CDB SEMINAR

Kenta Sumiyama

National Institute of Genetics, Division of Population Genetics

Tuesday, December 25, 2012

14:00~15:00 A7F Seminar Room

Cis-element evolution of the *Dlx* genes as an underlying mechanism in toolkit gene co-option in vertebrate appendages

Summary

Dlx genes in mammals are expressed and function in a variety of tissues, including neural plate boundary region, branchial arches, limb buds, mammary gland, placenta, and hair. It is of great interest how *Dlx* genes acquired novel regulatory system in such evolutionary novel appendages. We have been investigating *cis*-regulatory elements in *Dlx* gene clusters by comparing non-coding genomic sequences of various vertebrate species, and tested their functions in transgenic mouse system. We have identified *cis*-elements for branchial arch, limb and hair by transgenic mouse experiments, and analyzed their evolution of the *cis*-elements. We found that "limb" enhancer has been conserved between mammals and cephalochordates. Enhancer activity of this "ancient" *cis*-element was evaluated by using transgenic mouse system to explore its original function. We will argue about the possible underlying mechanism in *Dlx* gene co-option in mammalian appendages ("core motif" model) based on our evolutionary analysis of the cis-regulatory elements.

I also introduce our new *Tol2* transposase mediated mouse transgenesis technique. Transgenic mice are commonly created by microinjection of plasmid DNA into pronuclei of fertilized eggs. Since survival of injected embryos and integration of plasmid DNA are not efficient, the standard protocol requires microinjection of hundreds of eggs to obtain ~10 positive founders. To circumvent this problem, here we describe a novel method to create transgenic mice. We injected a plasmid DNA containing a foreign DNA cloned in a *Tol2*-transposon vector and the transposase mRNA into the cytoplasm of fertilized eggs. The *Tol2* transposon vector was transposed from the plasmid into the genome and transmitted to the next generation very efficiently. Also, the cytoplasmic injection gave rise to higher survival rates in comparison to the pronuclear injection. Thus, we demonstrate that the labor for mouse transgenesis can be drastically reduced by the *Tol2*-mediated cytoplasmic injection method (Tol2:CI). Considering its simplicity, stable expression of transgenes, and perfect compatibility with existing mice transgenic facilities designed for pronuclear injection, we propose that the Tol2:CI method is applicable to various experiments, including transgene expression studies and high throughput functional genomic studies of cis-regulatory elements.

References

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5. Kamioka, Sumiyama et al. Cell Structure and Function 2012 37(1), 65-73.

Host:
Mitsuru Morimoto
Lung Development, CDB
mmorimoto@cdb.riken.jp
Tel: 078-306-3199
(ext:1602)