An Investigation of Hair Follicle Cell Immortalisation and Hair Keratin Gene Regulation

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Thesis Summary

This thesis presents results from an investigation into the regulation of hair-specific gene expression, including attempts to produce an immortalised hair follicle cortical cell line for this purpose and the use of mouse transgenesis and *in vitro* gel mobility shift assays.

A major aim of this project was to produce an immortalised hair follicle cell line derived from differentiating cortical cells. To achieve this, SV-40 T antigen (TAg) expression was targeted to the hair follicles of transgenic mice using the promoter of a sheep type II intermediate filament wool keratin gene, *K2.10*. Expression of TAg in the follicle cortex altered the normal protein profile and disrupted normal fibre ultrastructure, producing a marked phenotypic effect. *K2.10-TAg* transgenic mice developed various other abnormalities including vertebral abnormalities and bladder, liver and intestinal tumours which resulted in reduced life expectancy. However, no follicle tumours or neoplasia were apparent and immortalisation of cortical cells could not be established in culture. *In situ* hybridisation studies in the hair follicle using histone H3 as a cell proliferation marker suggested that cell proliferation had ceased prior to commencement of *K2.10-TAg* expression and was not re-established in the differentiating cortical cells. Hence, TAg was unable to induce cell immortalisation at this stage of cortical cell differentiation. Given this unsuccessful attempt to produce an immortalised cortical cell line, the use of more appropriate gene promoters which would target TAg expression earlier in follicle cell differentiation is briefly discussed.

The second part of this thesis investigates the regulation of a hair-specific keratin intermediate filament gene, *K2.10*. Initially, mouse transgenesis was used to analyse a small series of promoter deletion constructs utilising the *lacZ* reporter gene to detect expression in the hair follicle. It was shown that 400 bp of 5' proximal *K2.10* promoter was sufficient to confer correct cortical-specific gene expression. Mutation of a highly conserved putative regulatory element (designated HK-1) within this region failed to abolish cortical-specific expression, although expression levels appeared reduced. No expression was observed when the promoter was shortened to 150 bp of 5' proximal *K2.10* sequence.

Further analysis of the HK-1 sequence in gel shift assays showed that a protein (or proteins) present in hair follicle nuclear extracts binds specifically to this sequence. Finally, a series of overlapping 35-mer oligonucleotides spanning the 400 bp *K2.10* promoter region was tested in gel

shift assays and a number of specific complexes were shown to be formed, suggesting that the regulation of this promoter may be quite complex.

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