purification, biochemical and molecular study of lipase producing from a newly thermoalkaliphilic Aeribacillus pallidus for oily wastewater treatment

Ameni Ktata[1], Najeh Krayem[1], Ahmed Aloulou[1], Sofiane Bezzine[1], Adel Sayari[1], Mohamed Chamkha[2] and Aida Karray[1]

[1]Laboratoire de Biochimie et de Génie Enzymatique des Lipases, ENIS Route de Soukra, km 3.5, université de Sfax-Tunisie, BP

1173 3038 Sfax, Tunisia, [2]Centre de Biotechnologie de Sfax, Route Sidi Mansour Km 6, BP 1177 3018 Sfax, Tunisia

Treatment of oily wastewater is constantly a challenge; biological wastewater treatment is an effective, cheap and eco-friendly technology. A newly thermostable, haloalkaline, solvent tolerant and non-induced lipase from *Aeribacillus pallidus* designated as GPL was purified and characterized of biochemical and molecular study for apply in wastewater treatment. The GPL showed a maximum activity at 65°C and pH 10 after 22 h of incubation, with preference to TC4 substrates. Pure enzyme was picked up after one chromatographic step. It displayed an important resistance at high temperature, pH, NaCl, at the presence of detergents and organic solvents. In fact, GPL exhibited a prominent stability in wide range of organic solvents at 50% (v/v) concentration for 2 h of incubation. The efficiency of the GPL in oil wastewater hydrolysis was established at 50°C for 1 h, the oil removal efficiency was established at 96, 11% and the oil biodegradation was confirmed through fourier transform infrared (FT-IR) spectroscopy. The gene that codes for this lipase was cloned and sequenced and its open reading frame encoded 236 amino acid residues. The deduced amino acids sequence of the GPL shows an important level of identity with *Geobacillus* lipases.

Keywords: Aeribacillus pallidus, lipase, oil biodegradation, thermo alkaliphilic

MOLECULAR BIOLOGY

Molecular Biology General

GRWD1 directly interacts with p53 and negatively regulates p53 transcriptional activity

Hiroki Fujiyama[1], Takahiro Tsuji[1], Kensuke Hironaka[1], Kazumasa Yoshida[1], Nozomi Sugimoto[1] and Masatoshi Fujita[1]

[1]Department of Cellular Biochemistry, Graduate School of Pharmaceutical Sciences, Kyushu University, 3–1–1 Maidashi, Higashiku, Fukuoka 812–8582, Japan

Glutamate-rich WD40 repeat containing 1 (GRWD1) functions as a histone chaperone to promote loading of the MCM replication helicase at replication origins. GRWD1 is overexpressed in several cancer cell lines, and GRWD1 overexpression confers tumorigenic potential in human cells. However, less is known concerning its oncogenic activity. Our previous analysis showed that GRWD1 negatively regulates the tumour suppressor p53 via the RPL11-MDM2-p53 and RPL23-MDM2-p53 axes. Here, we demonstrate that GRWD1 directly interacts with p53 via the p53 DNA-binding domain. Upon DNA damage, GRWD1 downregulation resulted in increased p21 expression. Conversely, GRWD1 co-expression suppressed several p53-regulated promoters. GRWD1 interacted with the p21 and MDM2 promoters, and these interactions required p53. By using the Human Cancer Genome Atlas database, we found that GRWD1 expression levels are inversely correlated with the expression levels of some p53-target genes. Interestingly, high GRWD1 expression in combination with low expression levels of some p53-target genes was significantly correlated with poor prognosis in skin melanoma patients with wild-type p53. Taken together, our findings suggest a novel oncogenic function of GRWD1 as a transcriptional regulator of p53 and that GRWD1 might be an attractive therapeutic target and prognostic marker in cancer therapy.

