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Effects of the *white* allele of the *mi* locus on coat pigmentation in chimeric mice

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Summary

To investigate the cellular action of the Mi^{wh} allele in the mouse with regard to its effects upon coat color patterns, we generated a series of aggregation chimeras, using embryos that differ in their *mi* locus genotype. We have obtained 11 chimeras $Mi^{wh}/+ C/C \leftrightarrow +/+ c/c$ and 8 chimeras $+/+ C/C \leftrightarrow +/+ c/c$. Chimerism was determined by coat and retinal pigment epithelium mosaicism and by the electrophoretic analysis of GPI-1 isoenzymes. In $Mi^{wh}/+ C/C \leftrightarrow +/+ c/c$ mice white coat color prevailed due to the higher percentage of unpigmented areas and the higher percentage of unpigmented hairs in pigmented areas. Our data indicate that a single Mi^{wh} gene dose decreases the melanoblast proliferative activity, causing the lightening of coat pigmentation. In $Mi^{wh}/+ C/C \leftrightarrow +/+ c/c$ mice a few pigmented hairs were often detected on the belly where $Mi^{wh}/+$ mice always had a white spot. This suggests that in the chimeras the presence of some non-Mi^{wh} cells in the skin of the belly allows pigment cells to develop. Using embryos of two substrains of Mi^{wh}/Mi^{wh} mice that differ in their Gpi-1 locus genotype we have produced 8 $Mi^{wh}/+ \leftrightarrow Mi^{wh}/Mi^{wh}$ chimeras. In all these chimeras coat color patterns resembled those of $Mi^{wh}/+$ heterozygotes despite the higher percentage of the Mi^{wh}/Mi^{wh} component in three chimeras. Mosaic hairs were absent in the chimeras. This shows that the chimeras have only one $Mi^{wh}/+$ melanoblast population which actively proliferates and colonizes almost all hair follicles. Thus the Mi^{wh}/Mi^{wh} dermis and epidermis do not suppress proliferation and differentiation of the $Mi^{wh}/+$ melanoblasts except the certain area on the belly.

1. Introduction

The white gene (Mi^{wh}) is one of the 9 mutant alleles of the *mi* locus and belongs to the so-called spotting genes. Mi^{wh}/Mi^{wh} homozygotes are white with slightly pigmented eyes and have no melanocytes in hair follicles (Markert & Silvers, 1956; Silvers, 1956; Konyukhov & Osipov, 1968). In $Mi^{wh}/+$ heterozygotes the pigmentary disturbance is detected as slightly diluted fur color with white spotting on the belly and on the feet and tail (Green, 1989).

Studies on chimeric mice have demonstrated that mutant alleles of the *mi* locus are expressed in melanoblasts, which results in a lack of melanocytes in hair follicles of homozygotes (Mintz, 1967, 1969; Kindyakov *et al.* 1984). The *mi* locus may control the formation of neural crest derivatives (Konyukhov & Osipov, 1968). Mutant alleles of the *mi* locus particularly affect ectomesenchymal cells; therefore in animals with mutant alleles at the *mi* locus the melanogenesis-stimulating activity of these cells and their growth-inhibiting action on the retinal pigment epithelium (RPE) may be abnormal (Konyukhov & Sazhina, 1980). Using aggregation mouse chimeras we have shown that the Mi^{wh} gene affects head ecto-mesenchyme, thus causing hyperplasia of RPE, and microphthalmia arises because the increased rate of proliferation in RPE causes failure of choroid fissure to close normally (Kindyakov *et al.* 1984; Konyukhov *et al.* 1986). Mintz (1970) believes that the data obtained from chimeras indicate that genetically programmed death of certain clones of melanoblasts is the cause of white spotting on the belly, feet and tail in $Mi^{wh}/+$ heterozygotes.

Pratt's (1982) data obtained in experiments with dermal–epidermal recombinant grafts with skin from embryos of Mi^{wh}/Mi^{wh}, $Mi^{wh}/+$ and $+/+$ genotypes indicate that $+/+$ melanoblasts cannot develop normally in skin of Mi^{wh}/Mi^{wh} and $Mi^{wh}/+$ mouse embryos. The author considers that Mi^{wh}/Mi^{wh} and

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$Mi^{wh}/+$ epidermis and dermis alter or block the development of $+/+$ melanoblasts. However, the data obtained on chimeric animals do not suggest any effect of this mutant gene on dermis or epidermis. In another work Pratt (1983) established that slightly diluted fur color in $Mi^{wh}/+$ heterozygous mice, in particular, was due to a reduction in the number of melanocytes in hair follicles.

