*Journal of Biochemistry*

**Vol. 172, No. 5**（2022 年 11 月 発 行）

# ダイジェスト

***JB REVIEW***

**The E-Id axis specifies adaptive and innate lymphoid lineage cell fates**

Masaki Miyazaki and Kazuko Miyazaki

Laboratory of Immunology, Institute for Life and Medical Sciences, Kyoto University, Kyoto, 606–8507, Japan

Our bodies are constantly threatened with the invasion of pathogens, such as bacteria and virus. Immune responses against pathogens are evoked in collaboration with adaptive and innate immune systems. Adaptive immune cells including T and B cells recognize various antigens from pathogens through the antigen recognition receptors such as immunoglobulin (*Ig*) and T-cell receptor (TCR), and they evoke antigen-specific immune responses to eliminate the pathogens. This specific recognition of a variety of antigens relies on the V(D)J DNA recombination of *Ig* and *TCR* genes, which is generated by the Rag (recombination activation gene) 1/Rag2 protein complex. The expression of *Rag1/2* genes is stringently controlled during the T and B cell development; *Rag1/2* gene expression indicates the commitment towards adaptive lymphocyte lineages. In this review article, we will discuss the developmental bifurcation between adaptive and innate lymphoid cells, and the role of transcription factors, especially the E and Id proteins, upon the lineage commitment, and the regulation of *Rag* gene locus.

Keywords: adaptive immunity, *E2A*, gene regulaton, *Rag1/Rag2* expression, T and B cell development

***BIOCHEMISTRY***

***Biochemistry General***

**Acute exposure of minimally oxLDL elicits survival responses by downregulating the mediators of NLRP3 inflammasome in cultured RAW 264.7 macrophages**

Finosh G Thankam, Bisma Khwaja, Megan Nguyen, Osama Ahsan and Devendra K Agrawal

Department of Translational Research, Western University of Health Sciences, Pomona, California 91766, USA

Lipid burden in macrophages driven by oxidized low-density lipoprotein (oxLDL) accelerates the foam cell formation and the activation of sterile inflammatory responses aggravating the atherosclerosis. However, there is limited information on the mediators and the pathways involved in the possible survival responses, especially at the initial phase, by lipid burden in macrophage cells on encountering oxLDL. The present study was designed to assess the expression status of major mediators involved in the NLRP3 inflammasome pathway of sterile inflammation and the cellular responses in oxLDL-challenged cultured RAW 264.7 macrophage cells. OxLDL-treated RAW 264.7 macrophage cells displayed a decreased expression of the key sterile inflammatory mediators, TLR4, TLR2, ASC, NLRP3 and IL-18 at protein and transcript levels; however, they displayed increased level of IL-1$\beta$, RAGE and TREM1 at protein level. Biological responses including lipid uptake, lipid peroxidation, cellular hypertrophy, mitochondrial density and mitochondrial membrane potential were significantly increased in oxLDL-treated macrophages. Moreover, superoxide production was significantly decreased in the oxLDL-treated macrophages compared to the control. Overall, the findings revealed the expression status of key sterile mediators and the macrophage response during the initial phase of oxLDL exposure tend towards the prevention of inflammation. Further understanding would open novel translational opportunities in the management of atherosclerosis.

Keywords: sterile inflammation, oxidized LDL, macrophages, lipid burden, atherosclerosis

**Biochemical characterization of medaka (Oryzias latipes) fibrinogen gamma and its gene disruption resulting in anemia as a model fish**

Qi Meng[1], Yuko Watanabe[1], Hideki Tatsukawa[1], Hisashi Hashimoto[2] and Kiyotaka Hitomi[1]

[1]Graduate School of Pharmaceutical Sciences, Nagoya University, Nagoya 464–8601, Japan, [2]Graduate School of Science, Nagoya University, Nagoya, 464–8602, Japan

At the final stages of blood coagulation, fibrinogen is processed into insoluble fibrin by thrombin resulting in fibril-like structure formation. Via further cross-linking reactions between the fibrin gamma subunit by the catalytic action of blood transglutaminase (Factor XIII), this molecule gains further physical stability. Meanwhile, since fibrinogen is expressed in various cells and tissues, this molecule can exhibit other functions apart from its role in blood coagulation. To create a system studying on aberrant coagulation and investigate the physiological functions, using a model fish medaka (*Oryzias latipes*), we established gene-deficient mutants of fibrinogen gamma subunit protein in parallel with its biochemical analysis, such as tissue distribution pattern and substrate properties. By genetic deletion via genome editing, two distinct mutants displayed retardation of blood coagulation. The mutants showed lower hematocrit with aberrant erythrocyte maturation, which indicates that fibrin deficiency caused severe anemia, and also appeared as a model for investigation of the fibrin function.

