The effects of the pink-eyed unstable gene on the retinal pigment epithelium of the mouse

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SUMMARY

Pink-eyed unstable (\(p^{un}\)) is an autosomal gene in the mouse, causing variegation of the coat. In some melanocytes it functions as the normal allele \(p^+\), producing dark pigment, and in others as the mutant \(p\), producing light pigment. As a study of another unstable gene at a different locus had shown that the instability was strongly influenced by the tissue environment, it seemed desirable to find out whether this also applied to \(p^{un}\).

An examination of the retinal pigment epithelium, the only structure in mammals in which it is practicable to determine the position of individual melanocytes, showed that the distribution of dark and light cells in \(p^{un}p^{un}\) animals was not random. The dark cells increased in frequency with the distance from the optic nerve, suggesting that the tissue environment was a factor in the instability of the gene (i.e., in its rate of mutation), although the increase was less striking than in the other mutant.

It is generally assumed that when \(p^{un}\) behaves as \(p^+\) it is a case of reversion, and that reversion can also occur in germ cells, the revertant \(p^+\) allele subsequently behaving as a stable gene. It is here argued that it is unlikely to be a case of reversion, and that the evidence for the involvement of the germ line is inconclusive. Further, it is suggested that the phenotype of \(p^{un}p^{un}\) animals is probably an instance of Position Effect variegation, the instability resulting from some chromosomal alteration, which is too small to be cytologically detectable.

INTRODUCTION

There are several instances in the mouse of what appear to be genes with an extremely high rate of somatic mutation (Silvers, 1979). They are generally referred to as unstable genes. They lead to mosaicism, which is inherited in the normal autosomal manner. Nearly all the unstable genes that have been discovered so far affect pigmentation, the reason presumably being that a variegated coat is a striking feature. One such gene, chinchilla-mottled (\(c^n\)), has been investigated in detail (Deol & Truslove, 1981). In this case the chinchilla (\(c^{ch}\)) allele seems to change into the albino (\(c\)) allele in a proportion of pigment cells. The frequency of this change shows clear regional differences. Not only is the belly of \(c^n c^n\) animals much lighter than the back, but when the entire coat is very light the proportion of unpigmented (\(cc\)) cells in the retinal pigment epithelium (RPE) is far lower than expected. Further, the vast majority of these unpigmented cells in the RPE are concentrated in the centre of the eye cup.

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especially around the optic nerve, there being few if any near the rim. These observations suggest that tissue environment has a significant influence on the rate of mutation. It seemed desirable to find out whether this is also true of other unstable genes, and pink-eyed unstable \( (p^{un}) \) appeared to be a suitable mutant for this purpose.

The gene \( p^{un} \) arose spontaneously in the inbred strain C57BL/6J (Wolfe, 1963), which is phenotypically black and genotypically normal at the pink-eyed locus \( (p^+p^+) \). In some melanocytes it produces dark pigment which is characteristic of the normal \( (p^+) \) allele, and in others light pigment which is characteristic of the mutant \( (p) \) allele. The resulting coat is an intermixture of normal, mutant type, and intermediate hairs, but as the gene is very sensitive to the genetic background, \( p^{un}p^{un} \) animals may have a wholly normal or a wholly mutant type coat. The eye colour shows a similar variation.

MATERIAL AND METHODS

The RPE, which extends into the ciliary body and the iris, is ideal for a study of the distribution of different types of melanocytes (Deol & Whitten, 1972; Deol & Truslove, 1981). It is the only structure in mammals in which the melanocytes form a continuous layer, a single cell thick. This makes it possible to determine the position of individual cells with considerable accuracy.

The gene was kindly supplied by Dr A. G. Searle, MRC Radiobiology Unit, Harwell. As the aim was to count and determine the position of cells of one particular type, the genetic background chosen was such that normal (dark) cells were sufficiently infrequent to make cell counting practicable. The coat in all cases appeared to be wholly of the mutant type. Altogether, 54 eyes were examined, 44 \( p^{un}p^{un} \) and 10 \( p^{un}p^{un} \). The whole head was fixed in Wittmaack’s fluid, and after decalcification, the eyes, with surrounding structures intact, were cut out. They were embedded in paraffin or celloidin and serially sectioned at 10 \( \mu \text{m} \). Some sections were stained with H & E, while others were simply cleared, as the normal dark cells stand out sharply among the light ones in unstained preparations.