In this report we have used the aggregation embryos technique to characterize the effects of the Mi^{wh} gene on melanoblast clones in $Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$ and $Mi^{wh}/+ \leftrightarrow Mi^{wh}/Mi^{wh}$ chimeric mice.

2. Materials and methods

(i) *Mouse strains and mutations*

The strains of mice, CC57BR/Mv-Mi^{wh} (genotype $Mi^{wh}/Mi^{wh}\ a/a\ b/b\ C/C$), C57BL/6J ($+/+\ a/a\ B/B\ C/C$) and BALB/cJLac ($+/+\ A/A\ b/b\ c/c$) were used. Mi^{wh}/Mi^{wh} mice are devoid of pigment in the retinal pigment epithelium (RPE) and coat, only the eye iris being pigmented. C57BL/6 mice are black and have an electrophoretically fast variant of glucose phosphate isomerase GPI-1B (genotype $Gpi\text{-}1^{bb}$), BALB/c mice are albino and have the slow-migrating GPI-1A ($Gpi\text{-}1^{aa}$). Two substrains of Mi^{wh}/Mi^{wh} mice that differ in their *Gpi-1* locus genotype ($Gpi\text{-}1^{aa}$ or $Gpi\text{-}1^{bb}$) were developed in our laboratory. Heterozygotes, $Mi^{wh}/+$, were obtained by mating C57BL/6 (for 1st series chimeras) and CC57BR/Mv (for 2nd series chimeras) female mice (genotype $+/+\ Gpi\text{-}1^{bb}$) to $Mi^{wh}/Mi^{wh}\ Gpi\text{-}1^{bb}$ males.

(ii) *Embryo manipulation and production of aggregation chimeras*

Mice were paired overnight with their respective males. The presence of a vaginal plug the following morning was taken as indication of successful mating, and this was designated day 0. On day 2, the oviduct and anterior portion of the uterine horn were flushed with warm incomplete Dulbecco's medium (about 37 °C).

Chimeras were obtained by aggregation of embryos according to Mintz (1971). 6–8-cell stage embryos devoid of zona pellucida were aggregated in pairs using phytohemagglutinin-P (Mintz *et al.* 1973). Aggregated embryos were cultured in small drops of Whitten's medium under paraffin oil which had been previously equilibrated with medium. The following day, fully aggregated chimeras, at the early blastocyst stage, were transferred into one horn of the uterus of a day 2 pseudopregnant B6CBF1 recipient female (C57BL/6 × CBA).

A total of 444 blastocysts were transferred, from which 68 mice were born. Among them 27 chimeras survived to the age of 20 days: 11 $Mi^{wh}/+\ C/C\ Gpi\text{-}1^{bb} \leftrightarrow +/+\ c/c\ Gpi\text{-}1^{aa}$ (BALB/c), 8 $+/+\ C/C\ Gpi\text{-}1^{bb}$ (C57BL/6) $\leftrightarrow +/+\ c/c\ Gpi\text{-}1^{aa}$ (BALB/c (1st series chimeras) and 8 $Mi^{wh}/+\ Gpi\text{-}1^{bb} \leftrightarrow Mi^{wh}/Mi^{wh}\ Gpi\text{-}1^{aa}$ (2nd series chimeras).

(iii) *Identification of chimeras genotypes and determination of genotypic proportions*

Chimerism of 20-day-old mice was determined by coat and RPE mosaicism and by the electrophoretic analysis of GPI-1 isoenzymes in blood, brain, heart and left kidney. Percentage of pigmented cells in RPE was estimated by nuclear counts in every 10th serial sagittal section through the eye. Eyes for histological analysis were fixed in Bouin's fluid and after proper treatment serial 6 μm paraffin sections were made. The sections were stained with hematoxylin-eosin.

The percentage of cells of the parental genotypes in the tissues and organs of the chimeras was determined by their content of GPI-1 isoenzymes. The tissues were homogenised in water and centrifuged, and the supernatants were subjected to GPI-1 electrophoresis to separate the A and B homodimer isozyme forms. Electrophoresis was carried out in 12% starch gel using Tris-citric buffers (Shows & Ruddle, 1968). After staining the gel was dried. The relative amounts of AA and BB isoenzymes were estimated from the intensity of staining of the two bands, measured by a Gilford densitometer. The percentage of the parental genotypes in the chimeric tissues was determined by weighting of the excised peaks of corresponding isoenzymes on densitograms. The error did not exceed 5%.

