Genes and the Pigment Cells of Mammals

Pigment cells provide unique material for studying the interactions of genetic determinants.

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Interest in the genetic aspects of mammalian pigmentation is almost as old as the science of genetics itself, for it was only shortly after the rebirth of Mendelism, at the beginning of this century, that W. E. Castle and his students, at the former Bussey Institute of Harvard University, initiated studies on the inheritance of specific coat-color types in guinea pigs, rats, rabbits, and mice. Although these workers were completely unaware of the anatomical basis and biochemistry of pigmentation, the genetic analyses resulting from their extensive breeding studies established that the production of coat-color pigment patterns involved a local interaction of specific gene products which was relatively unaffected by systemic or environmental factors. It remained for subsequent investigators to produce experimental evidence confirming the belief of some of the older histologists that melanogenesis is the sole prerogative of specialized branched or dendritic cells, now usually referred to as melanocytes, of neural-crest origin, which function as unicellular melanin-secreting glands in the epidermis (*1*). This elucidation of the cellular basis of pigment formation, made a little more than two decades ago, set the stage for extensive studies on the physiological genetics of pigmentation. These are directed toward answering the important question of how the genes which influence pigmentation produce their effects, and it is this that forms the principal subject matter of this article.

The genetic aspects of mammalian pigmentation have been more thoroughly worked out in the mouse than in any other species, for two reasons. Large numbers of coat-color mutations—that is, deviations from "wild type"—have occurred in this species (*2*), and the requisite stocks manifesting these mutations either are available or can be produced comparatively easily because of the large number of inbred strains in existence. Although most of what follows, therefore, concerns studies on the mouse, it may be emphasized that the general principles illustrated certainly apply with relatively few qualifications to other mammals.

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Recognition of Genes Concerned with Pigment Formation

Since the effect or effects of a particular genetic locus can only be established on the basis of variations (alleles) from the "wild type" that have been produced by mutations, it is obvious that the whole field of physiological genetics of pigment patterns has depended entirely upon the occurrence, recognition, and description of mutations, along with their preservation. For example, the so-called wild-type coat color in mice is probably best described as "grey-bellied agouti." Animals of this color have a yellow banding of the otherwise black hair on the dorsum and a yellow terminal ticking of the ventral pelage. It is evident, however, that if mice of only this one phenotype were available for pigment studies, very little, if anything, could be found out about the number and action of specific genes which are responsible for producing this pigment pattern. An understanding of the genetic factors concerned has been made possible by the occurrence of deviant coat-color types produced by gene mutations. The investigation of the individual effects of these mutations reveals which aspect of melanogenesis is controlled by the so-called wild-type allele.

It is obvious, therefore, that in the mouse there must still be a very large number of gene loci, in some way involved in pigment formation, about which we know nothing at present and about which we may learn something in the future only if mutations appear. Nevertheless, enough coat-color mutations have occurred in this species to make a systematic analysis of their effects one of the most fruitful means of exploring the many diverse ways in which gene action and gene interaction can influence the distribution, morphology, synthetic activity, and so on, of a single cell—the melanocyte.

Studies on Pigment Granules

Although casual examination of murine hair shafts might suggest either that the pigment is in the form of a solid mass or that it is homogeneously distributed, careful microscopic study reveals that the color of hairs results principally from the presence of very tightly packed clusters of pigment granules. When hairs are actively growing, the half-dozen or so melanocytes in the generative region of their follicles are continuously supplying pigment granules to the adjacent Malpighian cells of the upper bulb, which are to form the medulla and cortex of the hair shaft. There seems to be a direct transfer of the melanin which the melanocyte secretes, by way of the end processes of the cell's branches, into the cytoplasm of these neighboring cells (see Fig. 1). This apparently unique direct transfer of the secretory product of one cell into the cytoplasm of another has been aptly described by Masson (*3*) as "cytocrine" activity.

