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ダイジェスト

BIOCHEMISTRY

Biochemistry General

Gene disruption of medaka (Oryzias latipes) orthologue for mammalian tissue-type transglutaminase (TG2) causes movement retardation

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Transglutaminases are an enzyme family that catalyses protein cross-linking essential for several biological functions. In the previous studies, we characterized the orthologues of the mammalian transglutaminase family in medaka (*Oryzias latipes*), an established fish model. Among the human isozymes, tissue-type transglutaminase (TG2) has multiple functions that are involved in several biological phenomena. In this study, we established medaka mutants deficient for the orthologue of human TG2 using the CRISPR/Cas9 and transcription activator-like effector nucleases systems. Although apparent morphological changes in the phenotype were not observed, movement retardation was found in the mutant fish when evaluated by a tank-diving test. Furthermore, comparative immunohistochemistry analysis using in this fish model revealed that orthologue of human TG2 was expressed at the periventricular layer of the optic tectum. Our findings provide novel insight for the relationship between tissue-type transglutaminase and the nervous system and the associated behaviour.

Keywords; gene-editing, immunohistochemistry, medaka, movement, transglutaminase

Protein Structure

Crystal structure of adenylate kinase from an extremophilic archaeon Aeropyrum pernix with ATP and AMP

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The crystal structure of an adenylate kinase from an extremophilic archaeon *Aeropyrum pernix* was determined in complex with full ligands, ATP-Mg^{2+} and AMP, at a resolution of 2.0 Å. The protein forms a trimer as found for other adenylate kinases from archaea. Interestingly, the reacting three atoms, two phosph orus and one oxygen atoms, were located almost in line, support ing the S_N2 nucleophilic substitution reaction mechanism. Based on the crystal structure obtained, the reaction coordinate was estimated by the quantum mechanics calculations combined with molecular dynamics. It was found that the reaction undergoes two energy barriers; the steps for breaking the bond between the oxygen and γ-phosphorus atoms of ATP to produce a phosphoryl fragment and creating the bond between the phosphoryl fragment and the oxygen atom of the β-phosphate group of ADP. The reaction coordinate analysis also suggested the role of amino-acid residues for the catalysis of adenylate kinase.

Keywords; adenylate kinase, crystal structure, QM/MM calculation, reaction coordinate

Biochemical properties of human full-length aryl hydrocarbon receptor (AhR)

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The aryl hydrocarbon receptor (AhR) is a very unstable protein. AhR binds to the molecular chaperone complex (HSP90-p23-XAP2) to maintain a stable structure in the cytoplasm. After binding to ligands, such as dioxin, AhR translocates from the cytoplasm to the nucleus with a molecular chaperone complex. The protein forms a heterodimer with Arnt after nuclear transfer, functions as a transcription factor by binding to a xenobiotic responsive element (XRE), and induces the cytochrome P450 1A1 (CYP1A1). Because of the unstable protein, expression of the full-length AhR in the *E. coli* expression system is very difficu lt. Many studies investigated AhR using AhR domains *in vitro*. We expressed and purified the human full-length AhR in *E. coli* expression system. Furthermore, specific antibodies were prep ared. Purified full-length AhR could bind to ligand. In the presence of ligand, α-helix and random coil of AhR increased and β-sheet decreased on CD spectrum. Full-length AhR could bind to HSP90, XAP2 and p23 in the presence or absence of ligand. We now show the biochemical properties of full-length AhR.

Keywords; AhR, HSP, molecular chaperone, secondary structure

Enzymology

Predominant secretion of cellobiohydrolases and endo-*β*-1,4-glucanases in nutrient-limited medium by Aspergillus spp. Isolated from subtropical field

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Biological degradation of cellulose from dead plants in nature and plant biomass from agricultural and food-industry waste is important for sustainable carbon recirculation. This study aimed at searching diverse cellulose-degrading systems of wild filamentous fungi and obtaining fungal lines useful for cellooligosaccharide production from agro-industrial wastes. Fungal lines with cellulolytic activity were screened and isolated from stacked rice straw and soil in subtropical fields. Among 13 isolated lines, in liquid culture with a nutrition-limited cellulose-containing medium, four lines of ***Aspergillus*** spp. secreted 50–60 kDa proteins as markedly dominant components and gave clear activity bands of possible endo-*β*-1,4-glucanase in zymography. Mass spectroscopy (MS) analysis of the dominant components identified three endo-*β*-1,4-glucanases (GH5, GH7 and GH12) and two cellobiohydrolases (GH6 and GH7). Cellulose degradation by the secreted proteins was analysed by LC-MS-based measurement of derivatized reducing sugars. The enzymes from the four ***Aspergillus*** spp. produced cellobiose from crystalline cellulose and cellotriose at a low level compared with cellobiose. Moreover, though smaller than that from crystalline cellulose, the enzymes of two representative lines degraded powdered rice straw and produced cellobiose. These fungal lines and enzymes would be effective for production of cellooligosaccharides as cellulose degradation-intermediates with added value other than glucose.

