ARTICLE

The retinal pigment epithelium as a developmental regulator of the neural retina

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Abstract

Melanin-related agents regulate the development of the mammalian neural retina, because in albinos there are a range of retinal deficits including abnormal connections between the eye and brain, an underdeveloped central retina and a rod deficit. These deficits may arise because gradients of retinal development in the albino are delayed and the retina is abnormally proliferative, but also goes through a subsequent period of excessive cell death. This may be caused by a reduction in ocular DOPA in albinos as this is in the synthetic pathway of melanin and is a known cell cycle regulator.

Key words Albinism, Cell cycle, Development, Retina

A melanin-related agent in the mammalian retinal pigment epithelium (RPE) has a strong influence over the developing neural retina because when melanin is absent a range of retinal abnormalities are present at maturity. These include a systematic re-routing of chiasmatic fibres in favour of the crossed projection,[1] an underdevelopment of the central retina[2] and a deficit in rod numbers that leaves cone numbers and their mosaic distribution unaffected.[3]

Melanin production is controlled by the tyrosinase gene, which regulates melanin expression in both the eye and the skin, although it does not regulate melanin production at other locations such as the substantia nigra. Here, melanin production is regulated by tyrosine hydroxylase. A simplified diagram of the early stages of ocular melanin synthesis is given in Fig. 1. Tyrosinase acts as a catalyst both in the production of DOPA from tyrosine and in the subsequent production of dopaquinone from DOPA. The deficits found in albinos can be corrected in transgenic animals by introduction of a tyrosinase gene on an albino background.[4,5] Albino animals in the laboratory frequently lack a functional tyrosinase gene; however, it is possible to be albino with a functional tyrosinase gene. This is particularly true in some cases of human albinism, the genetic basis of which can be complex and has not been investigated.[6] Such individuals have the same retinal deficits as individuals who are tyrosinase negative. These cases demonstrate that disruption to the synthetic pathway of melanin in more than one place can have similar effects for the developing visual system.

Although Elschnig in 1913[2] was the first to clearly identify that the fovea was absent from a human albino eye and that the macular region was underdeveloped, and as such was the first to demonstrate a central nervous system deficit in albinos, the majority of scientific interest has focused on the abnormal chiasmatic projections that were revealed much later by Lund in 1965.[7] This was in the hope that this aspect of the abnormality would provide an insight into the mechanism of axon guidance at critical stages of neuronal pathway selection. In spite of the

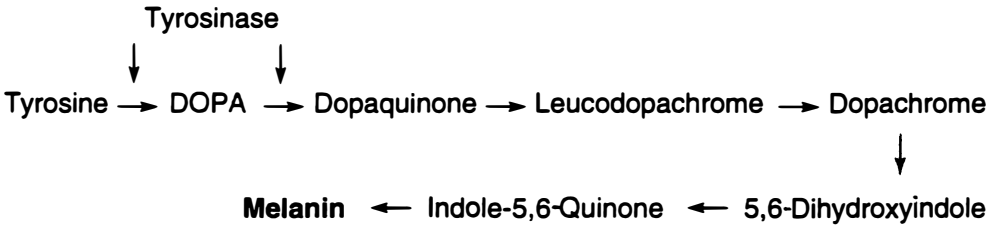

Fig. 1. *A simplified schematic representation of the synthetic pathway of melanin. In many forms of albinism, tyrosinase, which catalyses the reaction between tyrosine and DOPA and between DOPA and dopaquinone, is absent. However, it is possible for an organism to be hypopigmented with a functional tyrosinase gene. This can occur in humans, but the genetic basis of such cases is highly variable and complex.*

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considerable efforts made to unravel chiasmatic development in albino animals, little has been gleaned about the factors that shape this region. However, with a change of scientific attention towards an analysis of retinal development in albinos, advances are being made that might allow us to understand the mechanisms that generate the wide range of abnormalities found in albinism, and as such are revealing the role of melanin in sculpting the normal retina.

