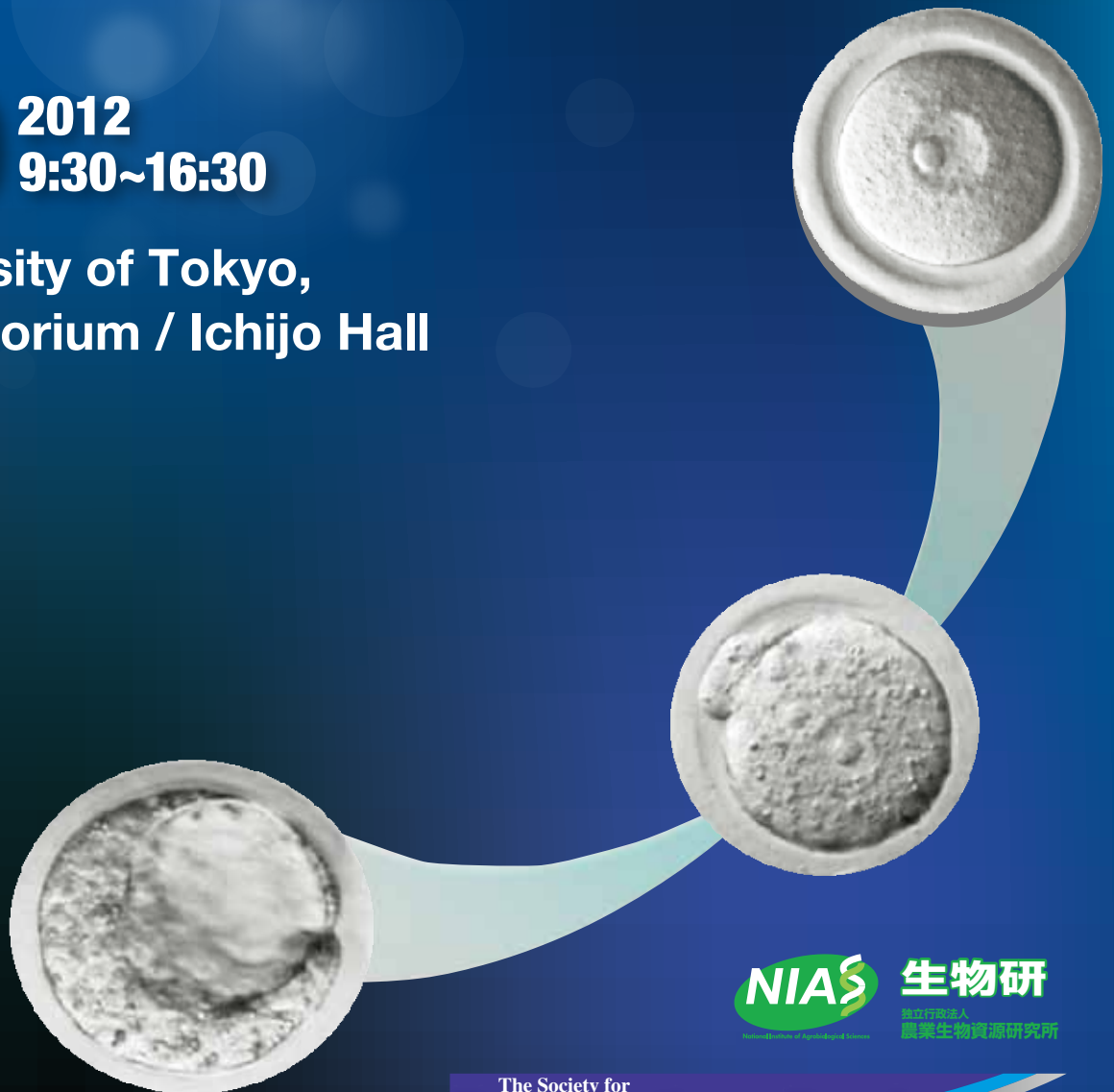


SRD-NIAS THE 2ND JAPAN-CZECH JOINT SYMPOSIUM

“Understanding the Germ Cell Potential”

