

## 学術論文抄録—2013 年発表

### Submicroscopic deletion involving the fibroblast growth factor receptor 1 gene in a patient with combined pituitary hormone deficiency

Fukami M, Iso M, Sato N, Igarashi M, Seo M\*, Kazukawa I, Kinoshita E, Dateki S, Ogata T.

Endocrine Journal; 60(8): 1013–1020 (2013. 5)

Combined pituitary hormone deficiency (CPHD), isolated hypogonadotropic hypogonadism (IHH), Kallmann syndrome (KS), and septo-optic dysplasia (SOD) are genetically related conditions caused by abnormal development of the anterior midline in the forebrain. Although mutations in the fibroblast growth factor receptor 1 (FGFR1) gene have been implicated in the development of IHH, KS, and SOD, the relevance of FGFR1 abnormalities to CPHD remains to be elucidated. Here, we report a Japanese female patient with CPHD and FGFR1 haploinsufficiency. The patient was identified through copy-number analyses and direct sequencing of FGFR1 performed for 69 patients with CPHD. The patient presented with a combined deficiency of GH, LH and FSH, and multiple neurological abnormalities. In addition, normal TSH values along with a low free T4 level indicated the presence of central hypothyroidism. Molecular analyses identified a heterozygous  $\sim 8.5$  Mb deletion involving 56 genes and pseudogenes. None of these genes except FGFR1 have been associated with brain development. No FGFR1 abnormalities were identified in the remaining 68 patients, although two patients carried nucleotide substitutions (p. V102I and p. S107L) that were assessed as benign polymorphism by in vitro functional assays. These results indicate a possible role of FGFR1 in anterior pituitary function and the rarity of FGFR1 abnormalities in patients with CPHD.

### Symmetries for Borchers lifts on Hilbert modular groups and Hirzebruch-Zagier divisors

Bernhard Heim and Atsushi Murase\*

International Journal of Mathematics, Vol. 24, No. 08, 2013

We show certain symmetries for Borchers lifts on the Hilbert modular group over a real quadratic field. We give two different proofs, the one analytic and the other arithmetic. The latter proof yields an explicit description of the action of Hecke operators on Borchers lifts.

## Erratum and addendum to “Commutators of $C^\infty$ -diffeomorphisms preserving a submanifold”

Kojun ABE and Kazuhiko FUKUI\*

Journal of the Mathematical Society of Japan, 65-4, 1329–1336 (2013)

Let  $D^\infty(M,N)$  be the group of  $C^\infty$ -diffeomorphisms of a compact manifold  $M$  preserving a submanifold  $N$ . We give a condition for  $D^\infty(M,N)$  to be uniformly perfect.

## Characterization of the uniform perfectness of diffeomorphism groups preserving a submanifold

Kojun ABE and Kazuhiko FUKUI\*

Foliations 2012, World Scientific, Singapore, 1–8 (2013)

In the previous paper we studied the conditions for the diffeomorphism groups of manifolds preserving a submanifold to be uniformly perfect. In this paper, applying the results we characterize the uniform perfectness of the diffeomorphism groups of surfaces preserving a union of circles, and give some extension to the cases of manifolds of higher dimensions.

## DNA damage response in plants: Conserved and variable response compared to animals

Kaoru O Yoshiyama, Kengo Sakaguchi, Seisuke Kimura\*

Biology, 2, 1338–1356 (2013.12)

The genome of an organism is under constant attack from endogenous and exogenous DNA damaging factors, such as reactive radicals, radiation, and genotoxins. Therefore, DNA damage response systems to sense DNA damage, arrest cell cycle, repair DNA lesions, and/or induce programmed cell death are crucial for maintenance of genomic integrity and survival of the organism. Genome sequences revealed that, although plants possess many of the DNA damage response factors that are present in the animal systems, they are missing some of the important regulators, such as the p53 tumor suppressor. These observations suggest differences in the DNA damage response mechanisms between plants and animals. In this review the DNA damage responses in plants and animals are compared and contrasted. In addition, the function of SUPPRESSOR OF GAMMA RESPONSE 1 (SOG1), a plant-specific transcription factor that governs the robust response to DNA damage, is discussed.

## ATM-mediated phosphorylation of SOG1 is essential for the DNA damage response in *Arabidopsis*

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Seisuke Kimura\*, Hisaji Maki, Masaaki Umeda

EMBO Reports, 14, 817–822 (2013.8)

*Arabidopsis* SOG1 (suppressor of gamma response 1) is a plant-specific transcription factor that governs the DNA damage response. Here we report that SOG1 is phosphorylated in response to DNA damage, and that this phosphorylation is mediated by the sensor kinase ataxia telangiectasia mutated (ATM). We show that SOG1 phosphorylation is crucial for the response to DNA damage, including transcriptional induction of downstream genes, transient arrest of cell division and programmed cell death. Although the amino-acid sequences of SOG1 and the mammalian tumour suppressor p53 show no similarity, this study demonstrates that ATM-mediated phosphorylation of a transcription factor has a pivotal role in the DNA damage response in both plants and mammals.