(iv) *Analysis of pigmentation patterns*

The percentage of the pigmented areas of coat was determined visually. Mosaicism within hairs was studied using preparations obtained as follows: hairs plucked from the central-lateral and/or central-dorsal areas of integument were placed on a glass slide smeared with a protein-glycerol mixture (1:1), then they were dried in a vacuum thermostat for 5 h and placed in medium prepared according to Berlese (see Gray, 1973). Afterwards hairs were covered with a cover glass and placed again in a vacuum thermostat for 2·5 h. After such treatment the air is completely removed from the hair cavities and pigment granules can be seen well.

In $Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$ and $+/+\ C/C \leftrightarrow +/+\ c/c$ chimeras the pigmentation of hairs from various areas of integument was studied. In $Mi^{wh}/+\ Gpi\text{-}1^{bb} \leftrightarrow Mi^{wh}/Mi^{wh}\ Gpi\text{-}1^{aa}$ chimeras and in the $Mi^{wh}/+$ mice a length of unpigmented portion of the apical segments, in percent of whole length of apical segment, in 200 zigzags plucked from the dorsal and lateral areas of the integument was measured. Apical segments were copied on paper using a Visapan microscope, and length of apical segments and their unpigmented portions were measured by a curvimeter.

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Table 1. *Effect of the* Mi^{wh} *gene on coat pigmentation in aggregation mouse chimeras*

Chimeras	Chimera no.	Pigmented cells in RPE, %	GPI-1B, %				Degree of pigmentation
			Blood	Kidney	Brain	Heart	
$Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$	1	4	nr	nr	nr	nr	±
	2	46	64	49	47	44	+
	3	69	64	59	59	68	+
	4	84	86	78	66	80	+
	5	89	nr	nr	nr	nr	+
	6	89	81	57	86	84	+
	7	91	63	54	64	65	+
	8	93	95	95	95	95	+
	9	93	95	95	95	95	++
	10	94	80	68	65	80	++
	11	94	73	52	86	70	++
$+/+\ C/C \leftrightarrow +/+\ c/c$	12	38	48	47	37	38	++
	13	52	64	34	43	54	++
	14	52	44	44	51	45	++
	15	81	61	60	70	75	+++
	16	82	71	77	74	70	+++
	17	95	90	90	86	83	++++
	18	95	85	87	92	78	++++
	19	97	95	95	95	95	++++

RPE, retinal pigment epithelium; GPI-1B, cell marker of the parental components of $Mi^{wh}/+$ and $+/+\ C/C$ genotypes; ±, +, ++, +++, ++++ correspond to 5%, 25–30%, 40–50%, 75% and 90% for pigmented coat areas; nr, not recorded.

The hairs were divided into 5 groups: I – apical segments are completely pigmented; II, III, IV, V – unpigmented portions of the apical segments are equal to 1–25, 26–50, 51–75 and 76–100%, respectively.

3. Results

(i) *Pigmentation patterns of* $Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$ *and* $+/+\ C/C \leftrightarrow +/+\ c/c$ *chimeras*

In eleven 20-day-old $Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$ and 8 $+/+\ C/C \leftrightarrow +/+\ c/c$ chimeras the coat color patterns were characterised by the transverse stripes of different widths and lengths, extending from the mid-dorsum to the mid-ventrum. In all tissues and organs of the chimeras examined, GPI-1 isoenzymes of both parental strains were found. These findings confirmed chimerism of mice with mosaic pigmentation of the coat (Table 1). In $Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$ mice the white color was predominant, due to the higher percentage of unpigmented areas and lightening of the pigmented ones. Pigmented coat areas did not exceed 40–50% (Table 1).

The percentage of cells of the mutant $Mi^{wh}/+$ genotype was very variable: it was 4%, 46% and 69% in chimeras nos 1–3, and 84–94% in the others. The content of GPI-1B (parental component of $Mi^{wh}/+$ genotype) in various tissues and organs corresponded approximately to the percentage of pigmented cells in RPE: if the percentage of pigmented cells in RPE was high then the content of GPI-1B was also high. In general, the degree of chimerism in various organs was similar (Table 1).

The data obtained indicate that in $Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$ chimeras the cells of the mutant ($Mi^{wh}/+$) and normal ($+/+$) genotypes are present in approximately equal proportions in various tissues and organs: RPE, blood, heart, kidney and brain. However, we observed a discrepancy between the degree of fur color and the content of the mutant component $Mi^{wh}/+$ in RPE and other studied organs. In all cases phenotypic expression of the coat pigmentation was much less as compared to the content of $Mi^{wh}/+$ cells in other tissues. For instance, chimera no. 2 had 46% of pigmented cells of the mutant genotype in RPE and only 25% pigmented coat areas. In chimeras nos 7–11 the percentage of pigmented cells in RPE was over 90% but pigmented areas of coat did not exceed 50%.

