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► To cite this version:

G. Prota, A. G. Searle. Biochemical sites of gene action for melanogenesis in mammals. *Annales de génétique et de sélection animale*, 1978, 10 (1), pp.1-8. <hal-00892944>

HAL Id: hal-00892944

<https://hal.science/hal-00892944v1>

Submitted on 11 May 2020

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Biochemical sites of gene action for melanogenesis in mammals

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Summary

Current information is reviewed on the biochemical and genetic basis for coat pigmentation in mammals. While the dark eumelanin pigments are regarded as a fairly homogeneous group, the lighter phaeomelanins are more diverse, including the trichochromes (of low molecular weight) as well as more complex sulphur-containing compounds. However, both eumelanins and phaeomelanins arise from a common metabolic pathway, with dopaquinone as the key intermediate. Six main allelomorphic series for the control of coat colour have been established in mammals, although many other loci affecting pigmentation are known in laboratory mammals. Of the six, the *Brown*, *Dilute* and *Pink-eyed Dilute* series seem to affect mainly the differentiation and fine structure of melanosomes (also melanocyte morphology in *Dilute*) while the *Agouti*, *Albino* and *Extension* series genes seem likely to control the basic enzymic steps of melanogenesis. Biochemical information suggests that for eumelanin formation at least one enzyme and one gene is required. One of these is presumably tyrosinase, mediated by the *Albino* locus. For formation of the phaeomelanins at least two enzymes and two genes are needed. Both the *Agouti* and the *Extension* loci are involved in phaeomelanin production, the former *via* the tissue environment and the latter within the melanocyte. However, more work is needed to determine the enzymes involved and the exact types of phaeomelanin produced.

Introduction

Recent advances in our knowledge of the structure and biosynthesis of melanin pigments in mammals (THOMSON, 1974; PROTA and THOMSON, 1976; JIMBOW *et al.*, 1976) have made it necessary to reconsider the probable sites of action of the main allelic series of genes involved in coat coloration. These allelic series are so widespread in mammals that some of the genes concerned are likely to control the basic enzymic steps in melanogenesis.

This review arose out of a Round-table discussion on "La correspondance gene-enzyme pour la pigmentation du pelage des Mammifères" organised by the Department of Animal Genetics of I.N.R.A., with the following participants: S. ADALSTEINSSON (Iceland), J. J. LAUVERGNE (France-Organiser), J. P. ORTONNE (France), G. PROTA (Italy), A. G. SEARLE (United Kingdom) and C. VOULOT (France).

Biochemical aspects

Melanin pigments can be roughly classified into those giving dark colours, namely black and brown and their derivatives, and those giving light ones, with a wide range from the bright yellow coat of some mice to the caroty red colour of some human hair. The dark colours are regarded as deriving from a fairly homogeneous group of insoluble nitrogenous pigments, the eumelanins, which are irregular polymers consisting mainly of 5,6-dihydroxyindole units at various oxidative levels (NICOLAUS, 1968). The light colours are usually described under the omnibus term of « phaeomelanins », but these include pigments with diverse chemical and physical properties. The best characterized group of these phaeomelanins is found in reddish human hair and in feathers and contains sulphur in addition to nitrogen (PROTA, 1972). Degradative studies (FATTORUSSO *et al.*, 1970) carried out on various pigments of this group isolated from hair of rabbits, sheep, and from red human hair, suggest that the building units which constitute these complex macromolecules are formed by benzothiazole, 1,4-benzothiazine and tetrahydroisoquinoline systems, thus being chemically quite different from eumelanins. Another group of phaeomelanin pigments consists of the trichochromes (PROTA and THOMSON, 1976), formerly named trichosiderins because thought to contain iron. These compounds of low molecular weight occur in certain types of yellow and reddish human hair and are the only « melanin » pigments with a well-defined structure, characterized by a novel nitrogen and sulphur containing chromophore

