SOME PHYSIOLOGICAL GENETIC ASPECTS OF MAMMALIAN MELANOGENESIS*

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Introduction: Some Recent Puzzling Findings

During the past several years we have been studying a number of melanogenic attributes using the skins of house mice afflicted by various types of genetic damage (Foster, 1956, 1959; Foster and Thomson, 1958). Our most recent study (Foster and Thomson, 1961, and unpublished data) involves a closer scrutiny of the effects of allelic interactions at a specific locus, *b* (brown). Briefly, by means of a combination of manometric and turbidimetric assays we were able to measure the following major sets of attributes: (1) tyrosinase and dopa oxidase activities, (2) initially present natural melanin content, as well as newly formed melanin within incubated skin subsamples, and (3) darkening of incubation media in respirometer vessels due to escape of melanogenic intermediates from incubated skin. These assay procedures are summarized in FIGURE 1.

The results most pertinent to our present discussion are summarized in TABLE 1, but it might be mentioned in passing that we were able to recognize the characteristic numerical profiles of all 10 homozygous and heterozygous genotypes involving the 4 alleles, "light" (B^{LT}), black (B), cordovan (b^c), and brown (b)—a kind of numerical "taxonomy under glass" for recognition of genotypes.

Focusing attention on the comparisons of black (BB) and brown (bb) homozygotes, as well as the black heterozygote (Bb), we find that, depending upon the sets of attributes used for genotypic comparisons, we could conclude that we are dealing with (1) genetic damage when allele b is present, (2) heterotic interaction or enzymic "hybrid vigor" (luxuriance), or (3) the superiority of b over B, at least when the skin is incubated with exogenous substrates. These separate and partly incompatible conclusions obviously involve different aspects of the whole truth. One approach involves taking the reasonable position that the organism is trying to tell us it is suffering from a genetic damage when it carries a single or double dose of the brown allele (b). This starting point is supported by the superiority of the black (BB or Bb) over brown (bb) skins with respect to the first set of pigmentary attributes listed in TABLE 1. Thus homozygous (BB) black skin has a higher natural melanin content than brown (bb) skin; it produces more melanin than does brown skin when left to its own endogenous resources (incubation in buffer); moreover, under conditions of substrate saturation (incubation with dopa) when brown skin seems superior to black, the total tissue-bound melanin, both initially present and newly synthesized, nevertheless is found to be greater in black skin. Thus the certain

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genetic damage afflicting the brown skin could involve both (1) some form of endogenous substrate limitation *and*, almost certainly, (2) a deficiency in the number or effectiveness of the melanin polymer binding sites of the protein granule matrix.

Discussion of Results

Let us tentatively assume that the brown locus specifies the synthesis of a matrix protein that could be concerned with (1) binding of tyrosinase to neighboring protein molecules in the granule matrix, or (2) provision of conjugation

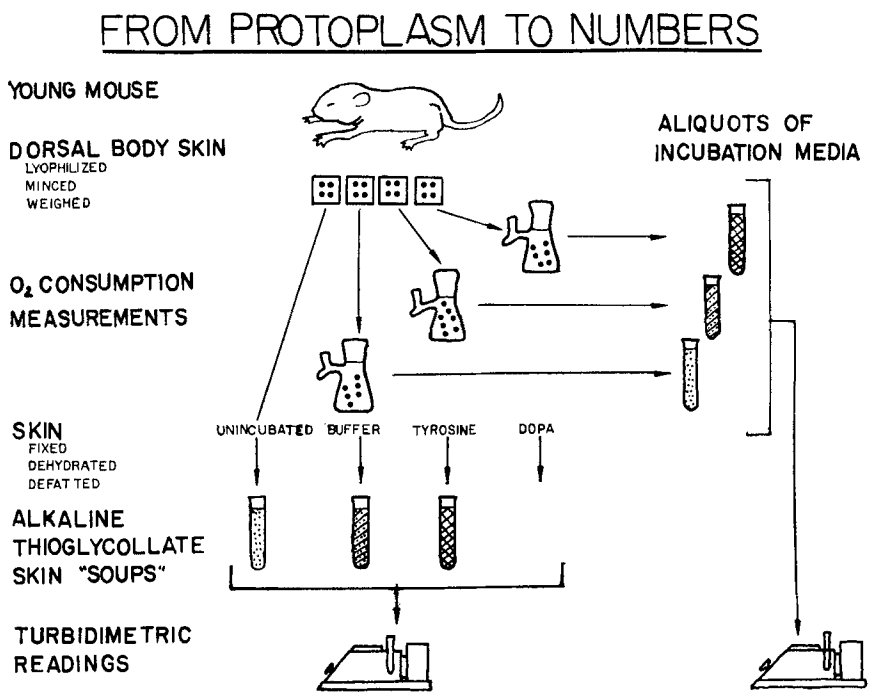

FIGURE 1.

sites for melanin polymers, or (3) the binding of matrix proteins to one another. Moreover, we might visualize a normal melanin granule as a tightly bound complex comprising tyrosinase, as well as other structural and functional proteins. Then a mutation at the brown locus could result in the production of a modified protein that might then *secondarily* cause an alteration of one or more of the following: (1) location, specific activity, or stability of tyrosinase; (2) number or binding effectiveness of melanin polymer conjugation sites; or (3) the structural integrity of the protein matrix, and hence of the pigment granule.

