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Retinal Cell Addition and Rod Production Depend on Early Stages of Ocular Melanin Synthesis

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ABSTRACT

Retinal mitosis is regulated by dopa, a melanin precursor present in the developing retinal pigment epithelium. Its absence results in retinal deficits including a failure of ≈30% of the rod population to develop. Here, ^{3}H-thymidine labelling is used to analyse patterns of cell addition spanning the main period of retinal development in rat litters containing both pigmented and albino phenotypes. Many more thymidine-labelled cells are found in each cellular layer at maturity in albinos than in their pigmented littermates. Normal spatial patterns of photoreceptor addition are seen in albinos during cone production and for most of the subsequent period of rod addition. However, abnormal spatial patterns of cell addition occur across the retinal when rod production peaks. A delay in the centre to periphery gradient of cell addition is apparent in both nuclear layers. These data are related to deficits in the mature architecture of the albino retina. The results are consistent with there being significant cell cycle and/or exit point irregularities in hypopigmented retinae. It is probable that reduced dopa levels in albinos result in the cell cycle rate not slowing appropriately with development, which may lead to cells missing their exit points. This produces abnormal patterns of cell addition at key stages and delays in the gradient of retinal maturation along with a large cell loss at critical stages of rod production. J. Comp. Neurol. 420:437–444, 2000.

Indexing terms: Indexing terms: mitosis; albino; retinal pigment epithelium

The presence of an early melanin precursor, probably dopa, is critical for normal retinal development. When initial stages of melanin biosynthesis in the retinal pigment epithelium (RPE) fail, deficits appear in the developing neural retina. Approximately 30% of the rod population does not develop, cell density in central regions is abnormally low, and the pattern of connections between the eye and the brain are systematically disrupted (Jeffery et al., 1997; Jeffery, 1997 review).

During albino retinal development, cell proliferation is elevated resulting in transient retinal thickening and excess cell death. These defective patterns can be corrected in vitro in whole organ culture by dopa (Ilia and Jeffery, 1999). Dopa is a known cell cycle regulator, with the ability to lengthen the cycle, and it is present in abnormally low levels in albino eyes (Wick, 1977; Akeo et al., 1994; Ilia and Jeffery, 1999). The elevation in mitosis probably arises because the cell cycle is not regulated appropriately. The cell cycle lengthens with time (Young, 1983; Alexiades and Cepko, 1996), and in the absence of dopa this may not happen. It is also possible that dopa acts to signal cell cycle exit via alterations in cycle length. A combination of these factors may be responsible for the albino deficits and could explain the delays found in patterns of retinal maturation in albinos as cells remain in the cycle longer than they should (Berman and Payne, 1985; Webster and Rowe, 1991; Ilia and Jeffery, 1996).

To determine how the increased levels of mitosis in albino retinae impact on patterns of cell addition and rod production, ^{3}H-thymidine has been pulse labelled in pigmented and albino littermates, and these data related to deficits in albino retinal architecture.

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MATERIALS AND METHODS

Developing animals

The use of animals in this study complied with both local and national British regulations for animal care. The methods used to generate animals used here have been published in detail (Ilia and Jeffery, 1996). Briefly, to produce litters with both pigmentation phenotypes and reducing maturational differences at defined stages, pigmented male DA rats were crossed with Lewis albinos (both Olac, UK) to produce DALEW F1's heterozygous for the albino gene. Males from these litters were crossed with female Sprague-Dawley rats (Olac, UK) that were homozygous for the albino gene.

Males were put with females overnight. Day 0 was the first 24 hours after plug identification. Birth occurred on about postconceptional day (PCD) 22. Pregnant animals were given intraperitoneal injections of 5 μCi of metyl-1,2-^{3}H-thymidine (Amersham International) per gram of maternal body weight at 11.00 a.m. on PCD 12, 14, 17, 19, or 21. Postnatal injections were made on PCD 23, 25, and 28. When the animals were ≈2 months old and weighed about 250 g, they were deeply anesthetised (60 mg/kg sodium pentobarbitone) and a stitch placed through the eyes dorsal limbus for orientation before being perfused transcardially with phosphate-buffered saline (PBS, pH 7.2) followed by 2% paraformaldehyde and 2% glutaraldehyde in PBS. Eyes were removed, the cornea and lens dissected free, and then dehydrated and embedded in Historesin (Leica, UK). These were sectioned horizontally at 5 μm and a 1:5 series was collected. Sections were mounted on slides, coated with Ilford K-2 emulsion, and stored at 4°C for 12 weeks. They were developed with Kodak D-19, stained, and coverslipped.

Photographs and/or outline drawings of every eighth section were made at ×25. These were compared with the section and the location of labelled cells marked on overlaid transparencies. Positive label was identified when the maximum number of grains were calculated and a cell found to have at least half this number.

To obtain a quantitative index of the spatial distribution of labelled cells through development in the inner nuclear layer (INL), an expanding series of annuli were placed on reconstructed retinal sections centred on the optic nerve head (ONH; Ilia and Jeffery, 1996). The position of labelled cells were marked in relation to which annulus they fell in, and these data were normalised to account for different numbers of labelled cells between retinae.

