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Stable integration and conditional expression of electroporated transgenes in chicken embryos

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Abstract

The in ovo electroporation in chicken embryos has widely been used as a powerful tool to study roles of genes during embryogenesis. However, the conventional electroporation technique fails to retain the expression of transgenes for more than several days because transgenes are not integrated into the genome. To overcome this shortcoming, we have developed a transposon-mediated gene transfer, a novel technique in chicken manipulations. It was previously reported that the transposon Tol2, originally found in medaka fish, facilitates an integration of a transgene into the genome when co-acting with Tol2 transposase. In this study, we co-electroporated a plasmid containing a CAGGS-EGFP cassette cloned in the Tol2 construct along with a transposase-encoding plasmid into early presomitic mesoderm or optic vesicles of chicken embryos. This resulted in persistent expression of EGFP at least until embryonic day 8 (E8) and E12 in somite-derived tissues and developing retina, respectively. The integration of the transgene was confirmed by genomic Southern blotting using chicken cultured cells. We further combined this transposon-mediated gene transfer with the tetracycline-dependent conditional expression system that we also developed recently. With this combined method, expression of a stably integrated transgene could be experimentally induced upon tetracycline administration at relatively late stages such as E6, where a variety of organogenesis are underway. Thus, the techniques proposed in this study provide a novel approach to study the mechanisms of late organogenesis, for which chickens are most suitable model animals.
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Keywords: Transposon; In ovo electroporation; Chicken; Genomic integration; Tetracycline

Introduction

The chicken embryo has been a remarkably important model system in developmental biology. Its usefulness has recently been expanded by the in ovo electroporation technique, developed in Nakamura's laboratory (Funahashi et al., 1999; Momose et al., 1999; Yasuda et al., 2000), which has allowed the molecular analyses of tissue interactions, cell differentiation, and pattern formation in these embryos. However, with the conventional technique of electroporation, the expression of a transgene is transient, lasting for merely two to three days in the embryonic cells, even though the electroporated transgene is driven by a ubiquitous promoter, such as CAGGS (Niwa et al., 1991). This failure of sustained expression is due to the inability of electroporated plasmids to become integrated into the chromosomes, and thus they are diluted as embryonic cells undergo proliferation. For instance, in an experiment where a developing presomitic mesoderm (PSM) is to be manipulated with transgenes, we need to electroporate the genes into the prospective PSM cells that ingress near the primitive streak of an embryo at stage 8. This technique enables a successful transgenesis in PSM and early somites where expression of the introduced gene remains active until early embryonic day 4 (E4). However, from E5 onward, the expression signal rapidly decreases in intensity, and by E6, the signal vanishes (see also

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below, Fig. 3). In addition, it is practically difficult to electroporate DNA into the somites of E4. Thus, the transient expression of the gene has precluded analyses of effects elicited by a transgene at stages later than E5.

To circumvent these problems, we here describe a novel method in chickens using a transposon-mediated gene transfer technique that enables an electroporated gene to be stably integrated into chicken chromosomes. Transposons are genetic elements that move from one locus in the genome to another, and have been used as powerful tools in model animals and plants. Recently, the *Tol2* transposable element, which was originally found in medaka (Koga et al., 1996), has been reported to be capable of undergoing efficient transposition in a wide variety of vertebrate species including zebrafish, frogs and mice (Kawakami et al., 2000; Kawakami et al., 2004a,b; Kawakami and Noda, 2004). When a DNA plasmid that contains a transposon construct carrying a gene expression cassette is introduced into vertebrate cells with the transposase activity, the transposon construct is excised from the plasmid, and the cassette is subsequently integrated into the host genome (Kawakami, 2005), see also Fig. 1A). In this study we exploited this unique character of the transposon to obtain stable integration of an electroporated gene into cultured chicken cells and also into chicken developing embryos. Indeed, the electroporation of a gene cassette cloned in the *Tol2* transposon vector has permitted stable expression of the gene in the chicken cells. We further combined this transposon-

