A hyperactive piggyBac transposon system is an easy-to-implement method for introducing foreign genes into mouse preimplantation embryos

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Abstract. Transgenic mice are important tools for genetic analysis. A current prominent method for producing transgenic mice involves pronuclear microinjection into 1-cell embryos. However, the total transgenic efficiency obtained using this method is less than 10%. Here, we demonstrate that highly efficient transgenesis in mice can be achieved by cytoplasmic microinjection using a hyperactive piggyBac system. In embryos in which hyPBase mRNA and pPB-CAG-TagRFP DNA were co-injected into the cytoplasm, TagRFP fluorescence was observed after the 2-cell stage; when 30 ng/µl pPB-CAG-TagRFP DNA and 30 ng/µl hyPBase mRNA were co-injected, 94.4% of blastocysts were TagRFP positive. Furthermore, a high concentration of hyPBase mRNA resulted in creation of mosaic embryos in which the TagRFP signals partially disappeared. However, suitable concentrations of injected DNA and hyPBase mRNA produced embryos in which almost all blastomeres were TagRFP positive. Thus, the hyperactive piggyBac transposon system is an easy-to-implement and highly effective method that can contribute to production of transgenic mice.

Key words: Cytoplasmic injection, Hyperactive piggyBac transposase, Transgenic mice
exclude the possibility that part of the TagRFP signals are derived from donor plasmid DNA. Furthermore, we determined when TagRFP fluorescence starts to emerge. Weak TagRFP fluorescence was already observed in 2-cell embryos co-injected with pPB-CAG-TagRFP DNA (30 ng/µl) and hyPBase mRNA (10, 30, 50 and 100 ng/µl), whereas it was scarcely observed in embryos injected without hyPBase mRNA (Fig. 2B). However, in embryos co-injected with pPB-CAG-TagRFP DNA (30 ng/µl) and low concentrations of hyPBase mRNA (10 and 30 ng/µl), TagRFP signals were observed in almost all blastomeres at the blastocyst stage; by contrast, in embryos co-injected with pPB-CAG-TagRFP DNA (30 ng/µl) and high concentrations of hyPBase mRNA (50 and 100 ng/µl), the TagRFP signals partially disappeared (Fig. 3). Thus, it is probable that excess hyPBase proteins act to remove integrated DNAs from the genome, resulting in the production of mosaic embryos. Because a second generation of transgenic mice could be obtained from mosaic embryos only when cells containing the target gene contributed to the germline, even mosaic embryos could be utilized to produce transgenic progeny. Additionally, TagRFP fluorescence in embryos injected with pPB-CAG-TagRFP DNA (30 ng/µl) and hyPBase mRNA (10 ng/µl) was stronger than that in embryos injected with pPB-CAG-TagRFP DNA (30 ng/µl) and hyPBase mRNA (10 ng/µl) (Fig. 2A and 3), suggesting that the copy number of donor DNA integrated into the genomic DNA depends on the concentration of hyPBase mRNA.

On the basis of these findings, we conclude that the hyperactive piggyBac transposon system is an easy and highly effective method for contributing to production of transgenic mice.

**Methods**

**Superovulation and embryo collection**

Eight- to ten-week-old ICR female mice (Japan SLC, Hamamatsu, Japan) were superovulated by injection of 5 IU of equine chorionic gonadotropin (eCG; ASUKA Pharmaceutical, Tokyo, Japan), followed by 5 IU of human chorionic gonadotropin (hCG; ASUKA Pharmaceutical) 48 h later. Unfertilized eggs were harvested 14 h after hCG injection and placed in a 90-µl droplet of HTF supplemented with 4 mg/ml BSA (A3311; Sigma-Aldrich, St. Louis, MO, USA) [13]. Spermatozoa were collected from the cauda epididymis of 11- to 15-week-old ICR male mice (Japan SLC) and cultured for 2 h in 100-µl droplets of HTF supplemented with 4 mg/ml BSA. After preincubation, sperm were introduced into fertilization droplets at a final concentration of 1 × 10⁶ cells/ml. After a 3-h incubation, fertilized 1-cell embryos were collected and washed 3 times in KSOM supplemented with amino acids [14] and 4 mg/ml BSA and then used for microinjection [15].
In vitro transcription, microinjection, embryo culture and observation

For construction of a hyPBase expression vector, the hyPBase ORF was amplified from pCMV-hyPBase [8] by PCR using specific primers (5′-GGGACCGGTATACGACTCACA TAGGAAATCGGCCACCATGGGC-3′, 5′-GGGCGGTACC GAAACCTCTGGCCACATGT-3′), and the SV40 polyadenylation signal was added to the amplicon. The resultant DNA fragment was used as a template for in vitro transcription. RNA synthesis and poly(A) tailing were performed with a MEGAscript T7 kit (Invitrogen, Carlsbad, CA, USA). Approximately 5–10 pl of 0, 10, 30, 50 and 100 ng/μl hyPBase mRNA and 30 ng/μl pPB-CAG-TagRFP [10] in DEPC water (Invitrogen) were microinjected into the cytoplasm of 1-cell embryos between 3 and 4 h after insemination. After injection, the embryos were cultured in KSOM medium supplemented with amino acids [14] and 4 mg/ml BSA under mineral oil (Sigma-Aldrich).
at 37 C in an atmosphere containing 5% CO2. To examine TagRFP fluorescence, embryos were observed at 38 and 108 h after insemination. At 108 h after insemination, embryos were collected and fixed in phosphate-buffered saline (PBS) containing 4% paraformaldehyde (Sigma-Aldrich) for 10 min. Stained embryos were mounted on slides in 50% glycerol/PBS, and fluorescent signals were detected using a fluorescence microscope (BX50, Olympus, Tokyo, Japan).

**Statistical analysis**

Each experiment was repeated at least three times. Statistical analysis of the data was performed by analysis of variance (ANOVA) with the Student’s t-test. P values < 0.05 were considered to be statistically significant.

**Ethical approval for the use of animals**

All animal experiments were approved by the Animal Research Committee of Kyoto University (permit number: 24–17) and performed in accordance with the guidelines of the committee.

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