Retinal deficits in albinos

In mammals that have specialised visual systems there is a relatively sharp vertical line dividing retinal ganglion cells in each eye that project to different sides of the brain: the naso-temporal division. This passes through the central retina, including the fovea in primates.[8] In albinos there is a systematic shift in this line such that ganglion cells in the temporal retina that are adjacent to the line and which should project ipsilaterally are re-routed at the optic chiasm and project contralaterally. As a consequence of this the regions of termination of ipsilaterally projecting fibres in the lateral geniculate nucleus and superior colliculus are significantly smaller in albinos than in pigmented animals. Such abnormal patterns of decussation result in a disruption of the pathways that subserve normal binocular vision, as the abnormal routing of optic fibres results in a disruption to pattern registration between retinal maps from each eye in the lateral geniculate nucleus.[1] In mammals with less specialised visual systems, such as rodents, ipsilaterally projecting cells are still confined to the temporal retina, but here they are mixed with cells that project contralaterally. In spite of the two populations being spatially mixed, in albino rodents there is a clear reduction in the number of cells that project ipsilaterally, which are located in a smaller region of the temporal retina.[9]

It was originally thought that the abnormal chiasmatic pathways arose because optic fibres in albinos adopted ectopic locations in the initial segment of the developing optic nerve, the optic stalk, because dorsal parts of the stalk are pigmented during the initial stages of axon outgrowth. In albinos this dorsal region of the stalk is not pigmented and it was thought that developing axons would be free to occupy regions that would otherwise contain melanocytes.[10] Hence, there would be regions containing optic fibres in albinos that were fibre-free in normally pigmented animals. The significance of this observation rested on the notion that spatial factors were critical for determining whether a fibre crossed or did not cross the chiasmatic midline. However, it is now clear that in the animal models used spatial factors do not play a significant role in pathway selection, and that when fibres are traced, no significant differences can be found in fibre order or the regions containing fibres between pigmented and albino animals. Further, not all pigmented mammals have a pigmented optic stalk in development, but albino strains of these animals exist with abnormal chiasmatic pathways.[11]

Explanations of the chiasmatic abnormality based upon differences in the spatial order of optic fibres have been further undermined by studies undertaken directly on the developing chiasm. In developing rodents, fibres from each eye interact with one another and midline chiasmatic glia, the processes of which span the depth of the chiasm. These interactions occur in specific time windows and are significant in determining pathway selection.[12] No differences have been found between pigmented and albino animals in either the developing architecture of this region or the patterns of axon growth through it. Also the capacity of midline glia to signal axon divergence has been tested *in vitro* by growing retinal explants from pigmented animals on dissociated chiasmatic cells from either pigmented or albino animals. No differences were found in patterns of axon growth between tissue from the different animal groups.[12] These experiments strongly indicate that the location where this aspect of the abnormality is specified is the retina and not the chiasm.

The retinal deficits found in albinos are present in each of the cellular layers of the retina. Cell density is abnormally low in the central retina in animal models of albinism, with ganglion cell numbers reduced by approximately 25%, resulting in reduced acuity.[13] Also, the inner nuclear layer is abnormally thin in central regions[14] and there is a panretinal rod deficit of the order of 30%. The latter deficit is particularly interesting in that although rod numbers are reduced the cone population is unaffected; hence within the outer nuclear layer the deficit is cell-specific. The reduction in rod numbers is not due to light damage because the deficit has been demonstrated in animals that have been dark-reared as well as those reared under normal light conditions.[3]

There is evidence from comparative studies that rods may play a special role in the development of the albino abnormality. Birds have a highly specialised central retina where there is a marked increase in cell density in all retinal layers, but their retinae, unlike those of most mammals, are cone-dominated not rod-dominated.[8] Surprisingly in albino birds there is no deficit in central retinal regions.[15] There are only two types of mammal that are known to have a cone-dominated retina: tree shrews and squirrels. Albino squirrels are rare but do exist in the wild. Here again, the central retina is relatively normal. Also, these animals are the only known examples of an albino mammal to survive and breed successfully in the wild (Esteve, personal communication), adding weight to the notion that they do not have significant visual deficits. These studies imply that a cone-dominated retina provides immunity from the retinal deficits found in albinism. But it does not explain why the ganglion cell layer and inner nuclear layer of these animals are normal.

Normal retinal development

If light is to be cast upon the melanin-related agent whose absence is responsible for the deficits found in albinism, it is important to trace retinal development in

both pigmented and albino animals to identify when abnormalities first appear in the albino eye and relate these to melanin or its synthesis. The normal retina develops with a rough centre-to-periphery gradient. In rodents and carnivores, which have been the main animal models in this area of research, the gradient is focused around the optic nerve head such that at each stage of development central regions are more mature than those peripheral to them.[16] The only exception to this is the accumulation of melanin in the RPE, which has a reverse gradient with melanin appearing first at the retinal margin and progressing developmentally towards the central retina.[17] In spite of this, it appears that the tyrosinase gene is expressed uniformly across the RPE early in development and does not reflect this reverse gradient.[18]

The RPE is developmentally advanced in relation to the neural retina, in that at any location cell addition in the RPE is complete before that in the neural retina.[19] During development nuclei of cells in the neural retina that are in the cell cycle move between the vitreal surface and the ventricular surface adjacent to the RPE. Cells undergo mitosis when they are adjacent to the relatively mature RPE,[17] and there is evidence that at this point they form transitory gap junctions with cells in the RPE.[20] This may represent a point at which the RPE has an opportunity to influence patterns of cell addition in the neural retina.