Keywords: GRWD1, p53, transcriptional activity, tumourigenesis

CRY2 suppresses trophoblast migration and invasion in recurrent spontaneous abortion

Lianzhi Wu[1], Biheng Cheng[1], Qian Liu[1], Ping Jiang[1] and Jing Yang[2]

[1]Department of Obstetric, Renmin Hospital of Wuhan University, No. 99 Zhangzhidong Road, Wuchang District, Wuhan, Hubei Province, China, [2]Reproductive Medical Center, Renmin Hospital of Wuhan University, No. 99 Zhangzhidong Road, Wuchang District, Wuhan, Hubei Province, China

Disruption of circadian rhythms is associated with aberrant trophoblast migration and invasion in recurrent spontaneous abortion (RSA). This study aims to explore the functional role and the mechanisms of cryptochrome 2 (CRY2), a fundamental component of the circadian clock, in regulating trophoblast migration and invasion. Human extravillous trophoblast cell line HTR-8/SVneo was used as a cell model. Cell migration and invasion were examined using wound healing assay and Transwell assay, respectively. The mRNA and protein levels were determined using quantitative real-time polymerase chain reaction and western blot, respectively. Luciferase reporter assay and chromatin immunoprecipitation assay were performed to explore the interaction between c-Myc to the brain and muscle ARNT-like protein 1 (BMAL1) promoter. CRY2 was highly expressed in human villous specimens of RSA. Furthermore, CRY2 overexpression impaired migration and invasion in HTR-8/SVneo cells, whereas CRY2 knockdown yielded the opposite results. Mechanistically, c-Myc bound to the BMAL1 promoter and induced BMAL1 transcription, both of which further activated matrix metalloproteinase 2/9 (MMP2/9) and facilitated migration and invasion in HTR-8/SVneo cells. CRY2 inhibited c-Myc-BMAL1 pathway and impaired migration and invasion of HTR-8/SVneo cells. Collectively, these findings demonstrate that CRY2 suppresses trophoblast migration and invasion via inhibiting c-Myc-BMAL1-MMP2/9 pathway.

Keywords: BMAL1, c-Myc, CRY2, recurrent spontaneous abortion, trophoblast

CELL

Cytoskeleton, Cell Motility, and Cell Shape

Nonmuscle myosin IIA and IIB differently suppress microtubule growth to stabilize cell morphology

Yuta Sato[1], Keiju Kamijo[2], Motosuke Tsutsumi[3], Yota Murakami[1,4]

and Masayuki Takahashi[1,4]
[1]Graduate School of Chemical Sciences and Engineering, Hokkaido University, Kita 13, Nishi 8, Kita-ku, Sapporo Hokkaido, Japan, [2]Division of Anatomy and Cell Biology, Faculty of Medicine, Tohoku Medical and Pharmaceutical University, 1–15–1 Fukumuro, Miyagino-ku, Sendai Miyagi, Japan, [3]Research Institute for Electronic Science, Hokkaido University, Kita 20, Nishi 10, Kita-ku, Sapporo Hokkaido, Japan, [4]Department of Chemistry, Faculty of Science, Hokkaido University, Kita 10, Nishi 8, Kita-ku, Sapporo Hokkaido, Japan

Precise regulation of cytoskeletal dynamics is important in many fundamental cellular processes such as cell shape determination. Actin and microtubule (MT) cytoskeletons mutually regulate their stability and dynamics. Nonmuscle myosin II (NMII) is a candidate protein that mediates the actin–MT crosstalk. NMII regulates the stability and dynamics of actin filaments to control cell morphology. Additionally, previous reports suggest that NMII-dependent cellular contractility regulates MT dynamics, and MTs also control cell morphology; however, the detailed mechanism whereby NMII regulates MT dynamics and the relationship among actin dynamics, MT dynamics and cell morphology remain unclear. The present study explores the roles of two well-characterized NMII isoforms, NMIIA and NMIIB, on the regulation of MT growth dynamics and cell morphology. We performed RNAi and drug experiments and demonstrated the NMII isoform-specific mechanisms—NMIIA-dependent cellular contractility upregulates the expression of some mammalian diaphanous-related formin (mDia) proteins that suppress MT dynamics; NMIIB-dependent inhibition of actin depolymerization suppresses MT growth independently of cellular contractility. The depletion of either NMIIA or NMIIB resulted in the increase in cellular morphological dynamicity, which was alleviated by the perturbation of MT dynamics. Thus, the NMII-dependent control of cell morphology significantly relies on MT dynamics.
Keywords: actin, cell morphology, cytoskeletal crosstalk, microtubule, nonmuscle myosin II

BIOTECHNOLOGY

Biotechnology General

iTRAQ-based quantitative proteomic analysis of two transgenic soybean lines and the corresponding non-genetically modifi ed isogenic variety