In $Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$ chimeras the feet remained unpigmented, but a few pigmented hairs were often detected on the belly where $Mi^{wh}/+$ mice always had a white spot. This suggests that in chimeras the presence of some non-Mi^{wh} cells in the skin of the belly allows pigment cells to develop.

In 8 $+/+\ C/C \leftrightarrow +/+\ c/c$ chimeras obtained by aggregation of C57BL/6 and BALB/c embryos the distribution of pigmented and unpigmented transverse stripes was similar to that in $Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$ mice. However, in $+/+\ C/C \leftrightarrow +/+\ c/c$ chimeras the feet were as a rule pigmented. There was correlation between the percentage of pigmented coat areas and the percentage of pigmented cells in RPE. Thus, in chimeras nos 13 and 14 the percentage of

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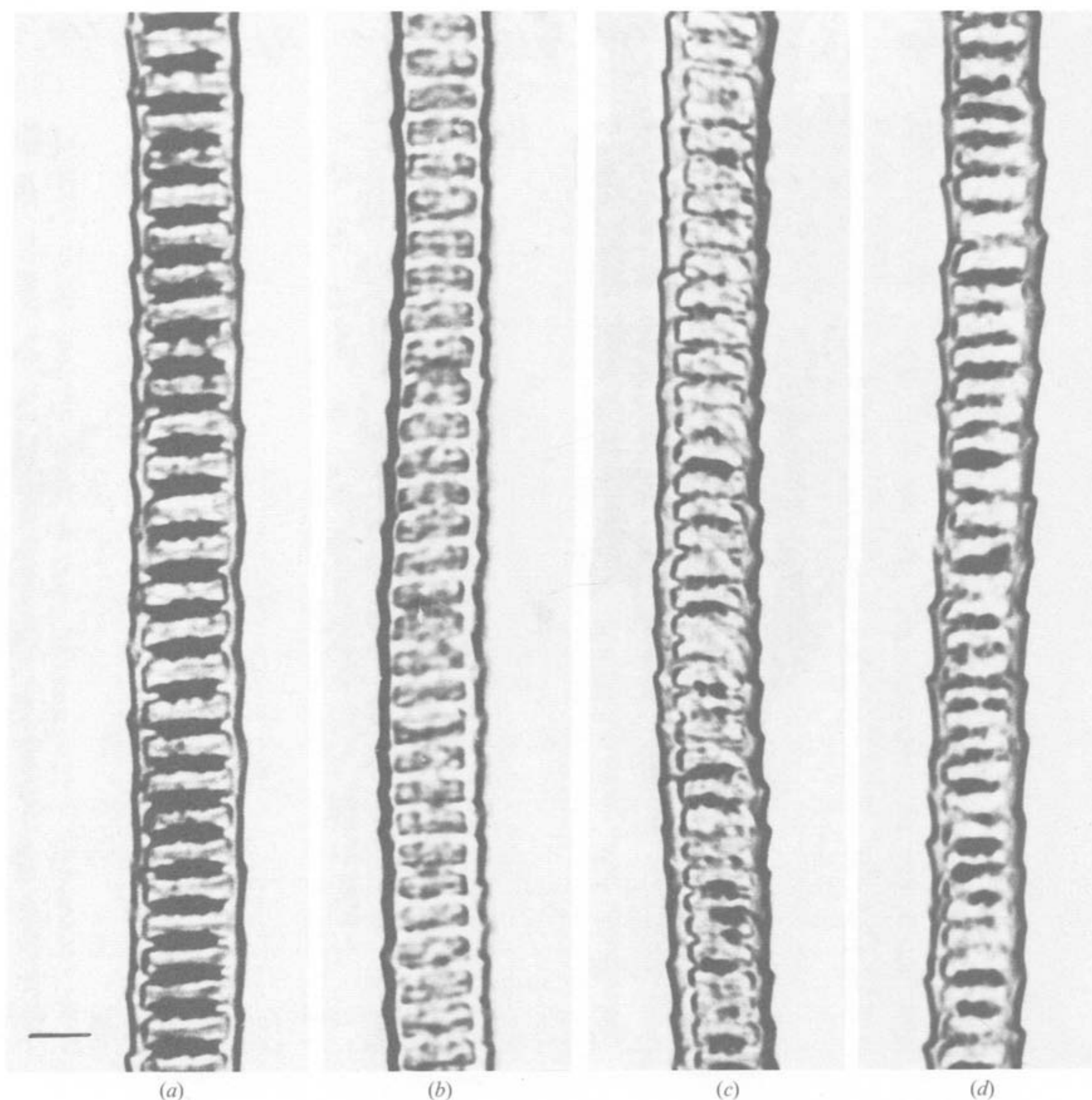