Cell addition in the neural retina occurs in two overlapping phases from a population of cells that are multi-potential. Cells produced in the first phase include cones, ganglions cells, horizontal cells and amacrine cells. The main wave of cell production occurs in the second phase when the more numerous rods and bipolar cells are produced along with horizontal cells and Müller glia. The retinal layers differentiate from the vitreal surface downwards within the centre-of-periphery gradient, such that the ganglion cell layer at central locations is the first layer to differentiate and the outer nuclear layer at the retinal periphery is the last. However, cell production and retinal differentiation are separate processes that overlap spatially, such that at any retinal location the ganglion cell layer may have differentiated but at the same location cells destined to become rods and bipolar cells are still within the cell cycle. Hence, retinal development occurs via a series of overlapping waves operating over a central-to-peripheral gradient.[21–23]

Development of the albino retina

There are data from a number of laboratories indicating that one of the main differences between pigmented and albino animals is a temporal lag in the centre-of-periphery pattern of retinal maturation in albinos, indicating that the significant agent missing in albinos may be regulating an aspect of the temporal domain in development. It has been shown that in postnatal albino rodents there is a delay in the formation of the plexiform layers and that these do not expand evenly but develop irregularly across the retinal surface. Also, the normal patterns of cell death appeared to be delayed.[24] These data are supported by an additional study that demonstrated quantitatively, using pulsed [^{3}H]thymidine, that there was a delay in patterns of cell addition in the ganglion cell layer of prenatal albino rodents of the order of 2 days when compared with pigmented animals. This was most marked later in development during the period when rod production was taking place.[25] Further, there is a delay in the innervation of retino-recipient nuclei in the thalamus and the brain stem of Siamese cats when compared with normally pigmented cats, consistent with the finding that there is a delay in ganglion cell production in hypopigmentation.[26]

The demonstration of a delay in retinal maturation may be of importance in analysing the abnormal chiasmatic pathways in albinos. Numerous factors influence pathway selection at the chiasm. One of these is the time at which ingrowing axons enter the chiasmatic environment. Although patterns of ganglion cell production follow a rough centre-to-periphery pattern, the cells destined to project ipsilaterally are generated prior to those that will give rise to the crossed projection from similar retinal regions. In rodents where the cells that give rise to both projections are mixed in the temporal retina, ipsilaterally projecting cells are generated approximately around embryological day 13, while those that give rise to the contralateral projection are generated around embryological day 15.[27] Hence, the probability of projecting ipsilaterally in the temporal retina declines with time. This difference of 2 days in the periods of cell production for the two populations is similar to the delay found in the patterns of cell production in the ganglion cell layer in albino rat retinae when compared with pigmented animals.[25]

This temporal correlation does not prove a functional relationship, but it does provide a framework within which future experiments might be undertaken. Unfortunately, it would not be possible to extend these studies in rodents by comparing patterns of ganglion cell production in pigmented and albino animals in relation to chiasmatic pathways because within the temporal retina these cells are spatially mixed.[9] Hence, it would not be possible to distinguish which cells have adopted an abnormal crossed chiasmatic pathway from those that normally project contralaterally. These experiments need to be undertaken in an animal that has a sharp naso-temporal division so that one can examine the birthdates of cells just temporal to the area centralis that have an aberrant contralateral projection.

A delay or temporal disruption in patterns of retinal cell production might also result in the underdevelopment of the central retina. It has been proposed that the mature gradient in cell density across the retina might depend on the spatio-temporal gradients in patterns of cell production.[28] If this were correct, and if the magnitude of the delays varied, it might explain why differences exist in cell density in the central retina between pigmented and albino strains. But until the development of the central retina is traced in a species

with a marked gradient in cell density and in which there is an established albino strain, it will not be possible to determine whether there is any validity in this hypothesis.