We have already demonstrated both increased and decreased levels of assayed enzyme activity toward exogenous substrate in the skins containing different mutant alleles; *i.e.*, brown (*bb*) skin shows increased enzyme activity, while the "light" homozygote ($B^{LT}B^{LT}$) shows reduced activity, when both of

these genotypes are compared with black skin. Moreover, it has been possible to show that these mutants are defective in their melanin-binding capacities under conditions of substrate saturation, *i.e.*, when the dopa incubation media for all genotypes show substantial darkening (intermediates leaking out of the tissue). Thus our observations are consistent with our visualization of the normal melanin granule and of the assumed role of the brown locus.

It still remains for us to try to explain the enzymic "hybrid vigor" (luxuriance) of the black (*Bb*) heterozygote. I can suggest at this point only that the melanin granule matrix of this genotype could contain 2 varieties of the same parent protein molecule, 1 normal and 1 abnormal (altered amino acid sequence), thereby leading to a modified molecular architectural arrangement that *secondarily* increases the specific activity of tyrosinase. Nevertheless, the end result is that the heterozygote produces less natural pigment than does the black homozygote (*BB*), perhaps because of the partially reduced number

TABLE 1
SUMMARIZED MELANOGENIC INEQUALITIES

Attribute	Genotype ranking	Conclusion
Natural melanin content Total tissue-bound melanin Skin darkening in buffer control medium	(1) BB ≥ Bb > bb	Genetic damage in single or double dose of *b*
Tyrosinase, dopa Oxidase Darkening of tyrosine and dopa incubation media	(2) Bb ≥ bb > BB	"Hybrid vigor"
Skin darkening in tyrosine and dopa (in excess of control incubated in buffer)	(3) bb ≥ Bb > BB	*b* superior to *B* in exogenous substrate

or effectiveness of melanin-binding sites in the "hybrid" granule protein matrix.

General Discussion

Introductory remarks. In our previous studies of melanogenesis we had often noted no simple correspondence between natural melanin content and other assayed melanogenic attributes. Moreover, the results previously obtained by us seemed amenable to reconciliation by *ad hoc* invocations of inhibitors, substrate restrictions, or reduced efficiency of terminal melanogenic phases. Such possibilities are still useful, but only partly so, in explaining our current apparently conflicting results.

A number of recently reported facts and ideas provide a basis for developing a broader perspective for interpreting observations concerning melanogenesis. Briefly, the considerations listed below range from postulated relations between genes and proteins to the possible functional consequences of altered macromolecular architecture. The resulting viewpoint is not intended to account for every case of mutational damage. It does, however, provide a basis

for accepting those genetic situations involving both diminished natural pigmentation and increased tyrosinase activity (assayed under conditions convenient for the observer).

Genetic considerations. A single gene seems to be concerned with specifying the amino acid sequence of a specific polypeptide. At the most only a very few genes contribute to the formation of the primary structure (amino acid sequence) of some specific protein (see review by Yanofsky and St. Lawrence, 1960). Thus a key agent in mammalian melanogenesis, the tyrosinase molecule, is synthesized either under the aegis of a single gene (perhaps the gene for albinism) or of only a very few genes. In *Neurospora*, for example, only a single gene, T, has so far been implicated in determining 4 structural variants of the tyrosinase molecule (Horowitz *et al.*, 1961). Many other genes affecting melanogenesis in laboratory mammals must then exert their effects by way of other polypeptides or proteins that in various ways regulate the microenvironments in which tyrosinase is synthesized, functions, or is rendered temporarily or permanently inactive.

At the level of the cell, transplantation studies (Silvers, 1961) have shown that at least in the house mouse a number of coat color genes apparently act autonomously within the melanocyte, *i.e.*, the *c* (albinism), *b* (brown), *p* (pink-eyed dilution), *d* (maltese dilution), and *ln* (leaden) loci. At least one other (agouti) locus, however, acts indirectly by controlling the melanocyte tissue environment (see also Cleffmann, 1953, 1954, 1961).

