F814 Studies on the Transfection System of Keratinocyte Using P Transposon Vector for Gene Therapy

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Gene therapy is a promising novel approach to treatment of variety of disorders including genetic, metabolic and neurologic disease, cancer. It has two essential elements which are vector system and tageting cell. In our studies, DNA sequences of the P transposable elements have been used in gene transfer vectors. P element-derived transfer vector system ,pUChsneo / p π 25.7wc Δ2-3, has a 31bp long terminal repeats essential to transfer. Keratinocytes are potentially appealing targeting cells for the delivery of secreted gene products because they can be transferred to human skin by relatively simple procedure of grafting. Gene transfer into cultured keratinocytes has been demonstrated by utilizing a variety of different foreign promoters to drive expression of varoius secreted products. we use transgenic technology to demonstrate that the activity of human keratin 14 promoter remains high in adult skin and we consructed a chimeric K14-Insulin as a transgene, examined the ability of transgenic skin keratinocytes to produce, process and secrete a 6kDa insulin using dot blotting. We confirmed helper P vector for stable transfection in keratinocytes using β-gal assay. These findings have important implications for considering the keratinocyte and P vector system as a possible vehicle for gene therapy.

F815 **Analysis of the Terminator Region of *groEx* Required for a Stable and Enhanced Expression of Foreign Genes in *groEx* Expression System**

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The *groEx* gene, a *groE* analogue of a symbiont X-bacteria in *Amoeba proteus* was highly expressed in *E. coli* by its own transcription and translation signal motives. The level of GroEL was over 50% of total protein. In this study we confirmed the terminator serve as an efficient transcription signal for foreign genes in addition to the promoter which had been evaluated to be a potent signal for large expression. It has peculiar characteristic of both a putative *rho*-independent (hairpin structures) and a *rho*-dependent structure (C-rich region). To confirm the putative sequences as terminator, the effect of deletion and site directed mutations on the expression of the *lacZ* was quantitatively analyzed. The second hairpin was assured to serve as an important signal. The mutations which was made to have less stable hairpin or loss of the hairpin structure significantly reduced the quantity of β-galactosidase to the extent of 40% and 28% of unmutated control (25,000 Miller units/mg protein), respectively. The decrease in the number of C residues in C-rich region by deletion or insertion of G bases reduced the expression to 64% and 56% of the control. By the deletion of the first hairpin structure the clone became very unstable and the expression level was less than 50% of the control. A slight reduction in the stability of the stem region by one base substitution resulted in a little unstable expression with a large standard deviation.