Keywords; agricultural waste, cellooligosaccharides, cellulose, filamentous fungi

MOLECULAR BIOLOGY

Molecular Biology General

A novel monoclonal antibody cross-reactive with both human and mouse *α*9 integrin useful for therapy against rheumatoid arthritis

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This study introduces a novel monoclonal anti-*α*9 integrin antibody (MA9-413) with human variable regions, isolated by phage display technology. MA9-413 specifically binds to both human and mouse *α*9 integrin by recognizing a conserved loop region designated as L1 (amino acids 104–122 of human *α*9 integrin). MA9-413 inhibits human and mouse *α*9 integrin-dependent cell adhesion to ligands and suppresses synovial inflammation and osteoclast activation in a mouse model of arthritis. This is the first monoclonal anti-*α*9 integrin antibody that can react with and functionally inhibit both human and mouse *α*9 integrin. MA9-413 allows data acquisition both in animal and human pharmacological studies without resorting to surrogate antibodies. Since MA9-413 showed certain therapeutic effects in the mouse arthritis model, it can be considered as a useful therapy against rheumatoid arthritis and other *α*9 integrin-associated diseases.

Keywords; anti-*α*9 integrin antibody, cross-reactivity, phage display technology, rheumatoid arthritis, therapeutic antibody

Microtubule elongation along actin fi laments induced by microtubule-associated protein 4 contributes to the formation of cellular protrusions

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Actin-microtubule crosstalk is implicated in the formation of cellular protrusions, but the mechanism remains unclear. In this study, we examined the regulation of cell protrusion involving a ubiquitously expressed microtubule-associated protein (MAP) 4, and its superfamily proteins, neuronal MAP2 and tau. Fluorescence microscopy revealed that these MAPs bound to F-actin and microtubules simultaneously, and formed F-actin/microtubule hybrid bundles. The hybrid bundle-forming activity was in the order of MAP2 > MAP4 ≫ tau. Interestingly, the microtubule assembly-promoting activity of MAP4 and MAP2, but not of tau, was upregulated by their interaction with F-actin. When MAP4 was overexpressed in NG108-15 cells, the number of cell processes and maximum process length of each cell increased

significantly by 28% and 30%, respectively. Super-resolution microscopy revealed that 95% of microtubules in cell processes colocalized with F-actin, and MAP4 was always found in their vicinity. These results suggest that microtubule elongation along F-actin induced by MAP4 contributes to the formation of cellular protrusions. Since MAP4, MAP2 and tau had different cross-talk activity between F-actin and microtubules, it is likely that the functional differentiation of these MAPs is a driving force for neural evolution, causing significant changes in cell morphology.
Keywords; cellular protrusion, MAP2, MAP4, super-resolution microscopy, tau

Gene Expression

MicroRNA-548-3p overexpression inhibits proliferation, migration and invasion in osteoblast-like cells by targeting STAT1 and MAFB

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Osteoporosis is the most common bone disease and a public health issue with increasing prevalence in Mexico. This disease is caused by an imbalance in the bone remodelling process mediated by osteoclast and osteoblast. MicroRNAs have emerged as key players during the differentiation of both types of cells specialized involved in bone metabolism. We found high expression levels of miR-548x-3p in circulating monocytes derived from postmenopausal osteoporotic women. This study aimed to analyse the functional characterization of miR-548x-3p roles in the bone remodelling process. We validated by RT-qPCR, the elevated levels of miR-548x-3p in circulating monocytes derived from osteoporosis women. Through bioinformatics analysis, we identify *MAFB* and *STAT1* as potential target genes for miR-548x-3p. Both genes showed low levels of expression in circul ating monocytes derived from osteoporotic women. In addition, we demonstrated the binding of miR-548x-3p to the 3′-UTR of both mRNAs. MiR-548x-3p was overexpressed in osteoblasts-like cell lines decreasing the levels of *MAFB* and *STAT1* mRNA and protein. We found that miR-548x-3p overexpression inhibi ts the proliferation, migration and invasion of the cell lines evalu ated. Our results identified, by the first time, the potential role of miR-548x-3p as a modulator of the bone remodelling process by regulating the expression of *MAFB* and *STAT1*.
Keywords; bone remodelling, gene expression, microRNA, osteoblast, osteoclast

CELL
Differentiation, Development, and Aging

Conditioned medium of the osteosarcoma cell line U2OS induces hBMSCs to exhibit characteristics of carcinoma-associated fi broblasts via activation of IL-6/STAT3 signalling

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As a research hotspot in recent years, bone mesenchymal stem cells (BMSCs) play an important role in the process of a variety of human diseases, including cancers. However, in osteosarcoma, the role of BMSCs and their communication with tumour cells are not clear. In this study, we validated the communication of osteosarcoma (OS) cells with BMSCs. The results showed that the conditioned medium of osteosarcoma cell line U2OS (U2OS-CM) induces the carcinoma-associated fibroblasts (CAFs)-like transformation of BMSCs and promotes the proliferation, migration and invasion of BMSCs. Mechanistically, treatment of human bone mesenchymal stem cells (hBMSCs) with U2OS-CM results in a significant increase in the IL-6 expression and phosphorylation of STAT3. Furthermore, blockade of the IL-6/STAT3 signalling in hBMSCs rescues the transformation of CAF phenotype induced by U2OS-CM. And, human IL-6 can directly increase the expression of the CAF marker genes in hMSCs. Meanwhile, IL-6/STAT3 signalling involves in promoting effects of U2OS-CM on the proliferation, migration and invasion of BMSCs. In summary, our results suggest that BMSCs communicate with OS cells through IL-6/STAT3 signalling and play an important role in the progress of osteosarcoma.
Keywords; carcinoma-associated fibroblasts, interleukin-6, mesenchymal stem cells, osteosarcoma, STAT3