Fig. 1. Portions of zigzags plucked from the central-dorsal area of integument of mice C57BL/6 (a), BALB/c (b), chimera no. 7 $Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$ (c) and chimera no. 14 $+/+\ C/C \leftrightarrow +/+\ c/c$ (d). The bar represents 10 μm.

pigmented cells in RPE and the percentage of pigmented coat areas were nearly 50%. In chimeras nos 17, 18 and 19 the percentage of pigmented coat areas estimated visually was about 90% and the percentage of pigmented cells in RPE and GPI-1B in different organs was also high. The degree of chimerism in various organs of these chimeras was similar. There was a correlation between the percentage of pigmented cells in RPE and the content of GPI-1B in blood, brain, heart and kidney.

Analysis of zigzags plucked from pigmented coat areas of $Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$ and $+/+\ C/C \leftrightarrow +/+\ c/c$ chimeras showed the presence of pigmented, unpigmented and mosaic hairs. Comparing hairs of the same type plucked from analogous pigmented coat areas we found that in the $Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$ and $+/+\ C/C \leftrightarrow +/+\ c/c$ chimeras the pigment in the hair shaft was distributed similarly: pigmented and unpigmented cells alternated stochastically (Fig. 1).

Study of hairs plucked from similar localized pigmented coat areas of $Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$ and $+/+\ C/C \leftrightarrow +/+\ c/c$ chimeras, in which the parental components were represented in approximately equal proportions, showed that the percentage of pigmented and unpigmented zigzags in those chimeras differed considerably (Table 2). For instance, in $+/+\ C/C \leftrightarrow +/+\ c/c$ chimera no. 13 the percentage of mosaic, pigmented and unpigmented hairs was equal to 78, 12 and 10 respectively. In $Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$ chimera no. 2 the percentage of mosaic hairs was about the same (75%), completely pigmented hairs were absent but the percentage of unpigmented hairs was 2·5 times higher than in chimera no. 13. In chimeras nos 7 and 14 the percent distribution of hairs was similar: in both types of chimeras the percentage of mosaic hairs was approximately equal but pigmented hairs prevailed considerably in $+/+\ C/C \leftrightarrow +/+\ c/c$ chimeras, and there were several times more unpigmented hairs

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Table 2. *Percentage of mosaic, pigmented and unpigmented zigzags hairs in chimeric mice*

Chimeras	Chimera no.	Pigmented cells in RPE, %	GPI-1B in blood, %	Mosaic	Pigmented	Unpigmented
$Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$	2	46	64	75	0	25
$+/+\ C/C \leftrightarrow +/+\ c/c$	13	52	64	78	12	10
$Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$	7	92	63	73	2	25
$+/+\ C/C \leftrightarrow +/+\ c/c$	14	52	44	60	33	7

In each chimera 100 zigzags plucked from the pigmented coat area were studied: in chimeras nos 2 and 13 – from the central-lateral area, and in chimeras nos 7 and 14 – from the central-dorsal area.

Table 3. *Percentage of zigzags hairs completely pigmented and with different lengths of unpigmented portion of the apical segments in* $Mi^{wh}/+$ Gpi-1$^{bb} \leftrightarrow Mi^{wh}/Mi^{wh}$ Gpi-1^{aa} *chimeras and* $Mi^{wh}/+$ *mouse*

		Body areas									
		Dorsal					Lateral				
Chimera no.	GPI-1A in blood, %	I	II	III	IV	V	I	II	III	IV	V
2	17	8	10	55	23	4	4	1	17	59	19
4	50	6	1	68	20	5	0	0	13	37	50
5	57	9	1	68	20	2	0	0	0	15	85
6	67	6	1	57	27	9	2	4	7	14	73
$Mi^{wh}/+$ mouse	0	39	1	44	16	0	6	1	32	45	16

In each area of the animal 100 zigzags were studied. GPI-1A – genetic marker of the Mi^{wh}/Mi^{wh} component. I – hairs are completely pigmented; II, III, IV, V – the unpigmented portion is equal to 1–25%, 26–50%, 51–75% and 76–100% of the length of the apical segment, respectively.

in $Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$ chimeras than in $+/+\ C/C \leftrightarrow +/+\ c/c$ animals (Table 2).