9.10 2012
9:30~16:30

The University of Tokyo,
Yayoi Auditorium / Ichijo Hall



NIAS 生物研
独立行政法人
農業生物資源研究所

The Society for
Reproduction and Development
日本繁殖生物学会

SRD-NIAS 第2回 日・チェコ交流シンポジウム

**SRD-NIAS the 2nd Japan-Czech Joint Symposium
“Understanding the Germ Cell Potential”**

Location: The University of Tokyo, Yayoi Auditorium/Ichijo Hall

Date & time: September 10 (Mon), 2012; 9:30-16:30

Society for Reproduction and Development (SRD)

<http://reproduction.jp/index-j.php>

National Institute of Agrobiological Sciences (NIAS)

<http://www.nias.affrc.go.jp/>

Program

President & Opening Remarks 9:30~10:00

Hiroshi SHINBO (*Vice-President of National Institute of Agrobiological Sciences, JP*)

Josef FULKA JR. (*Department of Biology of Reproduction, Institute of Animal Science, CZ*)

Session 1. Meiosis 10:00~11:00

(Chair: Takashi MIYANO & Sugako OGUSHI)

Petr SOLC (*Institute of Animal Physiology and Genetics, The Czech Academy of Sciences, CZ*);

Polo-like kinase I controls nuclear envelope break down and chromosome dynamics
in meiosis I. p. 1

Tomoya KITAJIMA (*Laboratory for Chromosome Segregation, Center for Developmental
Biology, RIKEN, JP*);

Meiotic chromosome dynamics in mammalian oocytes. p. 3

Coffee Break 11:00~11:20

Session 2. Maternal to Zygotic Reprogramming 11:20~12:20

(Chair: Petr SOLC & Noboru MANABE)

Tereza TORALOVA (*Institute of Animal Physiology and Genetics, The Czech Academy of
Sciences, CZ*);

The expression of nucleophosmin in bovine embryos before and after embryonic
genome activation. p. 5

Sugako OGUSHI (*The Hakubi Center for Advanced Research, Kyoto University, JP*);

Maternal nucleolus governs the higher chromatin organization after fertilization.
..... p. 7

Lunch 12:20~13:30

Session 3. Reprogramming to ES/iPS Cells 1 13:30~14:30

(Chair: Jan MOTLIK, Kazuhiro KIKUCHI & Takashi NAGAI)

Helena FULKA (*Department of Biology of Reproduction, Institute of Animal Science, CZ*);

Production of mouse embryonic stem cell lines from maturing oocytes by direct conversion of meiosis into mitosis. p. 9

Tadashi FURUSAWA (*Division of Animal Sciences, National Institute of Agrobiological Sciences, JP*);

Heterogeneity of embryonic stem cells and its application to generation of transgenic animals. p. 11

Coffee Break 14:30~14:50

Session 3. Reprogramming to ES/iPS Cells 2 14:50~15:50

(Chair: Helena FULKA & Katsuhiko HAYASHI)

Arata HONDA (*Organization for Promotion of Tenure Track, University of Miyazaki, JP*);

Development of ES/iPS cell research using rabbits p. 13

Jan MOTLIK (*Institute of Animal Physiology and Genetics, The Czech Academy of Sciences, CZ*);

Induced pluripotent stem cells derived from the minipig neural stem cells. p. 15

Session 4. From ES to Germ cells 15:50~16:20

(Chair: Josef FULKA & Koji SUGIURA)

Katsuhiko HAYASHI (*Graduate School of Medicine, Kyoto University, JP*);

Production of fertile gametes from mouse pluripotent stem cells. p. 17

Closing Remarks 16:20~16:30

Kei-ichiro MAEDA (*President of the Society for Reproduction and Development, JP*).

Banquet 17:00~

Abstracts

Polo-like kinase I controls nuclear envelope break down and chromosome dynamics in meiosis I

Petr SOLC^{1*}, Tomoya KITAJIMA^{2,4*}, Vladimir BARAN³, Adela BRZAKOVA¹,
Alexandra MAYER¹ Pavlina SAMALOVA¹, Jan MOTLIK¹ and Jan ELLENBERG²

¹ Department of Reproductive and Developmental Biology, Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, 277 21 Liběchov, Czech Republic

² Cell Biology and Biophysics Unit, European Molecular Biology Laboratory, Heidelberg D-69117, Germany

³ Institute of Animal Physiology, Slovak Academy of Sciences, 040 01 Kosice, Slovak Republic

⁴ Current address: Laboratory for Chromosome Segregation, RIKEN Center for Developmental Biology, Kobe, Hyogo 650-0047, Japan

* These authors contributed equally to this work.