Weixiao Liu[1], Zhe Zhang[1], Xuri Liu[2] and Wujun Jin[1]
[1]Biotechnology Research Institute, Chinese Agricultural and Academic Sciences, No. 12 Zhongguancun South St., Haidian District, Beijing, P.R. China, [2]Department of Food and Biological Engineering, Handan Polytechnic College, No.141 Zhuhe Road, Hanshan District, Handan, P.R. China

To investigate the unintended effects of genetically modified (GM) crops, an isobaric tags for relative and absolute quantitation (iTRAQ)-based comparative proteomic analysis was performed with seed cotyledons of two GM soybean lines, MON87705 and MON87701×MON89788, and the corresponding non-transgenic isogenic variety A3525. Thirty-five differentially abundant proteins (DAPs) were identified in MON87705/A3525, 27 of which were upregulated and 8 downregulated. Thirty-eight DAPs were identified from the MON87701×MON89788/A3525 sample, including 29 upregulated proteins and 9 downregulated proteins. Pathway analysis showed that most of these DAPs participate in protein processing in endoplasmic reticulum and in metabolic pathways. Protein–protein interaction analysis of these DAPs demonstrated that the main interacting proteins are associated with post-translational modification, protein turnover, chaperones and signal transduction mechanisms. Nevertheless, these DAPs were not identified as new unintended toxins or allergens and only showed changes in abundance. All these results suggest that the seed cotyledon proteomic profiles of the two GM soybean lines studied were not dramatically altered compared with that of their natural isogenic control.
Keywords: genetic modification, iTRAQ, qRT-PCR, quantitative proteomics, soybean seed cotyledons

RNA Technology

miR-146a promoted breast cancer proliferation and invasion by regulating NM23-H1

Jun Chen[1], Qiang Jiang[2], Xue-Qin Jiang[1], De-Quan Li[1], Xiao-Cheng Jiang[1], Xiao-Bo Wu[1] and Ya-Li Cao[1]
[1]Breast Tumor Department, 3rd Hospital of Nanchang, No. 2 Xiangshan South Road, Xihu District, Nanchang, Jiangxi, China, [2]Oncology Department, Jiangxi Provincial Chest Hospital, No. 346, Dieshan Road, East Lake District, Nanchang, Jiangxi, China

The study aimed to investigate the regulatory effect of miR-146a in proliferation, invasion and migration of breast cancer and its possible mechanism via *NM23-H1*. The expression levels of miR-146a in breast cancer with different pathological classification were significantly increased, while the expression levels of *NM23-H1* were significantly decreased, which were closely correlated. Double luciferase reporter gene was used to verify the target regulatory relationship between miR-146 and *NM23-H1* on a human breast cancer cell line. miR-146a was closely related to the proliferation and metastasis of breast cancer. miR-146a also promoted the growth of breast cancer *in vivo* via targeting *NM23-H1*. In conclusion, miR-146 can promote the proliferation and invasion of breast cancer by targeting *NM23-H1*.
Keywords: breast cancer, Hsa-miR-146a, invasion; NM23-H1, proliferation

Circ_0000218 plays a carcinogenic role in colorectal cancer progression by regulating miR-139-3p/RAB1A axis

Fu-Lai Pei[1], Ming-Zheng Cao[2] and Yue-Feng Li[1]
[1]Department of Oncology, Linyi Central Hospital, No. 17 Jiankang Road, Linyi, Shandong, China
[2]Department of General Surgery, Linyi Central Hospital, No. 17 Jiankang Road, Linyi, Shandong, China
Accumulating researches have confirmed that circRNA abnormal expression plays a prominent role in the progression of colorectal cancer (CRC). The role of circ_0000218 in CRC and its potential mechanism are not clear. In this study, real-time polymerase chain reaction (RT-PCR) was employed to measure the circ_0000218, miR-139-3p and RAB1A mRNA expression in CRC tissues and cells. Immunohistochemistry and western blot were conducted to determine the RAB1A expression in CRC tissues and cells, respectively. Colony formation assay and BrdU method were employed to monitor the effect of circ_0000218 on cell proliferation. Transwell assay was adopted to detect cell migration and invasion. Dual luciferase reporter assay and RNA immunoprecipitation assay were adopted to confirm the targeting relationship between circ_0000218 and miR-139-3p, miR-139-3p and RAB1A. We demonstrated that circ_0000218 was notably upregulated in CRC tissues and cell lines, and its high expression level was markedly linked to the increase of T staging and local lymph node metastasis. Circ_0000218 overexpression enhanced the proliferation and metastasis of CRC cells while knocking down circ_0000218 caused the opposite effects. We also observed that miR-139-3p was negatively regulated by circ_0000218, while RAB1A was positively regulated by it. Collectively, this study suggested that circ_0000218 upregulated RAB1A and promoted CRC proliferation and metastasis via sponging miR-139-3p.
Keywords: circ_0000218, CRC, miR-139-3p, RAB1A

