Journal of Biochemistry

Vol. 164, No.5 （2018 年 11 月 発 行）

ダイジェスト

JB Review

Featured article of the month. Epigenetic regulation of intragenic transposable elements: a two-edged sword

[1]Hidetoshi Saze

[1]Plant Epigenetics Unit, Okinawa Institute of Science and Technology Graduate University, 1919–1 Tancha, Onna-son, Okinawa, Japan

Genomes of animals and plants contain a large number of transposable elements (TEs). TEs often transpose into genic regions, affecting expression of surrounding genes. Intragenic TEs mostly reside in introns, and in much the same way as intergenic TEs, they are targeted by repressive epigenetic marks for transcriptional silencing. Silenced intragenic TEs generally co-repress expression of associated genes, while in some cases they significantly enhance splicing and transcript elongation. Genomes have evolved molecular mechanisms that allow the presence of silenced TEs within transcriptionally permissive chromatin environments. Epigenetic modulation of intragenic TEs often contributes to gene regulation, phenotypic expression, and genome evolution.

Keywords: epigenetics, heterochromatin, intron, plant, transposable elements

BIOCHEMISTRY

Protein Structure

Crystal structure of the central and the C-terminal RNase domains of colicin D implicated its translocation pathway through inner membrane of target cell

[1]Jung-Wei Chang, [2,3,4]Yusuke Sato, [1,5]Tetsuhiro Ogawa, [1,5]Takatoshi Arakawa, [2,3,4]Shuya Fukai, [1,5]Shinya Fushinobu and [1,4]Haruhiko Masaki

[1]Department of Biotechnology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1–1–1 Yayoi, Bunkyo-ku, Tokyo, Japan, [2]Synchrotron Radiation Research Organization, The University of Tokyo, Tokyo, Japan, [3]Institute for Quantitative Biosciences, The University of Tokyo, Tokyo, Japan, [4]Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo, 5–1–5 Kashiwanoha, Kashiwa, Chiba, Japan, [5]Collaborative Research Institute for Innovative Microbiology, The University of Tokyo, Tokyo, Japan

Colicins are protein toxins produced by and toxic to *Escherichia coli* strains. Colicin D consists of an N-terminal domain (NTD), central domain (CD) and C-terminal RNase domain (CRD). The cognate immunity protein, ImmD, is co-synthesized in producer cells to block the toxic tRNase activity of the CRD. Previous studies have reported the crystal structure of CRD/ImmD complex. Colicin D hijacks the surface receptor FepA and the energy transducer TonB system using the NTD for translocation across the outer membrane of the target cells. The CD is required for endoproteolytic processing and the translocation of CRD across the inner membrane, and the membrane-associated protease FtsH and the signal peptidase LepB are exploited in this process. Although several regions of the CD have been identified in interactions with the hijacked inner membrane system or immunity protein, the structural basis of the CD is unknown. In this study, we determined the crystal structure of colicin D, containing both the CD and CRD. The full-length colicin D/ImmD heterodimer structure was built by superimposing the CD-CRD structure with the previously determined partial structures. The overall translocation process of colicin D, including the interaction between CD and LepB, is discussed.

Keywords: bacteriocin, membrane transport, nuclease, signal peptidase, *X-ray crystallography*

Protein engineering for improving the thermostability of tryptophan oxidase and insights from structural analysis

[1]Hiroki Yamaguchi, [1]Moemi Tatsumi, [1]Kazutoshi Takahashi, [1]Uno Tagami, [1]Masayuki Sugiki, [1]Tatsuki Kashiwagi, [2,3,4]Masafumi Kameya, [2,3]Seiji Okazaki, [1]Toshimi Mizukoshi and [2,3]Yasuhisa Asano

[1]Fundamental Technology Labs., Institute for Innovation, Ajinomoto Co., Inc., Kawasaki, Japan, [2]Biotechnology Research Center and Department of Biotechnology, Toyama Prefectural University, Imizu, Japan [3]Asano Active Enzyme Molecule Project, ERATO, JST, 5180 Kurokawa, Imizu, Toyama, Japan [4]Department of Biotechnology, The University of Tokyo, Yayoi 1–1–1, Bunkyo-ku, Tokyo, Japan

