Epigenetics by DNA Methylation for Normal and Cloned Animal Development

Kunio Shiota

Cellular Biochemistry, Animal Resource Sciences / Veterinary Medical Sciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan (e-mail: ashiota@mail.ecc.u-tokyo.ac.jp. FAX +81-3-5841-8189)

"Epigenetics" means the study of heritable changes in gene activity without changes in DNA sequences. Methylation of the cytosine residue in a CpG dinucleotide sequence is a characteristic of the vertebrate genome. In vertebrates, methylation of DNA mainly occurs at the 5'-position of cytosine in a CpG dinucleotide forming 5-methylcytosine. Methylation of DNA plays a profound role in transcriptional repression of gene expression through several mechanisms. Generally, DNA of inactive genes is more heavily methylated than that of active ones; conversely, demethylation of DNA reactivates gene expression in vivo and in vitro.

CpG islands with tissue-dependent and differentially methylated region (T-DMR)

Sequences of CpGs are not evenly distributed in the mammalian genome. They appear at a 10 to 20 times higher density in selected regions than in other regions, and regions enriched with CpGs are known as CpG islands. These CpG islands are used as landmarks to find genomic regions in bulk DNA sequences, because CpG islands are generally found in transcription units. Generally, it has been recognized that CpG islands are unmethylated in normal tissues, except the CpG islands involved in X inactivation and genomic imprinting. However, most data on DNA methylation mediated gene repression concerns TATA-less and CG-rich promoters that are associated with CpG islands. The human genome project identified 30,000–40,000 protein coding genes, and there are
approximately 29,000 CpG islands. There are 30,000 genes and 15,000 
CpG islands in the mouse genome. Tissue-specific promoters revealed 
that 50% of CpG islands are linked to tissue-specific genes. The re-
main ing tissue-specific promoters do not associate with CpG islands.
Restriction Landmark Genomic Scanning (RLGS) can perform rapid 
analysis of methylation profiles of thousands of CpG islands associated 
with genes in parallel. Scanning of 1,500 CpG islands of genomes from 10 
different cell types and tissues, including ES, EG, TS cells before and after 
differentiation revealed 247 T-DMR. Considering that there are 15,000 CpG 
islands in the mouse genome, the total number of CpG islands with 
T-DMR will be much greater. It is clear that CpG islands having T-DMR 
were numerous and widespread in the genome. A recent study by 
restriction enzyme-based library cloning identified normally methylated 
CpG islands in the human genome (Liora Z. Strichman-Almashanu 
The T-DMR panel clearly indicates that DNA methylation is cell type 
specific. Consequently, it is clear that genes associated with CpG islands 
should be included in the list of genes investigated for DNA methylation 
mediated gene-silencing.

Abnormal DNA methylation status in cloned animals

The rate of cloned animal production is generally quite low average 
2–3% or less of reconstituted eggs develop into live offspring. It is most 
likely that incomplete DNA methylation does not allow cells of the embryo 
to correctly express genes required for development and survival of the 
embryo and fetus. Cloned animals that survived birth and beyond have 
early correct DNA methylation patterns as previously reported. In cloned 
mice, the DNA methylation pattern at T-DMR was 99% identical to the 
normal mated control. Therefore, the cloned animals that developed to full 
term have almost normal DNA methylation patterns and are fairly good 
copies of nuclear donor animals. So, the epigenetic system is more flexible 
than previous thought. However, cloned animals have a variety of abnormal 
symptoms at and after birth. It is important to note that all cloned mice 
have imperfect DNA methylation without exception. Each cloned animal has 
different DNA methylation aberrations, and the extent of abnormality and 
loci varies among different individuals.
Conclusions and feature directions

A single fertilized egg gives rise to a complex multi-cellular organism consisting of at least 200 differentiated cell types. Most cells differentiate without changes in DNA sequence through activation of a particular set of genes and inactivation of others. The molecular basis for the memory for activated or inactivated gene sets, which is inherited to the cell's next generations, is critical for differentiation and development of multicellular organisms. Epigenetic errors cause other diseases as suggested in cloned animals.

References