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In Vivo Gene Transfer to Mouse Spermatogenic Cells by DNA Injection into Seminiferous Tubules and Subsequent Electroporation

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Recently, in vivo gene transfer techniques have become popular as a tool for gene therapy and biological analysis at the whole organ level and several different methods have been developed thus far. One of non-viral methods, in vivo electroporation (EP), has been shown to be an efficient method for transferring genes to the tissues of living animals [1,2,3]. This system indiscriminately delivers DNA molecules into any type of tissue cell, and has a markedly higher transfer efficiency than other non-viral transfer systems. In this study, we investigated an in vivo EP method using the testes of living mice for foreign DNA delivery to spermatogenic cells.

Mice were anesthetized and plasmid DNA was injected into seminiferous tubules of the testes using injection glass pipettes. After DNA injection, EP was performed with an electroporetor. Testes were held between a tweezers-type electrode and pulses were applied eight times at 20-50V according to the procedure of Muramatsu et al. [3]. After EP treatment, the mice raised until analysis.

At first, transient expression was assayed. Transfer of the firefly luciferase reporting gene driven by the protamine-1 (Prm-1) enhancer region revealed significant increase in activity of the reporter enzyme (Fig. 1). Histological studies of transfer of the lacZ gene driven by Prm-1 enhancer showed the specific lacZ-expression only in haploid

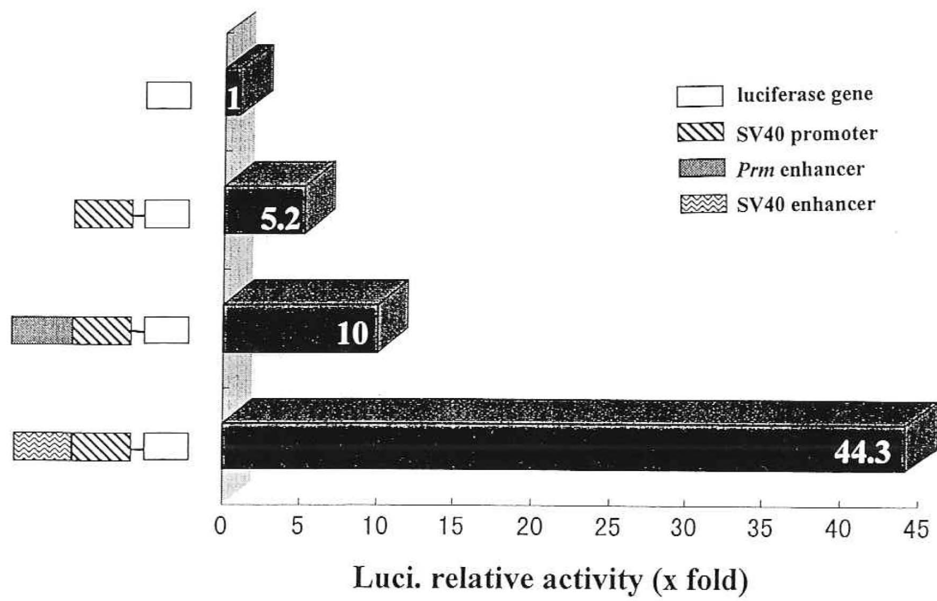

Fig.1. Transient expression assay of luciferase reporting transgene. At 18 hr after in vivo EP, each luciferase activity was measured.

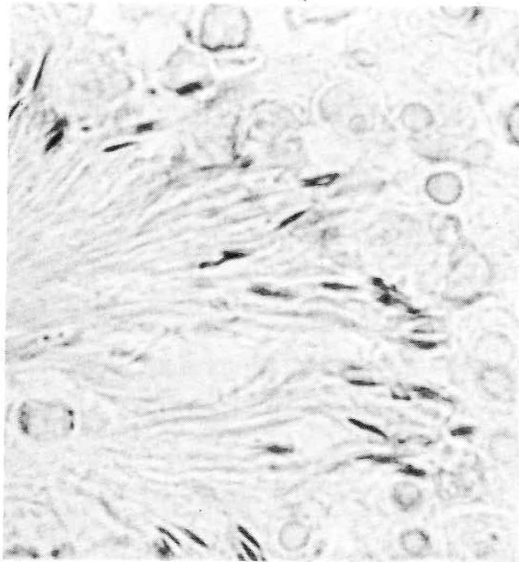

Fig.2. Spermatid-specific expression of Prm-lacZ transgene at 48 hr after EP. Nuclear localized β -gal expression is detected in the nuclei of the numerous elongating spermatids.

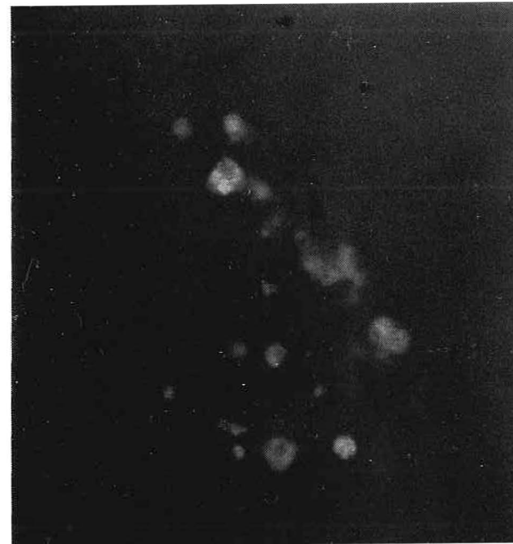

Fig.3. Analysis of long-lasting CMV-GFP transgene expression at two months after EP. GFP positive cells are found as a colony localized within the seminiferous tubules.

spermatid cells in the adult testis (Fig.2). This method can be used as a novel transient expression assay system for transcriptional regulatory elements of spermatogenic specific genes.

On the other hand, a group of spermatogenic differentiating cells maintained the transfected lacZ-expression after more than two months of transfection, suggesting that spermatogenic stem cells and/or spermatogonia could also incorporate foreign DNA and that the transgene could be transmitted to the progenitor cells derived from a transfected proliferating germ cell [4].

Furthermore, the possibility of this method to generate transgenic offspring via male germ cells was tested using green fluorescent protein (GFP) as a marker. We could detect the long-lasting transgene expression in the transfected spermatogenic cells. A group of spermatogenic differentiating cells maintained expression of the marker gene at two months after transfection (Fig.3). Fertilizable ability of the transfected males could be detected by natural mating with normal females, but transgenic offspring has not been obtained up to now.

References

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