L-Tryptophan oxidase, VioA from *Chromobacterium violaceum*, which has a high substrate specificity for tryptophan, is useful for quantitative assay of tryptophan. However, stability of wild type VioA is not enough for its application in clinical or industrial use. To improve the thermal stability of the enzyme, we developed a VioA (C395A) mutant, with higher stability than wild type VioA. The VioA (C395A) exhibited similar specificity and kinetic parameter for tryptophan to wild type. Conventionally, the quantity of tryptophan is determined by instrumental methods, such as high-performance liquid chromatography (HPLC) after pre-column-derivatization. Using the mutant enzyme, we succeeded in the tryptophan quantification in human plasma samples, to an accuracy of $<2.9\%$ when compared to the instrumental method, and to a precision of CV $<3.2\%$. To analyse the

improvement in storage stability and substrate specificity, we further determined the crystal structures of VioA (C395A) complexed with FAD, and with FAD and tryptophan at 1.8 Å resolution.

Keywords: protein engineering, protein–ligand complex, reaction mechanism, tryptophan oxidase, X-ray crystallography

Biochemistry of Proteolysis

Two Cdc48 cofactors Ubp3 and Ubx2 regulate mitochondrial morphology and protein turnover

[1,2]Abhijit Chowdhury, [1,2,3]Teru Ogura and [1,3]Masatoshi Esaki

[1]Department of Molecular Cell Biology, Institute of Molecular Embryology and Genetics, Kumamoto University, Honjo 2–2–1, Chuo-ku, Kumamoto, Japan, [2]Program for Leading Graduate Schools "HIGO Program", Kumamoto University, Honjo 1–1–1, Chuo-ku, Kumamoto, Japan, [3]Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Honcho 4–1–8, Kawaguchi-shi, Saitama, Japan

Mitochondria continuously undergo coordinated fusion and fission during vegetative growth to keep their homogeneity and to remove damaged components. A cytosolic AAA ATPase, Cdc48, is implicated in the mitochondrial fusion event and turnover of a fusion-responsible GTPase in the mitochondrial outer membrane, Fzo1, suggesting a possible linkage of mitochondrial fusion and Fzo1 turnover. Here, we identified two Cdc48 cofactor proteins, Ubp3 and Ubx2, involving mitochondria regulation. In the absence of *UBP3*, mitochondrial fragmentation and aggregation were observed. The turnover of Fzo1 was not affected in Δ*ubp3*, but instead a deubiquitylase Ubp12 that removes fusion-required polyubiquitin chains from Fzo1 was stabilized. Thus, excess amount of Ubp12 may lead to mitochondrial fragmentation by removal of fusion-competent ubiquitylated Fzo1. In contrast, deletion of *UBX2* perturbed disassembly of Fzo1 oligomers and their degradation without alteration of mitochondrial morphology. The *UBX2* deletion led to destabilization of Ubp2 that negatively regulates Fzo1 turnover by removing degradation-signalling polyubiquitin chains, suggesting that Ubx2 would directly facilitate Fzo1 degradation. These results indicated that two different Cdc48-cofactor complexes independently regulate mitochondrial fusion and Fzo1 turnover.

Keywords: Cdc48/p97, cofactor, membrane fusion, mitochondria, ubiquitin

CELL

Cell General

Chemical compounds that suppress hypoxia-induced stress granule formation enhance cancer drug sensitivity of human cervical cancer

[1]Shikshya Timalsina, [1]Kyoko Arimoto-Matsuzaki, [1,2]Masami Kitamura, [1,3]Xiaoyin Xu, [1]Qiu Wenzhe, [4]Mari Ishigami-Yuasa, [4,5]Hiroyuki Kagechika and [1,6]Yutaka Hata