Since pigment granules are the basic unit of pigmentation, all coat-color mutations, except those that produce white spotting, must necessarily produce their effect by altering in some way the various attributes of these granules. This became evident from the results of E. S. Russell's systematic histological investigation of the pigment of the hair shafts in different inbred color stocks of mice (*4*). Probably the most surprising outcome of

this study was that, in spite of all the different color hues to be seen in the coats of mice, only two basic kinds of pigment granules were found—oval or spherical black and brown granules, which are classified as eumelanin, and the much smaller, round, yellow granules known as pheomelanin.

In the light of recent work (*5*) it appears almost certain that both these melanins are protein conjugates. Eumelanin is formed by the coupling of a quinonoid polymer, indole-5,6-quinone, with protein. The polymerization and coupling processes occur on the surface of the subcellular cytoplasmic granule—the anlage of the mature pigment granule. The quinonoid polymer is derived from the amino acid tyrosine, by a chemical reaction catalyzed by the copper-containing enzyme tryosinase, which is attached to the granule. Not very much is known about the synthesis of pheomelanin, although it appears that tyrosine is also involved in its formation. In the hair-bulb melanocytes of mice, the so-called *A* or agouti locus determines which of these types of melanin granule will be produced.

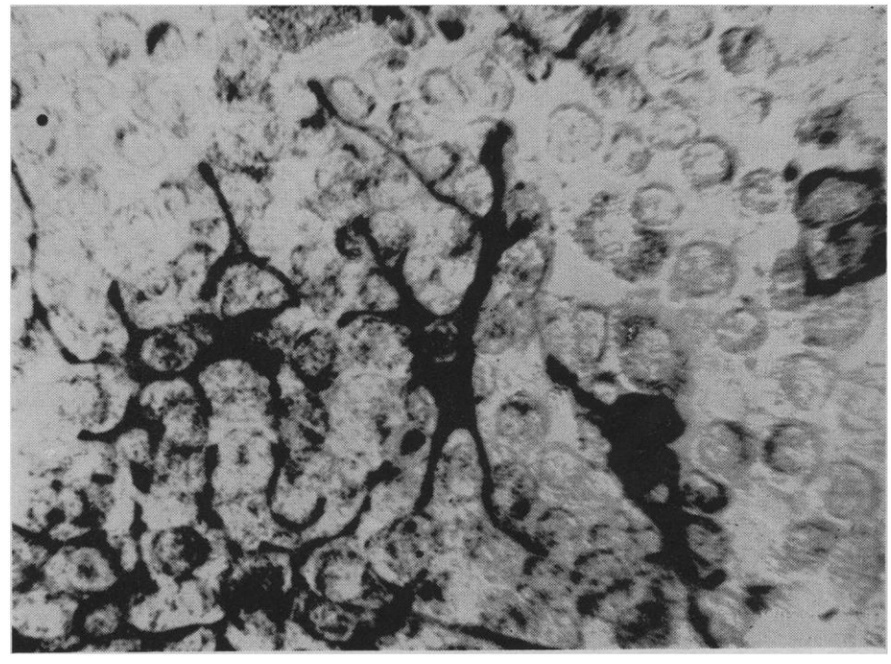

Fig. 1. Living melanocytes in a squashed preparation of epidermal cells prepared from the black ear skin of a guinea pig. Notice the characteristic branches originating from the perikaryon of the melanocytes and the characteristic end caps terminating the end processes. These end caps are applied to the cytoplasm of cells of the Malpighian series. (About × 500)

Although the nature (eumelanin or pheomelanin) of the melanin granules which occur in the hair shaft is extremely important in determining the coat-color phenotype, other genetically determined attributes of the melanin granules, such as their size, shape, and color density, also play important roles. Marked differences in coat coloration can also be traced to the influence of certain genes on the number of granules, as well as to the distribution of the granules in the hair shafts. There is no correlation in some of the attributes of pigment granules. For example, the nature, degree of granular clumping, and amount of pigmentation are determined independently; other attributes, such as the nature of granule color and the extent of variation in granule size, are interrelated.