Journal of Biochemistry

Vol.167, No.2（2020 年 2 月 発 行）

ダイジェスト

JB SPECIAL ISSUE—REDOX METABOLISM AND SIGNALING GUEST EDITORS: MOTOHIRO NISHIDA AND ALBERT VAN DER VLIET

JB Special Issue—Reviews

Dynamic regulation of subcellular mitochondrial position for localized metabolite levels

Haya Alshaabi[1], Meara Heininger[1] and Brian Cunniff[1]
[1]Department of Pathology and Laboratory Medicine, University of Vermont Cancer Center, Larner College of Medicine, Burlington, VT 05405, USA
Mitochondria are not passive bystanders aimlessly floating throughout our cell's cytoplasm. Instead, mitochondria actively move, anchor, divide, fuse, self-destruct and transfer between cells in a coordinated fashion, all to ensure proper structure and position supporting cell function. The existence of the mitochondria in our cells has long been appreciated, but their dynamic nature and interaction with other subcellular compartments has only recently been fully realized with the advancement of high-resolution live-cell microscopy and improved fractionization techniques. The how and why that dictates positioning of mitochondria to specific subcellular sites is an ever-expanding research area. Furthermore, the advent of new and improved functional probes, sensitive to changes in subcellular metabolite levels has increased our understanding of local mitochondrial populations. In this review, we will address the evidence for intentional mitochondrial positioning in supporting subcellular mitochondrial metabolite levels, including calcium, adenosine triphosphate and reactive oxygen species and the role mitochondrial metabolites play in dictating cell outcomes.
Keywords: metabolite gradients, mitochondrial contacts, mitochondrial dynamics, reactive oxygen species

Selenoprotein P as a signifi cant regulator of pancreatic *β* cell function

Yoshiro Saito[1]
[1]Laboratory of Molecular Biology and Metabolism, Graduate School of Pharmaceutical Sciences, Tohoku University, C301, 6–3 Aoba, Aramaki, Aoba-ku, Sendai, Miyagi 980–8578, Japan
Selenoprotein P (SeP; encoded by *SELENOP*) is selenium (Se)-rich plasma protein that is mainly produced in the liver. SeP functions as a Se-transport protein to deliver Se from the liver to other tissues, such as the brain and testis. The protein plays a pivotal role in Se metabolism and antioxidative defense, and it has been identified as a 'hepatokine' that causes insulin resistance in type 2 diabetes. SeP levels are increased in type 2 diabetes patients, and excess SeP impairs insulin signalling, promoting insulin resistance. Furthermore, increased levels of SeP disturb the functioning of pancreatic *β* cells and inhibit insulin secretion. This review focuses on the biological function of SeP and the molecular mechanisms associated with the adverse effects of excess SeP on pancreatic *β* cells' function, particularly with respect to redox reactions. Interactions between the liver and pancreas are also discussed.
Keywords: hepatokine, insulin secretion, pancreatic*β*, cell, selenium, Selenoprotein P

Persulphide-responsive transcriptional regulation and metabolism in bacteria

Takayuki Shimizu[1] and Shinji Masuda[2]
[1]Department of General Systems Studies, Graduate School of

Arts and Sciences, University of Tokyo, 3–8–1 Komana, Meguro-ku, Tokyo 153–8902, Japan, [2]Center for Biological Resources and Informatics, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama 226–8501, Japan