The outer nuclear layer (ONL) is much thicker than the INL. It was not meaningful to undertake the same analysis on the ONL as the INL because differences in the centre to periphery gradient were present, depending on the depth examined. To obtain a picture of ONL cell generation, it was divided into four equally spaced sublayers across its depth, layer one being the outer most. Labelled cells in the ONL were grouped into one of these sublayers. If a labelled cell straddled a line dividing two sublayers, it was placed in the sublayer in which the greater part of its cell body appeared to be located. In central locations each sublayer was about 12-μm thick, whereas in the periphery sublayers were about 8-μm thick, reflecting the gradient in layer thickness. Labelled cells were counted in each sublayer in each phenotype. The number of animals injected and analysed are shown in Table 1.

TABLE 1. Number of Pigmented and Albino Animals Injected and Used

Injection age (days)	Number of animals injected		Number of animals analysed	
	Pigmented	Albino	Pigmented	Albino
PCD 12	3	7	3	4
PCD 14	4	10	4	4
PCD 17	2	5	2	4
PCD 19	5	3	4	3
PCD 21	4	3	3	3
PCD 23	4	4	3	3
PCD 25	4	4	3	4
PCD 28	4	4	3	3

The number of animals in litters following ^{3}H-thymidine injections (left side) and the number analysed (right side). Differences in numbers between the two sides are accounted for by uneven emulsion or poor staining. Both eyes in each animal were used in the analysis.

Mature animals

Measurements of INL and ONL thickness were made in adult littermates from each pigmentation phenotype. To establish whether there were age-dependent differences in layer organisation between phenotypes, possibly compromising analysis of the adult abnormality, animals were anaesthetised and perfused as above at 3 and 6 months of age. The 3-month-old group consisted of five pigmented and five albinos, whereas the 6-month-old group consisted of four pigmented and four albinos. Their eyes were embedded in Historesin, sectioned at 5 μm, and a 1:7 series collected and stained. Also, five pigmented and five albino 3-month-old animals were anaesthetised and perfused with 10% formalin. The retinae were removed, whole-mounted ganglion cell layer up and stained.

Nuclear layer thickness was measured at ×600 with a graduated graticule 160 μm long. Ten equally spaced counts were made per section. These did not include regions < 1 mm from the retinal margin nor dorsal and ventral regions where curvature results in sections that are not truly transverse. Sections were separated by ≈240 μm. There were around 300 locations where layers were measured in each eye. Cell density measurements were made at these locations by counting cell bodies and corrections made for double counting (Ebbesson and Tang, 1965).

Counts were made of cell bodies in the central temporal retina in whole mounts to identify the location and density of cells at the area centralis in five pigmented and five albino retinae. These were made in a square measuring 0.028 mm^2, which was moved in a regular box grid manner with every other square counted in a chequerboard pattern. The counts were initiated in the centre of the temporal retina and extended outward. The box grid counts were maintained at increasing eccentricities, but regions close to radial cuts made during whole mounting and areas where there was clear differential shrinkage in the far periphery were avoided. For this reason the total number of squares counted varied between about 200and 300 in different retinae. These counts were made at a magnification of ×600.

Statistical comparisons across repeated measures were made between groups by using an ANOVA to determine whether pigmented and albino groups differed. Then post hoc tests were undertaken on paired data at specific time points.

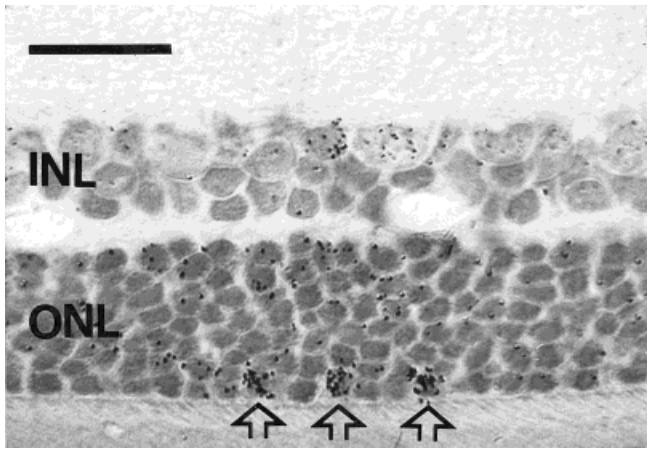

Fig. 1. The outer nuclear layer (ONL) and inner nuclear layer (INL) in the temporal periphery of an albino retina exposed to ^{3}H-thymidine on PCD 19. Positively labelled cells in the outer regions of the ONL are marked with arrows. Temporal is to the right. Scale bar = 100 μm.

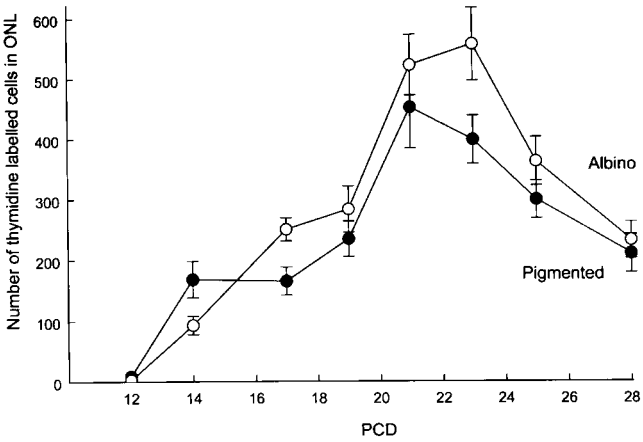

Fig. 2. Pooled data for number of ^{3}H-thymidine-labelled ONL cells at progressive postconceptional days (PCD) taken from the animals shown in Table 1. Although there are fewer labelled cells in albinos at PCD 12 and PCD 14, from PCD 17–28 more are present in albinos than in their pigmented littermates. The error bars represent standard deviations. The differences between pigmented and albino animals across these age ranges were statistically significant, and post hoc tests revealed significant differences between groups at PCD 14, 17, 23, and 25 (see text).

