AN INVESTIGATION OF FEATHER KERATIN GENE EXPRESSION

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by

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CHAPTER 2 MATERIALS AND METHODS

SUMMARY

This thesis presents the results of initial investigations toward understanding the elements regulating the transcription of chicken feather keratin genes. Results were also obtained relating to the transcription of the Herpes Simplex Virus thymidine kinase (HSV-tk) gene.

The **Xenopus** oocyte was chosen as the simplest available expression system. It was observed that the chicken feather keratin genes were transcribed at very low levels in the oocyte and primer extension reactions using selected primers showed that none of the detectable transcripts were spliced. When the single intron, located in the 5' untranslated region of all the known feather keratin genes was removed from feather keratin gene B to produce an intronless gene, a 5-fold increase was observed in transcript initiation from the **in vivo** cap site. This 5-fold increase was retained when the feather keratin intron was replaced with a similar-sized fragment from pBR322, suggesting that the intron sequence was specifically acting to reduce the number of correctly initiated transcripts. That this was so was reinforced by the finding that the low level of keratin transcripts produced from the intact feather keratin gene was not attributable to a degredation of partially-spliced transcripts. The role that this intron mediated repression of transcription may play in regulating feather keratin gene expression **in vivo** is discussed.

Although the level of correctly initiated transcript was increased by intron removal, the level of transcription remained very low when compared with that which occurs **in vivo**. Transcription from the intronless gene was at least an order of magnitude lower than that observed for other genes co-injected into **Xenopus** oocytes. To determine whether the low level of feather keratin gene transcription in oocytes was the result of inefficient functioning of the keratin promoter, gene hybrids were constructed using feather keratin gene B and the HSV-tk gene. The tk gene is expressed very efficiently in oocytes. When the tk promoter was linked to the protein coding region of feather keratin gene B, however, the level of transcript produced was similar to that obtained from the intronless keratin gene. Furthermore, when the keratin promoter was linked to the coding region of the tk gene, the level of transcript produced was 20-fold greater than that from the tk promoter/keratin coding region construct and approached that produced from the unmodified HSV-tk gene. Time-course experiments showed that these results were not due to a decreased stability of transcripts from constructs containing the feather keratin coding region. It was concluded that one of two possible mechanisms was acting, either the keratin coding region was acting to inhibit transcription from the tk promoter, or the tk coding region was acting to stimulate transcription from the keratin promoter.

To test whether the keratin coding region was acting to inhibit transcript initiation from the tk promoter, deletions were made in the keratin coding region of the tk promoter/keratin coding construct to define the location of

any potentially inhibitory regions. None of the gross deletions performed resulted in an increase in transcription from the tk promoter. When the tk promoter was linked to the coding region of the H2B histone gene (which is efficiently expressed in oocytes from its own promoter) a low level of transcription was again observed from the tk promoter. From these results it was concluded that the relatively high level of transcripts initiating from the keratin promoter when linked to the tk coding region was a result of a stimulatory action of the tk coding region on the keratin promoter. This conclusion can also be extended to a possible activation of the tk coding region on its own promoter when the tk gene is transcribed in oocytes.

Further investigations using the keratin promoter/tk gene construct, showed that the tk coding region stimulatory effect was distance dependent. A preliminary investigation to define the region was undertaken by inserting a variety of large fragments from the tk coding region into the coding region of the intronless keratin gene in a position dependent manner. Although the insertion of the entire tk coding region into the coding region of the intronless keratin gene resulted in transcription stimulation from the keratin promoter, inidivdual fragment insertion failed to define the stimulatory region. This was presumably related to disruption of the stimulatory region(s) when the selected fragments were isolated from the tk gene.

The tissue specific expression, and the critically timed activation of keratin gene transcription **in vivo** suggests a possible need for other, tissue specific factors. The lack of such factors in frog oocytes would provide an explanation for the low levels of keratin gene transcripts and the inability to detect spliced transcripts. The hypothetical need for tissue specific factors was further supported by the inability to detect correctly initiated keratin transcripts after the genes were transfected into a variety of non-feather tissue derived cell lines via calcium phosphate mediated DNA transfection.

Co-injection of keratin genes into oocytes with protein fractions purified from the nuclei of transcriptionally active feather cells was considered as a means of confirming the existence of specific activatory factors. Such co-injection experiments were thwarted by the extreme difficulty encountered in obtaining sufficient nuclei from developing feathers from which to extract chromatin proteins. Attention was subsequently focussed on culturing primary chick epidermal cells for DNA transfection purposes. Poor yields of viable cells and their propensity to rapidly keratinize in culture made these cells difficult to maintain for such studies.

The frog oocyte system has provided some interesting results on the expression of feather keratin genes and during the course of this work has revealed some important questions in relation to HSV-tk gene transcription. The need for an homologous expression system is considered paramount for future studies relating to feather keratin gene transcription. A proposal is given for an homologous expression system, which once established, should provide answers to questions pertaining not only to feather keratin gene expression but

also to the expression of the other genes known to be associated with avian keratinization. Preliminary work towards establishing this homologous expression system is presented.