Hydrogen sulphide (H2S) impacts on bacterial growth both positively and negatively; it is utilized as an electron donor for photosynthesis and respiration, and it inactivates terminal oxidases and iron-sulphur clusters. Therefore, bacteria have evolved H2S-responsive detoxification mechanisms for survival. Sulphur assimilation in bacteria has been well studied, and sulphide:quinone oxidoreductase, persulphide dioxygenase, rhodanese and sulphite oxidase were reported as major sulphide-oxidizing enzymes of sulphide assimilation and detoxification pathways. However, how bacteria sense sulphide availability to control H2S and sulphide metabolism remains largely unknown. Recent studies have identified several bacterial (per)sulphide-sensitive transcription factors that change DNA-binding affinity through persulphidation of specific cysteine residues in response to highly reactive sulphur-containing chemicals and reactive sulphur species (RSS). This review focuses on current understanding of the persulphide-responsive transcription factors and RSS metabolism regulated by RSS sensory proteins.

Keywords: bacterial transcription, cysteine modification, persulphide, reactive sulphur species, sulphur transfer

From germ cells to neonates: the beginning of life and the KEAP1–NRF2 system

Daisuke Matsumaru[1] and Hozumi Motohashi[1]

[1]Department of Gene Expression Regulation, Institute of Development, Aging and Cancer, Tohoku University, 4–1 Seiryo-cho, Aoba-ku, Sendai, Miyagi 980–8575, Japan

The Kelch-like ECH-associated protein 1(KEAP1)–NF-E2-related factor 2 (NRF2) system is one of the most studied environmental stress response systems. In the presence of oxidative and electrophilic insults, the thiols of cysteine residues in KEAP1 are modified, and subsequently stabilized NRF2 activates its target genes that are involved in detoxification and cytoprotection. A myriad of recent studies has revealed the broad range of contributions of the KEAP1–NRF2 system to physiological and pathological processes. However, its functions during gametic and embryonic development are still open for investigation. Although oxidative stress is harmful for embryos, *Nrf2*$^{-/-}$ mice do not show any apparent morphological abnormalities during development, probably because of the compensatory antioxidant functions of NF-E2-related factor 1 (NRF1). It can also be considered that the antioxidant system is essential for protecting germ cells during reproduction. The maturation processes of germ cells in both sexes are affected by *Nrf2* mutation. Hence, in this review, we focus on the stress response system related to reproduction and embryonic development through the functions of the KEAP1–NRF2 system.

Keywords: development, embryos, neonates, oxidative stress, reproduction

Chemical toolbox for ‘live’ biochemistry to understand enzymatic functions in living systems

Toru Komatsu[1] and Yasuteru Urano[1]

[1]Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7–3–1 Hongo, Bunkyo-ku, Tokyo 113–0033, Japan

In this review, we present an overview of the recent advances in chemical toolboxes that are used to provide insights into ‘live’ protein functions in living systems. Protein functions are mediated by various factors inside of cells, such as protein−protein interactions, posttranslational modifications, and they are also subject to environmental factors such as pH, redox states and crowding conditions. Obtaining a true understanding of protein functions in living systems is therefore a considerably difficult task. Recent advances in research tools have allowed us to consider ‘live’ biochemistry as a valid approach to precisely understand how proteins function in a live cell context.

Keywords: chemical biology, enzymes, fluorescent sensors

Redox regulation of tyrosine kinase signalling: more than meets the eye

Christopher M Dustin[1], David E Heppner[2,3], Miao-Chong J Lin[1] and Albert van der Vliet[1]

[1]Department of Pathology and Laboratory Medicine, University of Vermont, Burlington, VT, USA, [2]Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA, USA, [3]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA

Protein kinases are essential mediators of cellular signal transduction and are often dysregulated in disease. Among these, protein tyrosine kinases (PTKs) have received specific interest due to their common roles in various diseases including cancer, and emerging observations indicating that PTK signalling pathways are susceptible to regulation by reactive oxygen species (ROS), which are also frequently implicated in disease pathology. While it is well recognized that ROS can impact on tyrosine kinase signalling by inhibiting tyrosine phosphatases, more recent studies highlight additional modes of redox-based regulation of tyrosine kinase signalling by direct redox modification of non-catalytic cysteines within tyrosine kinases or other protein components of this signalling pathway. In this review, we will present recent advancements with respect to redox-based mechanisms in regulating PTK signalling, with a specific focus on recent studies demonstrating direct redox regulation of Src-family kinases and epidermal growth factor receptor kinases. Importantly, redox-based modulation of tyrosine kinases may be relevant for many other kinases and has implications for current approaches to de-

velop pharmacological inhibitors for these proteins.
Keywords: Redox, cysteine, Src; EGFR; NOX