RESULTS

Positive thymidine label was present in the retinae of both pigmentation phenotypes in each of the cellular layers. Its presence spanned the period over which animals were injected, although by PCD 28 it was restricted to the retinal periphery. Figure 1 shows positively labelled cells in the ONL.

Outer nuclear layer

The number of cells labelled at progressive stages in the ONL in pigmented and albino animals is shown in Figure 2. There were ≈20% more labelled cells over the period examined in the ONL of albinos compared with the normally pigmented phenotype. Very few labelled cells were present in the ONL in either phenotype after thymidine injections on PCD 12. A gradual increase in their number occurred with time in both animal types with the number of labelled cells peaking in both groups at PCD 23. From PCD 17 to PCD 28 many more labelled cells were present in albinos. By PCD 28 the number of labelled cells declined in both phenotypes, and these cells were present only in the retinal periphery, although a few were scattered at more central locations in albinos. The differences between the two groups shown in Figure 2 as determined by a two-way ANOVA were significant ($P < 0.05$). Differences at specific time points were significant at PCD 14 (Mann-Whitney U, $P < 0.01$), PCD 17 ($P < 0.01$), PCD 23 ($P < 0.01$), and PCD 25 ($P < 0.05$).

Because cells at different depths of the ONL are generated at different times (Carter-Dawson and La Vail, 1979), it was not possible to analyse a single centre to periphery gradient in this layer as in the ganglion cell layer (Ilia and Jeffery, 1996) or in the INL (see below). There were multiple waves of cell generation in the ONL following a centre to periphery pattern, which were apparent when it was subdivided across its depth into the four sublayers. It was apparent that there was a centre to periphery pattern for cells in layers 1 and 2 between PCD 12 and PCD 19/21 followed by a more uniform addition of cells to these layers later. A separate centre to periphery pattern was initiated between PCD 14/17 in layers 3 and 4, which became confined to the periphery by PCD 28 (Fig. 3).

Patterns of label across the depth of the ONL were consistent within each phenotype at each stage of development. But there were marked differences between phenotypes at two specific developmental stages. Figure 3 shows two representative plots for two eyes from each phenotype. At PCD 12 cell generation was almost exclusively confined to sublayer 1 in pigmented and albino animals. The patterns of neurogenesis marked at PCD 14 were also very similar between the phenotypes, with most of the labelled cell being present in the outer regions of the ONL spanning layers 1 and 2.

At PCD 17 and PCD 19 spatial patterns of label were similar within each group, but marked differences between phenotypes were apparent. Although label was present in each sublayer in both groups, there was no obvious relationship between the patterns in the two phenotypes. Differences were particularly marked at PCD 19 where >60% of the label in pigmented animals was confined to sublayer 4, with the remainder divided between the other layers. In albinos it was much more evenly distributed and partially reflected the pattern seen in the pigmented animals 2 days earlier at PCD 17, implying that there is a delay in the development of these spatial patterns in albinos.

From PCD 21 to PCD 28 the patterns of spatial label between the two groups come back into harmony, with even subtle features preserved between phenotypes, such as the small numbers of cells labelled in layers 1 and 4 at PCD 23 and PCD 28, respectively. At PCD 21 label is confined to layers 3 and 4, whereas from PCD 23 to PCD 28 it is present in each layer. The relative distribution of label between phenotypes follows very similar patterns between at PCD 25 and PCD 28. However, again there are indications that there may be delays in the albinos because the pattern seen in them at PCD 28 is similar to