### 植物の TWINKLE は葉緑体 DNA 複製に関わる DNA プライマーゼか？ —アミノ酸配列の比較による検討—

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京都産業大学総合学術研究所報, 8号, 49–55 (2013.8)

細胞内共生により生じた葉緑体は、核とは異なる独自の DNA 複製機構を持っている。これまで、葉緑体 DNA の複製開始に必要な DNA プライマーゼは同定されていなかった。筆者らは、T7 ファージの T7 bacteriophage gene 4 protei (n T7gp4) という DNA ヘリカーゼ/プライマーゼのホモログである TWINKLE が、植物では葉緑体に局在して DNA 複製を開始する DNA プライマーゼとして働いているのではないかという仮説を立てて研究を進めている。本研究では、ホモロジーモデリングなどの手法により T7gp4、動物および植物の TWINKLE のアミノ酸配列を比較し、植物では DNA プライマーゼドメインが高度に保存されていることを明らかにした。この結果は、植物の TWINKLE が葉緑体 DNA の複製に働く DNA プライマーゼであることを強く示唆する。

## Comparative transcriptomics reveals patterns of selection in domesticated and wild tomato

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Proc. Natl. Acad. Sci. USA, 110, E2655–2662 (2013.6)

Although applied over extremely short timescales, artificial selection has dramatically altered the form, physiology, and life history of cultivated plants. We have used RNAseq to define both gene sequence and expression divergence between cultivated tomato and five related wild species. Based on sequence differences, we detect footprints of positive selection in over 50 genes. We also document thousands of shifts in gene-expression level, many of which resulted from changes in selection pressure. These rapidly evolving genes are commonly associated with environmental response and stress tolerance. The importance of environmental inputs during evolution of gene expression is further highlighted by large-scale alteration of the light response coexpression network between wild and cultivated accessions. Human manipulation of the genome has heavily impacted the tomato transcriptome through directed admixture and by indirectly favoring nonsynonymous over synonymous substitutions. Taken together, our results shed light on the pervasive effects artificial and natural selection have had on the transcriptomes of tomato and its wild relatives.

## Fine genetic mapping of *RXopJ4*, a bacterial spot disease resistance locus from *Solanum pennellii* LA716

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Theoretical and Applied Genetics 126, 601–609 (2013.1)

The *RXopJ4* resistance locus from the wild accession *Solanum pennellii* (Sp) LA716 confers resistance to bacterial spot disease of tomato (*S. lycopersicum*, Sl) caused by *Xanthomonas perforans* (Xp). *RXopJ4* resistance depends on recognition of the pathogen type III effector protein XopJ4. We used a collection of Sp introgression lines (ILs) to narrow the *RXopJ4* locus to a 4.2-Mb segment on the long arm of chromosome 6, encompassed by the ILs 6-2 and 6-2-2. We then adapted or developed a collection of 14 molecular markers to map on a segregating F<sub>2</sub> population from a cross between the susceptible

parent SI FL8000 and the resistant parent RXopJ4 8000 OC<sub>7</sub>. In the F<sub>2</sub> population, a 190-kb segment between the markers J350 and J352 cosegregated with resistance. This fine mapping will enable both the identification of candidate genes and the detection of resistant plants using cosegregating markers. The RXopJ4 resistance gene(s), in combination with other recently characterized genes and a quantitative trait locus (QTL) for bacterial spot disease resistance, will likely be an effective tool for the development of durable resistance in cultivated tomato.

## 複葉の発生と進化

木村 成介\*

生物の科学 遺伝, 67, 50–56 (2013.1)

植物の葉は、1枚の葉が1つの葉身からなる単葉と、複数の葉身に分かれている複葉の2種類に分けることができる。維管束植物の祖先は単葉であったと考えられており、単葉から複葉への進化は独立して複数回起こっている。複葉という「形」は、どのように進化したのであるか？本稿では、複葉の発生と進化について、その研究の進展と現況を概説する。

## Comparison of Two Interval Models for Interval-valued Genetic Algorithm

H. Okada\*

Proc. of The 1st International Conference on Industrial Application Engineering (ICIAE) 2013, 152–157 (2013.03)

In this paper, we propose an extension of evolution strategy (ES) for evolving interval-valued neural networks. In the proposed ES, values in the genotypes are not real numbers but intervals. We apply our interval-valued ES (IES) to the approximate modeling of interval functions with interval-valued neural networks (INNs). Experimental results showed that INNs trained by our IES could well approximate a hidden test function, despite the fact that the learning was not supervised.

## Interval-valued Differential Evolution for Evolving Neural Networks with Interval Weights and Biases

H. Okada\*

Proc. of the 6th International Workshop on Computational Intelligence & Applications (IWCIA2013), 81–84 (2013.07)

The ordinary differential evolution (DE) algorithm employs real-valued vectors as genotypes. The author previously proposed an extension of DE which can handle interval-valued genotypes. In this paper, the proposed method is applied to evolution of neural networks with interval connection weights and biases. Experimental results show that the interval DE can evolve neural networks which model interval functions well despite that no training data is explicitly provided.