OBSERVATIONS

Identification of the two types of cells was easy (Fig. 1), and both types occurred in all the eyes examined, \( p^{un}p^{un} \) as well as \( p^{un}p^{un} \). Dark cells were counted and their position in the eye determined in 30 \( p^{un}p^{un} \) eyes. The RPE is naturally divisible into three clearly distinguishable regions: the iris, the ciliary body and the retina proper (Fig. 2). The retinal part was further arbitrarily subdivided into three approximately equal parts, proximal (PR), medial (MR) and distal (DR) with reference to the optic nerve. It was found that the frequency of dark cells increased with the distance from the optic nerve. Since the cell numbers and
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distribution were similar in all 30 eyes, the results were pooled and presented in Fig. 2. The total number of melanocytes in the retinal part of the RPE is estimated (from sections) at $5 \times 10^5$ approximately. For technical reasons the number of cells in the ciliary body and the iris is difficult to estimate reliably, but the impression is that it is considerably larger in the ciliary body.

As it is possible that the regional differences in cell numbers might reflect differences in rates of cell division, it seemed desirable to repeat these observations for patches of dark cells. The results were similar to those obtained for individual dark cells (Fig. 2). The patches had a strong tendency to be very small, mostly consisting of only one or two cells. As patch size could have an important bearing on the time of occurrence of the genetic event involved, the number and size of patches in all five regions are shown in Fig. 3.

Fig. 1. Transverse sections of $p^{un}p^{un}$ eyes showing dark cells in the retinal pigment epithelium (A), ciliary body (B) and the inner layer of the iris (C). The iris has two such cells, the others only one each. Bar = 0.05 mm.
The number of dark cells in $p^unp$ eyes was greatly reduced as would be expected, but there did not seem to be any other significant difference from $p^unp$un.

**DISCUSSION**

The distribution of the two types of cells in the RPE, whether we consider individual cells or patches of cells, does not appear to be a random one: pigmented cells are more likely to occur away from the optic nerve that near it. The regional differences, however, are not as striking as in the mutant chinchilla-mottled discussed above. Whatever the genetic event involved, it presumably is influenced by the tissue environment, as no regional differences were observed in mosaics resulting from X-chromosome inactivation or from the fusion of early embryos (Deol & Whitten, 1972; West, 1976; Sanyal & Zeilmaker, 1977). Moreover, as the majority of patches in all regions consist of one or two cells only, this genetic event probably occurs at a late stage in the development of the eye, close to the time of the last cell division in the RPE. However, as Searle's (1978) study
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shows, it can also occur at earlier stages, giving rise to large patches, presumably depending on the genetic background. The presence of some dark cells in all \( p^{un}p \) eyes, when the coat is usually wholly of the mutant type, shows that the absence of dark hairs in the coat is not a reliable guide to the true genotype of the animal. Similarly, it would seem possible that \( p^{un}p^{un} \) animals with a wholly normal coat may also have some light cells in the eyes.

