Bioresource Research Collaborative Group

Ishii Research Collaborative Group

Laboratory Head

Shunsuke ISHII, Ph.D.

Goal

Transcriptional control is a key step in the development, stress response and various diseases of humans. However, the mechanism of transcriptional control remains elusive, particularly how transcription is regulated via changes in the nuclear architecture and chromatin structure. Using molecular biology and whole-animal systems, we are investigating the mechanism of transcriptional control. In particular, we are generating knockout mice of various transcription factors to understand the physiological role of each transcription factor. Furthermore, we are analyzing the network of various transcription factors using the *Drosophila* genetic system.

Activities

1. Nuclear oncogene product as a transcriptional regulator
2. Gene expression extracellular stimuli
3. Genetic study of transcription using *Drosophila*

Members

Laboratory Head

Shunsuke ISHII, Ph. D. (1982.4~)

Vice Chief Scientist

Toshio MAEKAWA, Ph. D. (1982.6~)

Senior Research Scientist

Teruaki NOMURA, Ph. D. (1990.11~)

Tsuyoshi TAKAGI, Ph. D. (1997.4~)

Toshie SHINAGAWA, Ph. D. (2005.4~)

Specific Aims

1. Nuclear oncogene product as a transcriptional regulator

B-Myb is a member of the vertebrate Myb family of transcription factors and is expressed ubiquitously. B-Myb activates the transcription of a group of genes required for G2/M cell cycle transition by forming the dREAM/Myb-MuvB-like complex, which was originally identified in *Drosophila*. Mutants of zebrafish B-myb and *Drosophila* myb exhibit defects in cell cycle progression and genome instability. Although the genome instability caused by a loss of B-Myb has been speculated to be due to an abnormal cell cycle progression, the precise mechanism remains unknown. We have purified a B-Myb complex containing clathrin and filamin (Myb-Clafi complex). This complex is required for the normal localization of clathrin at mitotic spindles, which was previously reported to stabilize kinetochore fibres. The Myb-Clafi complex is not tightly associated with the mitotic spindles, suggesting that this complex ferries clathrin to mitotic spindles. Thus, the identification of the Myb-Clafi complex reveals a yet unrecognized function of B-Myb that may contribute to its role in chromosome stability, possibly, tumour suppression.

2. Gene expression regulation by extracellular stimuli

Adipocyte differentiation is an important step in obesity development; however, how hormonal cues mediate adipocyte differentiation remains elusive. BMP stimulates in vitro adipocyte differentiation, but the role of BMP in in vivo adipogenesis is unknown. *Drosophila* Schnurri (Shn) is required for the signaling of Decapentaplegic, a *Drosophila* BMP homolog, via interaction with the Mad/Medea transcription factors. Vertebrates have three Shn orthologs: Shn-1, Shn-2, and Shn-3. We have demonstrated that Shn-$2^{-/-}$ mice have a reduced amount of white adipose tissue and that Shn-$2^{-/-}$ mouse embryonic fibroblasts cannot efficiently differentiate into adipocytes in vitro. Shn-2 enters the nucleus upon BMP-2 stimulation and, in cooperation with Smad1/4 and C/EBPα, induces the expression of PPARγ2, a key transcription factor for adipocyte differentiation. Shn-2 directly interacts with both Smad1/4 and C/EBPα on the PPARγ2 promoter. These indicate that Shn-2-mediated BMP signaling has a critical role in adipogenesis.

3. Genetic study of transcription factor using *Drosophila*

ATF-2 is a member of the ATF/CREB family of transcription factors that is activated by stress-activated protein kinases such as p38. To analyze the physiological role of *Drosophila* ATF-2 (dATF-2), we generated dATF-2 knockdown flies using RNA interference. A reduced dATF-2 level in the fat body, the fly equivalent of the mammalian liver and adipose tissue, decreased survival under starvation condition. This was due to the smaller triglyceride reserves of the dATF-2 knockdown flies than of the control flies. Among multiple genes that control triglyceride levels, the *Drosophila* PEPCK (dPEPCK) gene had a markedly reduced expression level in the dATF-2 knockdown flies. PEPCK is a key enzyme for both gluconeogenesis and glyceroneogenesis, which is a pathway required for triglyceride synthesis via glycerol-3-phosphate. Although the blood sugar level of the dATF-2 knockdown flies was almost the same as that of the control flies, glyceroneogenesis activity was reduced in the fat bodies of the dATF-2 knockdown flies. Thus, reduced glyceroneogenesis activity might have at least partly contributed to the decreased amounts of triglyceride stores in the dATF-2 knockdown flies. Furthermore, we showed that dATF-2 positively regulated dPEPCK gene transcription via several CRE half-sites in the PEPCK promoter. Thus, dATF-2 is critical for fat metabolism regulation.

