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RETINOIC ACID MODULATES RETINAL DEVELOPMENT IN THE JUVENILES OF A TELEOST FISH

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Summary

Small (<30 g) juvenile rainbow trout (*Oncorhynchus mykiss*) possess retinal photoreceptor mechanisms sensitive to light in the near ultraviolet, short (blue), middle (green) and long (red) wavelengths. During normal development, the ultraviolet cone mechanism gradually disappears until, by approximately 60–80 g, individuals are no longer sensitive in the ultraviolet. This shift in spectral sensitivity is associated with the loss of a single class of photoreceptor cells – small accessory corner cones – from the retinal photoreceptor cell mosaic. Treating small (<15 g) rainbow trout with 10^{-6} mol l^{-1} all-*trans* retinoic acid (20 min exposure by immersion) induced a precocial loss of ultraviolet photosensitivity and an associated change in the retinal photoreceptor cell mosaic only 2 weeks after treatment. These changes were indistinguishable from the events that occur during normal development. Six weeks after exposure to retinoic acid, large (>90 g) rainbow trout, which had lost their ultraviolet cones during normal development, were once again ultraviolet-photosensitive and small accessory corner cones were found in their retinas. These results imply that the ultraviolet-sensitive cones, although lost at one point during development, can reappear at another time during the life history of the same individual. Retinoic acid is involved in these morphogenetic processes.

Introduction

Small (<30 g), juvenile rainbow trout [*Oncorhynchus mykiss* (Walbaum)] possess retinal photoreceptor mechanisms sensitive to near ultraviolet (UV), short (S), middle (M) and long (L) wavelengths (Hawryshyn *et al.* 1989; Hawryshyn and Harosi, 1994). During normal development, the sensitivity peak of the UV-cone mechanism shifts progressively towards the S-wavelengths (i.e. blue) until, by approximately 60–80 g, individuals are no longer sensitive in the UV (Hawryshyn *et al.* 1989; Beaudet *et al.* 1993). This shift in spectral sensitivity is associated with an almost complete disappearance of small accessory corner cones (ACCs) from the retinal photoreceptor cell

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mosaic (Browman and Hawryshyn, 1992; Beaudet *et al.* 1993). Further, the disappearance of ACCs is associated with a loss of UV-sensitive ganglion cell fibres from the optic nerve and of UV-sensitive single units from the optic tectum and torus semicircularis (Beaudet *et al.* 1993; Coughlin and Hawryshyn, 1994). Recent evidence indicates that rainbow trout ACCs contain a photopigment sensitive in the UV (Beaudet *et al.* 1993; Hawryshyn and Harosi, 1994). Thus, the loss of UV photosensitivity in rainbow trout apparently results from the disappearance of UV-sensitive cones from the retina and of UV-sensitive units from the optic centres of the brain. A similar developmental loss of the UV cone mechanism has been reported in at least four other fish species (Bowmaker and Kunz, 1987, for brown trout, *Salmo trutta*; Kunz, 1987, for Atlantic salmon, *Salmo salar*; Whitmore and Bowmaker, 1989, for rudd, *Scardinius erythrophthalmus*; Loew and Wahl, 1991, for yellow perch, *Perca flavescens*) and appears to be a size- and not age-dependent phenomenon (Browman and Hawryshyn, 1992).

We recently reported that treating small (<30 g) rainbow trout with thyroid hormone (T_4) induces a precocial loss of UV photosensitivity, and an associated change in the retinal photoreceptor cell mosaic (i.e. loss of ACCs), indistinguishable from the events that occur during normal development (Browman and Hawryshyn, 1992). Further, T_4 also induces a reappearance of UV photosensitivity and ACCs in large rainbow trout (approximately 100 g) that have lost their UV cone mechanism during normal development (Browman and Hawryshyn, 1994). In the research reported here, we examined the role of all-*trans* retinoic acid (RA) in the developmental loss and reappearance of UV photosensitivity in rainbow trout.