Presenting author: **Petr SOLC** (solc@iapg.cas.cz)

In somatic cells Polo-like kinase I (PLK1) is critical mitotic kinase that regulates processes such as centrosome maturation, mitotic entry and spindle formation. In mouse oocytes, PLK1 is firstly activated on microtubule organizing center (MTOC) before resumption of meiosis (nuclear envelope break down, NEBD). Later in prometaphase I and metaphase I, PLK1 activity is mainly targeted to chromosomes. Acute pharmacological PLK1 inhibition leads to the significant delay in NEBD. In normal control oocytes NEBD and chromosome condensation are highly synchronized processes when the beginning of the chromosome condensation precedes NEBD by few minutes. Using quantitative live cell imaging of oocytes from transgenic CAG::H2B mice we have shown that PLK1 activity is essential for synchronization of these two important processes because PLK1 inhibited oocytes fully condenses chromatin but nuclear envelope is still present. PLK1 is also essential for proper MTOCs biogenesis since it controls gamma-tubulin loading on MTOCs. Bipolar spindle formation is significantly delayed after PLK1 inhibition and oocytes remain arrested at metaphase I without ability to trigger anaphase onset. However, this arrest is not due to SAC activity because its silencing via MPS1 inhibition does not override it. Moreover, chromosomes at PLK1 inhibited metaphase I arrested oocytes are not fully pulled by microtubules. The acute inhibition of PLK1 during anaphase results in cytokinesis failure. In conclusion PLK1 is the key player in meiosis I when it controls NEBD, chromosome condensation and spindle formation, anaphase entry and cytokinesis.

Financial support: GACR 301/09/J036 and GACR P301/11/P081 to P.S.

Keywords: PLK1, GVBD, Spindle formation, Anaphase entry, SAC, Cytokinesis

Meiotic chromosome dynamics in mammalian oocytes

Tomoya KITAJIMA^{1,2} and Jan ELLENBERG²

¹ Laboratory for Chromosome Segregation, RIKEN Center for Developmental Biology, Kobe, Hyogo 650-0047, Japan

² Cell Biology and Biophysics Unit, European Molecular Biology Laboratory, Heidelberg D-69117, Germany

Presenting author: **Tomoya KITAJIMA** (tkitajima@cdb.riken.jp)

Meiotic division of an oocyte produces an egg, which differentiates into any kinds of cells after fertilization. During oocyte meiosis, chromosomes must be segregated properly into daughter cells. If errors occur in chromosome segregation, aneuploidy eggs are produced, and fertilization of such eggs would result in miscarriage or severe congenital diseases, such as Down's syndrome.

To achieve proper chromosome segregation, all chromosomes must establish stable biorientation prior to anaphase. We performed high-resolution confocal live imaging of kinetochores and chromosomes from nuclear envelop break down (NEBD) to anaphase onset of the first meiotic division in mouse oocytes. This provided us the complete 3D kinetochore tracking datasets throughout the first meiotic division. Our systematic quantitative analysis using this dataset showed that in the acentrosomal oocytes, chromosome congression results in formation of the prometaphase belt, which precedes chromosome biorientation and spindle elongation. The prometaphase belt is then transformed into the metaphase plate, by the chromosomes that invade the spindle center from the periphery. Concomitantly with spindle elongation, kinetochores start to be attached by microtubule bundles, inducing chromosome biorientation attempts, however, the initial attachment manner is predominantly lateral or merotelic. Due to these improper attachments, close to 90% of all chromosomes have to undergo one or more rounds of error correction of their kinetochore-microtubule attachments before achieving correct biorientation with amphitelic attachments. Our data provide quantitative evidences that homologous chromosome biorientation in mouse oocytes is a highly error-prone process.

