Specific Localization of DNMT1 in Mouse and Bovine Preimplantation Embryos

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DNA methylation is a covalent modification of DNA that can modulate gene expression and is now recognized as a major component of the epigenome. During evolution, the dinucleotide CpG has been progressively eliminated from the genome of higher eukaryotes and is present at only 5% to 10% of its predicted frequency. Approxymately 80% of the remaing CpG sites contain methylated cytosines in most vertebrates and they are distributed in a pattern that is unique in each tissue and is inversely correlated with gene expression. The pattern of methylation is faithfully maintained during cell division by the enzyme Dnmt1, the maintenance DNA methyltransferase, which catalyzes the transfer of a methyl group from S-adenosyl-methionine to the 5'-position of the cytosine ring. We have been identified bovine Dnmt1 cDNA full-length recently (AY173048). Little is known on the functions of Dnmt1 in bovine preimplantation embryos. Thus, we analyzed the specific pattern of Dnmt1 in in vitro derived/nuclear transfer bovine and in vivo derived mouse embryos to monitor the epigenetic reprogramming process. We investigated these process by using indirect immunofluresence with an antibody to Dnmt1.

According to other studies, Dnmt1 accumulates in nuclei of early growing oocytes but is sequestered in the cytoplasm of mature oocytes. In 2-cell and 4-cell embryos, Dnmt1 is cytoplasmic, but at the 8-cell stage, it is present only in the nucleus. By the blastocyst stage, Dnmt1o is again found only in the cytoplasm. Thus, nuclear lo-calization of Dnmt1o in preimplantation embryos is limited to the 8-cell stages. After implantation, Dnmt1 is localized in the nucleus in mouse. However, we have found different patterns of Dnmt1 nuclear localization. Though we used the common antibody, immuno-localization data revealed that Dnmt1 antibody have been detected at the nucleus in 1-cell to blastocyst embryos. Therefore, maybe we think that the functions of Dnmt1 between bovine and mice are different. In order to identify the mechanisms that regulate DNA methylation in bovine preimplantation embryo, we have plans on using bovine oocyte and somatic specific Dnmt1 antibodies.

Key words) DNMT1, DNA methylation, reprogramming, immuno-localiza tion