[1]Department of Medical Biochemistry, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan, [2]Diversity Diamond Unit Project Department, Student Support and Health Administration Organization, Tokyo Medical and Dental University, Tokyo, Japan, [3]China Department of Breast Surgery, The Second Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China, [4]Chemical Biology Screening Center, Tokyo Medical and Dental University, Tokyo, Japan, [5]Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Tokyo, Japan, [6]Center for Brain Integration Research, Tokyo Medical and Dental University, Tokyo, Japan

In eukaryotic cells, when exposed to certain types of stress including hypoxia, eIF2α is phosphorylated by several kinases including protein kinase R (PKR) and PKR-like endoplasmic reticulum kinase (PERK). Subsequently, protein translation is stopped and stress granules (SGs) are formed. Cancer cells form SGs under hypoxia. SGs accumulate apoptosis-related molecules and play anti-apoptotic roles. Thus, hypoxia-induced SG formation contributes to drug resistance in cancer cells. For this reason, inhibition of SG formation is expected to be beneficial in cancer therapy. To prove this concept, chemical reagents that inhibit SG formation are required as experimental tools. We searched for chemical compounds that suppress SG formation and identified that β-estradiol, progesterone, and stanolone (hereafter described as EPS) inhibit SG formation in human cervical cancer HeLa cells. As it turned out, EPS block PKR but not PERK, thus fail to suppress SG formation in most cancer cells, where SGs are formed *via* PERK. Nevertheless, in this study, we used HeLa cells as a model and demonstrated that EPS block hypoxia-induced SG formation in HeLa cells and consequently reduce drug resistance that HeLa cells acquire under hypoxia. Our findings support that inhibition of SG formation is a useful method to control cancers.

Keywords: cancer, drug resistance, hypoxia, protein kinases, stress granules

Stress Proteins and Molecular Chaperones

Chaperonin facilitates protein folding by avoiding initial polypeptide collapse

[1,2]Fumihiro Motojima, [1]Katsuya Fujii and [3]Masasuke Yoshida

[1]Department of Molecular Biosciences, Kyoto Sangyo University Kamigamo-Motoyama, Kita-ku, Kyoto, Japan, [2]Biotechnology Research Center and Department of Biotechnology, Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama, Japan, [3]Institute for Protein Dynamics, Kyoto Sangyo University, Kamigamo-Motoyama, Kita-ku, Kyoto, Japan

Chaperonins assist folding of many cellular proteins, including essential proteins for cell viability. However, it remains unclear

how chaperonin-assisted folding is different from spontaneous folding. Chaperonin GroEL/GroES facilitates folding of denatured protein encapsulated in its central cage but the denatured protein often escapes from the cage to the outside during reaction. Here, we show evidence that the in-cage-folding and the escape occur diverging from the same intermediate complex in which polypeptide is tethered loosely to the cage and partly protrudes out of the cage. Furthermore, denatured proteins in the chaperonin cage are kept in more extended conformation than those initially formed in spontaneous folding. We propose that the formation of tethered intermediate of polypeptide is necessary to prevent polypeptide collapse at the expense of polypeptide escape. The tethering of polypeptide would allow freely mobile portions of tethered polypeptide to fold segmentally.
Keywords: chaperonin, collapsed state, GroEL, molecular chaperone, protein folding

BIOTECHNOLOGY

Biotechnology General

A dual system using compartmentalized partnered replication for selection of arsenic-responsive transcriptional regulator

[1]Seaim Lwin Aye, [1]Kei Fujiwara and [1]Nobuhide Doi

[1]Department of Biosciences and Informatics, Keio University, 3–14–1 Hiyoshi, Yokohama, Japan

Engineering and design of genetic circuit in living cell is critical in accessing the beneficial application of synthetic biology. Directed evolution can avoid the complicated rational design of such circuit by screening or selecting functional circuit from non-functional one. Here, we proposed a positive–negative selection system for selecting a transcription factor that activates gene expression in response to arsenic in solution. First, we developed a whole cell biosensor for sensing arsenite in liquid using a regulator (ArsR) and a reporter (GFP), and evaluated its performance. Second, we developed a positive selection system for active ArsR using compartmentalized partnered replication that uses thermostable DNA polymerase as the reporter of activity. Third, we developed a negative selection system using sucrose-induced suicide gene SacB as the reporter for exclusion of inactive ArsR variants.
Keywords: arsenic contamination, biosensor, gene circuit, water-in-oil emulsion