Keywords: Oocyte, Meiosis, Chromosome, Kinetochore

The expression of nucleophosmin in bovine embryos before and after embryonic genome activation

Tereza TORALOVA¹, Veronika BENESOVA^{1,2}, Katerina Vodickova KEPKOVA¹,
Petr VODICKA¹ and Jiri KANKA¹

¹ Department of Reproductive and Developmental Biology, Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, 277 21 Liběchov, Czech Republic

² Faculty of Science, Charles University in Prague, 128 43 Prague, Czech Republic

Presenting author: **Tereza TORALOVA** (moravcova@iapg.cas.cz)

Nucleophosmin is an abundant nucleolar phosphoprotein that participates in many basic cellular processes like protein transport, ribosome biogenesis or RNA processing. At the beginning of preimplantation development there are no functional nucleoli in bovine embryos and only structures called nucleolar precursor bodies (NPBs) are present in the embryonic nucleus during the first three cycles. During the major wave of embryonic genome activation (EGA), transformation of the NPBs into fibrillo-granular nucleoli occurs and the typical rRNA synthesizing nucleolus is formed. We wanted to know, how does nucleophosmin expression correlate with the formation of functionally active nucleoli.

To determine the onset of nucleophosmin transcription, we analysed the mRNA expression during whole preimplantation development of bovine embryos. The mRNA level decreased from MII oocytes to 2-cell stage embryos and further slightly, however nonsignificantly, decreased until the early 8-cell stage. At late 8-cell stage the mRNA level increased and it remained approximately at the same level until the blastocyst stage. Even though the increase at late 8-cell stage was nonsignificant again, it was alpha-amanitin sensitive and hence likely represents the activation of nucleophosmin embryonic transcription. The level of nucleophosmin mRNA level in 4-cell stage embryos was not influenced by the alpha-amanitin treatment. On the other hand, the protein expression increased from MII oocytes to 4-cell stage embryos and even more from 4-cell stage to morula stage embryos. In MII oocytes the protein represented a slight mobility shift, which was shown to be caused by phosphorylation of nucleophosmin at this stage.

Localization of the protein copied the status of nucleoli formation. In pre-EGA embryos, it was dispersed throughout the nucleoplasm and during mitosis throughout whole cytoplasm. At early 8-cell stage, when the nucleoli are starting to be formed, ring shaped structures emerged inside the nuclei. From late 8-cell stage onwards, typical nucleoli staining could be seen in all embryos. We wanted to know whether the dissimilarities in protein localization before and after EGA may be caused by some differences between mRNA sequence of maternal and embryonic origin. Sequencing revealed 100% homology of mRNA of both origins.

After silencing of nucleophosmin mRNA, the protein is to great extent degraded at morula

stage, however there is still some residual protein which enables further development. During EGA, the relocalization of the protein to nucleoli is temporarily delayed in embryos with silenced nucleophosmin mRNA, however, yet at 16-cell stage, there were no differences between experimental and control groups in protein localization. This shows that even nucleophosmin of maternal origin is able to localize to nucleoli and the differences in protein localization before and after EGA are caused only by the different state of nucleoli formation.

Supported by GACR 523/09/1035; GACR 204/09/H084 and GAUK 43-251133

Keywords: Nucleophosmin, EGA, Nucleolus

Maternal nucleolus governs the higher chromatin organization after fertilization

Sugako OGUSHI^{1,2}, Kazuo YAMAGATA^{3,4}, Teruhiko WAKAYAMA^{3,5} and Mitinori SAITOU²

¹ The Hakubi Center for Advanced Research, Kyoto University, Kyoto, Kyoto 606-8302, Japan

² Graduate School of Medicine, Kyoto University, Kyoto, Kyoto 606-8315, Japan

³ Laboratory for Genomic Reprogramming, RIKEN Center for Developmental Biology, Kobe, Hyogo 650-0047, Japan

⁴ Current Address: Center for Genetic Analysis of Biological Sciences, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565-0871, Japan

⁵ Faculty of Life and Environmental Sciences, Yamanashi University, Kofu, Yamanashi 400-8510, Japan

Presenting author: **Sugako OGUSHI** (ogushi@anat2.med.kyoto-u.ac.jp)

The primary function of the nucleolus is in ribosome biogenesis. In mammals, a fully-grown oocyte has the nucleolus showing highly compact and fibrillar morphology. The oocyte ceases its entire transcription, including ribosomal RNA transcription, and the chromatin no longer penetrates into the nucleolus. This nucleolus seems inactive for ribosome biogenesis, but assembles/disassembles its structure in every cell cycle of early embryonic development. When we produced an embryo lacking this inactive nucleolus, the embryo failed to develop past the first few cleavages. These embryos, however, had the ability to synthesize the proteins and to replicate DNA as control embryos. Thus, it is clear that the oocyte nucleolus is required for preimplantation development, and might have other aspects of nucleolus function. Further detailed analysis revealed that the zygotes lacking nucleolus showed the drastic higher chromatin disorganization during interphase and had the defect in the first mitotic progression. These zygotes possessed the chromosomes with the abnormal heterochromatin formation. These results indicate that the oocyte nucleolus is involved in the functional higher chromatin organization in the zygote.