Thus, in $Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$ mice, in which the $Mi^{wh}/+$ component markedly prevailed, the fur color was much diluted as compared to that in $+/+\ C/C \leftrightarrow +/+\ c/c$ chimeras in which the percentages of cells of the parental genotypes were approximately equal. This was due to both a large number of unpigmented areas and a higher percentage of unpigmented hairs in pigmented areas in $Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$ mice as compared to that in $+/+\ C/C \leftrightarrow +/+\ c/c$ chimeras (Tables 1, 2).

(ii) *Pigmentation patterns of* $Mi^{wh}/+\ Gpi\text{-}1^{bb} \leftrightarrow Mi^{wh}/Mi^{wh}\ Gpi\text{-}1^{aa}$ *chimeras*

All eight 20-day-old $Mi^{wh}/+\ Gpi\text{-}1^{bb} \leftrightarrow Mi^{wh}/Mi^{wh}\ Gpi\text{-}1^{aa}$ chimeras had coat color patterns resembling those of $Mi^{wh}/+$ mice. The feet and tail tip were unpigmented, and there was a white spot on the belly. Almost all chimeras had also white spotting in the lumbar and femoral areas. Such white spotting in $Mi^{wh}/+$ mice occurs very seldom. There was a correlation between the white spotting and the content of unpigmented cells in RPE and GPI-1A in blood. For instance, chimera no. 6 (Table 3), in which the percentage of Mi^{wh}/Mi^{wh} cells was the highest (unpigmented cells in RPE – 63%, GPI-1A in blood – 67%), also had more white spotting than other chimeras.

Analysis of zigzags plucked from various areas of the integument (dorsal and lateral) showed that all chimeras had two types of hairs: completely and partly pigmented. As a rule, the distal part of the apical segment of zigzags was unpigmented, but in some cases the unpigmented part included also the middle or basal segment. In such chimeras we did not observe mosaic hairs when pigmented cells alternated with unpigmented ones.

In the dorsal area of the integument of the chimeras partly pigmented zigzags prevailed and relatively few were completely pigmented (6–9%). At the same time, the $Mi^{wh}/+$ heterozygote contained 39% of completely pigmented hairs in this area (Table 3). In the lateral area of the integument of the $Mi^{wh}/+ \leftrightarrow Mi^{wh}/Mi^{wh}$ chimeras many zigzags had no pigmentation of the whole apical segments but the percentage of hairs pigmented completely or having a small unpigmented distal portion of the apical segments decreased. In the $Mi^{wh}/+$ mice such transgression is less pronounced. This is especially clearly seen from comparison of hairs of a $Mi^{wh}/+$ mouse with chimeras nos 4 and 5 in which the parental components are present in equal or almost equal ratio. In the lateral

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area of the body of the chimeras the percentage of zigzags with completely unpigmented apical segments was several times higher than in $Mi^{wh}/+$ mice (Table 3).

4. Discussion

Inherited white spotting in mammals may have several causes. Mintz (1967, 1970) suggests the presence of some melanoblast clones genetically programmed to die prior to migration. Based on the experiments for the expression of white spotting genes, Schaible (1968, 1969) concluded that these genes could inhibit proliferation of stem melanoblasts. Other authors believe that melanoblasts having a normal proliferative activity and migration ability cannot differentiate in certain integument areas (Rawles, 1955; Markert & Silvers, 1956). Taking into account the many spotting genes described in the mouse (Green, 1989), we can strongly suggest that such mechanisms of the gene expression determining white spotting really take place.

The result of the present work shows that in all $Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$ chimeras the fur color is much lighter than in $+/+\ C/C \leftrightarrow +/+\ c/c$ mice. Even in the cases where in many tissues of $Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$ chimeras the $Mi^{wh}/+$ component was over 80%, pigmented coat areas did not exceed 50% and had a lighter color than those in $+/+\ C/C \leftrightarrow +/+\ c/c$ animals (Table 1). This is due to the presence of a lot of unpigmented hairs in pigmented areas in $Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$ (Table 2).

The data obtained suggest that the Mi^{wh} gene reduces the proliferative activity of melanoblasts. The reduction in the number of $Mi^{wh}/+\ C/C$ melanoblasts, as compared to the number of $+/+\ c/c$ melanoblasts, results in lightening of fur color in $Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$ chimeras as compared to $+/+\ C/C \leftrightarrow +/+\ c/c$ mice. This fact may also explain the lack of pigmentation on the feet both in $Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$ chimeras and in $Mi^{wh}/+$ mice. The results agree well with Pratt's (1983) data indicating that the Mi^{wh} gene in the heterozygous state decreases the number of melanocytes.