Journal of Biochemistry

Vol. 164, No. 6 （2018 年 12 月 発 行）

ダイジェスト

BIOCHEMISTRY

Immunochemistry

Generation of an anti-desmoglein 3 antibody without pathogenic activity of pemphigus vulgaris for therapeutic application to squamous cell carcinoma

[1]Shin-Ichi Funahashi, [1]Shigeto Kawai, [1,2]Etsuko Fujii, [2]Kenji Taniguchi, [1]Kiyotaka Nakano, [3]Shumpei Ishikawa, [3]Hiroyuki Aburatani and [1,2]Masami Suzuki

[1]Forerunner Pharma Research Co., Ltd., Komaba Open Laboratory, The University of Tokyo, 4–6–1 Komaba, Meguro-ku, Tokyo, Japan, [2]Chugai Pharmaceutical Co., Ltd., 200 Kajiwara, Kamakura, Kanagawa, Japan, [3]Genome Science, RCAST, The University of Tokyo, 4–6–1 Komaba, Meguro-ku, Tokyo, Japan

It is ideal for the target antigen of a cytotoxic therapeutic antibody against cancer to be cancer-specific, but such antigens are rare. Thus an alternative strategy for target selection is necessary. Desmoglein 3 (DSG3) is highly expressed in lung squamous cell carcinoma, while it is well-known that anti-DSG3 antibodies cause pemphigus vulgaris, an autoimmune disease. We evaluated DSG3 as a novel target by selecting an epitope that exerts efficacy against cancer with no pathogenic effects in normal tissues. Pathogenic anti-DSG3 antibodies induce skin blisters by inhibiting the cell–cell interaction in a Ca^{2+}-dependent manner. We screened anti-DSG3 antibodies that bind DGS3 independent of Ca^{2+} and have high antibody-dependent cell cytotoxicity (ADCC) activity against DSG3-expressing cells. These selected antibodies did not inhibit cell–cell interaction and showed ADCC activity against squamous cell carcinoma cell lines. Furthermore, one of the DSG3 antibodies showed anti-tumour activity in tumour mouse models but did not induce adverse effects such as blister formation in the skin. Thus it was possible to generate an antibody against DSG3 by using an appropriate epitope that retained efficacy with no pathogenicity. This approach of epitope selection may expand the variety of druggable target molecules.
Keywords: DSG3, epitope, monoclonal antibody, pemphigus vulgaris, squamous cell carcinoma

Biochemical Pharmacology

Hsp90 inhibitor AT-533 blocks HSV-1 nuclear egress and assembly

[1]Feng Li, [1]Fujun Jin, [1]Yiliang Wang, [1]Danlin Zheng, [1]Junwei Liu, [1]Zhen Zhang, [1]Rongze Wang, [1,2]Dong Dong, [3]Kai Zheng,

[1,4,5]Yifei Wang

[1]Institute of Biomedicine, College of Life Science and Technology, Jinan University, Guangzhou, China, [2]International Ocular Surface Research Center and Institute of Ophthalmology, College of Medicine, Jinan University Medical School, Guangzhou, China, [3]School of Pharmaceutical Sciences, Health Science Center Shenzhen University, Shenzhen, China, [4]Key Laboratory of Virology of Guangzhou, Jinan University, Guangzhou, China, [5]Key Laboratory of Bioengineering Medicine of Guangdong Province, Jinan University, Guangzhou, China