As the structure of the oocyte nucleolus is highly compact, the antibodies are not able to penetrate into its structure. Thus, the materials of the oocyte nucleolus remain elusive, except nucleoplasmin2 (Npm2). Now, we are trying to find the oocyte nucleolus materials that are important for early embryonic development, and will show our recent data in this symposium.

Keywords: Heterochromatin, Nucleolus, Zygote

Production of Mouse Embryonic Stem Cell Lines from Maturing Oocytes by Direct Conversion of Meiosis into Mitosis

Helena FULKA¹, Michiko HIROSE², Kimiko INOUE², Narumi OGONUKI²,
Noriko WAKISAKA^{2,3}, Shogo MATOBA², Atsuo OGURA², Tibor MOSKO¹, Tomas KOTT⁴,
and Josef FULKA, JR¹

¹ Department of Biology of Reproduction, Institute of Animal Science, 104 00 Prague, Czech Republic

² Bioresource Engineering Division, RIKEN BioResource Center, Tsukuba, Ibaraki 305-0074, Japan

³ Current address: Laboratory for Neurobiology of Synapse, RIKEN Brain Science Institute, Wako, Saitama 351-0198, Japan

⁴ Department of Molecular Genetics, Institute of Animal Science, 104 00 Prague, Czech Republic
Presenting author: **Helena FULKA** (hell.f@seznam.cz)

ESCs are most commonly derived from embryos originating from oocytes that reached metaphase II. We described a novel approach where ESCs with all pluripotency parameters were established from oocytes in which metaphase I was converted, from the cell cycle perspective, directly into metaphase II-like stage without the intervening anaphase to telophase I transition. The resulting embryos initiated development and reached the blastocyst stage from which the ESC lines were then established. Thus, our approach could represent an ethically acceptable method that can exploit oocytes that are typically discarded in *in-vitro* fertilization clinics. Moreover, our results also indicate that the meiotic cell cycle can be converted into mitosis by modulating chromosomal contacts that are typical for meiosis with subsequent licensing of chromatin for DNA replication.

Key Words: Oocytes, Mouse, Embryonic stem cells, Butyrolactone I

Heterogeneity of embryonic stem cells and its application to generation of transgenic animals

Tadashi FURUSAWA¹, Katsuhiko OHKOSHI¹, Kouji KIMURA², Shuichi MATSUYAMA², Satoshi AKAGI³, Masahiro KANEDA³, Mitsumi IKEDA¹, Misa HOSOE¹, Keiichiro KIZAKI⁴ and Tomoyuki TOKUNAGA¹

¹ Animal Development and Differentiation Research Unit, Division of Animal Sciences, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, 305-8602, Japan

² Animal Feeding and Management Division, National Institute of Livestock and Grassland Science, National Agriculture and Food Research Organization, Nasushiobara, Tochigi, 329-2793, Japan

³ Animal Breeding and Reproduction Division, National Institute of Livestock and Grassland Science, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, 305-8602, Japan

⁴ Laboratory of Veterinary Physiology, Iwate University, Morioka, Iwate, 020-8550, Japan

Presenting author: **Tadashi FURUSAWA** (tfuru@affrc.go.jp)

Mouse embryonic stem (ES) cells are now routinely used to generate knock-in and knock-out mice for elucidation of gene function. When generating chimera mice, we usually inject more than 10 ES cells into host embryos, but only around 30% of pups are born as chimeras: it means most of injected ES cells fail to incorporate into host embryos. Because it is difficult to assume that most of them died or lost pluripotency, it raises the possibility of the existence of status variations in ES cells that related to contribution to epiblast and unrelated to differentiation.