From such comparative studies on the distribution and qualities of pigment granules in hairs, the roles of some of the major genes concerned with pigment formation in the mouse have been deduced. For example, it was concluded that the principal effect of substituting the mutant genes *bb* (brown) for *B*- (black) at the so-called *B* locus involved a qualitative change which implicated only the formation of eumelanin, having no appreciable effect on pheomelanin synthesis. The four allelic genes at the albino locus (*C*, c^{ch}, c^{r}, and *c*) appeared to control in a purely quantitative manner the general level or intensity of pigmentation without affecting its nature. This suggested that this locus controlled the formation or availability of some substance, now known to be the enzyme tyrosinase, essential for the formation of all pigment. Substitution of *pp* (pink-eyed dilution) for *P*- (intense or wild type) altered both the size and the shape of the pigment granules and also reduced the level of pigmentation. Finally, the main effect of incorporating either *dd* (dilute) or *lnln* (leaden) genes into an otherwise wild-type genetic background was to alter the pattern of the deposition of pigment granules in the cells of the hair shaft: the granules formed large granular clumps, unevenly distributed among the septules, instead of being distributed in a more orderly pattern.

Current electron-microscope studies by Frank Moyer of Johns Hopkins University (*6*) may help to localize the primary effects of some of these loci on the fine structure of melanin granules. Moyer is working on the retinal pigment granules of embryonic and neonatal mice derived from the same color stocks that Russell used. Some interesting findings have already been disclosed. For example, Russell's observations that the melanin granules of *pp* mice are irregular in shape and smaller than those of the wild type has been shown to stem from an early difference between *pp* and *P*- animals in the formation of the pigment granule, the internal fibers of *pp* granules differing in their order of arrangement from those of the wild type. Furthermore, it has been shown that in the albino mouse (*cc*), although mature melanin granules are not formed, unpigmented "precursor granules" similar in appearance to granules of the corresponding developmental stage in the wild type do exist. It seems likely, therefore, that tyrosinase activity is blocked at this stage.

Further studies along these lines may even reveal the existence of subtle changes in the granules of animals heterozygous for some color factors that we regard at the moment as completely recessive.

Enzymatic Studies

Not only have detailed investigations been made of the pigment granules of coat-color mutants but considerable attention has also been devoted to the capacity of hair bulbs, or even of skin

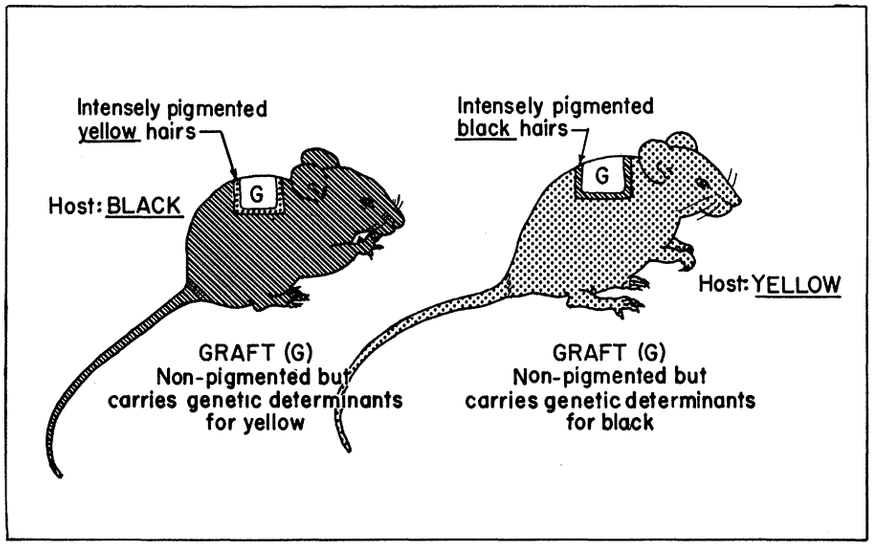

Fig. 2. Intensely pigmented black and yellow mice, grafted at birth with histocompatible skin from newborn genetically yellow (but nonpigmented) and genetically black (but nonpigmented) mice, respectively, exhibit some intensely pigmented hairs within the graft margin when grown. These hairs are pigmented by host melanocytes which have migrated into hair bulbs of the graft, where their functional behavior is dictated by the milieu (agouti-locus genotype) of the graft.