A unique mechanism for thiolation of serum albumins by disulphide molecules

Fumie Nakashima[1], Takahiro Shibata[1] and Koji Uchida[2]
[1]Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464–8601, Japan, [2]Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113–8657, Japan

Protein *S*-thiolation is a reversible oxidative modification that serves as an oxidative regulatory mechanism for certain enzymes and binding proteins with reactive cysteine residues. It is generally believed that the thiolation occurs at free sulphydryl group of cysteine residues. Meanwhile, despite the fact that disulphide linkages, serving structural and energetic roles in proteins, are stable and inert to oxidative modification, a recent study shows that the thiolation could also occur at protein disulphide linkages when human serum albumin (HSA) was treated with disulphide molecules, such as cystine and homocystine. A chain reaction mechanism has been proposed for the thiolation at disulphide linkages, in which free cysteine (Cys34) is involved in the reaction with disulphide molecules to form free thiols (cysteine or homocysteine) that further react with protein disulphide linkages to form the thiolated cysteine residues in the protein. This review focuses on the recent finding of this unique chain reaction mechanism of protein thiolation.
Keywords: disulphide molecules, protein thiolation, serum albumin

Pathological consequences of the unfolded protein response and downstream protein disulphide isomerases in pulmonary viral infection and disease

Nicolas Chamberlain[1] and Vikas Anathy[1]
[1]Department of Pathology and Laboratory Medicine, University of Vermont Larner College of Medicine, 149 Beaumont Ave, Burlington, VT 05405, USA

Protein folding within the endoplasmic reticulum (ER) exists in a delicate balance; perturbations of this balance can overload the folding capacity of the ER and disruptions of ER homoeostasis is implicated in numerous diseases. The unfolded protein response (UPR), a complex adaptive stress response, attempts to restore normal proteostasis, in part, through the up-regulation of various foldases and chaperone proteins including redox-active protein disulphide isomerases (PDIs). There are currently over 20 members of the PDI family each consisting of varying numbers of thioredoxin-like domains which, generally, assist in oxidative folding and disulphide bond rearrangement of peptides. While there is a large amount of redundancy in client proteins of the various PDIs, the size of the family would indicate more nuanced roles for the individual PDIs. However, the role of individual PDIs in disease pathogenesis remains uncertain. The following review briefly discusses recent findings of ER stress, the UPR and the role of individual PDIs in various respiratory disease states.
Keywords: disulphide bond, ER stress, PDI, pulmonary disease, UPR

BIOCHEMISTRY
Biochemistry General

Hypermetabolism of glutathione, glutamate and ornithine via redox imbalance in methylglyoxal-induced peritoneal injury rats

Ichiro Hirahara[1], Eiji Kusano[2], Denan Jin[1] and Shinji Takai[1]
[1]Department of Innovative Medicine, Graduate School of Medicine, Osaka Medical College, 2–7 Daigaku-machi, Takatsuki, Osaka 568–8686, [2]JCHO Utsunomiya Hospital, 11–17 Minamitakasago-chou, Utsunomiya, Tochigi 321–0143, Japan

Peritoneal dialysis (PD) is a blood purification treatment for patients with reduced renal function. However, the peritoneum is exposed to oxidative stress during PD and long-term PD results in peritoneal damage, leading to the termination of PD. Methylglyoxal (MGO) contained in commercial PD fluids is a source of strong oxidative stress. The aim of this study was to clarify the mechanism of MGO-induced peritoneal injury using metabolome analysis in rats. We prepared peritoneal fibrosis rats by intraperitoneal administration of PD fluids containing MGO for 21 days. As a result, MGO-induced excessive proliferation of mesenchymal cells with an accumulation of advanced glycation end-products (AGEs) at the surface of the thickened peritoneum in rats. The effluent levels of methionine sulfoxide, an oxidative stress marker and glutathione peroxidase activity were increased in the MGO-treated rats. The levels of glutathione, glutamate, aspartate, ornithine and AGEs were also increased in these rats. MGO upregulated the gene expression of transporters and enzymes related to the metabolism of glutathione, glutamate and ornithine in the peritoneum. These results suggest that MGO may induce peritoneal injury with mesenchymal cell proliferation via increased redox metabolism, directly or through the formation of AGEs during PD.
Keywords: glutaminolysis, glutathione, methylglyoxal, peritoneal dialysis, redox