## Comparison of Two Interval Models for Fuzzy-valued Evolution Strategy

H. Okada\*

Proc. of International Conference on Instrumentation, Control and Information Technology (SICE Annual Conference 2013), 757–760 (2013.09)

The author previously proposed an extension of evolution strategy (ES). The proposed method extends the processes of ES to handle fuzzy numbers as genotype values so that ES can be applied to fuzzy-valued optimization problems. For specifying symmetric triangular fuzzy numbers as genotype values, two interval models can be adopted, i.e., the lower and upper model or the center and width model. Ability of the fuzzy ES may depend on the model because the reproduction processes in the fuzzy ES are slightly different between the two models. This paper compares the two models to investigate which one contributes better. Experimental results show that the lower and upper model contributes better than the center and width model.

## Differential Evolution with Fuzzy Genotypes and its Application to Evolution of Fuzzy Neural Networks

H. Okada\*

Proc. of International Conference on Instrumentation, Control and Information Technology (SICE Annual Conference 2013), 767–770 (2013.09)

The author previously proposed an extension of DE which can handle fuzzy-valued genotypes. In

this paper, the proposed method is applied to evolution of neural networks with fuzzy-valued connection weights and biases. Experimental results show that the extended DE can evolve fuzzy neural networks which model fuzzy functions well despite that no training data is explicitly provided.

## Comparison of Two Interval Models for Fuzzy-valued Differential Evolution

H. Okada\*

3rd International Conference on Engineering and Applied Science (2013 ICEAS),  
1616–1622 (2013.11)

The author previously proposed an extension of differential evolution (DE). The proposed method extends the processes of DE to handle fuzzy numbers as genotype values so that DE can be applied directly to fuzzy-valued optimization problems. For specifying symmetric triangular fuzzy numbers as genotype values, two interval models can be adopted, i.e., the lower and upper model or the center and width model. Ability of the fuzzy DE may depend on the model because the search space with the LU model is not the same as that with the CW model. This paper compares the two models to investigate which one contributes better. Experimental results reveal that both contribute equally despite the inequality of the search spaces.

## Comparison of Two Interval Models for Interval-valued Particle Swarm Optimization

H. Okada\*

2013 IEEE/SICE International Symposium on System Integration (SII2013),  
885–888 (2013.12)

The author previously proposed an extension of particle swarm optimization (PSO). The proposed method extends the processes of PSO to handle interval numbers as genotype values so that PSO can be applied directly to interval-valued optimization problems. The interval PSO (IPSO) can employ either of two interval models, the lower and upper model or the center and width model, for specifying genotype values. Ability of the IPSO in searching for solutions may depend on the model. In this paper, the author compares the two models to investigate which model contributes better for the IPSO to find better solutions. IPSO is applied to evolutionary training of interval-valued neural networks. A result of preliminary study indicates that the CW model is slightly better than the LU model and both models contribute well: the IPSO with the LU/CW model could evolve neural networks with a small error.

## Evolving Neural Networks with Interval Weights by Means of Genetic Algorithm

H. Okada\*

Journal of the Institute of Industrial Applications Engineers, 1(1), 1–6 (2013.07)

In this paper, the author proposes an extension of genetic algorithm (GA) for evolving neural networks with interval-valued weights and biases. In the proposed extension, genotype values are not real numbers but intervals. Evolutionary processes in GA are extended so that the processes can handle interval-valued genotypes. Experimental results show that interval neural networks evolved by the proposed method can model target interval functions well despite the fact that no training data is explicitly provided.

## Alveolar macrophage functions and DNA damage in cigarette smoke-exposed mice

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Advances in Bioscience and Biotechnology, 4-8, 1–7 (2013.8)

Alveolar macrophages (AM) are known to play an essential role in lung defense through their ability to remove the foreign matters reaching the lung alveoli. Cigarette smoke (CS) is a critical risk factor for many lung diseases. CS is inhaled into the lung by respiration and affects AM. It has been previously reported that CS induces inhibition of cytokine production, cell surface receptor expression and antigen presentation in AM. However, the relationship of immune suppression and DNA damage caused by CS in AM is still unclear. Therefore, in this study, we investigated AM immune function and DNA damage in CS-exposed mice. Mice were exposed to CS of 20 cigarettes/day during 10 days using a Hamburg II smoking machine. After exposure, AM were obtained by bronchoalveolar lavage. The number of AM was significantly increased in CS-exposed mice compared with non-CS-exposed mice. Phagocytic activity of AM was significantly inhibited by CS exposure. Percentage of CD11b-, CD14-, toll like receptor (TLR)2- or TLR4-positive cells was significantly decreased in CS-exposed mice compared with non-CS-exposed mice. Interleukin-1 $\beta$  mRNA expression in lipopolysaccharide-stimulated AM was significantly inhibited by CS exposure. Intracellular reactive oxygen species (ROS) (O<sub>2</sub><sup>-</sup>, H<sub>2</sub>O<sub>2</sub>) production of AM was significantly increased, and DNA damage was induced by CS exposure. These results suggest that impaired immune functions by CS exposure may be related to DNA damage via excessive ROS induced by CS. These alterations of AM caused by CS could be associated with infection and development of pulmonary diseases.