extracts, of various genotypes to bring about the oxidative blackening of 3,4-dihydroxyphenylalanine (dopa), now known to be an intermediate product in the synthetic pathway that leads to synthesis of melanin. One interesting conclusion from these studies, principally carried out by Sewall Wright and his students (*7*), is that the intensity of the dopa reaction (the darkening of the extraction mixture when extracts are employed, or darkening of the cytoplasm of melanocytes when frozen sections are used) parallels very closely the intensity of the yellow pigmentation of the skin under study. In black and brown genotypes the intensity of the dark pigment produced corresponds with the amount of yellow that would have been produced if the animals had been genetically yellow.

Similarly designed experiments, in which tyrosine and tryptophane were used as substrates, have been carried out by Morris Foster of the University of Michigan (*8*). These suggest that trytophane or one of its metabolites may be a precursor of pheomelanin.

Sites of Gene Action in Pigment Patterns

Although the work just outlined has contributed greatly to our understanding of the way in which genes involved in pigmentation produce their effects (and there is certainly scope for further studies of this type), its shortcoming lies in its failure to reveal whether the specific effects which different genes have on the pigment granule are mediated through the melanocyte itself or whether these effects reflect a primary action on the part of the environment in which this cell occurs.

To illustrate this point let us return to the wild-type mouse and the agouti pattern which characterizes it, a pattern which involves the elaboration of both black and yellow pigment in the same hair, and which is determined by the *A* locus. Five different mutations have now been described at this locus, and when these various mutants are incorporated into an otherwise wild-type genetic constitution, phenotypes are produced which vary from animals that are all black (extreme nonagouti, $a^e a^e$) and animals that are all yellow (A^y-) to animals that have a black dorsum and a yellowish ventrum (black-and-tan, a^t-). Although this shows that the series of alleles at the *A* locus determines the nature of the melanin produced by the melanocytes of the hair bulb (that is, determines whether it is eumelanin, pheomelanin, or both), it does not indicate whether the alleles at this locus produce their effect by acting autonomously within the hair-bulb melanocytes themselves or by acting indirectly in conditioning the follicular environment in some manner which, in turn, affects the behavior of the melanocyte. These alternatives are important, since the former indicates that each agouti-series genotype differs from the others at the level of its melanocytes, whereas the latter alternative implies a similarity in melanocytes but a difference in tissue environments.

Determination of the primary site of action of the genes at the *A* locus requires a study of the behavior of melanocytes of one agouti-locus genotype experimentally incorporated into developing hair follicles composed of cells of a different agouti-locus constitution. This experimental condition can be achieved by transplanting compatible grafts of ventral or dorsal skin between newborn mice differing with respect to the nature (the agouti-locus constitution) and intensity (governed by other loci) of their future pigmentation. It takes advantage of the fact that some host melanocytes migrate across the graft boundary and establish themselves in developing follicles of the alien genotype (see Fig. 2). A difference in intensity of pigmentation between host and graft is of course essential in order that hairs of the graft which are pigmented by host melanocytes can be recognized.

It has been found that the agouti-locus genotype of the graft always determines whether eumelanin, pheomelanin, or both are produced by the "foreign" pigment cells of host origin (*9*). Thus, when potentially black melanocytes migrate into dorsal or ventral hair bulbs which, although phenotypically nonpigmented, are genetically yellow, intensely pigmented yellow hairs are produced (Fig. 2). When similar melanocytes are introduced into agouti (but nonpigmented) hair bulbs, the typical wild-type or agouti hair pattern results. Additional findings suggest that this expression of genic activity is dependent not only upon the genotype of the follicular environment but also upon the location of this environment on the integument. For example, when either genotypically yellow or genotypically black pigment cells invade a dorsal, nonpigmented, black-and-tan graft they produce intensely pigmented black hairs, whereas when they are incorporated into ventral follicles of the same genotype, yellow hairs with black bases (characteristic of the ventral hairs of intensely pigmented black-and-tan mice) result. This finding, in

conjunction with the observation that black-and-tan melanocytes are also able to respond completely to the agouti-locus genotype of the receiving hair follicle, implies that, in black-and-tan mice, ventrality and dorsality of location are not important *per se* but that, together with their genetic constitution, they present different follicular environments which influence the expression of the melanocyte.