MOLECULAR BIOLOGY
Genetic Engineering

Improving the catalytic effi ciency of thermostable Geobacillus stearothermophilus xylanase XT6 by single-amino acid substitution

Rasha A M Azouz[1], Usama M Hegazy[1], Mahmoud M Said[2], Roqaya I Bassuiny[1], Ahmed M Salem[2] and Afaf S Fahmy[1]
[1]Genetic Engineering and Biotechnology Research Division,

Molecular Biology Department, National Research Centre, El-Behouth Street, Dokki, 12622 Giza, Egypt, [2]Faculty of Science, Department of Biochemistry, Ain Shams University, El-Khalyfa El-Mamoun Street, Abbasya, 11566 Cairo, Egypt

Directed evolution using error-prone polymerase chain reaction was employed in the current study to enhance the catalytic efficiency of a thermostable *Geobacillus stearothermophilus* xylanase XT6 parent. High-throughput screening identified two variants with enhanced activity. Sequencing analysis revealed the presence of a single-amino acid substitution (P209L or V161L) in each variant. The maximum activity of mutant V161L and P209L was at 85°C and 70°C, respectively. Both mutants exhibited maximum activity at pH 7. The thermal and alkaline tolerance of mutant V161L only were markedly improved. The two mutants were more resistant to ethanol inhibition than the parent. Substrate specificity of the two mutants was shifted from beechwood xylan to birchwood xylan. The potential of the two mutants to hydrolyze rice straw and sugarcane bagasse increased. Both turnover number (k_{cat}) and catalytic efficiency (k_{cat}/k_M) increased 12.2- and 5.7-folds for variant P209L and 13- and 6.5-folds for variant V161L, respectively, towards birchwood xylan. Based on the previously published crystal structure of extracellular *G. stearothermophilus* xylanase XT6, V161L and P209L mutation locate on $\beta\alpha$-loops. Conformational changes of the respective loops could potentiate the loop swinging, product release and consequently result in enhancement of the catalytic performance.

Keywords: biotechnology, enzyme, genetic engineering, lignocellulose, structure–function relationship

CELL

Cell Cycle

Aurora kinase A-mediated phosphorylation of mPOU at a specifi c site drives skeletal muscle differentiation

Dhanasekan Karthigeyan[1], Arnab Bose[1], Ramachandran Boopathi[1], Vinay Jaya Rao[1], Hiroki Shima[2], Narendra Bharathy[3], Kazuhiko Igarashi[2], Reshma Taneja[3], Arun Kumar Trivedi[4] and Tapas K Kundu[1]

[1]Transcription and Disease Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, Karnataka, India, [2]Department of Biochemistry, Tohoku University Graduate School of Medicine, Seiryo-machi 2–1, Aoba-ku, Sendai 980–8575, Japan, [3]Department of Physiology, Cellular Differentiation and Apoptosis, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, [4]Division of Cancer Biology, CSIR-Central Drug Research Institute (CSIR-CDRI), Sector-10, Jankipuram Extension, Lucknow 226031, Uttar Pradesh, India

Aurora kinases are Ser/Thr-directed protein kinases which play pivotal roles in mitosis. Recent evidences highlight the importance of these kinases in multiple biological events including skeletal muscle differentiation. Our earlier study identified the transcription factor POU6F1 (or mPOU) as a novel Aurora kinase (Aurk) A substrate. Here, we report that Aurora kinase A phosphorylates mPOU at Ser197 and inhibit its DNA-binding ability. Delving into mPOU physiology, we find that the phospho-mimic (S197D) mPOU mutant exhibits enhancement, while the wild type or the phospho-deficient mutant shows retardation in C2C12 myoblast differentiation. Interestingly, POU6F1 depletion phenocopies S197D-mPOU overexpression in the differentiation context. Collectively, our results signify mPOU as a negative regulator of skeletal muscle differentiation and strengthen the importance of AurkA in skeletal myogenesis.

Keywords: Aurora kinase A, DNA binding, mitosis, mPOU, skeletal muscle differentiation