Heat shock protein 90 (Hsp90) has been identified as an essential host factor for the infection and replication of several viruses, including HSV-1. Recent works have clearly shown that Hsp90 plays a role in the early stages of HSV-1 infection, including nuclear import and DNA replication. However, the role of Hsp90 in the late stages of HSV-1 infection remains unclear. In this study, we found that Hsp90 was up-regulated during late viral infection. Treatment with the Hsp90 inhibitor AT-533 significantly decreased the intracellular and extracellular virus titers, and strongly inhibited nucleocapsid egress from the nucleus. More detailed studies revealed that AT-533 inhibited the nuclear egress of the viral nucleocapsid by suppressing the expression and translocation of nuclear-associated proteins pUL31 and pUL34. In addition, we found that AT-533 hindered the assembly of virus particles possibly though affecting the localization of glycoproteins in the endoplasmic reticulum and Golgi apparatus. These results thus invoke a new role for Hsp90 in the nucleocapsid egress and viral maturation of HSV-1, and further promote the development of Hsp90 inhibitors as potential anti-HSV-1 drugs.

Keywords: assembly, egress, Hsp90, Hsp90 inhibitor, HSV-1

Metabolomics approach in lung tissue of septic rats and the interventional effects of Xuebijing injection using UHPLC-QOrbitrap-HRMS

[1,2]Tanye Xu, [1]Lin Zhou, [1]Yingying Shi, [1]Liwei Liu, [1]Lihua Zuo, [1]Qingquan Jia, [1]Shuzhang Du, [1]Jian Kang and [1]Xiaojian Zhang, [1]Zhi Sun

[1]Department of Pharmacy, The First Affiliated Hospital of Zhengzhou University, No.1 Jianshe East Road, Erqi District, Zhengzhou, Henan Province, PR China, [2]College of Food Science and Engineering, Dalian Ocean University, No. 52 Heishijiao Street, Shahekou District, Dalian, Liaoning Province, PR China

Sepsis is the dysregulated host response to an infection which leads to life-threatening organ dysfunction. Metabolomic profiling in bio-fluid or tissue is vital for elucidating the pathogenesis of sepsis and evaluating therapeutic effects of medication. In this study, an untargeted metabolomics approach was applied to study the metabolic changes in lung tissue of septic rats induced by cecal ligation and puncture (CLP) and investigate the treatment effects of Xubijing injection (XBJ). Metabolomics analyses were performed on ultra-high performance liquid chromatography-Q Exactive hybrid quadrupole-orbitrap high-resolution accurate mass spectrometry (UHPLC-Q-Orbitrap-HRMS) together with multivariate statistical analysis. A total of 26 differential metabolites between CLP and sham-operated group were identified. The altered metabolic pathways included energy metabolism, amino metabolism, lipid metabolism, fatty acid metabolism and hormone metabolism. Among the 26-varied metabolites, 15 were significantly regulated after XBJ treatment. The metabolic pathway network of sepsis was drawn to interpret the pathological feature of lung damage caused by sepsis and the underlying regulating mechanism of XBJ on the molecular levels. Our findings display that LC-MS-based metabolomics is a useful tool for uncovering the underlying molecular mechanism of sepsis, and XBJ may exert therapeutic effect by regulating multiple metabolic pathways.

Keywords: lung tissue, metabolomics, sepsis, UHPLC-Q-Orbitrap-HRMS, Xuebijing injection

MOLECULAR BIOLOGY

Molecular Biology General

Long non-coding RNA Linc00662 promotes cell invasion and contributes to cancer stem cell-like phenotypes in lung cancer cells

[1]Wenjing Gong, [2]Yi Su, [3]Yan Liu, [1]Ping Sun and [1]Xiumei Wang

[1]Department of Oncology, The Affiliated Yantai Yuhuangding Hospital of Qingdao University Institution, 20 Yudong Road, Zhifu District, Yantai, Shandong Province, China, [2]Department of Radiotherapy, The Affiliated Yantai Yuhuangding Hospital of Qingdao University Institution, 20 Yudong Road, Zhifu District, Yantai, Shandong Province, China, [3]Department of Infectious Disease, The Affiliated Yantai Yuhuangding Hospital of Qingdao University Institution, 20 Yudong Road, Zhifu District, Yantai, Shandong Province, China

