Members

Professor  Ryoichiro Kageyama
Associate Professor Toshiyuki Ohtsuka
Assistant Professor Taeko Kobayashi
Hakubi Associate Professor Itaru Imayoshi
Hakubi Assistant Professor Tomoko Tateya
iCeMS Assistant Professor Hiromi Shimojo
Research Fellow Akihiro Isomura
Yukiko Harima
Takahiko Matsuda
Tsuyoshi Hirashima
Graduate Student Naoki Watanabe
Mitsushige Ando
Shama Ratiram Bansod
Kumiko Kobayashi
Kyogo Kawaguchi
Yuki Maeda
Anna Araki
Chihiro Masumoto

Introduction

The research interest of this laboratory is to understand the molecular mechanism of cell differentiation and organogenesis. Particularly, we are interested in basic helix-loop-helix (bHLH) transcription factors that regulate various developmental processes including neural development and somite formation. We are characterizing the functions of bHLH genes by misexpressing the genes with virus vectors and electroporation (gain-of-function study) and by generating knock-out mice (loss-of-function study). We previously showed that bHLH proneural genes such as Ascl1 (also called Mash1) and Math3 promote neuronal versus glial fate determination, whereas the bHLH repressor genes Hes1 and Hes5 regulate maintenance of neural stem cells by repressing proneural gene expression. These results indicate that the balance between bHLH proneural and bHLH repressor genes is important for a choice favoring neuronal differentiation or neural stem cell proliferation. It was recently shown however that the proneural gene Ascl1 not only promotes neuronal fate determination but also induces proliferation of neural stem cells. In addition, it was
found that the bHLH gene *Olig2* regulates both oligodendrocyte fate determination and neural stem cell proliferation. Our group found that Hes1 promotes astrocyte formation as well as neural stem cell proliferation. Thus, each bHLH fate determination factor has contradictory functions, neural stem cell proliferation and specific cell fate determination, but the detailed mechanism of how these bHLH factors regulate such contradictory functions is unknown.

We found that in neural stem cells, Hes1 expression oscillates by negative feedback with a period of about 2-3 hours. Hes1 oscillations drive cyclic expression of the proneural genes *Ascl1* and *Neurog2* and the Notch ligand gene *Deltalike1 (Dll1)*. In contrast, the expression of *Ascl1*, *Neurog2* and *Dll1* is sustained (non-oscillatory) in postmitotic differentiating neurons. We also found that although Hes1 expression oscillates in neural stem cells, it becomes up-regulated and sustained during astrocyte formation. Similarly, the bHLH factor Olig2 expression oscillates in neural stem cells but becomes up-regulated and sustained during oligodendrocyte formation. Our results therefore suggest that the multipotency is a state of oscillatory expression of multiple fate determination factors, while the fate determination is a process of dominant expression of a selected single bHLH factor, which represses the other fate determination factors. We further showed by a new optogenetics approach that sustained expression of the proneural gene *Ascl1* induces neuronal fate determination, whereas oscillatory expression of Ascl1 activates proliferation of neural stem cells. Thus, the expression dynamics are very important for a choice between neural stem cell proliferation and specific cell fate determination.

**Topics**

1) **Oscillatory control of factors determining multipotency and fate in mouse neural progenitors**: I. IMAYOSHI, A. ISOMURA, Y. HARIMA, K. KAWAGUCHI, H. KORI, H. MIYACHI, T. FUJIWARA, F. ISHIDATE and R. KAGEYAMA

The basic helix-loop-helix transcription factors Ascl1/Mash1, Hes1, and Olig2 regulate fate choice of neurons, astrocytes, and oligodendrocytes, respectively. These same factors are coexpressed by neural progenitor cells. Here, we found by time-lapse imaging that these factors are expressed in an oscillatory manner by mouse neural progenitor cells. In each differentiation lineage, one of the factors becomes dominant. We used optogenetics to control expression of Ascl1 and found that, although sustained Ascl1 expression promotes neuronal fate determination, oscillatory Ascl1 expression maintains proliferating neural progenitor cells. Thus, the multipotent state correlates with oscillatory expression of several fate-determination factors, whereas the differentiated state correlates with sustained expression of a single factor.
2) Hedgehog signaling regulates prosensory cell properties during the basal-to-apical wave of hair cell differentiation in the mammalian cochlea: T. TATEYA, I. IMAYOSHI, I. TATEYA, K. HAMAGUCHI, H. TORII, J. ITO and R. KAGEYAMA