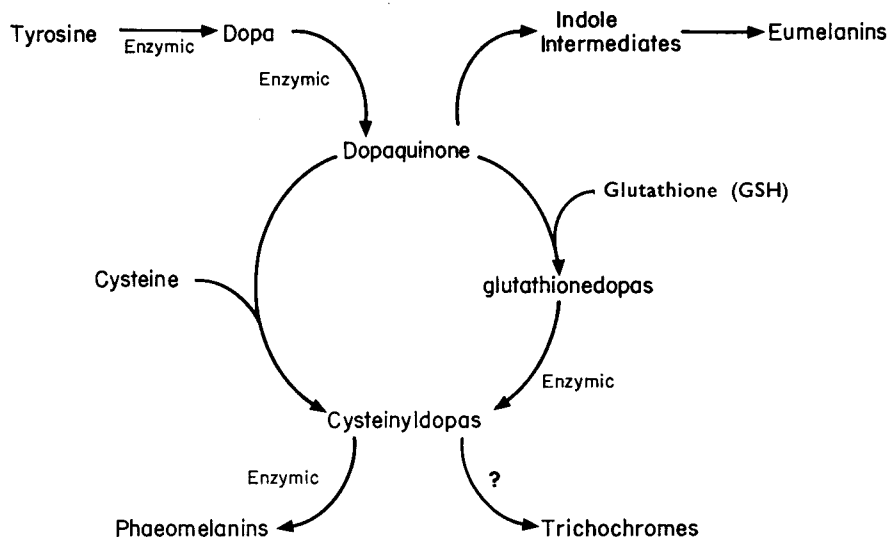


FIG. 1. — Tentative scheme of the probable metabolic pathways leading to formation of the different kinds of melanin pigment. « Phaeomelanins » refers to the complex phaeomelanin pigments as distinct from the simpler trichochromes.

Schéma possible des voies métaboliques qui conduisent à la formation des différents types de pigments mélaniques. Par phaeomelanines on désigne les pigments phaeomélaniques complexes qui se distinguent des trichochromes plus simples.

consisting of two conjugated 1,4-benzothiazine units. Despite their evident differences in molecular size and general properties, it is now well established that eumelanins, complex phaeomelanins, and trichochromes are biogenetically related, arising from a common metabolic pathway (fig. 1) in which dopaquinone is the key intermediate.

In the melanocytes of dark-coloured forms dopaquinone is converted into eumelanin by a complex series of spontaneous reactions which involve cyclization and oxidative polymerization. However, a switch mechanism exists which leads to the production of the S-containing phaeomelanins instead, through the intervention of sulphhydryl compounds such as glutathione and/or cysteine. This latter combines with dopaquinone in a non-enzymic very fast reaction to give the cysteinyl dopas which, by further oxidation, are converted into phaeomelanins and/or trichochromes. It is not yet clear whether the cysteinyl dopas required for phaeomelanin formation are derived by (i) direct addition of free cysteine to dopaquinone or (ii) by reaction of the latter with glutathione, followed by enzymic hydrolysis of the resultant glutathionedopa, or (iii) by both mechanisms. In this context, it is noteworthy that both cysteinyl dopa (BIORKLUND *et al.*, 1972) and glutathionedopa (AGRUP *et al.*, 1975) occur in melanoma tissues which seem to contain also the hydrolytic enzymes (γ -glutamyl transferase and a peptidase) capable of converting the latter into the former.

The same phenolase complex, known as tyrosinase (s), is thought to catalyse the conversion of tyrosine to dopaquinone in each of the pathways. Once formed, the subsequent fate of dopaquinone is the result of its own reactivity coupled with the chemical environment within the melanocyte, which is under genetic control. While the formation of eumelanin from this intermediate compound does not require any specific enzymic assistance, the formation of the S-containing phaeomelanins *via* cysteinyl dopa requires at least an additional enzymic control of the concentration of cysteine (or glutathione) in the melanocyte. *In vitro* biosynthetic experiments (FATTORUSSO *et al.*, 1969) suggest that the subsequent conversion of cysteinyl dopa to the pigmentary end-products may again be mediated by the tyrosinase complex in the presence of dopa as co-factor. However, an additional enzyme may be needed for trichochrome production.

