

[THE ZOOLOGICAL SOCIETY PRIZE]

GENE EXPRESSION INVOLVED IN MELANOCYTE DIFFERENTIATION IN THE MOUSE

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One of the best-suited cell types for the study of cell differentiation is the melanocyte for a number of reasons. 1) It possesses a biochemical marker, tyrosinase, that is the key enzyme for melanogenesis and is the specific protein to the melanocyte. 2) It possesses a morphological marker, melanosome, that is the specific organelle to the melanocyte. 3) Moreover, a number of mutants related to melanocyte differentiation and expression have been found. This means that there exist various gene loci, each of which controls a step of melanogenesis or melanocyte differentiation.

Genes involved in melanocyte differentiation can be classified into three groups. One consists of genes that control intercellular communication in early differentiation of melanoblasts from neural crest cells. One of the white spotting genes, *W*, has been shown to code for a tyrosine-kinase type receptor known as *c-kit*, while the Steel (*Sl*) locus is demonstrated to be the ligand for the *c-kit* receptor. The second category consists of genes that control proteins specific to the function of the melanocyte. This group of genes includes the *c* locus which encodes tyrosinase and the *b* locus for the melanosome protein. The third group consists of genes that control signal transduction involved in the hair pattern formation. Genes at the agouti (*a*) locus and the extension (*e*) locus determine the type of melanin synthesized in the hair follicle melanocytes, thereby controlling the hair pattern formation.

In order to elucidate the structure of the tyrosinase gene (*c*) and the mechanism of regulation of gene expression, we cloned the tyrosinase cDNA and the genomic 5'-flanking sequences. One of the cDNA cloned was found to consist of

1978 bp. The open reading frame is shown to encode 533 amino acids with two putative copper binding sites. We then constructed a minigene in which the cDNA was ligated with the genomic 5' flanking sequence of 2.6 kb, and transfected cultured albino melanocytes with the construct. We found that the minigene expressed and directed the production of melanin pigments in the albino melanocytes. This result indicates that both the cDNA and the 5'-flanking sequence are functional.

We also microinjected the minigene into fertilized eggs of albino mice and demonstrated that the transgenic mice produced melanin pigments. In order to verify the cell-type specific expression of the transgene, histological examinations were performed on various organs of the transgenic mice. Melanin pigments were observed only in hair bulbs, hair shafts, choroid and pigment epithelium, whereas the transgenes were detected in various tissues examined by Southern blot analysis using a 1-kb fragment of the cDNA as the probe. It seems that the 5'-flanking sequence deriving from the genomic tyrosinase gene contains *cis* elements responsible for the cell typespecific protein factors even if the transgenes are integrated randomly among chromosomes.

By crossing the founder mice with albino mice, transgenic lines and sublines were established. Each subline expressed a characteristic phenotype with its respective band patterns in Southern blot analysis. The difference in phenotypes among sublines is probably due to the position effect of the chromatin where the transgene is integrated.

On the other hand, two highly homologous sequences were found in the 5'-flanking regions of the *c*-gene and the *b*-gene. These two sequences

are designated as p-MSE (10 bp) and d-MSE (13 bp). The gel retardation assay showed that the mobility of two retarded bands was similar for both genes. This indicates that at least two different proteins interact with the 5'-regulatory regions of both the *c*-gene and the *b* gene. South-Western

blotting analysis demonstrated two major proteins (43 kD and 50 kD) that were common to the regulatory regions of both genes. Therefore, it seems reasonable to assume that multiple genes are regulated coordinately to produce melanin in the melanocyte as a "regulon".