## Cigarette Smoke Induce Alteration of Structure and Function in Alveolar Macrophages

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International Journal of Bioscience, Biochemistry and Bioinformatics, 3-2, 125–128  
(2013.3)

Cigarette smoke (CS) is released into the atmosphere, and impact lung health in non-smoker but not smoker. CS is inhaled into the lung by respiration and affects alveolar macrophages (AM). AM play an important role of immune system in the lung. In this study, we investigated the effect of CS on DNA damage and immune function in AM. The number of AM was significantly increased in CS exposed mice compared with non CS-exposed mice. Expressions of CD11b, TLR-2 and CD14 on AM were significantly inhibited in CS exposed mice but not CD16. Phagocytic activity of AM was significantly inhibited in CS exposed mice. Both of tail moment and tail length of AM as indicator of DNA damage were significantly increased in CS exposed mice. CS was a risk factor for DNA damage of AM and induced inhibition of immunological functions in AM mediated with DNA damage. These results suggest that changes of intracellular structure, inhibition of phagocytosis and TLR expression and induced-DNA damage of AM by CS may result in easily infection of bacteria or virus and carcinogenesis.

## Effect of Hot Water Extract from Agaricus Blazei Murill on Chemotaxis of Neutrophils

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Journal of Cosmetics, Dermatological Sciences and Applications, 3-1, 12–17 (2013.1)

Hot water extract from the edible Brazilian mushroom, Agaricus Blazei Murill (ABM), is used for both traditional and alternative medicine. ABM is reported to stimulate anti-tumor, anti-infection, and immune activity. However, there are few reports of how ABM affects neutrophils. Therefore, in this study, we examined the effect of hot water ABM extract on neutrophil migration, phagocytosis, and reactive oxygen species production using neutrophils from guinea pig. Mi-gratory direction and velocity as indicators of chemotactic activity of neutrophils were significantly ( $p < 0.001$ ) in-creased at concentration of 50 and 100 mg/ml in ABM extract compared with control. Phagocytic activity of neutrophil was significantly ( $p < 0.01$ ) increased at concentration of 5 mg/ml in ABM extract compared with control. Production of reactive oxygen species (ROS: H<sub>2</sub>O<sub>2</sub> or O<sub>2</sub><sup>-</sup>) by neutrophils was significantly ( $p < 0.01$ ) increased at concentration of 5 mg/ml in ABM extract compared with control. These results suggest that enhancement in neutrophil chemotactic activity, phagocytic activity and ROS production are mechanisms by which ABM extract inhibits bacterial infection in the skin and dermatitis.

## Spontaneous occurrence of photoageing-like phenotypes in the dorsal skin of old SAMP1 mice, an oxidative stress model

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Experimental Dermatology, 22-1, 54–80 (2013.1)

Skin photoageing is a complex, multifactorial process and both intrinsic and extrinsic factors may contribute to its pathogenesis. The ultraviolet-irradiated hairless mouse has been used as an animal model for photoageing, but this model mimics only the 'extrinsic' aspects. Here, we show that skin from old SAMP1 mice, a model for higher oxidative stress and senescence acceleration, exhibited histological and gene expression changes similar to those in human photoaged skin without ultraviolet irradiation. These changes include an increase in elastic fibre and glycosaminoglycan histologically, an upregulation of several proinflammatory cytokines and matrix metalloproteinases, and an increase in lipid peroxide. We propose that SAMP1 mice are a spontaneous animal model for photoageing caused by an exaggerated intrinsic mechanism, namely, higher oxidative status. This mouse model is useful to explore the link between oxidative stress and photoageing, and to evaluate the efficacy of antioxidants.

## ジャングルハニーによる抗体産生機能への影響とその機構について

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京都産業大学論集自然科学系列第 42 号, 21–52 (2013.3)

ジャングルハニーは、ナイジェリアの熱帯雨林に生息する野生の蜜蜂が長期にわたり樹木や花から集めてきた蜂蜜である。ナイジェリアではこの蜂蜜が健康や美容の他、風邪、皮膚炎、火傷の治療薬、疾患予防薬として利用されてきた。そのため、生体への免疫作用に対する効果があると考えられる。我々は、ジャングルハニーによる好中球数の増加、好中球機能の増強、活性酸素産生による抗腫瘍作用を報告している。しかし、ジャングルハニーによる免疫作用についての詳細な機構はまだ解明されていない。そこで、ジャングルハニーによる抗体産生機能への影響とその機構について検討した。抗体産生機能については、SRBC (Sheep red blood cell) を抗原とし、抗体産生誘導期と発現期に及ぼす影響について PFC (Plaque forming cell) 法により検討した。抗体産生誘導期では、ジャングルハニーの腹腔内投与及び経口投与により、コントロール [PBS (-) 投与群] と比べ、ジャングルハニー投与群で抗体産生細胞である PFC 数の有意な増加が認められたが、発現期では有意な差は認められなかった。また、脾臓細胞数はジャングルハニーの腹腔内投与により、有意な増加が認められた。貪食細胞陽性比率は、ジャングルハニー投与による影響は認められなかった。腹腔マクロファージの MHC class II, CD86, 脾臓細胞の TCR, CD28, CD3, CD4 の陽性細胞比率は、ジャングルハニー投与群で差は認められなかったが、脾臓細胞の CD19 陽性細胞比率のみ有意な増加が認められた。IL-1 $\beta$  mRNA 発現比率は、腹腔細胞、腹腔マクロファージ及び肺胞マクロファージにおいて、ジャングルハニーにより有意な増加が認められた。IL-6 mRNA 発現比率は、腹腔細胞と腹腔マクロファージにおいて