Thus biochemical information suggests that for the formation of eumelanins from tyrosine at least one enzyme, and therefore one gene locus, is required while for the formation of the complex phaeomelanins or trichochromes from tyrosine at least two enzymes and two loci are needed. In formation of the melanosomes or pigment granules the melanins are laid down on a protein framework; for the formation of this framework other enzymes may be needed. It is interesting to note that the organization and shape of the phaeomelanosomes are different from that of the eumelanosomes (MOYER, 1966).

Genetic aspects

Six main series of multiple alleles (*A*, *B*, *C*, *D*, *E*, *P*) concerned with coat colour are known to be widely distributed in mammals while a number of other possibly homologous loci have been described (SEARLE, 1968). In addition, genetic studies on the mouse and other mammals have revealed many loci affecting coat colour for which no homologies have yet been postulated. Table 1 lists the main allelic

TABLE I

Characteristics of the main allelic series determining coat colour in mammals
Caractéristiques des principales séries alléliques
déterminant la couleur du pelage des mammifères

Series	Name	Main alleles	Effects and mode of action	Site of action
Albino . . .	<i>C</i>	<i>C</i> ⁺ , <i>c</i> ^{ch} , <i>c</i> ^h , <i>c</i>	Reduces intensity of pigmentation, first yellow and then black, until none is left in <i>cc</i> (albino).	Within melanocyte
Agouti . . .	<i>A</i>	<i>A</i> ^y , <i>A</i> ^w , <i>A</i> ⁺ , <i>a</i> ^t , <i>a</i> , <i>a</i> ^e	Controls regional distribution of black and yellow pigments in body and in individual hairs, from all yellow (dominant in mouse) to all black.	Outside melanocyte
Extension .	<i>E</i>	<i>E</i> ^d , <i>E</i> ⁺ , <i>e</i> ^{br} , <i>e</i>	Extends black (dominant) or yellow (recessive) pigment in body as a whole, with <i>e</i> ^{br} giving a black/yellow variegation.	Within melanocyte
Brown . . .	<i>B</i>	<i>B</i> ^{lt} , <i>B</i> ⁺ , <i>b</i>	Affects eumelanins, changing black to brown in <i>bb</i> . May lighten eyes (<i>bb</i>) and under-fur (<i>B</i> ^{lt}).	Within melanocyte
Dilute . . .	<i>D</i>	<i>D</i> ⁺ , <i>d</i> , <i>d</i> ^l	Dilutes both black and yellow colours by clumping pigment granules. Dilute lethals (<i>d</i> ^l) have convulsions.	Within melanocyte, changing shape
Pink-eyed .	<i>P</i>	<i>P</i> ⁺ , <i>p</i> , <i>p</i> ^s	Main effect on eumelanosomes, with dark colours much more diluted than light ones. Retinal pigmentation removed in <i>p</i> and <i>p</i> ^s . <i>p</i> ^s also causes male sterility.	Within melanocyte

series, with what is known about the mode of action of the genes concerned. Since genes affecting trichochromes have not yet been studied in experimental mammals these pigments will be included under phaeomelanins in the remarks which follow.

Albino

This is thought to be the structural locus for tyrosinase (FOSTER, 1965), since the bottom recessive (*c*) leads to absence of all melanin, while intermediate alleles reduce the amounts of yellow and dark pigments. The effect on yellow pigment

is more pronounced. The belief that this is a structural rather than a regulatory gene is strengthened by the identification of a « himalayan » or acromelanistic allele in a number of species, in which the amount of pigmentation depends on skin temperature (see SEARLE, 1968). Thus this mutant allele presumably controls the formation of a different, thermolabile, form of the tyrosinase enzyme. HEARING (1973), however, reported a low level of tyrosinase activity in albino mice and suggested that the *c* locus is a regulator rather than a structural one.

It should be pointed out that albinism can also result from the failure of neural crest derived and optic cup derived melanocytes to reach their destinations in epidermis and retina. The mutant *Microphthalmia* (*mi*) seems to be an example of this.

