DIVISION OF CELL DIFFERENTIATION

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Cell and tissue differentiation proceeds systematically based on a number of gene expressions that commence successively along with the passage of time. As the consequence, a fertilised egg develops into a variety of tissues and organs comprising specialised cells in terms of their structures and functions. Accordingly, it is no doubt that investigation of the mechanisms underlying the cell and tissue-specific gene expression at a molecular base is essential for obtaining a proper understanding of the process of tissue differentiation. In our division of Cell Differentiation, two distinct but closely correlated studies at their central concepts have proceeded. One of them is the study for comprehensive understanding of sex differentiation at the levels from the gonad function to reproductive behavior, and the other is the study focussing on head formation through characterization of the function of head organizer.

I. Gene regulatory cascade in the steroidogenic tissue differentiation

When a differentiation process of a tissue is considered, it is reasonable to assume a tissue-specific gene regulatory cascade in which certain genes encoding transcription factors are involved as the critical components. In the cascades required for adrenal and gonadal differentiation, Ad4BP/SF-1 is locates upstream of 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 through out the tissue differentiation and Ad4BP/SF-1 is an essential transcription factor in the gonadal cascade, identification of the components consisting the cascade as well as their genetical relationship is essential for fully understanding the mechanisms of the tissue differentiation.

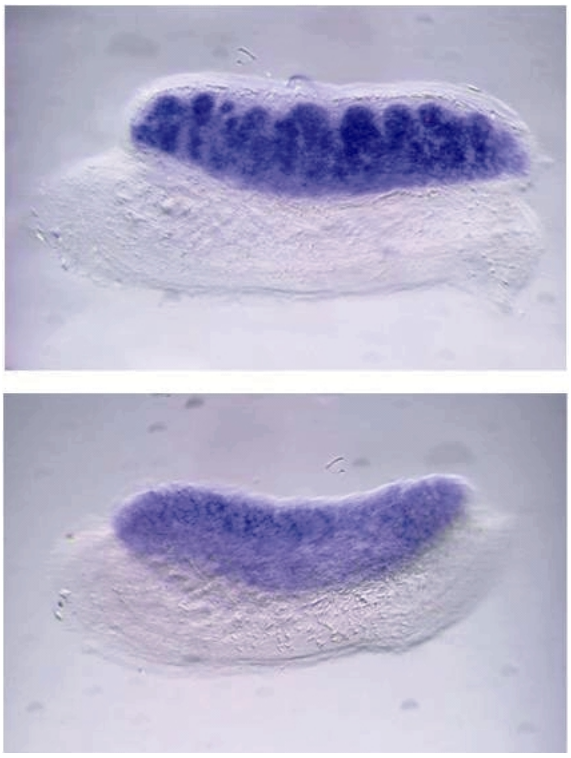
Based on the aspect above, the regulatory region of the *Ad4BP/SF-1* gene has been analysed *in vivo* by making transgenic mice. However, our *in vivo* study in a recent few years has not yet been successful, probably because the regulatory region locates far upstream or far downstream from the structural gene of *Ad4BP/SF-1*. On the contrary, our in vitro study with cultured cells provided a novel mechanism regulating Ad4BP/SF-1 gene, in which an activating signal from one of growth factors, Wnt, is implicated. Although the fine mechanism has been under investigation, the study from this aspect will give us a novel insight into gonad differentiation.

Dax-1 is another transcription factor of our interest, which is also implicated in the steroidogenic tissue differentiation. Our previous study revealed that the factor acts as a suppressor of Ad4BP/SF-1. However, regulation of the suppressive effect has remained to be clarified at the molecular level. We recently uncovered the function of the amino terminal half of Dax-1 containing a unique repeated sequence. When Dax-1 functions as the suppressor, a certain amino acid sequence in the repeats is essential for interaction with target nuclear receptors, and thereby the transcription mediated by the nuclear receptors are largely inhibited. Although it remains unclear how the inhibition activity is regulated in a variety of physiological conditions, this interaction gave us a cue to address it.

In addition to these transcription factors, Sox-9, Wt-1, Emx-2, and GATA-4 are known to be implicated in the gonad development. In order to isolate novel factors interacting with all these transcription factors above, yeast two-hybrid screening has been performed using a cDNA library constructed with an mRNA prepared from mouse fetal gonads. Extensive screening resulted in isolation of molecules including coactivators and other type of transcription factors. Some of them are novel factors and have interesting structures making us to anticipate novel regulations of the transcription. 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



Expression of Factor X, which was isolated by two-hybrid screening as a molecule interacting with Ad4BP/SF-1. Factor X is expressed in the developing gonads (E12.5 testis (upper) and ovary (lower)).

discrepancy between the two methods, close examination was carried out with a series of adrenal cortex of both sexes at several developing stages from fetal 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 androgen 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.

III. Molecular mechanism for head formation

It has been clarified that the anterior visceral endoderm (AVE) and mesendoderm (AME) plays critical roles for head formation by the induction of specific gene expressions in anterior neuroectoderm. Lim1, a LIM homeodomain-containing transcription factor, is expressed at the AVE and primitive streak-derived cells during gastrulation. Since a striking function of Lim1 was revealed by making gene disrupted mice which displayed headless phenotype, molecular events after commencing the *Lim1* expression have been investigated through identifying a set of downstream genes of the transcription factor. For identification, differential screen was performed using subtraction between cDNA pools generated from a small number of cells corresponding to the AVE of wild and KO individuals. A number of candidate genes showing a loss of expression in the KO and a close correlation with Lim1 in terms of their expression profiles have been isolated so far. The functions of these genes are investigating extensively by gene disruption studies.

Publication List:

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