Publications

【Original Papers】(*Peer reviewed journals)

1. Jin W., Takagi T., Kanesashi S., Kurahashi T., Nomura T., Harada J., Ishii S.: “Schnurri-2 controls BMP-dependent adipogenesis via interaction with Smad proteins.” Dev. Cell 10, 461-471 (2006).*

2. Takagi T., Jin W., Taya K., Watanabe G., Mori K., Ishii S.: “Schnurri-2 mutant mice are hypersensitive to stress and hyperactive.” Brain Res. 1108, 88-97 (2006).*

3. Makino C., Sano Y., Shinagawa T., Millar J. B., Ishii S.: “Sin1 binds to both ATF-2 and p38 and enhances ATF-2-dependent transcription in an SAPK signaling pathway.” Genes Cells 11, 1239-1251 (2006).*

4. Zhou H., Kim S., Ishii S., Boyer T. G.: “Mediator modulates Gli3-dependent Sonic hedgehog signaling.” Mol. Cell. Biol. 26, 8667-8682 (2006).*

5. Kim S., Song Y. J., Higuchi D. A., Kang H. P., Pratt J. R., Yang L., Hong C. M., Poursine-Laurent J., Iizuka K., French A. R., Sunwoo J. B., Ishii S., Reimold A. M., Yokoyama W. M.: “Arrested natural killer cell development associated with transgene insertion into the Atf2 locus.” Blood 107, 1024-1030 (2006).*

6. Maekawa T., Shinagawa T., Sano Y., Sakuma T., Nomura S., Nagasaki K., Miki Y., Saito-Ohara F., Inazawa J., Kohno T., Yokota J., Ishii S.: “Reduced levels of ATF-2

predispose mice to mammary tumors." Mol. Cell. Biol. 27, 1730-1744 (2007).*

7. Okamura T., Shimizu H., Nagao T., Ueda R., Ishii S.: "ATF-2 regulates fat metabolism in Drosophila." Mol. Biol. Cell. 18, 1519-1529 (2007).*

8. Kajino T., Omori E., Ishii S., Matsumoto K., Ninomiya-Tsuji J.: "TAK1 MAPKKK mediates TGF-β signaling by targeting SnoN oncoprotein for degradation." J. Biol. Chem. 282, 9475-9481 (2007).*

9. Saita Y., Takagi T., Kitahara K., Usui M., Ezura Y., Nakashima K., Kurosawa H., Ishii S., Noda M.: "Lack of Schnurri-2 expression associates with reduced bone remodeling and osteopenia." J. Biol. Chem. 282, 12907-12915 (2007).*

10. Kimura M., Iwamura C., Suzuki A., Miki T., Hasegawa A., Sugaya K., Yamashita M., Ishii S., Nakayama T.: "Schnurri-2 controls memory Th1 and Th2 cell numbers *in vivo*. J. Immunol. 178, 4926-4936 (2007).*

11. Yamauchi T., Keough R. A., Gonda T. J., Ishii S.: "Ribosomal stress induces processing of Mybbp1a and its translocation from the nucleolus to the nucleoplasm." Genes Cells 13, 27-39 (2008).*

12. Maekawa T., Sano Y., Shinagawa T., Sakuma T., Nomura S., Licht J. D., Ishii S.: "ATF-2 controls transcription of Maspin and GADD45α genes independently from p53 to suppress mammary tumors." Oncogene 27, 1045-1054 (2008).*

13. Yamauchi T., Ishidao T., Nomura T., Shinagawa T., Tanaka Y., Yonemura S., Ishii S.: "A B-Myb complex containing clathrin and filamin is required for mitotic spindle function." EMBO J. 27, 1852-1862 (2008).*

14. Shimizu H., Shimoda M., Yamaguchi T., Seong K. H., Ishii S.: "*Drosophila* ATF-2 regulates sleep and locomotor activity in pacemaker neurons." Mol. Cell. Biol. (*in press*).*