Agouti

This locus is concerned with the regional distribution of dark and light pigments within individual hairs and in the coat as a whole. The same melanocyte can produce both types of pigment, as shown by the formation of a yellow (agouti) band in an otherwise dark hair. In fact there is evidence that both types of pigment granule (eumelanosomes and phaeomelanosomes) can be present at the same time in a melanocyte (SAKURAI *et al.*, 1975). Action at the *Agouti* locus is *via* the tissue (follicular) environment (BILLINGHAM and SILVERS, 1960) and should be primarily concerned with the regulation of the synthesis of the cysteinyl-dopas (fig. 1). However, work is needed to provide a more precise metabolic meaning for the gene action at this locus.

Extension

This locus is also involved in phaeomelanin production, since different alleles extend or restrict the amount of yellow pigment in the coat. Homozygotes for the bottom recessive allele, *e* have a yellow coat similar to that of heterozygotes for *A^y*. However, it tends to be rather sooty (with admixture of eumelanin) in young *ee* mice, more so than in *A^y*. Moreover, *A^y* + *ee* mice resemble *ee* in coat colour.

Gene action of *e* is within the melanocyte, but it seems that phaeomelanin can only be produced in the environment of the hair follicle (LAMOREUX and MAYER, 1975). Action of the allele for partial extension of yellow, which gives a variegated phenotype, is exactly what would be expected if there were separate clones of black-forming and yellow-forming melanocytes, with random inactivation of alleles for production of one or other colour (SEARLE, 1968). Thus there are no quantitatively intermediate alleles of the type found in the albino series.

The marked difference in the effects and mode of action of the *E* locus with respect to the agouti locus makes it uncertain whether the nature and formation of the phaeomelanins observed in the extension series correspond to those of the apparently similar pigment in the agouti series.

Brown

The best known allele is *recessive brown* (*b*) which acts on eumelanin pigmentation only, but a dominant allele (*B^u*) which lightens the coat, especially the underfur, has been found in the mouse. Melanosomes of brown mice are rounded rather than ovoid, but also have rather a disordered internal structure, with coarsely granular melanin (MOYER, 1963). This gene is known to act within the melanocyte, but it is not yet clear whether the altered structure of mutant granules result from formation of an abnormal type of melanin or not. Indeed, little is known on the chemistry of the brown melanins.

Pink-eyed dilution

Alleles at this locus tend to affect eumelanin pigmentation much more than phaeomelanin. In *pp* mice, eumelanosomes become very abnormal, being small and shred-like with an irregular matrix and a marked reduction in cross-linking of fibres (MOYER, 1963), while retinal pigment granules are not formed. Phaeomelanosomes, however, have a normal structure. Action of the gene is again within the melanocyte (SILVERS, 1957) and one could postulate that it is concerned with the final stages in formation of the eumelanosome.

Dilute

In *recessive dilution* (and in its mimic *leaden* in the mouse) the epidermal (but not the retinal) melanocyte changes its shape, from nucleofugal to nucleopetal (MARKERT and SILVERS, 1956), and its pigment granules are deposited in clumps, whether eumelanic or phaeomelanic. Thus this interesting gene must help to determine the morphology of the epidermal pigment cell derived from the neural crest, rather than any stages in melanogenesis. Homozygotes for another allele, *dilute lethal* (*d^l*), suffer from convulsions (SEARLE, 1952) associated with extensive demyelination of certain tracts of nerve fibres (KELTON and RAUCH, 1962). Biochemical defects associated with phenylalanine have also been reported in these mutants, but it seems likely from later work that these were secondary results of the disease process (WOOLF, 1970). It should be pointed out that although diluting mutants which clump pigment granules in species other than the mouse are regarded as homologous with dilute (*d*) in the mouse, homology with the mimic gene *leaden* (*ln*) is another possibility.

