DIVISION OF BEHAVIORAL BIOLOGY (ADJUNCT)

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In mammals, several social behaviors are dependent on sex. The sex-dependent patterns of behaviors must be acquired through highly irreversible processes during development. We hypothesize that the long-term effects of sex steroids at the perinatal stage on behaviors after puberty are somehow marked at the genome level. We are currently investigating the epigenetic status of the critical brain subareas responsible for the sex difference of the behaviors.

I. DNA methylation of nuclear receptors

In mammals, DNA methylation, mainly occurring on CG dinucleotides, is a fundamental mechanism that differentiates the gene expression pattern in respective cell. DNA methylation is a restraint of the pluripotency because once the pattern is established during development it is maintained through cell division. On the other hand, for example, some fish that contain much less methylation activity are found to easily and reversibly change the sex status according to the environmental context. In rodents, endocrine disturbance at the fetal and/or postnatal stage irreversibly changes the behaviors such as the lordosis (in females) and the mounting (in males) after the pubertal stage that are normally dependent on genetic sex. In some cases, lordosis can be observed even in males and *vice versa*. These clearly indicate that sex-dependent patterns of behaviors are not directly dependent on "sex-specific" genes but rather established through epigenetic processes. We have found the sex-dependent DNA methylation pattern of steroid receptor genes (estrogen receptor α, ERα; androgen receptor, AR; progesterone receptor, PR) in the male and female rat brain subareas. In this year of 2005, we report herein the DNA methylation analyses of the steroid receptor genes and identification of the naturally occurring antisense RNAs as potent and specific epigenetic regulators in rats and mice.

1-1 Sex-dependent DNA methylation

In general, the binding affinity of the transcription factors is reduced by hypermethylation that are frequently observed on promoter region of either ERα, AR or PR in tumor cells. We analyzed the DNA methylation status of their 5'-flanking regions in the rat using the bisulfite sequencing method. DNA methylation patterns differed depending on brain subarea. For ERα, no differences were found within male rat brain subareas examined. In contrast, hypermethylation was specifically observed in the bed nucleus of the stria terminalis (BNST) of female rats. The sex-dependent DNA methylation was also found in the mouse BNST. It was to be noted that methylation pattern differed depending on brain subarea examined even in male mice. These suggested the species difference of the transcriptional regulation of the ERα. Nonetheless, sex-dependent difference was specifically observed in the BNST, suggesting that the BNST is an area important for the sexual differentiation of the brain in rats and mice.

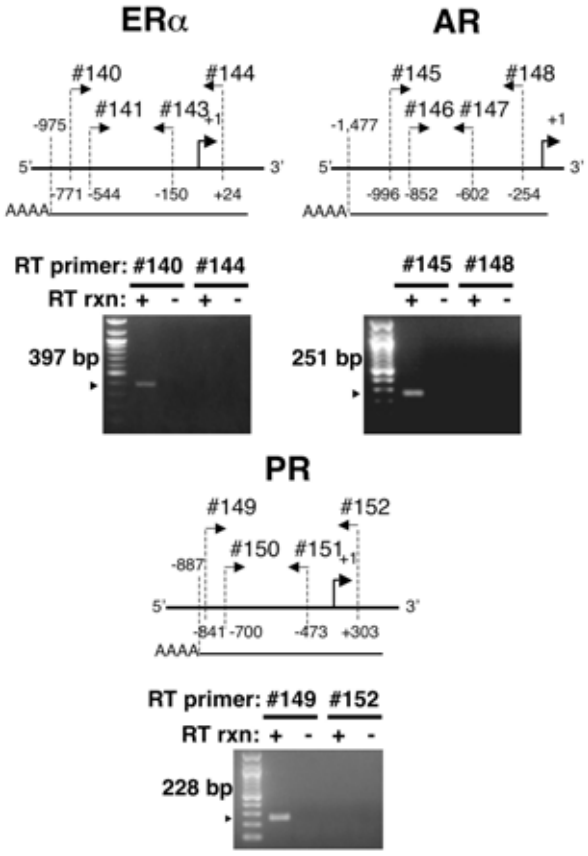

Figure 1. Detection of ERα-as, AR-as and PR-as. Each upper panel shows the genomic structure of ERα, AR, or PR. Arrows denote the primers used for the PCR amplification. Each lower panel shows the strand-specific RT-PCR. Note that specific bands were observed when sense primers were used for RT reaction.

1-2 Identification of naturally occurring antisense RNAs

The number of mammalian non-coding RNA genes is rapidly expanding. We found naturally occurring antisense RNAs for rat and mouse ERα, AR, and PR, tentatively named ERα-as, AR-as, and PR-as, respectively (Figure 1). Neither of the antisense RNAs showed obvious open reading frames, and possible amino acid motifs, raising the possibility that these function without translation. Each RNA overlapped with the respective promoter region including the sex-dependent differentially methylated region. In adult tissues, expression patterns were tissue- and/or sex-specific. For example, ERα-as and PR-as were predominantly expressed in the ovary. In the testis, these expressions were under the detectable level. In contrast, AR-as was highly expressed in the testis, but not in the ovary. It was to be noted that expressions in the brain were also area-

and/or sex-dependent. These structural features led us to investigate the possible involvement of these non-coding RNAs on the establishment of the specific DNA methylation pattern.

We used a rat pheochromocytoma cell line, PC12, that differentiates in response to NGF, as a model system (Figure 2). Most of the CG sites located on the sex-dependent differentially methylated region of ERα became methylated after the NGF treatment, confirming that the examined region was methylatable according to the cellular status. We introduced an expression vector driving a fragment of rat ERα into PC12 cells by transient transfection. Overexpression of a fragment of ERα-as containing the differentially methylated region dramatically diminished genomic methylation at the corresponding CG sites. This effect was gene-specific, because a PR-as fragment did not reverse the DNA methylation of ERα. We are currently investigating the expression profile of the novel antisense RNAs and their potential use for DNA methylation manipulation at the perinatal stage, that is thought critical period of sexual differentiation of the brain.

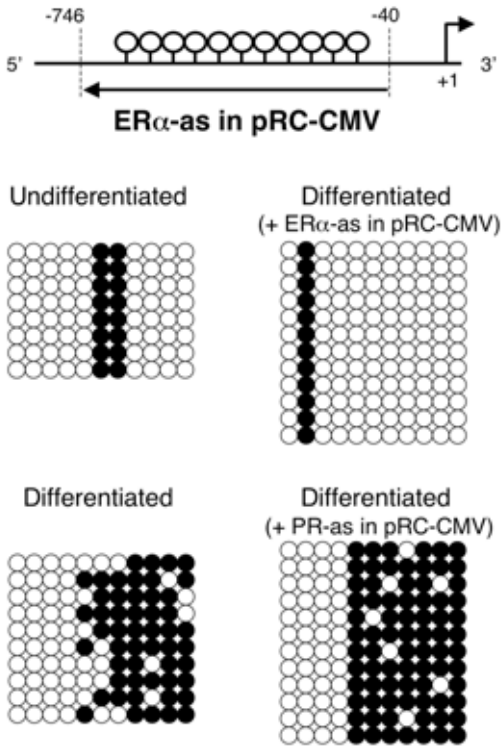

Figure 2. Methylation analysis of the ERα promoter in PC12 cells. Upper panel shows the location of CG sites and the fragment introduced into the cells. In the lower panel, each row of circles represents a single cloned allele with open circles for non-methylated cytosines and filled circles for methylated cytosines.

Publication List:

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