

Reconstruction of a large DNA fragment from coinjected small fragments by homologous recombination in fertilized mouse eggs

K.Shimoda, X.Cai, T.Kuhara[1] and K.Maejima
Laboratory Animal Center, Keio University School of Medicine, 35 Shinanomachi, Shinjuku, Tokyo 160 and [1]Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi, Tokyo 173, Japan

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Transgenic mice are now becoming a powerful tool for the investigation of gene function in the whole body. Entire genes or minigene constructs are usually used for microinjection into fertilized mouse eggs. Since the regulation of gene expression in transgenic mice is determined by much more complex mechanisms than in cultured cells (1), it is preferable to use large genomic sequences which include more information to obtain precise expression with temporal and spatial specificity. However, the capacity of vectors for inserting foreign DNAs is limited to 10, 25 and 45 kb in plasmids, λ phages and cosmids, respectively (2). Since homologous recombination between introduced DNA fragments was reported to be a common event in cultured cells (3, 4), we attempted to reconstruct a large DNA molecule from smaller fragments which shared overlapping regions by homologous recombination in transgenic mice.

A pSy14 plasmid (10.6 kb) was digested with 3 pairs of restriction enzymes (Figure 1a) and each fragment (B, E and H) was purified by agarose gel electrophoresis. Individual fragments were separately injected into fertilized mouse eggs and no homologous recombinants were observed (Figure 1b). Three fragments were mixed together with equal molar amounts (total 5 μg/ml) and coinjected into eggs as described previously (5). Tail DNAs obtained from the mice were examined by Southern blot hybridization.

Ten transgenic mice were obtained from 42 pups which were developed from 131 eggs implanted into oviducts of pseudo-pregnant female mice. Southern blot hybridization analysis of tail DNAs digested with PstI and other enzymes showed that the patterns of major bands hybridized were identical with that of pSy14 in 8 out of 10 transgenic mice. Plasmid rescue experiments of tail DNAs into *Escherichia coli* DH5αMCR (methylation−restriction mutant; 6) revealed that plasmids recovered from the transformants were indistinguishable from pSy14. Two fragments, E and H, were also introduced into mice and homologous recombinants between fragments were observed. The animals tested can transfer the transgenes to their offspring in accordance with Mendel's law. These results indicate that the same DNA sequences as the original plasmid pSy14 were reconstructed from coinjected fragments by homologous recombination at the overlapping regions and then integrated into mouse chromosomes.

Although we introduced only 10.6 kb of foreign DNA (pSy14) into mouse germ lines, it is expected that 100 to 1,000 kb fragments can be integrated into mouse chromosomes because transgenic mice generally possess many copies of transgenes as hemizygote. The present method would be useful for the investigation of large genes and gene families which usually extend far and wide on chromosomes.

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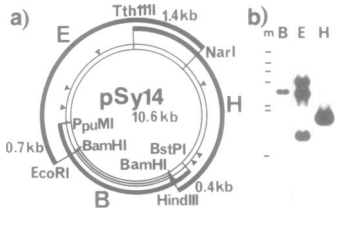

Figure 1. Structure of three overlapping fragments. a) pSy14 (10.6 kb) was digested with BstPI/PpuMI, EcoRI/NarI and HindIII/TthIIII, respectively. Each fragment (filled box), designated B (3.7 Kb), E (4.9 Kb) and H (4.5 Kb), was purified by agarose gel electrophoresis. HPV-16 sequences (open box) are inserted into the BamHI site of pUC19 (striped box). Triangles indicate PstI sites on pSy14. b) The fragments were separately injected into mouse eggs. DNAs obtained from transgenic embryos were digested with PstI and hybridized to pSy14 as a probe. Lanes B, E and H indicate injections of fragment B, E and H, respectively. Lane m contains bacteriophage λ DNA digested with HindIII (23.1, 9.4, 6.6, 4.4, 2.3, 2.0 and 0.6 Kb).