有意な増加が認められた。NF- $\kappa$ B mRNA 発現比率は、ジャングルハニーにより有意な増加が認められた。また、脾臓細胞の IL-4 mRNA 発現比率もジャングルハニーにより有意な増加が認められた。脾臓細胞の増殖は、ジャングルハニーにより有意な増加が認められたが、脾臓細胞の非付着細胞と B 細胞の細胞増殖は、ジャングルハニーによる変化は認められなかった。ジャングルハニーによる抗体産生機能、サイトカイン産生の増強及び脾臓細胞の増加が LPS の受容体である TLR4 を認識している可能性について、TLR4 欠損マウスを用いて抗体産生機能への影響を検討した。PFC 数は、野生型マウスと同様にジャングルハニー投与で有意な増加が認められた。ジャングルハニーの有効成分に関しては、ジャングルハニー全分画を HPLC で 6 つの分画 (Fr. 1~6) に分け、各分画の抗体産生機能とサイトカイン mRNA 発現への影響について検討した。PFC 数は、Fr. 2 投与群で有意な増加が認められ、その他の分画で増加は認められなかった。IL-1 $\beta$  と IL-6 mRNA 発現比率は、Fr. 2 により有意な増加が認められ、分子量約 206~393 の熱に安定な物質であった。以上より、ジャングルハニーはマクロファージを活性化し、NF- $\kappa$ B を介して IL-1 $\beta$  と IL-6 mRNA 発現を増強させ、マクロファージから産生された IL-1 $\beta$  が Th2 細胞に作用し、IL-4 の産生を増強させ、CD19 陽性細胞が増加し、IL-6 を介して B 細胞の初期段階に作用し、抗体産生細胞へと分化させ、抗体産生機能を増強させた可能性が示唆された。

## A remarkable $\sigma$ -finite measure unifying supremum penalisations for a stable Lévy process

Yuko Yano\*

Ann. Inst. H. Poincaré Probab. Statist. Vol. 49, No. 4, 1014–1032 (2013)

The  $\sigma$ -finite measure which unifies supremum penalisations for a stable Lévy process is introduced. Silverstein's coinvariant and coharmonic functions for Lévy processes and Chaumont's  $h$ -transform processes with respect to these functions are utilized for the construction of the measure.

## The pH sensitivity of murine hsp47 binding to collagen is affected by mutations in the breach histidine cluster

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J. Biol. Chem. 288(6): 4452–4461 (2013)

Heat shock protein 47 (HSP47) is a single-substrate molecular chaperone crucial for collagen biosynthesis. Although its function is well established, the molecular mechanisms that govern binding to pro-collagen peptides and triple helices in the endoplasmic reticulum (followed by controlled release in the Golgi) are unclear. HSP47 binds procollagen at a neutral pH but releases at a pH similar to the pK(a) of the imidazole side chain of histidine residues. It thus seems likely that these residues are involved in this

pH-dependent mechanism. Murine HSP47 has 14 histidine residues grouped into three clusters, known as the breach, gate, and shutter. Here, we report the use of histidine mutagenesis to demonstrate the relative contribution of these three clusters to HSP47 structure and the “pH switch.” Many of the tested mutants are silent; however, breach mutants H197A and H198A show binding but no apparent pH switch and are unable to control release. Another breach mutant, H191A, shows perturbed collagen release characteristics, consistent with observed perturbations in pH-driven trans-conformational changes. Thus, His-198, His-197 and His-191 are important (if not central) to HSP47 mechanism of binding/release to collagen. This is consistent with the breach cluster residues being well conserved across the HSP47 family.

## Serum heat shock protein 47 levels are elevated in acute exacerbation of idiopathic pulmonary fibrosis

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Cell Stress and Chaperones 18: 581–590 (2013)