Based on an analysis of $Mi^{wh}/Mi^{wh} \leftrightarrow +/+$ chimeras, Mintz (1970) supposed that the white spotting in $Mi^{wh}/+$ mice was due to a genetically programmed death of some clones of $Mi^{wh}/+$ melanoblasts. However, we have observed the presence of few pigmented hairs on the belly of $Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$ chimeras where an unpigmented spot is usually located in $Mi^{wh}/+$ heterozygotes. These data demonstrate that a single dose of the Mi^{wh} gene does not affect the rate of migration or survival of particular melanoblast clones. This suggests that in $Mi^{wh}/+\ C/C \leftrightarrow +/+\ c/c$ chimeras the presence of some non-Mi^{wh} cells in the skin of the belly allows $Mi^{wh}/+$ pigment cells to develop.

In hair follicles of Mi^{wh}/Mi^{wh} mice there are no melanocytes. This is probably due to the blockage of the proliferation of stem melanoblasts in the homozygotes for mutant alleles of the *mi* locus. It should be noted that in $Mi^{wh}/+ \leftrightarrow Mi^{wh}/Mi^{wh}$ mice there are no mosaic hairs because only one population of $Mi^{wh}/+$ melanoblasts is present.

Our findings show that despite the high percent of the Mi^{wh}/Mi^{wh} component in some $Mi^{wh}/+ \leftrightarrow Mi^{wh}/Mi^{wh}$ chimeras they have the white spotting phenotype seen in $Mi^{wh}/+$ mice. It may indicate that in most skin areas, except the certain area on the belly, neither the dermis nor the epidermis of the Mi^{wh}/Mi^{wh} genotype is able to suppress the proliferation or differentiation of the $Mi^{wh}/+$ melanoblasts (melanoblasts of the Mi^{wh}/Mi^{wh} genotype are absent in such chimeric mice). These data do not agree with the observation of Pratt (1982) who concluded that embryonic Mi^{wh}/Mi^{wh} skin could block the development of $+/+$ melanoblasts. This is most consistent with the interpretation that in such chimeras the $Mi^{wh}/+$ melanoblasts actively proliferate and spread through the dermis as if in an empty space because other melanoblast clones are absent.

Similar results have been obtained by other authors who generated aggregation chimeras using embryos that differ in their *W* locus genotype. The pigmentation patterns in $W/W \leftrightarrow +/+$ chimeras are similar to those in heterozygotes for *W* gene (Mintz, 1970; Stephenson *et al.* 1985; Nakayama *et al.* 1990). The lack of coat pigmentation in *W* mutants results from the absence of an endogenous melanoblast population (Silvers, 1956; Mayer & Green, 1968; Huszar *et al.* 1991).

It is interesting to note that in $Mi^{wh}/+ \leftrightarrow Mi^{wh}/Mi^{wh}$ chimeras there are many partly pigmented hairs: basal and middle segments of zigzags are pigmented but the apical segments are partly or completely unpigmented. Also, both the number of partly pigmented hairs and the length of the unpigmented portion of apical segments in them increase with increase of the Mi^{wh}/Mi^{wh} component in the chimeric organism. In such chimeras the percentage of zigzags with completely unpigmented apical segments was several times higher than in $Mi^{wh}/+$ mice.

The simplest explanation is that the number of $Mi^{wh}/+$ stem melanoblasts in $Mi^{wh}/+ \leftrightarrow Mi^{wh}/Mi^{wh}$ embryos is less than in $Mi^{wh}/+$ embryo. Therefore the $Mi^{wh}/+$ melanoblasts in the chimeras finish mitotic divisions later than in $Mi^{wh}/+$ mice because the required number of cells which form the fixed melanoblast population is provided by proliferative cell cycles. An active transition of melanoblasts to the differentiated state and the beginning of the melanin synthesis take place also later in the hair follicles of $Mi^{wh}/+ \leftrightarrow Mi^{wh}/Mi^{wh}$ chimeras as compared to the $Mi^{wh}/+$ mice. This may be the cause determining the lack of pigmentation of apical segments in many zigzags of the chimeras. The lack of the feet

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pigmentation in $Mi^{wh}/+ \leftrightarrow Mi^{wh}/Mi^{wh}$ mice may also be due to a deficiency in the melanocytes.

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References

Gray, P. (Ed) (1973). *The Encyclopedia of Microscopy and Microtechnique* p. 338. Cincinnati, Toronto, London, Melbourne: Van Nostrand Reinhold.