The importance of the hair follicle in promoting the elaboration of pheomelanin is also emphasized by the fact that in yellow mice it is only in the hair follicle that pheomelanin is produced. In all parts of the animal where extrafollicular melanocytes are found—for example, the ear skin, tail skin, or eye—only eumelanin is synthesized (*10*). It is therefore evident that the inherent capacity of melanocytes of all genotypes is to produce eumelanin, but that in the local milieu of "physiologically" appropriate hair bulbs they produce pheomelanin. The exact part played by the alleles at the *A* locus in promoting this physiologically appropriate environment still remains to be determined. When this has been determined we will be one step closer to understanding the primary gene action of these alleles.

The experimental design described above has also served in investigating whether other loci involved in melanin formation in the mouse act within the melanocyte or act through the neighboring cells of the Malpighian system composing the environment. For example, in the agouti-locus study it was found that the *C*-locus genotype of the receiving hair follicle had no influence on the immigrant melanocytes of host origin, the intense pigmentation of the host (*C*-) expressing itself in the genotypically albino (*cc*) or lightly pigmented ($c^{e}c^{e}$) hair bulbs of the graft. This locus, therefore, apparently acts autonomously within the melanocyte. Similar studies involving host-donor combinations which differed at the *B* and *P* loci also indicate that these loci act within the pigment cell.

It has already been mentioned that the main effect of both *dd* (dilute) and *lnln* (leaden) genes on the granules of the hair is to cause the formation of large, unevenly distributed granular clumps, which are responsible for the lighter appearance of these genotypes. In other investigations the origin of this clumping effect has been traced to a difference in the morphology of *dd* and *lnln* melanocytes as compared with melanocytes of the wild type (*DDLnLn*) (*10*) (see Fig. 3). Whereas the melanocytes of wild-type animals are characterized by the possession of long, relatively thick dendritic processes, in *lnln* and *dd* animals the pigment cells have fewer and finer dendrites. Because of this altered morphology the melanin granules are largely clumped around the nucleus in the body of the cell. Since the dendrites are the channels through which the granules are secreted, the reduction in size and number of the processes in *dd* and *lnln* mice may interfere with the transfer of pigment granules to the epidermal cells; this interference could account for the irregular distribution of the granules in the hair shafts of these mice.

To determine the site of action of the genes at these loci, Clement L. Markert and I (*11*) transplanted embryonic tissues containing potential melanocytes (melanoblasts) from normal, leaden, and dilute animals into the anterior chambers of the eyes of adult albino or pink-eyed mice having the same or different *Ln* and *D* constitution as the graft. The results were consistent with the hypothesis that, although the genes at both the *D* and *Ln* loci exert their activity from within the developing melanoblast, the number and size of dendritic extensions of a pigment cell is probably another function of the environment in which the cell resides. Melanocytes of *dd* and *lnln* genotypes have an innately weak capacity for extending dendrites, as reflected by their altered morphology in the rather compact tissue environments where they normally occur. However, in the less restrictive tissue

