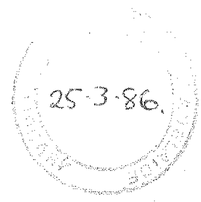


# STUDIES ON HAIR KERATIN GENES.

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by

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SUMMARY

This thesis was undertaken as part of a major project directed towards the elucidation of the processes involved in the keratinization of epithelial tissues in general, and the follicle in particular.

The work is divided into two sections: (i) the isolation and characterization of guinea pig hair follicle mRNA and (ii) a study of two BIIIB high-sulphur keratin genes isolated from sheep genomic DNA libraries. Techniques used in the course of the work included mRNA isolation, translation in cell-free systems, cDNA cloning, genomic DNA library screening, gene mapping and DNA sequencing.

The following results were obtained:

1. Guinea pig hair follicle RNA was isolated, fractionated and translated in wheat-germ and rabbit reticulocyte lysate cell-free systems. Examination of translation products by single and two-dimensional electrophoresis indicated that proteins representative of the major keratin protein classes were being synthesized. The presence of a disproportionate amount of low molecular weight products suggested that the mRNA was partially degraded.

2. An RNA subfraction was used to prime the synthesis of dscDNA. The dscDNA was annealed to the plasmid vector pBR322 and the hybrid DNA was used to transform E.coli. Only a small number of cDNA clones was obtained. Three clones were sequenced but only one had an open reading-frame of significant length. On the basis of the derived amino acid sequence and composition it was unlikely that the clone coded for a component of a major keratin protein class. This result, together with the partially degraded nature of the follicle mRNA, made it impractical to attempt further characterization of the mRNA species from which the cDNA clone was derived.

3. A novel hair follicle harvesting method was developed utilizing a cold-cure acrylic resin to replace the commonly used wax sheet. This

method had the major advantage of enabling hair follicles to be harvested from live guinea pigs and led to the isolation of undegraded RNA.

While studies with guinea pig hair follicle mRNA were in progress, a number of sheep keratin cDNA clones were isolated by other workers. The availability of these clones and of a sheep genomic DNA library made further studies on the guinea pig system unwarranted.

4. The cDNA clone pSWK18, which codes for a BIIIB high-sulphur component was used to screen a Charon 4A sheep genomic DNA library. Seven identical clones (λSWK50) were isolated. Sequencing studies showed that the 13.2 kb insert contained a BIIIB pseudogene which was homologous to pSWK18. The pseudogene (ψ50BIIIB) had a number of features, including an intact TATA sequence, mutated initiation codon, in-phase stop codons and a 23 bp deletion near the 3' end of the coding region. The derived amino acid sequence indicated that the pseudogene originally coded for a BIIIB3-like protein. Hybridization studies suggested that no other keratin-coding genes were present in λSWK50 but that there were three regions containing repeated DNA sequences.

5. Screening of λ1059 and λgt10 sheep genomic DNA libraries failed to detect any pSWK18-positive clones. A number of positives were isolated from a Charon 28 library. Restriction mapping, subcloning and limited DNA sequencing of a single clone (λSWK61) indicated that the 9.6 kb insert contained the same BIIIB pseudogene as λSWK50.

6. A mixed synthetic oligonucleotide probe based on three cDNA sequences and corresponding to a 20 bp fragment deleted from the pseudogene failed to hybridize with any of the Charon 28-pSWK18-positive clones. The library was re-screened using pSWK18 and the 20-mer to probe duplicate filters. Only one clone hybridized with both probes. This clone, λSWK96, contained a 13.8 kb insert with a single BIIIB gene. The derived amino acid sequence showed that the gene product differed at only two positions from the protein BIIIB4.

7. Comparisons of the non-coding and flanking regions of the

$\lambda$SWK50 and $\lambda$SWK96 BIIIB genes revealed some extensive homologies. The sequences were homologous for 120 bp 5' to the initiation codon. In the 3' non-coding region there were two blocks of homology ; 50 bp immediately following the termination codon and 70 bp surrounding the polyadenylation addition sequence. The same 3' homologies were found in two cDNA clones coding for BIIIB3- and 4-like proteins but not in a BIIIB2 cDNA clone. These results support amino acid sequence data which indicate that BIIIB3 and 4 are more closely related to each other than to BIIIB2.

8. The 5' non-coding and flanking regions of $\lambda$96BIIIB and $\psi$50BIIIB shared a 70% homology over approximately 160 bp and both genes had limited homologies with the 5' non-coding sequences of B2, BIIIA and high-glycine-tyrosine genes. Interestingly, $\lambda$96BIIIB and B2A shared a 65% homology over a 100 bp region located in different 5' flanking positions, while a 17 bp sequence was conserved in the BIIIB gene and three other high-sulphur genes. These data, in addition to the protein sequence studies of other workers, suggest that the major high-sulphur keratin gene families have arisen from a single ancestral gene.

9. A pHC79 cosmid library was screened with pSWK18 and a single positive clone was isolated. Limited restriction mapping indicated that the insert ($\simeq$32 kb) contained only 1-2 BIIIB genes on a 5 kb HindIII fragment and that the genes were not the same as those isolated from the bacteriophage $\lambda$ sheep genomic DNA libraries.