Keywords: anemia, coagulation, fibrinogen, genome editing, medaka

**Distribution and evolution of the serine/aspartate racemase family in invertebrates. II. Frequent and widespread parallel evolution of aspartate racemase**

Kouji Uda[1] and Luke A. Moe[2]

[1]Laboratory of Biochemistry, Faculty of Science and Technology, Kochi University, Kochi 780–8520, Japan, [2]Department of Plant and Soil Sciences, 311 Plant Science Building, University of Kentucky, Lexington, KY 40546–0312, USA

Our previous studies showed that invertebrate animal serine racemase (SerR) and aspartate racemase (AspR) evolved from a common ancestral gene and are widely distributed. However, the overall molecular evolutionary background of these genes has remained unclear. In the present study, we have cloned, expressed and characterized five SerR and three AspR genes from six invertebrate species. The coexistence of SerR and AspR paralogs has been observed in some species, and the presence of both SerR and AspR is here confirmed in the flatworm *Macrostomum lignano*, the feather star *Anneissia japonica*, the ark shell *Anadara broughtonii* and the sea hare *Aplysia californica*. Comparison of the gene structures revealed the evolution of SerR and AspR. The ancestral species of metazoans probably had a single SerR gene, and the first gene duplication in the common ancestor species of the eumetazoans occurred after the divergence of porifera and eumetazoans, yielding two SerR genes. Most eumetazoans lost one of the two SerR genes, while the echinoderm *A. japonica* retained both genes. Furthermore, it is clear that invertebrate AspR genes arose through parallel evolution by duplication of the SerR gene followed by substitution of amino acid residues necessary for substrate recognition in multiple lineages.

**Bioluminescence imaging using D -luciferin and its analogs for visualizing Bdnf expression in living mice; different patterns of bioluminescence signals using distinct luciferase substrates**

Mamoru Fukuchi[1], Satoru Mitazaki[1], Ryohei Saito-Moriya[2,3,4], Nobuo Kitada[2,5], Shojiro A. Maki[2], Hironori Izumi[6] and Hisashi Mori[6,7,8]

[1]Laboratory of Molecular Neuroscience, Faculty of Pharmacy, Takasaki University of Health and Welfare, 60 Nakaorui-machi, Takasaki, Gunma 370–0033, Japan, [2]Department of Engineering Science, Graduate School of Informatics and Engineering, The University of Electro-Communications, 1–5–1 Chofugaoka, Chofu, Tokyo 182–8585, Japan, [3]School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432–1 Horinouchi, Hachioji, Tokyo 192–0392, Japan, [4]Department of Chemical and Biological Sciences, Faculty of Science, Japan Women's University, 2–8–1 Mejirodai, Bunkyo-ku, Tokyo 112–8681, Japan, [5]Coordinated Center for UEC Research Facilities, The University of Electro-Communications, 1–5–1 Chofugaoka, Chofu, Tokyo 182–8585, Japan, [6]Department of Molecular Neuroscience, Faculty of Medicine, University of Toyama, 2630 Sugitani, Toyama 930–0194, Japan, [7]Research Center for Idling Brain Science, University of Toyama, 2630 Sugitani, Toyama 930–0194, Japan, [8]Research Center for Pre-Disease Science, University of Toyama, 2630 Sugitani, Toyama 930–0194, Japan

Brain-derived neurotrophic factor (BDNF) plays a crucial role in numerous brain functions, including memory consolidation. Previously, we generated a *Bdnf-Luciferase* transgenic (*Bdnf-Luc*) mouse strain to visualize changes in *Bdnf* expression using *in vivo* bioluminescence imaging. We successfully visualized activity-dependent *Bdnf* induction in living mouse brains using a *D*-luciferin analog, TokeOni, which distributes to the brain and produces near-infrared bioluminescence. In this study, we compared the patterns of bioluminescence signals within the whole body of the *Bdnf-Luc* mice produced by *D*-luciferin, TokeOni and seMpai, another *D*-luciferin analog that produces a near-infrared light. As recently reported, hepatic background signals were observed in wild-type mice when using TokeOni. Bioluminescence signals were strongly observed from the region containing the liver when using *D*-luciferin and TokeOni. Additionally, we detected signals from the brain when using TokeOni. Compared with *D*-luciferin and TokeOni, signals were widely detected in the whole body of *Bdnf-Luc* mice by seMpai. The signals produced by seMpai were strong in the regions containing skeletal muscles in particular. Taken together, the patterns of bioluminescence signals in *Bdnf-Luc* mice vary when using different luciferase substrates. Therefore, the expression of *Bdnf* in tissues and organs of interest could be visualized by selecting an appropriate substrate.