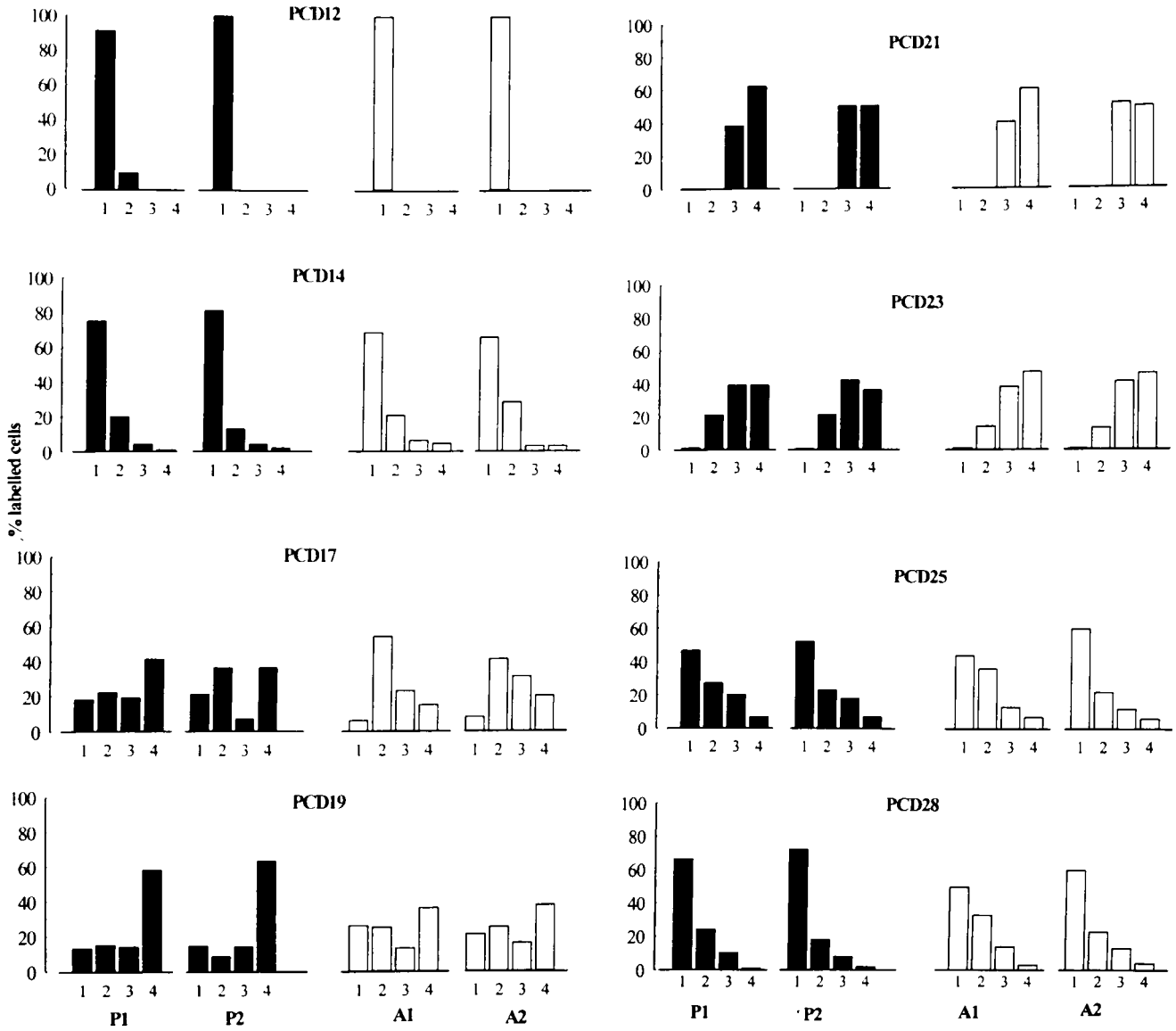

Fig. 3. Histograms showing the distribution of ^{3}H-thymidine labelled cells at progressive postconceptional days (PCD) across the depth of the ONL layer in two pigmented (P1, P2, black histograms) and two albino (A1, A2, empty histograms) eyes. The ONL has been divided into four equally spaced layers with layer 1 being the outermost. The distribution of label across the depth of the ONL is very similar between animals in each group across the stages injected (PCD 12–28) and very similar between the phenotypes for all stages except PCD 17 and PCD 19. Here there are marked differences between the phenotypes that occur close to the time when rod production peaks. The differences in the distribution of labelled cells between the phenotypes was statistically significant at PCD 17 and PCD 19 but not at other stages (see text).

that seen in the pigmented animal at PCD 25. Furthermore, at PCD 25 a clear region occupying around 30% of the central retina is free of label in pigmented animals, whereas the region of cessation of cell division in the albino is smaller.

Despite minor differences, the overall pattern of label across the layers is very similar for all stages except PCD 17 and 19. If the graphs shown in Figure 3 are combined for each phenotype at each stage, there is approximately a 90% overlap between the spatial distribution of label in the two phenotypes at all stages except PCD 17 and 19. Here the degree of overlap falls to ≈50%, which is what might be expected from comparing random patterns of distribution. Statistical comparisons from the pooled data fail to reveal any significant differences between the groups for PCD 12 and 14, and for PCD 21-28 (chi squared, $P > 0.05$ in each case). However, significant differences were found at PCD 17 and 19 ($P < 0.05$ in each case).

Inner nuclear layer

In the INL there were ≈40% more cells thymidine labelled in the albinos than in pigmented animals. Pooled data for each phenotype are shown graphically in Figure 4. An increase in the number of labelled cells in albinos

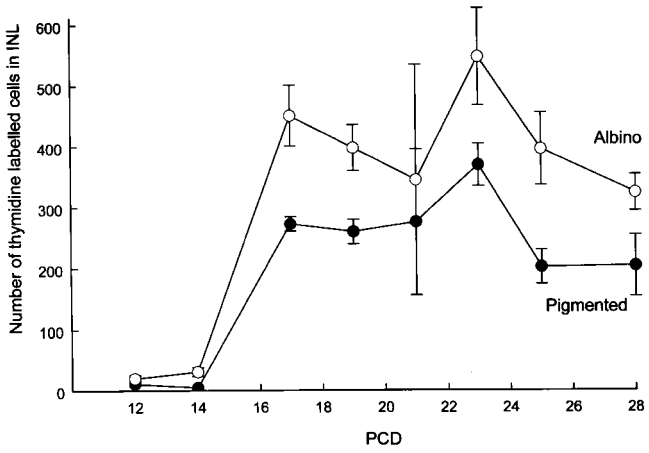

Fig. 4. Pooled data for the number of ^{3}H-thymidine-labelled cells at progressive postconceptional day (PCD) in the INL from animals shown in Table 1. More labelled cells are present in albinos compared with pigmented animals for each stage examined. These differences became marked after PCD 14. Error bars represent standard deviations. The differences between the two groups over the period examined were statistically significant, as were the differences between groups at each time point from PCD 14 except for PCD 21 (see text).