Sex-linked yellow

Although it is not a member of a well established allelic series, one other locus should be mentioned because it may well be directly involved in melanin formation. This is *sex-linked yellow*, known only in the cat (orange, *O*) and the Syrian hamster

(tortoiseshell, *To*) (see SEARLE, 1968). There is good reason to believe that this locus is not homologous with that for *extension of yellow* (*e*) because (i) the Syrian hamster appears to have both loci and (ii) this locus is on the X-chromosome, which OHNO (1967) suggests has been conserved in mammalian evolution, while the extension locus is autosomal. The *orange* allele at the sex-linked *Yellow* locus in the cat behaves like recessive yellow in giving full extension of phaeomelanin pigments, so that the effect of *Agouti* alleles cannot show up. The wild type allele allows normal expression of *Agouti* etc., while females heterozygous for *orange* show the well-known tortoiseshell pattern. This results from the presence of clones of yellow and non-yellow melanocytes, arising from random inactivation of one or other X-chromosome (LVON, 1961). Thus this gene exists in only two alternative forms and may well be regulatory in nature. Possibly it is the regulator gene for the structural extension locus. If so, it is strange that so few species are known to possess it.

Concluding remarks

Of the six main allelic series concerned with mammalian coat coloration, three, namely *B*, *D* and *P*, seem to affect mainly the differentiation and the fine structure of melanosomes, while *A*, *C* and *E* are likely to control the basic enzymic steps of melanogenesis. Mutations leading to albinism even in the presence of pigment cells occur at the *C* locus which therefore should contain the structural gene for tyrosinase, the key enzyme of melanogenesis. It is generally agreed that genes at the *A* and *E* loci are involved in the control of phaeomelanin biosynthesis but details of the mechanism of their action are needed as apparently they allow the formation of the same type of pigment in two very different ways. In the *Agouti* series, the biochemical message favouring the formation of phaeomelanins, presumably *via* cysteinyl-dopas, comes from outside the melanocyte and its action is determined by the *Agouti* allele present, by regional influences and by such factors as MSH. In the extension series, gene action is autonomous and uninfluenced by external factors. Therefore it seems probable that these genes control distinct enzyme systems, the possible nature of which remains indefinite because of lack of experimental evidence regarding primarily (i) the nature and chemical identity of the phaeomelanins observed in the *Agouti* and extension series, and (ii) the actual metabolic pathway leading to the formation of the cysteinyl-dopas.

Reçu pour publication en avril 1978.

Résumé

Localisations biochimiques de l'action des gènes qui contrôlent la mélanogenèse des Mammifères

L'information concernant les bases biochimiques et génétiques de la couleur du pelage est passée en revue. Alors que l'on peut considérer que les pigments eumélaniques sombres forment un groupe assez homogène, les phaeomélanines plus claires sont plus diverses, comprenant des trichochromes (de faible poids moléculaire) aussi bien que des composés sulfurés plus complexes. Cependant à la fois les eumélanines et les phaeomélanines proviennent d'une voie métabolique commune avec, comme intermédiaire clé, la dopaquinone. Six principales séries alléomorphiques qui contrôlent la couleur du pelage ont été mises en évidence chez les Mammifères. De ces six séries les séries *Brun* (*B*), *Dilution* (*D*) et *Dilution à œil rose* (*P*) affectent, semble-t-il, principale-

ment la différenciation et la structure fine des mélanosomes (ainsi que celle des mélanocytes pour la *Dilution*) alors que les gènes des séries *Agouti* (A), *Albinos* (C) et *Extension* (E) semblent surtout contrôler les voies enzymatiques de la mélanogénèse. Ce que l'on sait de la biochimie suggère que, pour la formation de l'eumélanine au moins, un de ces enzymes est probablement la tyrosinase, contrôlée par le locus *Albinos*. Pour la formation des phaeomélanines il faut au moins deux enzymes, donc deux gènes. Les loci *Agouti* et *Extension* semblent tous les deux impliqués dans cette production de phaeomélanine, le premier à travers le tissu environnant et le second à l'intérieur du mélanocyte. De nouvelles études sont encore nécessaires pour déterminer quels enzymes sont impliqués et les types exacts de phaeomélanine produite.

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