Although pulsed [^{3}H]thymidine has revealed a delay in the centre-to-periphery gradient of maturation in albinos,[25] such studies suffer from the fact that they label cells in development but examine them at maturity. Hence, they are only a reflection of developmental events. A very different story has been revealed by examining patterns of mitosis directly in prenatal albino retinae. This has shown that there are many more mitotic profiles during development in albinos compared with pigmented animals in both the neural retina and the RPE. These follow a centre-to-periphery gradient, with the peak in mitotic profile numbers occurring at the same time in the two animal types, but there are almost twice as many profiles in albinos at each stage of development when compared with pigmented animals. This results in an abnormal thickening of the albino retina followed by the presence of an elevated number of pyknotic profiles that bring cell numbers down to the reduced levels found in the adult. The elevated patterns of mitosis are not simply due to strain differences independent of melanin, as significantly more mitotic figures are present in a range of albino rats. Further, they are peculiar to the eye as there are no differences in patterns of mitosis in the brains of these animals. These results show that the melanin-related agent that is missing in albinism influences not only the pace of mitosis but also its magnitude.[29]

In both pigmented and albino rats the peak in the number of mitotic figures occurs around the day of birth, which is post-conceptional day 22. At this stage the majority of cells coming out of the cell cycle are destined to become rods and bipolar cells.[22,23] The excessive mitosis found in bipolar retinae at this stage and the resulting retinal thickening and elevated cell death may be the explanation for the reduction in thickness of the inner nuclear layer and the reduction in rod numbers.[3,14] It would also explain why cones are not affected, as these are generated in the first phase of retinal cell production when proliferative abnormalities are less marked.[22,29]

It is unlikely that melanin itself could be the agent influencing these events because significantly increased patterns of mitosis are found in albino rats from post-conceptional day 13 in the neural retina and post-conceptional day 12 in the RPE.[29] At these stages there is little or no melanin present in the retina or eye, although the tyrosinase gene is expressed then.[18] Hence, the agent has to be in the synthetic pathway of melanin or associated with elements in it.

The majority of chemical agents in the synthetic pathway of melanin are relatively inactive. However, DOPA, which is present at very early stages, is a pharmacologically active agent. Interestingly the histological test used to determine whether individuals are tyrosinase negative or positive is actually the test used to identify DOPA with which tyrosinase is associated.[30] High-performance liquid chromatography measurements have confirmed that DOPA levels are significantly reduced in the developing and mature albino eye.[29]

Experiments undertaken over a number of years have revealed that DOPA plays a significant role in regulating the cell cycle and that its effects differ depending on whether cells are pigmented or not. When DOPA is applied *in vitro* to cultures of developing RPE cells it lengthens the cell cycle from 19 to 27 h. The cell cycle is arrested in a dose-dependent manner in the S phase and there is a decrease in the number of cells in the G_1 phase.[31] Furthermore, DOPA has been used as an anti-mitotic agent in the treatment of some forms of experimental cancer. When DOPA is applied to human and mouse melanoma cell lines, where the synthetic pathway of melanin is the same as in the RPE, it is a highly selective inhibitor of growth.[32]

When these results are taken together with those showing that the albino retina is excessively mitotic and lacking in DOPA, a pattern emerges that may explain the abnormalities found during development. If there is a relationship between retinal DOPA and mitosis *in vivo*, then it should be possible to arrest the excess proliferation in the albino by DOPA administration. Experiments addressing this question have not produced a clear result. In part this is due to the difficulty of administering the drug to pregnant albino rats such that a known and fixed amount enters the ocular environment throughout retinal maturation. In spite of this significant results have been obtained by removing eyes when mitotic activity is at its peak and maintaining them in organ culture to which the drug is added. In this situation it is possible to reduce mitotic activity in albinos to that found in normal pigmented controls in less than 7 h.[29] Hence, DOPA is regulating patterns of mitotic activity in the retina.

Although considerable advances have been made in our understanding of the influence that the RPE has over the developing neural retina, many questions remain unresolved. No proof has been supplied as yet to show that abnormal patterns of mitosis give rise to the abnormalities found in the mature albino. Further, although it is clear that there is a relationship between DOPA and retinal mitosis, the mechanism by which this occurs is obscure. It is not known whether DOPA regulates the cell cycle alone or whether it acts as a signalling mechanism to initiate withdrawal from the cell cycle and induce cellular differentiation. Whichever of these aspects it regulates, how is it acting? The vast majority of DOPA in the central nervous system will be contained within cells rather than in the extracellular environment. Perhaps it influences mitosis by regulating the opening and closing of the gap junctional connections that are known to exist between RPE cells and mitotic profiles in the neural retina. Many of these questions will only be answered when there is a deeper understanding of the chemical environment of the developing retina and a better understanding of the signals that regulate cell cycle events.

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