Chemical and cytological considerations. At macromolecular levels one deals with surface-spread protein monolayers, with enzyme-substrate complexes, and with tightly knit architectural relations between a specific enzyme and its large molecular neighbors. Under such conditions of macromolecular architecture, the modified physical state of the enzyme molecule (when compared with the soluble, coiled, "native" state) can be reflected in altered specific activity, stability, or even the degree of intracellular localization (Hayashi, 1952, 1953; Hayashi and Edison, 1950; Kaplan 1952*a*,*b*).

It is thus possible to enlarge the repertoire of an enzyme molecule's behavior by virtue of this enzyme's regulation by its macromolecular microenvironment. Such functional alterations could of course be independent of changes in the enzyme's primary amino acid sequence. Thus a number of intracellular organelles (*i.e.*, chromosomes, ribonucleoprotein granules, mitochondria, and, of course, melanin granules) provide a basis for regulatory interactions between some key substances (such as nucleic acids or enzyme proteins) and the proteins with which these key substances are conjugated (see also Nanney, 1960, and Markert, 1958).

The possibility of such regulatory interactions is intriguing because immediate neighborhood proteins could provide the vehicles, and specific organelles could provide the foci, for numerous patterns of genic interactions. Indeed it seems conceivable that a critical stage in biochemical evolution or in cellular differentiation might in some cases depend, respectively, upon the origin or "activation" ("turning on") of a single gene controlling the synthesis of some specific "architectural" protein. Such an "architectural" protein, by virtue of its binding sites, could convert a somewhat disordered array of different

large key molecules into the highly ordered arrangement characteristic of a normal intracellular organelle (see also von Wettstein, 1959).

Thus evolutionary or epigenetic complexity could depend as much upon the variety of stablized *patterns* of genic, and consequently of protein, interactions as upon the absolute number of different genes and of corresponding proteins.

At the organelle level of melanin granule construction, electron microscope studies (Birbeck and Barnicot, 1959; Seiji *et al.*, 1961; Birbeck, 1961; Wellings and Siegel, 1961; Moyer, 1961) are consistent with the following presumed sequence of events: (1) the initial synthesis and assembling of granule matrix proteins, (2) the localized attachment of the already synthesized tyrosinase molecule to particular binding sites on the internal membranes of the protein matrix or "premelanin" granule, (3) the spatially organized enzymic conversion of amino acid precursor(s) to melanin polymers, and (4) the conjugation of melanin polymers with specific binding sites of the protein matrix.

The melanin granule and melanogenesis. In view of the foregoing, we might visualize a normal melanin granule consisting of a number of different proteins, including tyrosinase. A number of genes could act within the melanocyte by controlling the synthesis of different granule matrix proteins. These proteins could assume different roles: (1) to localize tyrosinase and melanin polymers, (2) to bind matrix proteins together, (3) to provide melanogenic substrates. A gene mutation resulting in a modified matrix protein could then *simultaneously* produce a number of secondary consequences: (1) altered tyrosinase activity, (2) changed availability of endogenous substrates, (3) modified degree of melanization, (4) altered melanin granule structure.

It is thus possible now to harmonize our apparently conflicting sets of observations obtained from the same tissue.

Concluding Remarks

Our visualization of architectural and functional relations within the normal melanin granule, and the possible consequences arising from genetic damage, have at least helped us transfer a group of observations from a category of interesting curiosities into a framework sufficiently broad to embrace a number of unexpected correlated findings. Such a framework seems almost too broad—too many *ad hoc* explanations seem possible, and too few specific predictions.

It seems conceivable, however, that one assumption might lend itself to testing in the foreseeable future, namely, that of the heterogeneous protein composition of the granule matrix. Perhaps, too, the combined powerful approaches of electron microscopy and of physical biochemistry, when applied to detailed comparisons between normal and genetically controlled abnormal situations, might lead to a sharper viewpoint with greater predictive value.

Acknowledgment

On the occasion of L. S. Dunn's retirement from the Department of Zoology at Columbia University, I express my great indebtedness to him for his continuing stimulation and encouragement, and for his exemplary embodiment of the finest attributes of the teaching scholar.

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Discussion of the Paper

F. H. MOYER, JR. (*Johns Hopkins University Medical School, Baltimore, Md.*): Based on electron microscope pictures that we have obtained, we think that the protein matrix of the melanin granule is composed of at least three subunits, since in the albino animal we find a well-organized but unmelanized matrix; whereas in the pink-eyed animal we see melanization but a disorganized matrix. In black and brown animals, both organization and melanization occur, but the melanin deposited on the matrix is different.

This clearly implies to us that there are three structural subunits under different genetic control. They are probably controlled by distinct genetic loci on three separate chromosomes.

M. FOSTER (*University of Michigan, Ann Arbor, Mich.*): According to Dr. Silver, we can now also entertain the notion that there are at least five gene-controlled proteins in a matrix, as shown by his grafting experiments.