Partial Sequencing and Characterization of Porcine DNA Methyltransferase I cDNA

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DNA methylation is involved in epigenetic processes such as X-chromosome inactivation, imprinting and silencing of transposons. DNA methylation is a highly plastic and critical component of mammalian development. The DNA methyltransferases (Dnmts) are responsible for the generation of genomic methylation patterns, which lead to transcriptional silencing. The maintenance DNA methyltransferase enzyme, Dnmt 1, and the *de novo* methyltransferase, Dnmt3a and Dnmt3b, are indispensable for development because mice homozygous for the targeted disruption of any of these genes are not viable. The occurrence of DNA methylation is not random, and it can result in gene silencing. The mechanisms underlying these processes are poorly understood. It is well established that DNA methylation and histone deacetylation operate along a common mechanistic pathway to repress transcription through the action of methyl-binding domain proteins (MBDs), which are components of, or recruit, histone deacetylase (HDAC) complexes to methylated DNA.

As a basis for future studies on the role of the DNA-methyl-transferase in porcine devleopment, we have isolated and characterized a partial cDNA coding for the porcine Dnmt1. Total RNA of testis, lung and ovary was isolated with TRIzol according to the manufacture's specifications. 5 ug of total RNA was reverse transcribed with Super Script II in the presence of porcine Dnmt 1 specific primers. Standard PCRs were performed in a total volume of 50 ul with cDNA as template. Two DNA fragmenets in different position were produced about 700bp, 1500bp and were cloned into pCR II-TOPO according to the manufacture's specification. Assembly of all sequences resulted in a cDNA from 158bp of 5' to 4861bp of 3' compare with the known human maintenance methyltransfease. Now, we are cloning the unknown Dnmt 1 region by 5'-RACE method and expression of Dnmt 1 in tissues from adult porcine animals.

Key words) DNA methyltransferase I, porcine