Long non-coding RNAs (lncRNAs) is essential in regulation of cancer cell and cancer stem cells (CSCs) behaviour. Linc00662 is a newly identified human lung cancer related lncRNA. In this study, we aimed to explore the function of Linc00662 in human lung cancer. The expression level of Linc00662 was analysed by quantitative real-time PCR. Cell metastasis and invasive ability were detected by transwell and scratch wound healing assays. The stemness of CSCs was shown by tumorsphere formation assay and flow cytometry. The interaction between Linc00662 and Lin28 was confirmed by RNA immunoprecipitation and RNA pulldown assay. Overexpression of Linc00662 promoted the poor prognosis of lung cancer. Cell invasion, metastasis and CSCs stemness in H1299 and A549 could be influenced by Linc00662. Linc00662 had direct interaction with Lin28, and the Linc00662 function was dependent on Lin28. We demonstrate that overexpression of Linc00662 enhances lung cancer cell me-

tastasis and CSC stemness by interacting with Lin28 in human lung cancer, which could be utilized as a potential diagnostic and therapeutic target for lung cancer patients.

Keywords: cancer stem cells (CSCs), Linc00662, Lin28, long non-coding RNAs (lncRNAs), lung cancer

Replication and Recombination

Function of the amino-terminal region of human MCM4 in helicase activity

[1]Xuan Wang and [1]Yukio Ishimi

[1]College of Science, Ibaraki University, Mito, Ibaraki, Japan

The amino-terminal region of eukaryotic MCM4 is characteristic of the presence of a number of phosphorylation sites for CDK and DDK, suggesting that the region plays regulatory roles in the MCM2-7 helicase function. However, the roles are not fully understood. We analyzed the role of the amino-terminal region of human MCM4 by using MCM4/6/7 helicase as a model for MCM2-7 helicase. First we found that deletion of 35 amino acids at the amino-terminal end resulted in inhibition of DNA helicase activity of the MCM4/6/7 complex. Conversion of arginine at amino acid no. 10 and 11 to alanine had similar effect to the deletion mutant of Δ1-35, suggesting that these arginine play a role in the DNA helicase activity. The data suggest that expression of these mutant MCM4 in HeLa cells perturbed the progression of the S phase. Substitution of six CDK phosphorylation sites (3, 7, 19, 32, 54 and 110) in the amino-terminal region by phospho-mimetic glutamic acids affected the hexamer formation of the MCM4/6/7 complex. MCM4 phosphorylation by CDK may play a role in DNA replication licensing system, and the present results suggest that the phosphorylation interferes MCM function by lowering stability of MCM complex.

Keywords: DNA, helicase, phosphorylation, protein kinases/CDKs, replication

Gene Expression

C1D is not directly involved in the repair of UV-damaged DNA but protects cells from oxidative stress by regulating gene expressions in human cell lines

[1]Takeshi Tomita, [1]Katsuaki Ieguchi, [1]Morichika Takita, [1]Fujiko Tsukahara, [2]Masayuki Yamada, [3]Jean-Marc Egly and [1]Yoshiro Maru

[1]Department of Pharmacology, Tokyo Women's Medical University, 8–1 Kawada, Shinjuku, Tokyo, Japan, [2]Center for Medical Education, Graduate School of Medicine, Kyoto University, Yoshida-Konoe-cho, Sakyo-ku, Kyoto, Japan, [3]Institut de Génétique et de Biologie Moléculaire et Cellulaire CNRS/INSERM/UdS 1, rue Laurent Fries, BP163 F-67404 Illkirch Cedex, France

A small nuclear protein, C1D, has roles in various cellular processes, transcription regulation, genome stability surveillance, DNA repair and RNA processing, all of which are required to maintain the host life cycles. In the previous report, C1D directly interacts with XPB, a component of the nucleotide excision repair complex, and C1D knockdown reduced cell survival of 27–1 cells, CHO derivative cells, after UV irradiation. To find out the role of C1D in UV-damaged cells, we used human cell lines with siRNA or shRNA to knockdown C1D. C1D knockdown reduced cell survival rates of LU99 and 786-O after UV irradiation, although C1D knockdown did not affect the efficiency of the nucleotide excision repair. Immunostaining data support that C1D is not directly involved in the DNA repair process in UV-damaged cells. However, H2O2 treatment reduced cell viability in LU99 and 786-O cells. We also found that C1D knockdown upregulated DDIT3 expression in LU99 cells and downregulated APEX1 in 786-O cells, suggesting that C1D functions as a co-repressor/activator. The data accounts for the reduction of cell survival rates upon UV irradiation.