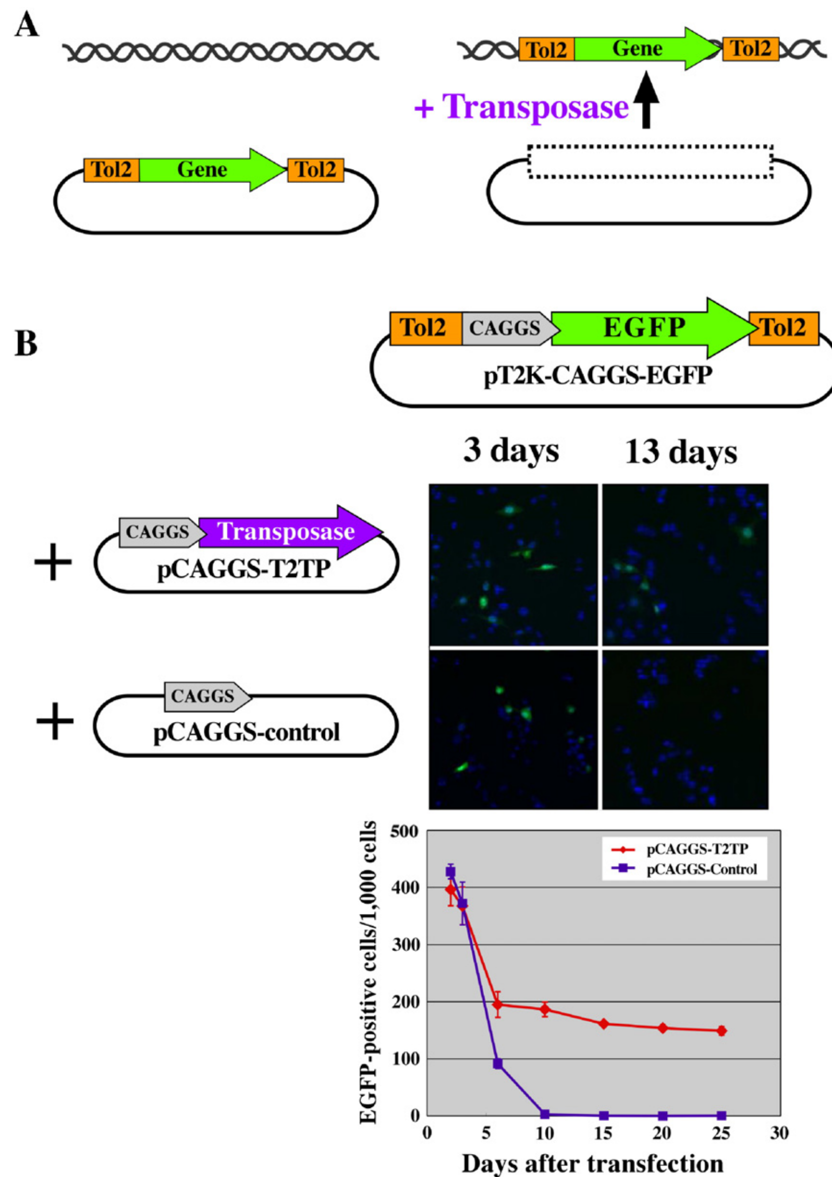

Fig. 1. Transposition of Tol2-flanked DNA by transposase. (A) When a DNA is cloned in a *Tol2* construct of a plasmid, the *Tol2*-flanked cassette is excised from the plasmid, and is transposed and integrated into a host genome by a transposase activity. (B) Transfection into DF1 chicken cultured cells with *Tol2*-flanked CAGGS-EGFP (pT2K-CAGGS-EGFP) and CAGGS-transposase (pCAGGS-T2TP). Until three days after the transfection, a relative ratio of EGFP-positive cells in culture was similar between with and without transposase. However, at later stages such as 25 days, the cells co-transfected with pT2K-CAGGS-EGFP and pCAGGS-T2TP retained EGFP signals in approximately 15% of total population, whereas almost no cells exhibited EGFP in control where pCAGGS-control was co-transfected with pT2K-CAGGS-EGFP.

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