DIVISION OF CELL DIFFERENTIATION

Professor:	*Ken-ichirou Morohashi*
Research Associate:	*Akihiko Shimono*
	Satoru Ishihara
Technical Staff:	*Sanae Oka*
	Hisae Tsuboi
	Yuko Shinohara [1)]
	Yoshie Takeda
	Masatomo Kusaka
	Tomoko Imura [2)]
Postdoctoral Fellow:	*Akiko Iizuka* [3)]
	Naohito Nakamura [4)]
Graduate Student:	*Ken Kawabe* [5)]
	Tokuo Mukai [5)]
	Hirofumi Mizusaki
	Ryouji Matsushima [6)]
	Akira Namba [7)]
Visiting Fellow:	*Yoshiyuki Kojima* [8)]

[1)] *JSPS technical staff*
[2)] *JSPS secretary*
[3)] *NIBB Postdoctoral Fellow*
[4)] *JSPS Postdoctoral Fellow*
[5)] *Graduate School of Kyushu University*
[6)] *Graduate School of Nagoya University*
[7)] *Graduate School of Tokyo University*
[8)] *Nagoya City University*

From an aspect that differentiation of cells is regulated at least in part by the functions of cell type- or tissue-specific transcription factors, mechanisms underlying differentiation of the steroidogenic tissues such as the gonads (testis and ovary) and adrenal cortex have been under investigation in our division. Based on observations obtained in the past decade, we started in Okazaki new projects to understand mechanisms underlying sex-differentiation of the gonads and mechanisms of sex-differentiation of brain functions necessary for reproductive behaviour. To address these issues, we have focussed our attention on the mechanisms how steroidogenic-tissue specific transcription factors regulate their target genes which are essential for the tissue differentiation and functions, and how the genes encoding the transcription factors are regulated during the tissue differentiation. In addition to the studies above, Dr. Shimono joined to our division as a research associate from April 1999 and has started to investigate mechanisms underlying head formation.

I. Gene regulatory cascade in the steroidogenic tissue differentiation

When a differentiation process of a tissue is considered, it is reasonable to assume a gene regulatory cascade specific for each tissue in which certain genes encoding transcription factors are involved as the components. In the cascades required for adrenal and gonadal differentiation, Ad4BP/SF-1 is locates upstream of some tissue-specific genes, including the steroidogenic CYP genes, and locates downstream of other transcription factors regulating the Ad4BP/SF-1 gene. Considering that the cascade flows from upstream to downstream during the tissue differentiation and Ad4BP/SF-1 is an essential transcription factor for the tissue differentiation, identification of the components consisting the cascade as well as their genetical relationship in the cascade are essential for fully understanding the mechanisms of the tissue differentiation.

From these points of view, the regulatory region of the Ad4BP/SF-1 gene was analysed by making transgenic mice or with cultured cells. However, our in vivo investigation in a recent few years has not yet been successful, probably because the regulatory region locates far upstream or far downstream from the structural gene of Ad4BP/SF-1. On the contrary, our in vitro study with cultured cells provided a novel mechanism regulating the Ad4BP/SF-1 gene, which will be expected to give us a new insight into metabolism of biologically active lipophylic compounds.

Dax-1 is another transcription factor of our interest, which is also implicated in the steroidogenic tissue differentiation. Although our previous study revealed that the factor acts as a suppressor of Ad4BP/SF-1, regulation of the suppressive effect has not yet clarified at the molecular level. We recently uncovered the function of the amino terminal half of Dax-1 containing a unique repeated sequence instead of Zn-finger DNA binding domain, which enables us to understand how Dax-1 suppresses the function of Ad4BP/SF-1 and how the activity of Dax-1 is regulated.

In addition to these transcription factors, factors such as Sox-9, Wt-1, Emx-2, and GATA-4 are known to be implicated in the gonad development. In order to isolate other factors interacting with the transcription factors above, yeast two-hybrid screening was performed with a cDNA library constructed with an mRNA prepared from mouse foetal gonads. Extensive screening resulted in isolation of interacting molecules including coactivators and other type of transcription factors, some of which were novel factors. Distributions and functions of these interacting molecules have been examined.

