Efficient genetic modification and germ-line transmission of primordial germ cells using piggyBac and Tol2 transposons

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AUTHOR SUMMARY

Genetically modified chickens have wide-ranging applications in academic research, biotechnology, and agriculture (1). Transgenesis in chicken has proven to be technically difficult in comparison with other animal species. In vertebrates, spermatogonia and oocytes originate from an embryonic stem cell population called “primordial germ cells” (PGCs). In chicken, as well as in other avian species, PGCs form very early in embryonic development (2). Chickens are unique, because PGCs from this species can propagated for extended periods in vitro while maintaining germ cell function (3). Unfortunately, chicken PGCs resist deliberate genetic modification, likely by silencing the introduced genes in the genome (4). Here, we describe a technique using mobile genetic elements, called “transposons,” to modify PGCs that overcomes this problem and therefore promises to exploit fully the economic, scientific, and therapeutic potential of the chicken egg.

In this study, we used piggyBac and Tol2 transposons to modify PGCs stably. DNA transposons are naturally occurring mobile genetic elements that “cut and paste” themselves to move from one genomic location to another unique site within the host genome. A transposon-encoded transposase recognizes the inverted terminal repeats flanking a transposon and catalyses the transposition of the element into the genome (Fig. P1). We found that the Tol2 transposon was fivefold more efficient than the piggyBac transposon in modifying chicken PGCs. Previous studies had shown that insulator elements, DNA sequences that shield regions of DNA from epigenetic silencing, were required for a transgene expression in chicken PGCs. We found that insulators were not required in the integrated transposon for transgene gene expression. PGCs containing integrated transposons also were able to colonize the gonad of host embryos and form functional gametes that produced transgenic offspring (Fig. P1).

We mapped the integration sites of the Tol2 transposon and determined that insertions were spread throughout the chicken genome, located in both the macro- and microchromosomes. The random distribution of transposons throughout the chicken genome suggested that a gene-trap mutational screen was feasible in PGCs. In gene-trap screens using transposons, the integrated transposon captures or “traps” an endogenous mRNA transcript, simultaneously mutating the encoded protein. To address this possibility we constructed a gene-trap variant of the Tol2 transposon vector and determined that endogenous transcripts could be trapped and mutated successfully in PGCs.

Genetically modified chickens serve as models for studying developmental biology, as bio-reactors for the production of therapeutic proteins, and as models of disease resistance to enhance agricultural production. Our results show that PGCs can be manipulated efficiently using transposon vectors, thus providing a cell-based tool for transgenesis and mutagenic screens in the chicken. The transgenic chickens that now can be generated using this technique should become important contributors to health, science, and agriculture.


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