Green, M. C. (1989). Catalog of mutant genes and polymorphic loci. In *Genetic Variants and Strains of the Laboratory Mouse*, 2nd edn. (ed. M. F. Lyon and A. G. Searle), pp. 12–404. New York: Oxford University Press.

Huszar, D., Sharpe, A. & Jaenisch, R. (1991). Migration and proliferation of cultured neural crest cells in *W* mutant neural crest chimeras. *Development* **112**, 131–141.

Kindyakov, B. N., Konyukhov, B. V. & Malinina, N. A. (1984). Study of mutant gene expression in mouse aggregation chimeras. 1. The *white* gene. *Ontogenez* **15**, 153–162. (in Russian).

Konyukhov, B. V., Kindyakov, B. N., Malinina, N. A. & Sazhina, M. V. (1986). A study of mutant gene expression in mouse aggregation chimeras. 6. Analysis of the *white* gene effects by a histochemical marker. *Ontogenez* **17**, 389–395. (in Russian).

Konyukhov, B. V. & Osipov, V. V. (1968). Interallelic complementation of the genes *microphthalmia* and *white* in mouse. *Genetika* **4** (11), 65–75. (in Russian).

Konyukhov, B. V. & Sazhina, M. V. (1980). Allelic complementation at the *mi*-locus of the mouse: partial normalization of the retinal pigment epithelium development. Biologisches Zentralblatt **99**, 571–584.

Markert, C. L. & Silvers, W. K. (1956). The effects of genotype and cell environment on melanoblast differentiation in the house mouse. *Genetics* **41**, 429–450.

Mayer, T. C. & Green, M. C. (1968). An experimental analysis of the pigment defect caused by mutations at the *W* and *Sl* loci in mice. *Developmental Biology* **18**, 62–75.

Mintz, B. (1967). Gene control of mammalian pigmentary differentiation. 1. Clonal origin of melanocytes. *Proceedings of the National Academy of Sciences U.S.A.* **58**, 344–351.

Mintz, B. (1969). Gene control of the mouse pigmentary system. *Genetics* **61**, (Supplement), 41.

Mintz, B. (1970). Gene expression in allophenic mice. In *Control Mechanisms in the Expression of Cellular Phenotypes* (ed. H. A. Padykula), pp. 15–42. New York: Academic Press.

Mintz, B. (1971). Allophenic mice of multi-embryo origin. In *Methods in Mammalian Embryology*, (ed. J. C. Daniel), pp. 186–214. San Francisco: Freeman.

Mintz, B., Gearhart, Y. D. & Guymont, A. O. (1973). Phytohemagglutinin mediated blastomere aggregation and development of allophenic mice. *Developmental Biology* **31**, 195–199.

Nakayama, H., Ru, X.-M., Fujita, J., Kasugai, T., Onoue, H., Hirote, S., Kuroda, H. & Kitamura, Y. (1990). Growth competition between *W* mutant and wild-type cells in mouse aggregation chimeras. *Development, Growth and Differentiation* **32**, 255–261.

Pratt, B. M. (1982). Site of gene action of the *white* allele (Mi^{wh}) of the *microphthalmia* locus: a dermal-epidermal recombination study. *Journal of Experimental Zoology* **220**, 93–101.

Pratt, B. M. (1983). Effects of microphthalmic *white* (Mi^{wh}) on the number function of cutaneous melanocytes. *Journal of Experimental Zoology* **225**, 329–335.

Rawles, M. E. (1955). Pigmentation in autoplastic and homoplastic grafts of skin from fetal and newborn hooded rats. *American Journal of Anatomy* **97**, 79–128.

Schaible, R. (1968). Development of transitory piebald-spotted and completely pigmented patterns in the chicken. *Developmental Biology* **18**, 117–148.

Schaible, R. H. (1969). Clonal distribution of melanocytes in piebald-spotted and variegated mice. *Journal of Experimental Zoology* **172**, 181–199.

Shows, T. B. & Ruddle, F. H. (1968). Malate dehydrogenase: Evidence for tetrameric structure in *Mus musculus*. *Science* **160**, 1356–1357.

Silvers, W. K. (1956). Pigment cells: occurrence in hair follicles. *Journal of Morphology* **99**, 41–55.

Stephenson, D. A., Glenister, P. H. & Hornby, J. E. (1985). Site of the *beige* (*bg*) and *leaden* (*ln*) pigment gene expression determined by recombinant embryonic skin grafts and aggregation mouse chimeras employing *sash* (W^{sh}) homozygotes. *Genetical Research* **46**, 193–205.

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