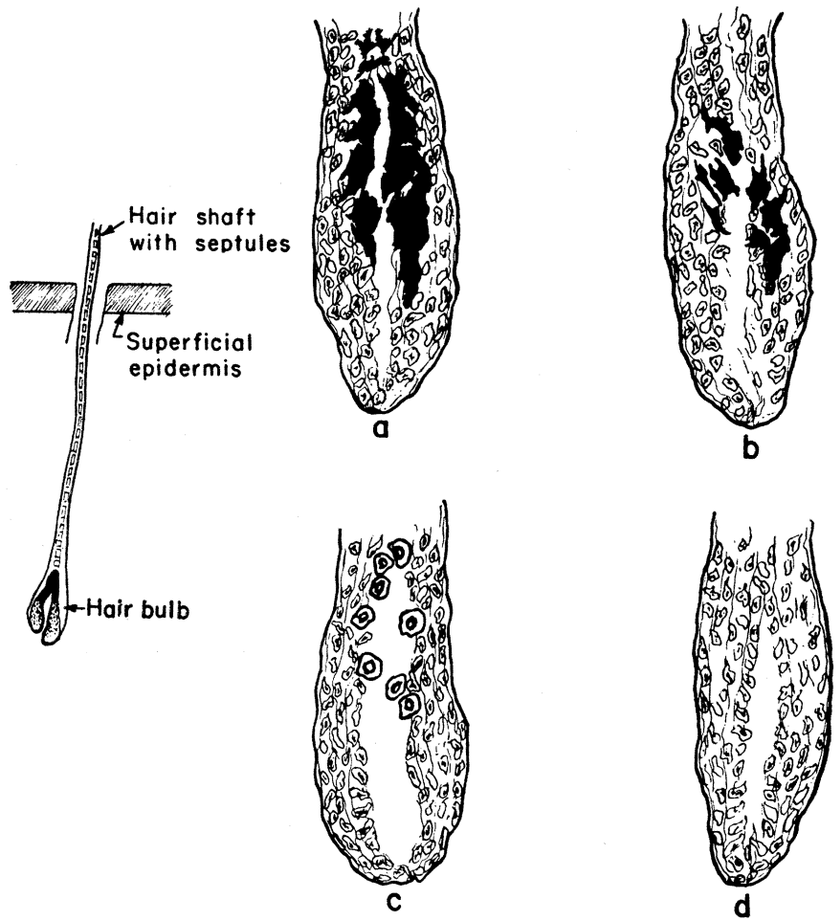

Fig. 3. Hair-bulb regions of follicles of different genotypes, illustrating differences in their melanocyte components. In the wild type (*a*) the many dendritic processes of the melanocytes of the bulb almost completely obscure the matrix, whereas in *dd* and *lnln* genotypes (*b*) the clumped arrangement of melanin granules around the nucleus of the cell makes it possible to identify individual pigment cells. The albino, or *cc*, genotype is characterized by the occurrence of large clear cells (*c*), which have been shown to be amelanotic melanocytes. These cells do not occur in hair bulbs originating in white spots (*d*).

environments in the eye, *dd* and *lnln* melanocytes do extend more and longer dendritic processes and in many instances are indistinguishable from the melanocytes of the wild type. Thus it appears that the *Ln* and *D* genotype of the pigment cell is expressed distinctly only in suitably compact tissue environments.

Genetic Factors in Melanoblast Differentiation

So far I have attempted to describe some of the experimental methods used to determine how certain loci, known to be involved in the formation of the pigment granule and its deposition into the growing hair, produce their effect. These methods have revealed that some of these loci act autonomously within the melanocyte, regardless of the tissue environment in which the melanocyte occurs; others exert their activity from within the developing melanoblast but produce their effect only in a suitable tissue environment; and still others produce their effect by altering in some way a particular tissue environment which, in turn, affects the behavior of the pigment cell.

The fact remains, however, that in all these cases we are dealing only with various ways in which specific genes can affect the product of a differentiated cell—the pigment granule. There is, in addition, a whole series of gene loci which, instead of influencing the product of the melanocyte, exert their effect on pigmentation at a more fundamental level by controlling some aspect of melanoblast differentiation. Probably the best examples of genetic factors in this category are those associated with white spotting—a character which is widespread among mammalian groups.

As background for a discussion of the physiological genetics of white spotting, I shall give a brief introductory account of the origin, early embryology, and occurrence of melanocytes.