II. Sex-differentiation observed in adrenal cortex

Our previous study indicated that Ad4BP/SF-1 is expressed in all three zones of the adrenal cortex while Dax-1 is expressed in only outer zone, the zona glomerulosa, but not in inner zones, the zona fasciculata and reticularis. However, this distribution revealed by immunohistochemistry was quite distinct from that obtained with in situ hybridization. To explain the discrepancy between the two methods, close examination was carried out with a series of adrenal cortex of both sexes at several developing stages from foetal to adult. Although the distribution of Dax-1 was identical between the two sexes before puberty, distinct distribution was clearly observed after sexual maturation. This sexually dimorphic expression disappeared by castration and emerged again after testosterone replacement. Injection of testosterone into female mice make the expression profile altered into that of male. Taken together, our in vivo studies suggested that an-

drogen and its receptor downregulate Dax-1 gene transcription, which is interestingly inconsistent with a common understanding that androgen receptor activates target gene transcription in a ligand dependent manner. The mechanism of suppression of the Dax-1 expression by androgen receptor and its ligand is further investigating at a molecular level.

Investigations of head formation and brain sex differentiation are included as the targets of our study. These studies, same as the studies described above, have been performed based on examination of the functions of transcription factors. Uncovering the molecular mechanisms will be elucidated by these efforts in the near feature.

Publication List:

Morohashi, K., Tsuboi-Asai, H., Matsushita, S., Suda, M., Nakashima, M., Sasano, H., Hataba, Y., Li, C-L., Fukuta, J., Irie, J., Watanabe, T., Nagura, H., & Li, E. (1999) Structural and Functional Abnormalities in the Spleen of *mFtz-F1* Gene Disrupted Mouse. *Blood* **93**, 1586-1594.

Kawabe, K., Shikayama, T., Tsuboi, H., Oka, S., Oba, K., Yanase, T., Nawata, H., & Morohashi, K. (1999) *Dax-1* as One of the Target Genes of Ad4BP/SF-1. *Mol. Endocrinol*. **13**, 1267-1284.

Kabe, Y., Goto, M., Shima, D., Imai, T., Wada, T., Morohashi, K., Shirakawa, M., Hirose, H., & Handa, H. (1999) The Role of Human MBF1 as a Transcriptional Coactivator. *J. Biol. Chem.* **274**, 34196-34202.

Nagamine, C.M., Morohashi, K., Carlisle, C., & Chang, D.K. (1999) Sex Reversal Caused by *Mus musculus domesticus* Y Chromosomes Linked to Variant Expression of the Testis-Determining Gene *Sry*. *Dev. Biol*. **216**, 182-194.

Morohashi, K. (1999) Gonadal and Extragonadal Functions of Ad4BP/SF-1 -Developmental Aspects-. *Trends in Endocrinol. Metab*. **10**, 169-173.

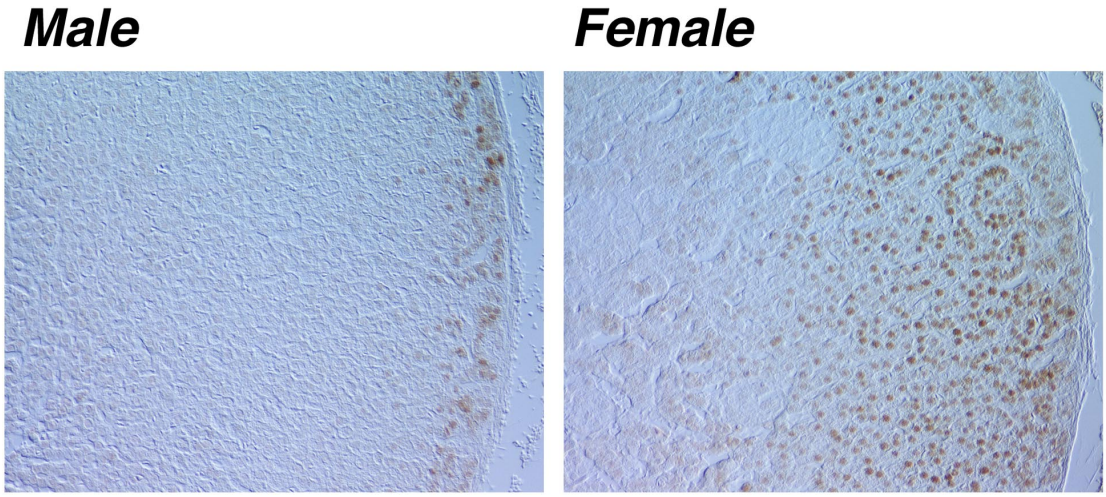

Fig. Sex-dependent distribution of Dax-1 in mouse adrenal cortex.
Adult mouse adrenal glands from both sexes were immunostained with an antiserum for Dax-1.