To evaluate this hypothesis, we searched surface markers that expressed on a particular population of ES cells, and found widely variable expression of platelet endothelial cell adhesion molecule 1 (PECAM-1) and stage-specific embryonic antigen (SSEA)-1. The expression level of PECAM1 was positively correlated with the pluripotency and PECAM1 positive ES cells could be divided into two subpopulations according to the expression of SSEA-1. SSEA-1 positive and negative cells demonstrated similar proliferation and entirely reciprocal repopulation in culture, however, SSEA-1 expressing cells predominantly differentiated into the epiblast of chimeric embryos. Microarray analysis revealed that they had quite similar gene expression profiles, but several epigenetically regulated genes identified as different expressed genes. These results imply that epigenetic heterogeneity in ES cells may cause the varied contribution of ES cells to chimeric embryos.

I also would like to present our current work in attempting to establish bovine ES cells.

Keywords: ES cells, Heterogeneity

Development of ES/iPS cell research using rabbits

Arata HONDA^{1,2,3}, Michiko HIROSE¹, Masanori HATORI¹, and Atsuo OGURA^{1,4,5}

¹ Bioresource Engineering Division, RIKEN BioResource Center, Tsukuba, Ibaraki 305-0074, Japan

² Organization for Promotion of Tenure Track, University of Miyazaki, Kiyotake, Miyazaki, 889-1692, Japan

³ PRESTO, Japan Science and Technology Agency, Kawaguchi, Saitama, Japan

⁴ Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Ibaraki, 305-0071, Japan

⁵ The Center for Disease Biology and Integrative Medicine, University of Tokyo, Bunkyo-ku, Tokyo, 113-0033, Japan

Presenting author: **Arata HONDA** (a-honda@med.miyazaki-u.ac.jp)

“Why do you use rabbits?”

I have been asked this question repeatedly. Rabbits pose fewer demands as laboratory animals than mice or rats. Once we noticed the congeniality between rabbit and pluripotent stem cell research, this combination became a very attractive analysis tool. Rabbit embryonic stem (ES) cells and induced pluripotent stem (iPS) cells have been established and share important major characteristics with human pluripotent stem cells. In addition, rabbit iPS cells fulfill all the requirements for the acquisition of a fully reprogrammed state, thus showing strong similarity to their ES cell counterparts. However, although the global gene expression profile of rabbit iPS cells becomes closer to that of the rabbit ES cells as the number of cell passages increases, a slight but rigid difference between the two types of rabbit pluripotent stem cells remains. Recently, we compared the differentiation capacities of rabbit pluripotent stem cell lines using an *in vitro* neural differentiation system. The quality of iPS cells may be inherently poorer than that of ES cells, but this can be improved by several treatments to attain a stem cell quality indistinguishable from that of ES cells.

We expect that rabbit pluripotent stem cells will provide unique, easily accessible animal models for assessing the efficacy and safety of new pluripotent stem cell-based treatments. Moreover, this rabbit model should enable us to compare iPS cells and ES cells under the same standardized conditions when evaluating their ultimate feasibility in cell-based regenerative medicine. We will be glad if you change your mind from “Why rabbits?” to “Therefore, rabbits!”

Keywords: ES cells, iPS cells, Rabbit

Induced Pluripotent Stem Cells Derived from The Minipig Neural Stem Cells

Jan MOTLÍK¹, Eliška SVOBODOVÁ¹, Irena LIŠKOVÁ¹ and Petr VODIČKA¹

¹Laboratory of Cell Regeneration and Plasticity, Institute of Animal Physiology and Genetics, 277 21 Liběchov, Czech Republic

Presenting author: **Jan MOTLIK** (motlik@iapg.cas.cz)