Little is known about the pathophysiology of acute exacerbation (AE) of idiopathic pulmonary fibrosis (IPF). Heat shock protein 47 (HSP47), a collagen-specific molecular chaperone, is essential for biosynthesis and secretion of collagen molecules. Previous studies in experimental animal fibrosis models have shown that downregulation of HSP47 expression reduces collagen production and diminishes fibrosis progression. In this study, serum HSP47 levels were evaluated to elucidate pathogenic differences involving HSP47 between AE-IPF and stable (S)-IPF. Subjects comprised 20 AE-IPF and 33 S-IPF patients. Serum levels of HSP47, Krebs von den Lungen-6 (KL-6), surfactant protein (SP)-A, SP-D, and lactate dehydrogenase (LDH) were measured. Immunohistochemical analysis of lung HSP47 expression was determined in biopsy and autopsy tissues diagnosed as diffuse alveolar damage (DAD) and usual interstitial pneumonia (UIP). Serum levels of HSP47 were significantly higher in AE-IPF than in S-IPF patients, whereas serum levels of KL-6, SP-A, and SP-D did not differ significantly. Receiver operating characteristic curves revealed that HSP47 was superior for discriminating AE-IPF and S-IPF. The cutoff for HSP47 resulting in the highest diagnostic accuracy was 559.4 pg/mL; sensitivity, specificity, and diagnostic accuracy were 100.0%, 93.9%, and 96.2%, respectively. Immunohistochemical analysis revealed that pulmonary HSP47 expression was greater in DAD than UIP tissues. Serum HSP47 was significantly higher in AE-IPF than in S-IPF patients, suggesting that underlying fibrogenic mechanisms involving HSP47 differ in the two conditions.

## Endoplasmic reticulum lectin XTP3-B inhibits endoplasmic reticulum-associated degradation of a misfolded $\alpha$ 1-antitrypsin variant

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The endoplasmic reticulum (ER) is an organelle that synthesizes many secretory and membrane proteins. However, proteins often fold incorrectly. Terminally misfolded polypeptides in the ER are retrotranslocated to the cytosol, where they are ultimately degraded by the ubiquitin-proteasome system, a process termed ER-associated degradation (ERAD). By recognizing the specific structures of N-linked oligosaccharides attached to polypeptides, lectins play an important role in the quality control of glycoproteins in the ER. Mammalian OS-9 and XTP3-B are ER-resident lectins that contain mannose 6-phosphate receptor homology (MRH) domains, which recognize sugar moieties; OS-9 has one MRH domain and XTP3-B has two. Both are involved in ERAD, but the functional differences between the two are poorly understood. The present study analyzed the function of human XTP3-B, and found, by frontal affinity chromatography analysis, that its C-terminal MRH domain specifically recognized the Man<sub>9</sub>GlcNAc<sub>2</sub> (M9) glycan in vitro and M9 glycans on an ERAD substrate NHK, a terminally misfolded  $\alpha$ 1-antitrypsin variant, in vivo. Furthermore, endogenous XTP3-B was a component of the HRD1-SEL1L membrane-embedded ubiquitin ligase complex, an association that was stabilized by a direct interaction with SEL1L. The lectin activity of XTP3-B was required for its binding to NHK, but not for its association with SEL1L. Unlike OS-9, XTP3-B did not enhance the degradation of misfolded glycoproteins, but instead inhibited the degradation of NHK bearing M9 oligosaccharides. Therefore, we propose that XTP3-B recognizes M9 glycans on unfolded polypeptides, thereby acting as a negative regulator of ERAD, and also protects newly synthesized immature polypeptides from premature degradation.

## Ero1- $\alpha$ and PDIs constitute a hierarchical electron transfer network of endoplasmic reticulum oxidoreductases

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Ero1- $\alpha$  and endoplasmic reticulum (ER) oxidoreductases of the protein disulfide isomerase (PDI) family promote the efficient introduction of disulfide bonds into nascent polypeptides in the ER. However, the hierarchy of electron transfer among these oxidoreductases is poorly understood. In this paper, Ero1- $\alpha$ -associated oxidoreductases were identified by proteomic analysis and further confirmed by surface plasmon resonance. Ero1- $\alpha$  and PDI were found to constitute a regulatory hub, whereby PDI induced conformational flexibility in an Ero1- $\alpha$  shuttle cysteine (Cys99) facilitated intramolecular electron transfer to

the active site. In isolation, Ero1- $\alpha$  also oxidized ERp46, ERp57, and P5; however, kinetic measurements and redox equilibrium analysis revealed that PDI preferentially oxidized other oxidoreductases. PDI accepted electrons from the other oxidoreductases via its a' domain, bypassing the a domain, which serves as the electron acceptor from reduced glutathione. These observations provide an integrated picture of the hierarchy of cooperative redox interactions among ER oxidoreductases in mammalian cells.

## Glycosylation-independent ERAD pathway serves as a backup system under ER stress

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During endoplasmic reticulum (ER)-associated degradation (ERAD), terminally misfolded proteins are retrotranslocated from the ER to the cytosol and degraded by the ubiquitin-proteasome system. Misfolded glycoproteins are recognized by calnexin and transferred to EDEM1, followed by the ER disulfide reductase ERdj5 and the BiP complex. The mechanisms involved in ERAD of nonglycoproteins, however, are poorly understood. Here we show that nonglycoprotein substrates are captured by BiP and then transferred to ERdj5 without going through the calnexin/EDEM1 pathway; after cleavage of disulfide bonds by ERdj5, the nonglycoproteins are transferred to the ERAD scaffold protein SEL1L by the aid of BiP for dislocation into the cytosol. When glucose trimming of the N-glycan groups of the substrates is inhibited, glycoproteins are also targeted to the nonglycoprotein ERAD pathway. These results indicate that two distinct pathways for ERAD of glycoproteins and nonglycoproteins exist in mammalian cells, and these pathways are interchangeable under ER stress conditions.