The neural crest of the embryo, which originates between the junction of the neural tube and its overlying ectoderm, besides forming melanocytes, gives rise to cells which form the dorsal root ganglia of the spinal nerves, the adrenal medulla and other chromaffin tissue, and the Schwann cells of all peripheral nerves. Initially the neural crest is continuous from front to back, but as development proceeds its constituent cells migrate ventrolaterally on either side of the spinal cord and at the same time become segmentally clustered. In the mouse, this anterior-to-posterior and mediolateral migration of neural-crest cells from their place of origin to their definitive positions takes place during the 8th to 12th day of embryonic development (the gestation period of the mouse is about 21 days), as demonstrated in the classic experiments of Rawles (*12*).

In addition to the melanocytes finally located in the basal layer of the superficial epidermis that are responsible for skin pigmentation, and those in the hair bulbs that are responsible for hair pigmentation, there are other melanocytes responsible for the pigmentation of the choroid and iris (retinal pigment cells have a different embryological origin, coming from the outer wall of the optic cup). Melanocytes may also be found in the leptomeninges and in other regions of the brain; they occur in at least some areas of the dermis in nearly all mammals, including man. Indeed, melanocytes have been observed in the parathyroid, the thymus, the ovary, the submucosal connective tissue of the uterus, the sheaths of the tubules of the epididymus, the spleen, the adrenal medulla, and the regional lymph nodes, as well as in many other locations in some species, especially rodents (*1*).

Whereas the absence of pigmentation in albino animals apparently stems from an inherited metabolic defect in the synthesis of tyrosinase in melanocytes which are normal in respect to their numbers and distribution, it is obvious that white spotting must have a different etiology, inasmuch as some, at least, of the cells of the body in white-spotted animals have the ability to synthesize melanin. This became even more apparent from the results of histological studies in which the white skin of the albino was compared with the white skin of piebald animals (*13*). In all species so far investigated—mouse, rat, guinea pig, rabbit—the hair bulbs of white-spotted areas are characterized by matrices consisting of regularly arranged cells of equal size, but albino hair follicles contain, in addition, many large "clear" cells in their upper bulb region (see Fig. 3). These large cells with an apparently hyaline cytoplasm are unquestionably amelanotic melanocytes (melanocytes which are in every respect normal except for their ability to synthesize melanin); there is much anatomical evidence consistent with this interpretation, and in the mouse these cells have been shown to be of neural-crest origin (*14*).

The matrices of hair follicles arising in white-spotted areas are indistinguishable from those of follicles of artificially whitened hairs produced by x-irradiating pigmented skin with dosages known to cause permanent destruction of the melanocytes of the hair bulb (*15*), and indistinguishable from hair follicles which develop in skin experimentally deprived of neural-crest derivatives (*14*). These observations suggest that the piebald condition results either from a complete absence of melanoblasts in affected areas or from the failure of melanoblasts to differentiate locally. Moreover, since there are so many loci associated with white spotting—more than 14 in the mouse alone—it seems reasonable to suppose that both these hypotheses are valid, according to the loci involved, and that either of the mechanisms may be accounted for in a number of different ways. For example, an absence of melanoblasts could be attributed (i) to a genetic disturbance in the neural-crest region specifically affecting the differentiation of neural-crest cells into melanoblasts; (ii) to a genetic effect on melanoblast migration, or to a general metabolic disturbance which occurred during melanoblast migration, so that these cells did not reach all areas of the epidermis; or (iii) to a failure of melanoblasts to survive in the "spotted environment." A failure of melanoblast differentiation might result either from a genetic suppression within the melanoblasts themselves or from a genetic effect which was expressed only in certain areas of the epidermis. This last possibility is somewhat analogous to the situation in the black-and-tan mouse, where the hair bulbs in different regions of the integument promote the synthesis of different kinds of melanin.