As to the nature of the change involved, Melvold (1971) thought \( p^{un} \) might be an instance of somatic recombination. This is unlikely since apparent reversion of the mutant to the normal allele can take place in the homozygote. Searle (1978) and Silvers (1979) treat it as a case of somatic mutation. This is a possibility, but it has to be reconciled with extremely high rates of mutation (well over 90% of cells may be of the mutant type). Whitney & Lamoreux (1982) suggest that a transposable element might be involved, the mutant phenotype resulting when the element is present and the activity of the normal allele \( (p^+) \) is suppressed, and the normal phenotype appearing when the element is excised and the gene can express itself. As there is no evidence for this, it seems equally
reasonable to assume that this may simply be a case of Position Effect variegation of the type long known in *Drosophila* (Baker, 1968): some chromosomal alteration, too small to be cytologically detectable, may have placed the *p*+ allele adjacent to a heterochromatic region, with the result that it became subject to inactivation in a proportion of cells. When this happens in both the genes, the cell is naturally of the mutant type. The cis-dominance proof of Position Effect (Baker, 1968), that the variegating gene must be on the same chromosome as the breakpoint, is irrelevant since it is implicit in the assumption made. However, there is some circumstantial evidence in favour of this explanation. In *Drosophila*, Position Effect variegation is known to be strongly influenced by the genotype of the mother, being much greater if the mother is a heterozygote (Spofford, 1976). The present mutant behaves in a comparable manner: the 'reversion' rates from the *p*un to the *p*+ allele are more than twice as high in *punpun* animals with heterozygous parents as in those with homozygous ones (Melvold, 1971; Silvers, 1979). Further, Position Effect variegation is strongly influenced by modifying genes (Spofford, 1976), and the same is true of the effects of the *p*un gene (Searle, 1978; Silvers, 1979). Again, it has been seen that tissue environment plays a significant role in the expression of the *p*un gene. The same applies to Position Effect variegation in *Drosophila*: not only are different organs affected to different extents, but there can be clear regional differences within the same organ (Spofford, 1976). Finally, two other alleles at the same locus and with similar phenotypical effects (*p*ml, *p*m2) have been described by Russell (1964), and the fact that these occurred in radiation experiments points towards chromosomal alterations. As Position Effect and transposable elements both involve suppression of gene activity, it would be difficult to distinguish them on the basis of phenotypical evidence alone.

The genetic event underlying the phenotypical effects of the *p*un gene is generally referred to as 'reversion' (Searle, 1978; Silvers, 1979), the assumption being that the unaltered gene is the mutant allele *p* and the resultant gene is the normal allele *p*+. This view does not appear to be justified by the history of the *p*un gene, which suggests forward mutation. The original mutation occurred in a *p*+*p*+ strain (C57BL/6J), and of the two assumptions; (1) that this mutation altered one *p*+ allele in such a way that it now changes into *p* in some cells, or (2) that the original mutation turned *p*+ into *p* which now reverts to *p*+ in some cells, the first appears to be the simpler. It it is indeed a reversion then its extremely high frequency would suggest that the chemical change involved is such as to be readily rectifiable. As the alleles *p*ml and *p*m2, mentioned above, are phenotypically similar, the same would apply to them.

Melvold (1971) and Searle (1978) found two totally dark animals among the offspring of *punpun* parents without any indication of dark pigment in the coat, and Searle (1978) found that a high proportion of high-grade mosaics (with over 30% of the coat dark) produced completely dark offspring, often in large numbers. This is regarded as evidence of gonadal involvement (Searle, 1978). If this
implies that the change from $p^{un}$ to $p^+$ can occur in the germ-line then the evidence must be regarded as inconclusive, because these completely dark animals were either not progeny tested or tested in a manner that is not incontrovertible, and their eyes were not examined for the presence of light cells. The assumption that the germ-line is involved would be valid if such wholly dark animals are bred with $pp$ animals from at least two different stocks, and the offspring are either wholly dark or wholly mutant type, and never mosaic. Should it turn out that the germ-line is affected, the Position Effect explanation would naturally not be valid. However, in that case, according to the view presented here (that the mosaic phenotype results from the conversion of $p^+$ into $p$), it would be the wholly mutant type animals, not the dark ones, that would represent the affected gametes.

Whitney & Lamoreux (1982) have listed a number of genes in the mouse that might be instances of transposable elements. Whether we are here dealing with transposable elements or Position Effect variegation or somatic mutations of some other type, the phenomenon probably occurs in other mammals as well. But such genes are only likely to be identified if their action is cell autonomous (or otherwise localized), the resulting phenotype is easily distinguishable, and large samples are examined routinely. In man these considerations are best exemplified by the locus governing the ABO blood-group system. A search of the literature revealed seven families in which mosaicism of this system is inherited, and for which no conventional explanation can be found (Race & Sanger, 1975; Marsh, Nichols, Øyen, Decary, Whitsett & Ethridge, 1975; Bird, Wingham, Watkins, Greenwell & Cameron, 1978). All these cases are consistent with suppression of gene activity of the same type as discussed here.

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REFERENCES


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