A Study of Sheep Epithelial Intermediate Filament Gene Expression.

or

(sK1.15 and Friends)

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Abstract

This thesis presents results from a molecular investigation of sheep epithelial intermediate (IF) keratin genes. A cDNA library was constructed from sheep rumen epithelium mRNA from which two type I and two type II keratin IF cDNA clones were isolated. The clones have been called K1.15, K1.4, K2.5 and K2.6 according to a new nomenclature suggested by Powell and Rogers in Keratinocyte Handbook, (Leigh, I., Watt, F., and Lane, B., eds., book in preparation, (briefly K2.5=K5, K1.15=K15). The cDNA clones were sequenced and their expression patterns studied in several tissues (rumen, oesophagus, tongue, skin and wool follicle) using cRNA in situ hybridization. K2.5 and K1.15 are expressed in the basal layers of the rumen epithelium and K2.6 and K1.4 in the suprabasal layers. Two of the cDNA clones, K2.5 and K2.6, are the sheep equivalents to keratins K2.5 and K2.6 identified in other species and the expression patterns of the sheep genes resemble those identified for these keratins in human tissues. One cDNA clone, K1.4, appears to code for a novel type I keratin IF, having the highest identity to a mouse hair-related type I IF (86% in the 2B coil with the mouse 48K IF) but little homology in the variable C-terminal domain. K1.4 is not expressed in the wool follicle but is expressed in the suprabasal layers of rumen epithelium and in tongue.

A more detailed study of the sheep K1.15 gene was undertaken and it was isolated from a cosmid library and completely sequenced. In sequence it is almost identical to the human K1.15 gene but in oesophagus, the only tissue for which human K1.15 in situ hybridization expression data are available, the expression of the human and sheep genes differ. Sheep K1.15 is expressed in the outer root sheath of the wool follicle and in the germinative cells of many epithelia (tongue, oesophagus, hoof, skin) including those cells in the follicle bulb which are in contact with the basement membrane that separates the dermal papilla. To date, no other IF gene expressed in those follicle bulb cells has been conclusively identified.

To study the control of the K1.15 gene, with a focus on the regulation of its follicle expression, transgenic mice were produced using a fragment containing the entire K1.15 gene and including 8kb of 5' flanking and 600bp of 3' flanking DNA. A total of ten transgenic mice were created and of these seven were found to express the transgene in a pattern resembling that seen in the sheep. The expression pattern of the transgene in the hair follicle bulb appears
slightly different to that in the wool follicle, with expression being more patchy in the proliferating bulb cells of the mouse hair during anagen. A very high level of expression of the transgene occurs in the cells surrounding the club end of the dormant hair during the catagen and telogen phases of the hair cycle.

Initial studies of the promoter of the K1.15 gene were undertaken using a 2.5kb fragment containing the promoter and 5' flanking DNA in a construct linking it to a reporter gene. Transient transfection of CHO cells demonstrated the ability of this promoter to drive expression in those cells. This work, in conjunction with the transgenic mouse experiments represents the beginning to defining the regulatory elements that direct expression of the K1.15 gene in epithelial tissues.
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