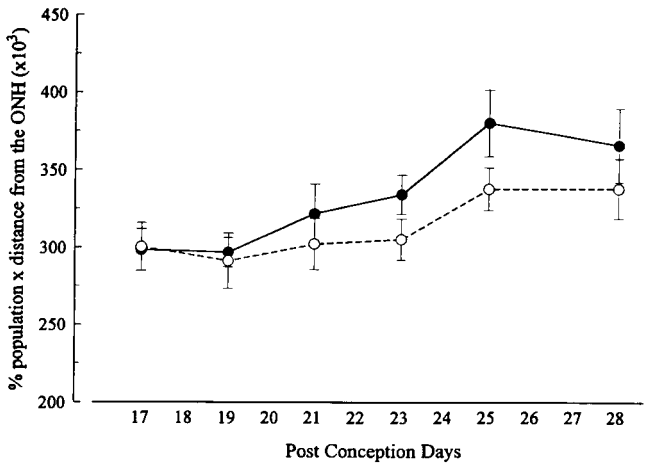

Fig. 5. The distance of the percentage of the population of ^{3}H-thymidine labelled cells in the INL from the optic nerve head (ONH) at progressive postconceptional days (PCD). Data are drawn from all of the animals shown in Table 1. From PCD 19 onward the labelled cells in the pigmented animals (filled circles) are consistently further from the ONH than in their albino littermates (open circles), implying that there is a delay in the centre to periphery gradient of development in albinos. The difference between these two groups is significant. Differences between individual groups were significant at PCD 23 and 25 (see text). Error bars represent standard deviations.

was present across the entire period over which these animals were injected (PCD 12–PCD 28). At PCD 12 and PCD 14 relatively few cells were labelled in either group. These cells were always located at the outer INL margin and had distinct large cell bodies, consistent with being horizontal cells. None of these were labelled after PCD 17. Their distribution followed a centre to periphery gradient in both phenotypes. Approximately three times as many of these labelled cells were found in albinos compared with pigmented animals. The peak in their number was at PCD 12 in pigmented and PCD 14 in the albino animals. There was a marked increase in the number of labelled cells in the INL following thymidine injections from PCD 17 to PCD 23. These cells did not have the distinctive large cell bodies of those seen earlier. The peak in the number of labelled cells in both phenotypes was found at PCD 23. From this stage onward numbers declined. The difference between the two groups over time was statistically significant (two-way ANOVA, $P < 0.01$). Statistical differences between groups at each time point were significantly different from PCD 14 onward (Mann-Whitney U, $P < 0.01$ for each case) with the exception of PCD 21 ($P > 0.05$).

To obtain a quantitative picture of the relative centre to periphery progression of cell addition in the INL between phenotypes, distances between each labelled cell and the ONH were measured in reconstructed retinae. Before PCD 17 there were too few labelled cells in the pigmented animals for this comparison to be meaningful. The relative distribution of labelled cells in the two groups in terms of their distance from the ONH was similar at PCD 17. But from PCD 19 to PCD 28 the mean distance between labelled cells and the ONH was consistently greater in pigmented animals than in the albinos, implying that there was a temporal delay in the centre to periphery pattern of cell addition in the albinos compared with their pigmented littermates (Fig. 5). This difference between phenotypes over the age ranges injected was statistically significant (two-way ANOVA, $P < 0.01$). Post hoc testing demonstrated that differences between groups were significant at PCD 23 and 25 (Mann Whitney U, $P < 0.01$), whereas differences at other ages failed to reach significance. By PCD 28, label in both phenotypes was confined to the retinal periphery.

Ganglion cell layer

There is a clear delay in the centre to periphery gradient of cell production in the ganglion cell layer (Ilia and Jeffery, 1996). However, data on the number of cells labelled has not been presented, because Ilia and Jeffery (1996) normalised cell numbers to account for differences in their number between retinae.

Cells in the ganglion cell layer were labelled between PCD 12 and 21. Overall, ≈20% more cells were labelled in albinos than in pigmented animals (Fig. 6). The peak in the number of labelled cells in both phenotypes was around PCD 14, but differences between the groups were not marked until after this stage. This difference was statistically significant (two-way ANOVA, $P < 0.05$). Post hoc testing revealed that differences between groups were significant at PCD 12 (Mann-Whitney U, $P < 0.05$) and at PCD 17–21 ($P < 0.01$ in each case), but not at PCD 14 ($P > 0.05$).

