Jpn. J. Vet. Res. **44**(4) : 226–227 (1997)

DISSECTION OF MAMMALIAN DEVELOPMENT BY GENETIC APPROACHES

Ken-ichi YAMAMURA

Institute of Molecular Embryology and Genetics,
Kumamoto University School of Medicine,
Kuhonji 4–24–1, Kumamoto 862, Japan

A strategy to monitor transcriptionally active regions of the genome was first described in bacteria in 1979. It involves the introduction of reporter constructs into the genome that require the acquisition of cis-acting DNA sequences to activate reporter gene expression. This approach has been applied more recently in higher organisms using modified vectors suitable for eukaryotic transcription units. In the mouse unknown genes have been identified by insertional mutation in transgenic mice generated by retroviral infection or DNA microinjection. However, this approach is laborious and time-consuming. Through the use of embryonic stem (ES) cells, the enhancer traps as well as the gene trap are now possible in the mouse. We adopted the gene trap method to identify new genes and to produce knock out mice at the same time. One essential point to identify a new gene efficiently is the screenig system. As we sought to find genes involved in the early steps of development, we analyzed whether the embryoid formation in vitro can be used for screenig system. So far, we cloned and sequenced 4 DNA fragments and three of these were known genes suggesting that this system traps genes quite efficiently. In addtion, we succeeded to develop exchangeable gene trap method in which a new DNA segment can be inserted using a Cre-loxP system. This system will greatly enhance the use of gene trap method as a tool in saturation mutagenesis.

Mouse quaking (qk) mutation shows pleiotropic phenotypes including tremor, embryonic lethality, and male sterility. qk locus locates within the T/t complex of mouse chromosome 17. There are at least two alleles in the qk locus, i.e. qk^v (viable) and qk^e (ENU-induced). qk^v/qk^v or qk^v/qk^e shows dysmyelinating phenotype, resulting in a tremor developed by postnatal day 10. However, qk^e is a recessive embryonic lethal mutation at midgestation stage. qk^v genome has a relatively large deletion spanning 1 Mb. A candidate gene for qk mutation has been cloned adjacent to the deletion break point, and designated as qkl. This gene encodes a putative RNA binding protein and show predominant expression in myelinating brain and in embryos. However, qkl gene itself is not deleted in the qk^v genome, and the level of qkl gene expression is not altered in qk^v/qk^v according to Northem analysis. To prove that qkl is the responsible gene for qk mutation, we have produced qkl gene knockout mice. Compound heterozygote mice for qk^v and this qkl-KO allele de-

veloped tremor, severer than qk^v/qk^v, thus demonstrating the direct association of the qkl gene with the quaking phenotype. However, they did not show any abnormality in the histological section of testis. We are now analyzing whether the homozygous mouse for qkl-KO shows embryonic lethailty.