## Dynamic regulation of Ero1 $\alpha$ and Peroxiredoxin-4 localization in the secretory pathway

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In the early secretory compartment (ESC), a network of chaperones and enzymes assists oxidative folding of nascent proteins. Ero1 flavoproteins oxidize protein disulfide isomerase (PDI), generating H<sub>2</sub>O<sub>2</sub> as a byproduct. Peroxiredoxin 4 (Prx4) can utilize luminal H<sub>2</sub>O<sub>2</sub> to oxidize PDI, thus favoring oxidative folding while limiting oxidative stress. Interestingly, neither ER oxidase contains known ER retention signal(s), raising the question of how cells prevent their secretion. Here we show that the two proteins share similar intracellular localization mechanisms. Their secretion is prevented by sequential interactions with PDI and ERp44, two resident proteins of the ESC-bearing KDEL-like motifs. PDI binds

preferentially Ero1 $\alpha$ , whereas ERp44 equally retains Ero1 $\alpha$  and Prx4. The different binding properties of Ero1 $\alpha$  and Prx4 increase the robustness of ER redox homeostasis.

## Serum heat shock protein 47 levels in patients with drug-induced lung disease

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**BACKGROUND:** Heat shock protein (HSP) 47 is a collagen-specific molecular chaperone that is required for molecular maturation of various types of collagens. We recently reported that HSP47 serum levels were markedly higher in patients with acute exacerbations of idiopathic pulmonary fibrosis (IPF) when compared with patients with stable IPF, suggesting that serum HSP47 levels correlate with interstitial pneumonia activity. The aim of this study was to evaluate serum HSP47 levels in patients with drug-induced lung disease (DILD). **METHODS:** Findings from high-resolution computed tomographic chest scans of 47 patients with DILD were classified into one of four predominant patterns: organizing pneumonia (OP) (n = 4), nonspecific interstitial pneumonia (NSIP) (n = 24), hypersensitivity pneumonitis (HP) (n = 11), and diffuse alveolar damage (DAD) (n = 8). Serum levels of HSP47, Krebs von den Lungen-6 (KL-6), surfactant protein (SP)-A, and SP-D were measured in these patients. **RESULTS:** The PaO<sub>2</sub>/fraction of inspired oxygen (FiO<sub>2</sub>) (P/F) ratios were significantly lower and the alveolar-arterial difference of oxygen (A-a DO<sub>2</sub>) was significantly higher in the DAD group than in the other groups. Patients with DAD had the worst outcomes among the different subgroups. Patients in the DAD group had significantly higher serum HSP47 levels than those in other groups. Receiver operating characteristic curves revealed that HSP47 was superior to KL-6, SP-A, and SP-D for discriminating between the DAD group and the other groups. The cut-off level for HSP47 that resulted in the highest diagnostic accuracy was 1711.5 pg/mL. The sensitivity, specificity, and diagnostic accuracy were 87.5%, 97.4%, and 95.7%, respectively. Serum levels of HSP47 in the group of patients requiring glucocorticoids were significantly higher than those in patients who experienced clinical improvement without glucocorticoid administration. Serum HSP47 levels also significantly correlated with various respiratory parameters. **CONCLUSION:** This study demonstrated that serum HSP47 levels were elevated in patients with DILD with a DAD pattern who had the worst outcomes among the different subgroups, and that this was correlated with P/F ratio and A-a DO<sub>2</sub>.

## Roles of M<sub>2</sub> and M<sub>3</sub> muscarinic receptors in the generation of rhythmic motor activity in mouse small intestine

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Peristalsis plays a central role in propulsions of the luminal contents forward in the gastrointestinal tract. It is well known that the intrinsic cholinergic nerves play an important role in generations of peristalsis through muscarinic receptors. However, little is known about roles of M<sub>2</sub> and M<sub>3</sub> muscarinic receptor subtypes and involvement of interstitial cells of Cajal which exists in the myenteric plexus (ICC-MY) in the generation of peristalsis remain to be elucidated. Thus, in the present study, we used M<sub>2</sub> and M<sub>3</sub> receptor single knockout (KO), M<sub>2</sub>/M<sub>3</sub> receptor double KO, and W/W<sup>V</sup> mice which lack ICC-MY as novel experimental tools. Our results suggest that both of M<sub>2</sub> and M<sub>3</sub> receptors differentially regulate the intestinal peristalsis in different manner. M<sub>2</sub> receptors play an essential role for generating gut peristalsis. On the other hand, M<sub>3</sub> receptors have a modulatory role for the controlling periodicity of the peristalsis together with ICC-MY. These findings may contribute to elucidate the pathophysiological conditions of functional gastrointestinal disorders and to develop a novel effective medicine for the diseases.