Adult retinal structure

Cellular deficits were found in all three retinal layers in adult albino animals compared with their pigmented littermates. The mean (±SD) peak in cell numbers in the ganglion cell layer of pigmented animals was 8,937 ± 154 cells mm^2, whereas in albinos it was only 8,508 ± 234 cells mm^2. This difference was statistically significant (t-test, $P < 0.01$). There were no obvious differences in cell density in the ganglion cell layer between the groups beyond the area centralis, where cell densities declined gradually toward the periphery.

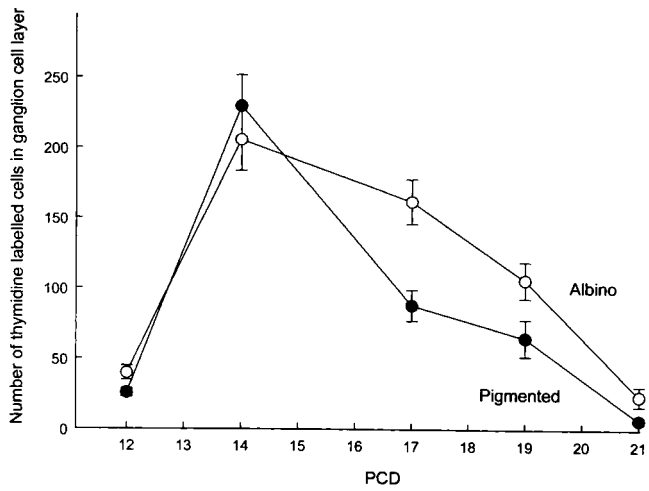

Fig. 6. Pooled data for number of ^{3}H-thymidine labelled cells at progressive postconceptional days (PCD) in the ganglion cell layer. More labelled cells are present in albinos compared with pigmented animals for each stage except PCD 14. Error bars represent standard deviations. The difference between the groups was statistically significant across the age range examined as were specific differences at PCD 12, 17, 19, and 21 (see text).

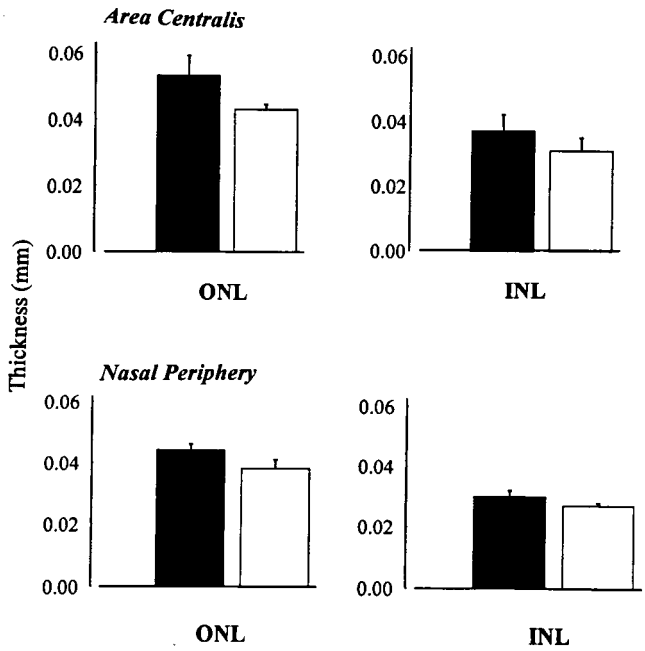

Fig. 7. Thickness of the outer nuclear layer (ONL) and inner nuclear layer (INL) in pigmented (filled bars) and albino animals (empty bars). Measurements were made at the area centralis and at identical locations in the nasal periphery. The ONL was thicker than the INL. Both layers at each location were significantly thinner in the albinos than in their pigmented littermates (see text). Error bars represent standard deviations.

There were no significant differences in the thickness of either the INL or the ONL between animals at 3 and 6 months of age within each phenotype (ANOVA, $P > 0.05$ in both cases). Also, there were no significant differences in cell density in these layers (ANOVA, $P > 0.05$ in both cases). Hence, any differences between phenotypes are unlikely to be the result of differential age related cell loss.

In both groups of animals the ONL was thicker than the INL, but both layers were thinner in albinos than in pigmented animals. Figure 7 shows layer thickness at the area centralis and in the nasal periphery in pigmented and albino animals. In both layers the differences between the phenotypes are statistically significant at the area centralis (t-test, ONL $P < 0.05$; INL $P < 0.01$). Likewise, in identical locations in the nasal periphery layer thickness is lower in albinos than in pigmented animals. (t-test, ONL $P < 0.05$; INL $P < 0.01$). Measurements at other locations confirmed that these differences were consistent.

Cell density measurements provided a more variable pattern of results (Fig. 8). Data from the area centralis showed a clear significant deficit in albinos (t-test, ONL $P < 0.01$; INL, $P < 0.05$). Cell density measurements at other locations were generally lower in albinos, but not statistically significant (nasal retina t-test ONL and INL $P > 0.05$).

DISCUSSION

This study demonstrates abnormalities in albino retinal organisation, and more importantly, that the abnormally low rod numbers in albinos are associated with disruptions in the spatial patterns of cell addition in the ONL at key stages of photoreceptor production. It shows that ^{3}H-thymidine injections in both pigmentation phenotypes result in many more labelled cells in albinos than pigmented animals, implying that cell cycle rates differ between the phenotypes, with that in the albinos being shorter than in pigmented animals. Furthermore, it shows that there is clear delay in the centre to periphery pattern of maturation in the INL and provides evidence for a similar process in the ONL.