A major barrier to research on neurodegenerative diseases was inaccessibility of diseased tissue for study. A potential solution is to utilize reprogramming technology and to derive iPS cells from patients and differentiate them into neurons affected by a specific disease. This model represents a new experimental system to identify compounds that reduce levels of α -synuclein or mutated huntingtin, and to investigate the molecular mechanism of neurodegeneration caused by mutated protein dysfunction. Miniature pigs are steadily gaining importance as large animal models in the field of regenerative medicine and in stem cell research. Several somatic stem cell populations, including mesenchymal stem cells or neural stem cells were successfully isolated in pigs, but establishment of proper embryonic stem (ES) cell line, fulfilling all classical ES cells landmarks, including germ-line transmission remains so far elusive. Discovery of induced pluripotency makes preparation of pig induced iPS cells an attractive alternative to ES cells. First, we have taken advantage of our experience with the isolation and culture of fetal porcine neural progenitors (NPC) and used them as a starting population for preparation of minipig iPS cells. The minipig neural stem cells (pNSCs) and embryonal fibroblasts (pEF) were isolated from 40 days old fetuses either control or transgenic for the N-terminal part of human mutated huntingtin. All cells were frozen and after thawing they were used in the following experiments: To generate pig induced stem cells (piPSCs), cells were transfected with the expression vectors coding for either four (Oct4, Sox2, Klf4 and c-Myc), three (Oct4, Klf4 and c-Myc) and one (Oct4) transcription vector. All generated piPSC lines were dependent on transgene expression as viral promoters tend not to be silenced in reprogrammed piPSCs. Moreover, in the case of inducible expression cells differentiated after switching off the exogenous expression. On the base of that previous experience, we have chosen the system which combines excisable Piggybac vector expressing ESC-specific micro RNAs 302-307 under the control of inducible promoter and reprogramming media containing small inhibitory molecules to further inhibit differentiation pathways and restore the pluripotency programme. We have optimized expression conditions for MiRplasmid either in the minipig neural stem cells and embryonal fibroblasts. Our experiments indicate that Piggybac vector expression is the right choice for generation of porcine iPSCs independent of transgene expression and capable of robust neural differentiation.

Keywords: iPS cells, Piggybac, Pig

Production of fertile gametes from mouse pluripotent stem cells

Katsuhiko HAYASHI^{1,2,3}, Sugako OGUSHI^{1,4}, So SHIMAMOTO¹ and Mitinori SAITOU^{1,3,5,6}

¹ Graduate School of Medicine, Kyoto University, Kyoto 606-8501, Japan

² PRESTO, Japan Science and Technology Agency, Saitama 332-0012, Japan

³ CiRA, Graduate School of Medicine, Kyoto University, Kyoto 606-8507, Japan

⁴ The Hakubi Center for Advanced Research, Kyoto University, Kyoto 606-8302, Japan

⁵ ERATO, Japan Science and Technology Agency, Kyoto 606-8501, Japan

⁶ WPI-iCeMS, Kyoto University, Kyoto 606-8502, Japan

Presenting author: **Katsuhiko HAYASHI** (khayashi@anat2.med.kyoto-u.ac.jp)

The germ cell lineage ensures the creation of new individuals, thereby perpetuating the genomic and epigenetic information across the generations. In mice, primordial germ cells (PGCs), origin of the germ cell lineage, arise from epiblast cells in response to BMP4 secreted by adjacent extraembryonic ectoderm. We recently reconstituted the PGC specification *in vitro* using mouse embryonic stem cells (mESCs) as well as induced pluripotent stem cells (iPSCs). In the culture system, mESCs/iPSCs first differentiate into epiblast-like cells (EpiLCs) and then induce PGC-like cells from the EpiLCs. The manner of differentiation from mESCs to PGCs reproduces the manner of PGC specification *in vivo*. PGCs produced from mESCs, termed PGC-like cells (PGCLCs), are fully potent, since they differentiate into spermatozoa and in turn the fertilized eggs with the spermatozoa give rise to healthy individuals. The progress was made by accumulated knowledge of the manner of PGC specification *in vivo*, the nature of self-renewing pluripotent stem cells, and growth factors endowing EpiLC formation and PGCLC induction *in vitro*. We are developing further the culture system toward reconstitution of the entire process of germ cell development. We will show recent advance in reconstitution of germ cell development *in vitro*.

Keywords: Primordial germ cells, Pluripotent stem cells, Epiblast

SRD-NIAS 第2回 日・チェコ交流シンポジウム 資料

編集・発行 独立行政法人 農業生物資源研究所
広報室

Tel. 029-838-8469 Fax. 029-838-7408

〒305-8602 茨城県つくば観音台2-1-2

発行日 平成24年9月1日

印刷所 佐藤印刷株式会社

ISBN 978 - 4 - 931511 - 23 - 1