## Cholinergic neuromuscular transmission mediated by interstitial cells of Cajal in the myenteric layer in mouse ileal longitudinal smooth muscles

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Cholinergic nerves mainly regulate the contractile activities in gastrointestinal smooth muscles. Recently, interstitial cells of Cajal (ICC) have been suggested to play an important role in regulating gut motility. However, little is known about the involvement of ICC which exist in the myenteric layer (ICC-MY) in cholinergic neuromuscular transmission in ileal longitudinal smooth muscles. In the present study, we investigated cholinergic nerve-induced contractions and excitatory junction potentials in the ileal longitudinal smooth muscles of W/W<sup>V</sup> mutant mice, that lacked ICC-MY, and compared with those in +/+ control mice. Our results suggest that ICC-MY mediate the cholinergic neuromuscular transmission in mouse ileal longitudinal smooth muscles. In addition, the direct pathway in which acetylcholine released from motor nerves can concomitantly act on longitudinal smooth muscles is likely to operate.



## A calcium-based simple model of multiple spike interactions in spike-timing dependent plasticity

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Neural Computation, Vol. 25, pp. 1853–1869 (2013.7)

Spike-timing-dependent plasticity (STDP) is a form of synaptic modification that depends on the relative timings of presynaptic and postsynaptic spikes. In this letter, we proposed a calcium-based simple STDP model, described by an ordinary differential equation having only three state variables: one represents the density of intracellular calcium, one represents a fraction of open state NMDARs, and one represents the synaptic weight. We shown that in spite of its simplicity, the model can reproduce the properties of the plasticity that have been experimentally measured in various brain areas (e.g., layer 2/3 and 5 visual cortical slices, hippocampal cultures, and layer 2/3 somatosensory cortical slices) with respect to various patterns of presynaptic and postsynaptic spikes. In addition, comparisons with other STDP models are made, and the significance and advantages of the proposed model are discussed.

## Asynchronous Cellular Automaton Based Neuron: Theoretical Analysis and On-FPGA Learning

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IEEE Transactions on Neural Networks and Learning Systems,  
Vol. 24, No. 5, pp. 736–748 (2013.5)

A generalized asynchronous cellular automaton based neuron model is a special kind of cellular automaton that is designed to mimic the nonlinear dynamics of neurons. The model can be implemented as an asynchronous sequential logic circuit and its control parameter is the pattern of wires among the circuit elements that is adjustable after implementation in a field-programmable gate array (FPGA) device. In this paper, a novel theoretical analysis method for the model is presented. Using this method, stabilities of neuron-like orbits and occurrence mechanisms of neuron-like bifurcations of the model are clarified theoretically. Also, a novel learning algorithm for the model is presented. An equivalent experiment shows that an FPGA-implemented learning algorithm enables an FPGA-implemented model to automatically reproduce typical nonlinear responses and occurrence mechanisms observed in biological and model neurons.

## Ghost Stochastic Resonance from Asynchronous Cellular Automaton Neuron Model

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IEEE Transactions on Circuits and Systems Part II, Vol. 60, No. 2, pp. 111–115  
(2013.2)

A novel electronic circuit neuron model whose nonlinear dynamics is described by an asynchronous cellular automaton is proposed. The model exhibits a so-called ghost stochastic resonance, which is suggested to be utilized in a nonlinear pitch extraction function in a biological auditory system. It is shown that, by tuning parameters, characteristics of the resonance can be enhanced so that it is suited to the pitch extraction function. Also, field programmable gate array (FPGA) experiments validate occurrences of the resonance and realization of the pitch extraction function.

## Bifurcation-based Synthesis of Asynchronous Cellular Automaton Based Neuron

Takashi Matsubara and Hiroyuki Torikai\*

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A spiking neuron model described by an asynchronous cellular automaton is introduced. Our model can be implemented as an asynchronous sequential logic circuit and its control parameter is adjustable after implementation in an FPGA. It is shown that our model can reproduce twenty types of dynamic response behaviors observed in biological and other model neurons. It is also shown that our model can reproduce the features of four groups into which biological and other model neurons are classified. In addition, underlying bifurcations of the four groups are analyzed, and the results yield basic guides to the synthesis of our model.

## Green fluorescent protein (GFP) expression patterns in the olfactory epithelium of GFP transgenic cloned Jinhua pigs

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Domestic pigs possess a well-developed sense of smell. However, the morphology of the porcine olfactory epithelium (OE) is poorly understood. Recently, several strains of transgenic cloned pigs that are

presumed to ubiquitously express green fluorescent protein (GFP) have been created. Thus, the purpose of this study was to elucidate the features of porcine OE using the tissues of GFP transgenic cloned pigs. Based on observations of Hematoxylin and Eosin staining and measurements of thickness, porcine OE tissue portions were classified into three categories (thick, standard, and thin). Cryosections revealed that the prominent GFP signals were expressed in olfactory sensory neurons (OSN), Bowman's glands, and olfactory nerve. A few GFP-expressing sustentacular cells were seen; however, the intensity of GFP fluorescence was slight. In the thick portion, numerous GFP-expressing polygonal OSN that did not possess dendrites were found. In the standard portions, GFP-expressing cells had longitudinal dendrites. A few GFP-expressing cells were found in the thin portion. In the thick and standard portions, most of the prominent GFP-expressing cells were positive for olfactory marker protein. Moreover, double immunofluorescence staining with boiled GFP and Sox2 antibody revealed that GFP expression patterns in OSN are synchronized with Sox2 immunoreactive patterns.