The analysis of adult retinae demonstrate that the albino rats used here possessed similar deficits to those described in other albino mammals (Jeffery and Kinsella, 1992; Stone et al., 1978). That the rod population is severely disrupted is not only reflected by ONL deficits but also by a recent study using absorbance spectrophotometry measurements of ocular rhodopsin in the same phenotypes and strains as used here. These show a significant reduction in rhodopsin content in albinos that is present from PCD 29, when the first reliable rhodopsin measurements can be made with this method (Grant et al., submitted manuscript). Although the above data are not unexpected, they are critical for the interpretation of developmental events and their impact on the developing retinal architecture.

It has been demonstrated in the same rat strains and phenotypes as those used here that during development there are abnormally high numbers of mitotic figures in albino retinae, resulting in transitory retinal thickening and an elevated phase of cell death that depletes cell numbers. Dopa application in vitro normalises the excess mitosis (Ilia and Jeffery, 1999). The peak in mitotic figures in these animals occurs around birth, which is when rod production peaks (Young, 1983; Alexiades and Cepko, 1996) and is close to the time when there are disruptions to the spatial addition of ONL cells. No spatial disruptions to patterns of cell addition are found earlier when rod

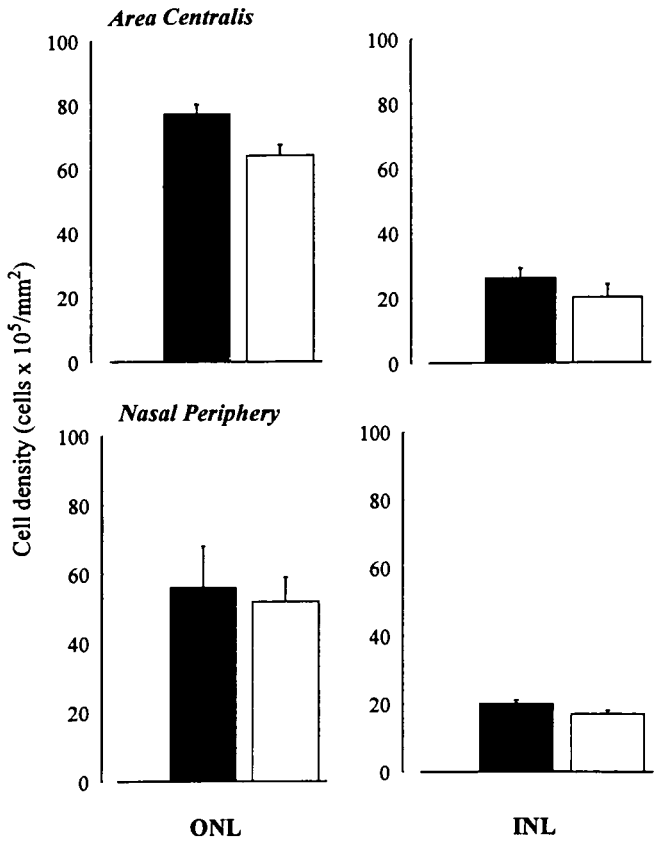

Fig. 8. Measurements of cell density in the outer nuclear layer (ONL) and inner nuclear layer (INL) in pigmented (filled bars) and albino animals (empty bars). Measurements were made at the area centralis and at identical locations in the nasal periphery. Generally, cell density was lower in albinos than in pigmented animals; however, although these differences were statistically significant at the area centralis, they were not always significant at other locations (see text). Error bars represent standard deviation.

production is still accelerating and cones are being produced (Young, 1983).

Early stages of melanin synthesis depend on dopa, an established cell cycle regulator (Wick, 1977; Akeo et al., 1994) that is present in abnormally low levels in albino eyes (Ilia and Jeffery, 1999). During development the cell cycle becomes longer as the progenitor pool is depleted (Young, 1983). That many more cells are ^{3}H-thymidine labelled in albinos than in their pigmented littermates after identical injections strongly suggests that the cell cycle rate is significantly shorter in albinos from PCD 17 onward. Dopa application in vitro lengthens the cell cycle (Akeo et al., 1994). Hence, it is likely that reduced dopa levels found in these albino rats (Ilia and Jeffery, 1999) result in an acclimation of cells whose cycle rate has not been extended. This would account for the increased number of mitotic profiles. That this may result in these cells missing their cell cycle exit points and leaving abnormally late may account for the delay found in the centre to periphery gradient of retinal maturation shown here and in other studies (Webster and Rowe, 1991; Ilia and Jeffery, 1996) and in the delayed innervation of central visual structures (Berman and Pyne, 1985).

Although there are more mitotic profiles in albino rats from PCD 12 onward, the elevation in their number does not become statistically significant until PCD 17 (Ilia and Jeffery, 1999). This is when differences first appear in patterns of ONL cell addition. These abnormal patterns span PCD 17–19. By this stage, photoreceptor addition is purely rod. The very small rodent cone population, accounting for only ≈3% of photoreceptors is generated by PCD 16 (Carter-Dawson and LaVail, 1979; Young, 1983). Cone nuclei are located in the outer regions of the ONL, which is consistent with the pattern of label being confined almost exclusively to sublayer 1 (outermost ONL region) at PCD 12 and heavily biassed toward it at PCD 14.