Keywords: bioluminescence imaging, brain-derived neurotrophic factor, luciferase, luciferin, luciferin analog

***Biochemistry of Proteolysis***

**Purification and characterization of protease M, a yeast mitochondrial nucleotide-stimulated metal protease: its identification as CYM1 gene product, a mitochondrial presequence peptidase**

Toshimasa Yasuhara[1],Toshiki Nakai[2] and Yukio Fujiki[3,4]

[1]Innovation Division, Co-Creation Bureau, Osaka University, 2–8 Yamadaoka, Suita, Osaka 565–0871, Japan, [2]Radioisotope Research Center, Yokohama City University School of Medicine, 3–9 Fukuura, Kanazawa-ku, Yokohama 236–0004, Japan, [3]Institute of Rheological Functions of Food-Kyushu University Collaboration Program, Kyushu University, 3–1–1 Maidashi, Higashi-ku, Fukuoka 812–8582, Japan, [4]Graduate School of Science, University of Hyogo, Kamigori, Hyogo 678–1297, Japan

A chelator-sensitive protease in the mitochondrial matrix of the yeast, *Saccharomyces cerevisiae* (*Biochem. Biophys. Res. Commun*. 144, 277, 1987), was purified and characterized. The purified enzyme, termed protease M, specifically hydrolyzes peptide

substrates on the N-side of the paired basic residues. When mastoparan was used as substrate, it cleaved $Ala^{8}$–$Leu^{9}$ and $Lys^{11}$–$Lys^{12}$ bonds as well as the N-side of $Lys^{11}$–$Lys^{12}$ residues. Nucleotide triphosphates stimulated the activity 3-fold at 2.5 mM. The genomic DNA sequence showed that protease M was a gene product of *CYM1* known as mitochondrial presequence protease homologue in *S. cerevisiae*, encoding a 989-amino acid-long precursor protein. The N-terminal sequence of the purified enzyme indicated that protease M has 16-residue signal sequence and the 'mature' protein consists of 973 amino acids with a molecular mass of 110 kDa. Protease M contained consensus sequence motifs of ATP-binding site very near the carboxyl terminus. The alignment of the two ATP-binding motifs is an inverted version of the common alignment. Gene disruption of the enzyme generates mixed subunits in tetrameric MnSOD formed with 23-kDa mature and 24-kDa partial presequence-containing subunits. This report describes newly identified enzyme properties of the *CYM1* gene product, protease M and abnormal MnSOD complex formation of the disruption mutant.

Keywords: *yeast Saccharomyces cerevisiae,* PreP, MnSODprocessing, mitochondrial presequence protease, *Cym1* gene product

***BIOTECHNOLOGY***

***Gene Delivery Systems***

**Effective plasmid delivery to a plasmid-free Bacillus natto strain by a conjugational transfer system**

Mitsuhiro Itaya[1,2], MitsuruSato[2], Satoru Watanabe[3] and Masakazu Kataoka[1]

[1]Department of Biomedical Engineering, Graduate School of Science and Technology, Shinshu University, Wakasato 4–17–1, Nagano-shi, Nagano 380–8553, Japan, [2]Institute for Advanced Biosciences, Keio University, Nipponkoku, Tsuruoka, Yamagata 997–0017, Japan, [3]Department of Bioscience, Tokyo University of Agriculture, Sakuragaoka, Setagaya-ku, Tokyo 156–8502, Japan

In this study, a *Bacillus natto* strain named NEST141 was constructed. The strain carries no plasmids and is an authentic proline auxotroph—a feature that confers effective selection conditions for plasmids transferred from a donor, such as *Bacillus subtilis* 168, via a pLS20-based conjugational transfer system. We have provided a standard effective protocol for the delivery of plasmids larger than 50 kilobase pairs. These results indicate that the *B. natto* NEST141 strain can become a standard model, like *B. subtilis* 168, for extensive genetic engineering with diverse applications.

Keywords: genetic engineering, conjugational transfer, *Bacillus subtilis*, *Bacillus natto*, *antibiotics*