Mechanosensory hair cells and supporting cells develop from common precursors located in the prosensory domain of the developing cochlear epithelium. Prosensory cell differentiation into hair cells or supporting cells proceeds from the basal to the apical region of the cochleae, but the mechanism and significance of this basal-to-apical wave of differentiation remain to be elucidated. Here, we investigated the role of Hedgehog (Hh) signaling in cochlear development by examining the effects of up- and downregulation of Hh signaling in vivo. The Hh effector smoothened (Smo) was genetically activated or inactivated specifically in the developing cochlear epithelium after prosensory domain formation. Cochlea expressing a constitutively active allele of Smo showed only one row of inner hair cells with no outer hair cells (OHCs); abnormal undifferentiated prosensory-like cells were present in the lateral compartment instead of OHCs and their adjacent supporting cells. This suggests that Hh signaling inhibits prosensory cell differentiation into hair cells or supporting cells and maintains their properties as prosensory cells. Conversely, in cochlea with the Smo conditional knockout (Smo CKO), hair cell differentiation was preferentially accelerated in the apical region. Smo CKO mice survived after birth, and exhibited hair cell disarrangement in the apical region, a decrease in hair cell number, and hearing impairment. These results indicate that Hh signaling delays hair cell and supporting cell differentiation in the apical region, which forms the basal-to-apical wave of development, and is required for the proper differentiation, arrangement and survival of hair cells and for hearing ability.

3) Control of Hes7 expression by Tbx6, the Wnt pathway and the chemical Gsk3 inhibitor LiCl in the mouse segmentation clock: A. GONZALEZ, I. MANOSALVA, T. LIU and R. KAGEYAMA

The mouse segmentation is established from somites, which are iteratively induced every two hours from the presomitic mesoderm (PSM) by a system known as the segmentation clock. A crucial component of the segmentation clock is the gene Hes7, which is regulated by the Notch and Fgf/Mapk pathways, but its relation to other pathways is unknown. In addition, chemical alteration of the Wnt pathway changes the segmentation clock period but the mechanism is unclear. To clarify these questions, we have carried out Hes7 promoter analysis in transgenic mouse embryos and have identified an essential 400 bp region, which contains binding sites of Tbx6 and the Wnt signaling effector Lef1. We have found that the Hes7 promoter is activated by Tbx6, and normal activity of the Hes7 promoter in the mouse PSM requires Tbx6 binding sites. Our results demonstrate that Wnt pathway molecules activate the Hes7 promoter cooperatively with Tbx6 in cell culture and are
necessary for its proper expression in the mouse PSM. Furthermore, it is shown that the chemical Gsk3 inhibitor LiCl lengthens the oscillatory period of Hes7 promoter activity. Our data suggest that Tbx6 and the Wnt pathway cooperatively regulate proper Hes7 expression. Furthermore, proper Hes7 promoter activity and expression is important for the normal pace of oscillation.

4) Hes1 in the somatic cells of the murine ovary is necessary for oocyte survival and maturation: I. MANOSALVA, A. GONZALEZ and R. KAGEYAMA