Because the number of mitotic figures is significantly elevated from PCD 17–28 (Ilia and Jeffery, 1999) it is surprising that abnormal patterns of cell addition in the ONL are confined to only two of the stages sampled here (PCD 17 and 19). However, the increased mitosis in albinos results in abnormal retinal thickening that peaks around the day of birth. It is probable that at this time the retina becomes increasingly congested as the nuclei of cells in the cell cycle are forced to move between the ever-widening inner and outer retinal margins. A critical point may be reached about this time, giving rise to a breakdown in normal patterns of cell addition. This seems likely because there is a dramatic increase at this point in the number of dying cells (Ilia and Jeffery, 1999).

There are apparent differences in the temporal focus of the abnormality depending on the methods used to investigate it. Peaks in mitotic numbers, retinal thickness, and dying cells occur around birth (Ilia and Jeffery, 1999), whereas spatial disruptions in thymidine labelling in the ONL are found earlier (PCD 17 and 19). Thymidine-positive cells incorporate label before passing through their final division. They then leave the cycle and migrate to an appropriate position. It is not clear how long this takes, but the cell cycle rate alone is ≈30–40 hours around the time of birth (Young, 1985; Alexiades and Cepko, 1996). Hence, cells going through their final division on PCD 17–19 will encounter an increasingly congested retina as they attempt to differentiate and locate some time after label incorporation while mitotic numbers are increasing. Cells incorporating label on the day of birth will subsequently encounter an environment in which retinal mitosis and thickness are declining as they differentiate and locate. This may account for the apparent difference in the temporal focus of the abnormality.

The results of this study add to our understanding of the interactions between the RPE and the neural retina during development and probably reveal why albinos have a rod deficit. But they cast no light on their other abnormalities found in albinism. It is not clear how abnormal cell cycle rates late in development influence chiasmatic pathways that form much earlier (Drager, 1985), although it is possible that there are subtle differences in cycle rates earlier that have not been revealed here, which exert an influence on pathway selection. Nor is it clear how abnormal patterns of photoreceptor production give rise to reductions in cell density in the ganglion cell layer at the area centralis. The answer to this question probably lies in a detailed analysis of the area centralis at the time when the retina is developing its pattern of connectivity between layers.

A clear challenge for this field of research is to determine whether it is possible to correct retinal abnormalities in vivo with dopa administration. Current projects are making significant advances in this direction and showing that dopa may not only regulate the pace of the cell cycle but also specifically signal cell cycle exit.

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LITERATURE CITED

Akeo K, Tanaka Y, Okisaka S. 1994. A comparison between melanotic and amelanotic retinal pigment epithelial cells in vitro concerning the effects of L-dopa and oxygen on cell cycle. Pigment Cell Res 7:145–141.

Alexiades RM, Cepko C. 1996. Quantitative analysis of proliferation and cell cycle length during development of the rat retina. Dev Dyn 205: 293–307.

Berman NEJ, Payne BR. 1985. An exuberant retinocollicular pathway in Siamese kittens: effects of abnormal activity on its maturation. Brain Res Dev Brain Res 22:197–209.

Carter-Dawson DL, LaVail MM. 1979. Rods and cones in the mouse retina. II. Autoradiograhic analysis of cell generation using tritiated thymidine. J Comp Neurol 188:263–272.

Drager U. 1985. Birthdates of retinal ganglion cells giving rise to the crossed and uncrossed optic projection in the mouse. Proc R Soc Lond B 224:57–77.

Ebbesson SOE, Tang D. 1965. A method for estimating the number of cells in histological sections. J R Micros Soc 84:449–464.

Ilia M, Jeffery G. 1996. Delayed neurogenesis in the albino retina: evidence of a role for melanin in regulating the pace of cell generation. Brain Res Dev Brain Res 95:176–183.

Ilia M, Jeffery G. 1999. Retinal mitosis is regulated by dopa, a melanin precursor that may influence the time at which cells exit the cell cycle: analysis of patterns of cell production in pigmented and albino retinae. J Comp Neurol 405:394–405.

Jeffery G. 1997. The albino retina: an abnormality that provides insight into normal retinal development. Trends Neurosci 20:165–169.

Jeffery G, Kinsella B. 1992. Translaminar deficits in the retinae of albinos. J Comp Neurol 326:637–644.

Jeffery G, Brem G, Montoliu L. 1997. Correction of retinal abnormalities found in albinism by introduction of a functional tyrosinase gene in transgenic mice and rabbits. Brain Res Dev Brain Res 99:95–102.

Stone J, Rowe MH, Leicester J. 1978. Retinal abnormalities in the Siamese cat. J Comp Neurol 180:773–782.

Webster ME, Rowe MH. 1991. Disruption of developmental timing in the albino rat retina. J Comp Neurol 307:460–474.

Wick MM. 1977. I-Dopa methyl ester as a new antitumour agent. Nature 269:512–513.

Young RW. 1983. The life history of retinal cells. Trans Am Ophthal Soc 51:193–228.

Young RW. 1985. Cell proliferation during postnatal development of the retina in the mouse. Brain Res Dev Brain Res 21:229–239.