Keywords: C1D, DNA damage, reactive oxygen species, XPB, gene regulation

CELL

Biomembranes

A newly isolated Pex7-binding, atypical PTS2 protein P7BP2 is a novel dynein-type AAA+ protein

[1]Hajime Niwa, [2]Yasuhiro Miyauchi-Nanri, [2]Kanji Okumoto, [2]Satoru Mukai, [3,4]Kentaro Noi, [3,4]Teru Ogura and [1]Yukio Fujiki

[1]Medical Institute of Bioregulation, Kyushu University, 3–1–1 Maidashi, Higashi-ku, Fukuoka, Japan, [2]Department of Biology, Faculty of Sciences, Kyushu University, 744 Motooka Nishi-ku, Fukuoka, Japan, [3]Department of Molecular Cell Biology, Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto, Japan, [4]Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Saitama, Japan

A newly isolated binding protein of peroxisomal targeting signal type 2 (PTS2) receptor Pex7, termed P7BP2, is transported into peroxisomes by binding to the longer isoform of Pex5p, Pex5pL, via Pex7p. The binding to Pex7p and peroxisomal localization of P7BP2 depends on the cleavable PTS2 in the N-terminal region, suggesting that P7BP2 is a new PTS2 protein. By search on human database, three AAA+ domains are found in the N-terminal half of P7BP2. Protein sequence alignment and motif search reveal that in the C-terminal region P7BP2 contains additional structural domains featuring weak but sufficient homology to AAA+ domain. P7BP2 behaves as a monomer in gel-filtration chromatography and the single molecule observed under atomic force microscope shapes a disc-like ring. Collectively, these results suggest that P7BP2 is a novel dynein-type AAA+ family protein, of which domains are arranged into a pseudo-hexameric ring structure.

Keywords: AFM, dynein-type AAA+, matrix protein import, peroxisome, Pex7p-binding protein

BIOTECHNOLOGY

Biomaterials

A novel protocol for the preparation of active recombinant human pancreatic lipase from *Escherichia coli*

[1]Nanami Kawaguchi, [2]Kimie Date, [3]Yusuke Suzuki, [1]Chihiro Tomita, [1]Rina Naradate, [1]Tomoko Higami, [1]Kosuke Nakamura, [1,2]Kyoko Aikawa and [1,2]Haruko Ogawa

[1]Chemistry and Biochemistry, Division of Advanced Sciences, Graduate School of Humanities and Sciences, [2]Glycoscience Division, Institute for Human Life Innovation, Ochanomizu University, 2–1–1 Otsuka, Bunkyo-ku, Tokyo, Japan, [3]Department of Materials and Applied Chemistry, College of Science and Technology, Nihon University, 1–5–1 Kanda Surugadai, Chiyoda-ku, Tokyo, Japan

An active recombinant human pancreatic lipase (recHPL) was successfully prepared for the first time from the *Escherichia coli* expression system using short Strep-tag II (ST II). The recHPL–ST II was solubilized using 8 M urea from *E.coli* lysate and purified on a Strep-Tactin-Sepharose column. After refolding by stepwise dialyses in the presence of glycerol and Ca^{2+} for 2 days followed by gel filtration, 1.8–6 mg of active recHPL–ST II was obtained from 1 L of culture. The recHPL was non-glycosylated, but showed almost equal specific activity, pH-dependency and time-dependent stability compared to those of native porcine pancreatic lipase (PPL) at 37°C. However, the recHPL lost its lipolytic activity above 50°C, showing a lower heat-stability than that of native PPL, which retained half its activity at this temperature.

Keywords: Escherichia coli, human pancreatic lipase, lipolytic activity, refolding, Strep-tag II