The Notch pathway plays an important role in ovary development in invertebrates like Drosophila. However its role for the mammalian ovary is unclear. Mammalian Hes genes encode transcriptional factors that mediate many of the activities of the Notch pathway. Here, we have studied the function of Hes1 during embryonic development of the mouse ovary. We find that Hes1 protein is present in somatic cells and oocyte cytoplasm and decreases between E15.5 and P0. Conventional Hes1 knock-out (KO), Hes1 conditional KO in the ovarian somatic, and chemical inhibition of Notch signaling decrease the total number, size and maturation of oocytes and increase the number of pregranulosa cells at P0. These defects correlate with abnormal proliferation and enhanced apoptosis. Expression of the proapoptotic gene Inhbb is increased, while the levels of the antiapoptotic and oocyte maturation marker Kit are decreased in the Hes1 KO ovaries. Conversely, overactivation of the Notch pathway in ovarian somatic cells increases the number of mature oocytes and decreases the number of pregranulosa cells. Fertility is also reduced by either Hes1 deletion or Notch pathway overactivation. In conclusion, our data suggest that the Notch-Hes1 pathway regulates ovarian somatic cell development, which is necessary for oocyte survival and maturation.

5) Oscillatory links of Fgf signaling and Hes7 in the segmentation clock: Y. HARIMA and R. KAGEYAMA

Somitogenesis is controlled by the segmentation clock, where the oscillatory expression of cyclic genes such as Hes7 leads to the periodic expression of Mesp2, a master gene for somite formation. Fgf signaling induces the oscillatory expression of Hes7 while Hes7 drives coupled oscillations in Fgf and Notch signaling, which inhibits and activates Mesp2 expression, respectively. Because of different oscillatory dynamics, oscillation in Fgf signaling dissociates from oscillation in Notch signaling in S-1, a prospective somite region, where Notch signaling induces Mesp2 expression when Fgf signaling becomes off. Thus, oscillation in Fgf signaling regulates the timing of Mesp2 expression and the pace of somitogenesis. In addition, Fgf signaling was found to be a primary target for hypoxia, which causes phenotypic variations of heterozygous mutations in Hes7 or Mesp2, suggesting gene-environment interaction through this signaling.
List of Publications


影山龍一郎: 短周期遺伝子発現振動の動作原理と意義、東北大学脳センターシンポジウム、仙台、2013年3月7日

影山龍一郎：大人の脳で新たに生まれる神経細胞とその不思議な役割、第8回京都大学附属研究所・センターシンポジウム、札幌、2013年3月16日


影山龍一郎：体節形成と遺伝子発現振動、第 14 回運動器科学研究会、東京、2013 年 9 月 13-14 日

Imayoshi, I.: Oscillatory expression of bHLH transcriptional factors in neural stem cells. Neurogenesis 2013 in Matsushima. 松島、2013 年 10 月 16-18 日

Kageyama, R.: Oscillatory control of determination factors for multipotency versus fate choice in mouse neural progenitors. OIST Symposium on Gradients and Signalling. 沖縄、2013 年 11 月 11-15 日


影山龍一郎：多分化能と運命決定における神経分化決定遺伝子のダイナミックな制御、第 36 回日本分子生物学会年会、神戸、2013 年 12 月 3-6 日

下條博美、播磨有希子、前田勇樹、大塚俊之、宮地 均、影山龍一郎：神経発生過程における遺伝子発現ダイナミクスによる神経分化制御機構の解明、第 36 回日本分子生物学会年会、神戸、2013 年 12 月 3-6 日

Imayoshi, I.: Continuous postnatal neurogenesis contributes to formation and maintenance of the functional olfactory bulb neural circuits. International Symposium on “Sensory Systems and Neural Circuits” Celebrating the 22nd Anniversary of Odorant-Receptor Gene Discovery. 東京、2013 年 2 月 11-12 日

Harima, Y., Takashima, Y., Ueda, Y., Ohtsuka, T., Kageyama, R.: Accelerated tempo of the segmentation clock by reducing the number of introns in the Hes7 gene. CDB Symposium 2013 “Making of Vertebrate”. 神戸、2013 年 3 月 4-6 日

Harima, Y., Takashima, Y., Ueda, Y., Ohtsuka, T., Kageyama, R.: Accelerated tempo of the segmentation clock by reducing the number of introns in the Hes7 gene. The 20th East Asia Joint Symposium. 東京、2013 年 11 月 5-8 日