In mice there are some genes—for example, W, W^v, W^j ("dominant spotting"), and Mi^{wh} ("dominant white")—which, when heterozygous, produce white spotting but when homozygous produce animals which are completely white except for retinal pigmentation. Examination of the skins of these animals has shown them to be essentially

"one big spot," since their hair follicles do not possess any demonstrable amelanotic melanocytes. To determine why these mice are nonpigmented, Markert and I transplanted embryonic tissue containing neural crest from animals destined to be completely white (that is, "one big spot") into an environment that is known to be favorable for melanoblast differentiation and melanin synthesis—the anterior chamber of the eye. Our reason was this: if in such an environment the explants did produce melanocytes, the inability to form pigment in the homozygous mutant could be attributed to action of the environment. In no instance were any pigment cells ever obtained from these grafts (*16*). These results tend to corroborate the hypothesis that the deficiency in the nonpigmented genotypes lies in the failure of the neural crest to give rise to cells capable of becoming melanocytes.

While this hypothesis seems to explain the complete absence of melanocytes in animals homozygous for these particular mutations, it is insufficient as an explanation of the localized, well-defined white-spotted areas, usually limited to the belly, of animals heterozygous for these same factors. Assuming that an autonomous effect within the melanoblasts themselves is responsible for the all-white condition of, for example, *WW* mice, and that one dose of *W* (*Ww*) genes interfered with the differentiation of some melanoblasts, one might anticipate that the heterozygous condition would result in a "silvering" pattern where pigmented, partially pigmented, and nonpigmented hairs were intermingled, rather than in the localized white spot which occurs. The mutation known as "splotch" in the mouse is pertinent here. Animals heterozygous for this mutation (*Spsp*) have a white belly spot and white extremities, while the homozygote (*SpSp*) dies in utero at approximately the 14th day of gestation, with abnormalities occurring in regions of the neural tube and the neural crest and their derivatives. Robert Auerbach, who studied this mutant (*17*) by implanting presumptive neural crest of *SpSp* embryos into the coelom of chick embryos, found that these grafts yielded no pigment, although corresponding grafts from heterozygotes did. The "splotch syndrome" is evidently caused by a disturbance of the region of the developing embryo which includes the neural crest. It therefore appears that although there is little doubt that the primary effect of the *Sp* gene in the homozygous condition is on the neural crest, animals heterozygous for this factor still exhibit localized white spotting.

It is obvious that white spotting still remains one of the most fascinating problems for those interested in gene action and gene interaction. Before leaving this subject I will mention just a few of the many other observations concerned with white spotting that await explanation. Individual mice heterozygous for two loci each of which is involved in white spotting lack pigment in more areas than one would expect merely on the basis of the sum total of the individual effects of each heterogyzote. This synergistic effect may produce phenotypes which are almost completely white. Moreover, the amount of white spotting is much greater on a black background than on a co-isogenic yellow background (*18*). In the guinea pig, the tortoise-shell ($e^{p}e^{p}$) pattern, characterized by an irregular intermingling of yellow with black hairs, is greatly affected by the presence of spotting factors. Not only is there an increase in the amount of yellow in tortoise-shell genotypes but, in addition, there is a tendency toward segregation of yellow and black so that a tricolored yellow, black, and white phenotype results (*19*).

Conclusions

In order to exemplify the many diverse ways in which gene action can influence or suppress pigment production, I have been mainly concerned in this discussion with analyzing how a few well-studied coat-color factors of the mouse produce their phenotypic effect. This analysis demonstrates that melanoblast differentiation and melanin synthesis proceed through an orderly sequence of genetically controlled steps, any one of which can be influenced in various ways. While some coat-color genes are involved in early steps in melanoblast differentiation (*W*, Mi^{wh}, *Sp*), melanocyte morphology (*D*, *Ln*), or the basic protein structure of the melanin granule (*P*), others produce their effect by controlling tyrosinase synthesis (*C*) or the polymerization of melanin (*A*, *B*). Although much work still remains to be done in tracing the phenotypic effects of these specific loci even farther back, to the time and place of their primary action, these studies have already contributed much to an understanding of how gene action and gene interaction can influence a single mammalian character (*20*).

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