MITOL dysfunction causes dwarfi sm with anterior pituitary hypoplasia

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In mitochondrial disorders, short stature and growth failure are common symptoms, but their underlying mechanism remains unknown. In this study, we examined the cause of growth failure of mice induced by nestin promoter-driven knockout of the mi-

tochondrial ubiquitin ligase MITOL (MARCH5), a key regulator of mitochondrial function. MITOL-knockout mice have congenital hypoplasia of the anterior pituitary caused by decreased expression of pituitary transcript factor 1 (Pit1). Consistently, both mRNA levels of growth hormone (GH) and prolactin levels were markedly decreased in the anterior pituitary of mutant mice. Growth failure of mutant mice was partly rescued by hypodermic injection of recombinant GH. To clarify whether this abnormality was induced by the primary effect of MITOL knockdown in the anterior pituitary or a secondary effect of other lesions, we performed lentiviral-mediated knockdown of MITOL on cultured rat pituitary GH3 cells, which secrete GH. GH production was severely compromised in MITOL-knockdown GH3 cells. In conclusion, MITOL plays a critical role in the development of the anterior pituitary; therefore, mice with MITOL dysfunction exhibited pituitary dwarfism caused by anterior pituitary hypoplasia. Our findings suggest that mitochondrial dysfunction is commonly involved in the unknown pathogenesis of pituitary dwarfism.

Keywords; dwarfism, growth hormone, mitochondrial ubiquitin ligase MITOL, Pit1, pituitary

BIOTECHNOLOGY

Biomaterials

Convenient method of producing cyclic single-chain Fv antibodies by split-intein-mediated protein ligation and chaperone co-expression

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Single-chain Fv (scFv) is a recombinant antibody in which the variable regions of the heavy chain (VH) and light chain (VL) are connected by a short flexible polypeptide linker. Compared with monoclonal antibodies, scFvs have the advantages of low-cost production using *Escherichia coli* and easy genetic mani pulation. ScFvs are, therefore, regarded as useful modules for producing next-generation medical antibodies. The practical use of scFvs has been limited due to their aggregation propensity mediated by interchain VH–VL interactions. To overcome this problem, we recently reported a cyclic scFv whose N-terminus and C-terminus were connected by sortase A-mediated ligation. Preparation of cyclic scFv is, however, a time-consuming process. To accelerate the application study of cyclic scFv, we developed a method to produce cyclic scFv by the combined use of a protein ligation technique based on protein *trans*-splicing reaction (PTS) by split intein and a chaperone co-expression system. This method allows for the preparation of active cyclic scFv from the cytoplasm of *E. coli.* The present method was app lied to the production of cyclic 73MuL9-scFv, a GA-pyridine ant ibody, as a kind of advanced glycation end-product. These findin gs are expected to evoke further application study of cyclic scFv.

Keywords; antibody engineering, cyclic scFv, protein trans-splicing, single-chain Fv, split inteins

RNA Technology

Application of solid-phase DNA probe method with cleavage by deoxyribozyme for analysis of long non-coding RNAs

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The solid-phase DNA probe method is a well-established technique for tRNA purification. We have applied this method for purification and analysis of other non-coding RNAs. Three columns for purification of $tRNA^{Phe}$, transfer-messenger RNA (tmRNA) and 16S rRNA from ***Thermus thermophilus*** were connected in tandem and purifications were performed. From each column, $tRNA^{Phe}$, tmRNA and 16S rRNA could be purified in a single step. This is the first report of purification of native tmRNA from ***T. thermophilus*** and the purification demonstrates that the solid-phase DNA probe method is applicable to non-coding RNA, which is present in lower amounts than tRNA. Furthermore, if a long non-coding RNA is cleaved site-specifically and the fragment can be purified by the solid-phase DNA probe method, modified nucleosides in the long non-coding RNA can be analysed. Therefore, we designed a deoxyribozyme (DNAzyme) to perform site-specific cleavage of 16S rRNA, examined optimum conditions and purified the resulting RNA fragment. Sequencing of complimentary DNA and mass spectrometric analysis revealed that the purified RNA corresponded to the targeted fragment of 16S rRNA. Thus, the combination of DNAzyme cleavage and purification using solid-phase DNA probe methodology can be a useful technique for analysis of modified nucleosides in long non-coding RNAs.

Keywords; DNAzyme, rRNA, solid-phase DNA probe, tmRNA, tRNA

JB号ダイジェスト10月は発行遅延により次号に掲載します.