We chose to evaluate the potential role of RA in these processes for the following reasons. (1) RA is a potent morphogen and teratogen, known to have profound effects on cell growth and differentiation, and it is essential for the normal programme of gene expression during development (Saurat, 1991; Maden and Holder, 1992; Morriss-Kay, 1992*a*,*b*). (2) RA is an inducer of cell differentiation, and cell death, in juveniles and adults (Glass and Rosenfeld, 1991; Petkovich, 1992). (3) The effects of RA and thyroid hormones on gene transcription and expression are linked (reviewed by Glass and Rosenfeld, 1991). (4) Retinoids, RA binding proteins and RA receptors are found throughout the retina, including the photoreceptor cell layer (Eisenfeld *et al.* 1985; De Leeuw *et al.* 1990; Milam *et al.* 1990; Stumpf *et al.* 1991; McCaffery, 1992). (5) RA affects cell proliferation and differentiation in the retina of fishes, amphibians and chicks (Manns and Fritzsch, 1991; Hyatt *et al.* 1992; Macaione *et al.* 1992; Kelley and Reh, 1993). (6) Finally, the great majority of work on the teratogenic and morphogenic effects of RA deals with embyrogenesis, particularly aspects of pattern formation and cellular differentiation (Bryant and Gardiner, 1992; Maden and Holder, 1992; Morriss-Kay, 1992*a*,*b*). Far less is known about the effects of RA on the nervous systems of juveniles or adults (though see Quinn and De Boni, 1991; Halevy and Lerman, 1993).

Following from the above, we evaluated the ability of RA to induce a precocial loss of the UV photoreceptor mechanism in small juvenile rainbow trout, as well as its ability to induce a reappearance of these photoreceptors in larger individuals.

Materials and methods

Animals, retinoic acid treatment and control groups

An undomesticated non-anadromous population of rainbow trout was used for this study. Details on these animals, and on their maintenance prior to and during experiments (e.g. water temperature, lighting conditions), have been published elsewhere (Browman and Hawryshyn, 1992).

Experimental animals were immersed for 20 min in water containing all-*trans* retinoic acid (Sigma Chemical Laboratories, R2625) dissolved in 100 % ethanol at a final concentration of 10^{-6} mol l^{-1}. The ethanol:water ratio (by volume) was $1:10^4$. Fish were placed into 20 l aquaria immediately after exposure. This manner of RA exposure has been used in several recent studies on the effects of RA on the development of the amphibian and fish visual systems (e.g. Manns and Fritzsch, 1991; Hyatt *et al.* 1992). Control fish were handled in an identical manner, but no RA was added to the water in which they were immersed. In order to minimize weight gain during the experiment, fish were fed on a maintenance diet.

Handling and maintenance of animals was in accordance with the guidelines set out by the Canadian Council on Animal Care.

Spectral sensitivity experiments

Spectral sensitivity curves were obtained using heart-rate conditioning. Animals were conditioned by pairing a 300 ms, 2–3 mA shock (delivered to the caudal peduncle) with monochromatic visual stimuli (Hawryshyn and Beauchamp, 1985). The methods and equipment used to obtain the spectral sensitivity data reported here were identical to those described in a previous study (Browman and Hawryshyn, 1992). Details of the immobilization procedure, fish set-up, optical system, conditioning protocol and threshold determination can be found therein.

Chromatic adaptation was used to isolate the spectral sensitivity of the UV cone mechanism. The illumination used to achieve this effect consisted of a yellow background (550 nm long-pass cut-off filter, Corion), which differentially light-adapted the cone mechanisms sensitive to M and L wavelengths, and a narrow-band blue background (460 nm narrow-band interference filter, Corion), which light-adapted the cone mechanisms sensitive to S wavelengths. The same background conditions were used during all training sessions and experiments. Fish were allowed a minimum of 60 min to adapt to the background conditions before initiation of a training session or experiment.

Since fish generally survived the heart-rate conditioning experiments, spectral sensitivity curves were obtained from the same individuals at the intervals defined below.

The visual pigment absorption curves fitted to our data were generated by an eighth-order polynomial template for vertebrate cone visual pigments, corrected for ocular media absorption (see Browman and Hawryshyn, 1992, for a complete description).

Experiments with small fish

Spectral sensitivity curves were obtained from 11 small (<16 g) rainbow trout of similar chronological age (±20 days), prior to the initiation of a control or treatment group

experiment. Five of these fish were allocated to the control group, and their spectral sensitivity was measured again after 6 weeks. One of these individuals died before its spectral sensitivity could be remeasured. After obtaining post-treatment spectral sensitivity curves, three of the remaining four control fish were killed for histological examination of their retinas. The other six individuals were exposed to RA (as described above), and their spectral sensitivity was measured again 6 weeks later. One of these individuals died before its spectral sensitivity could be remeasured. After obtaining post-treatment spectral sensitivity curves, three of these five fish were killed for histological examination of their retinas. An additional two small fish were killed for histological examination of pre-treatment retinas.

Experiments with large fish

Spectral sensitivity curves were obtained from eight large (>90 g) rainbow trout of similar chronological age (±20 days, and of the same chronological age as the small fish described above), prior to the initiation of a control or RA treatment experiment. Four of these fish were allocated to the control group, and their spectral sensitivity was measured again after 6 weeks. These fish were then killed for histological examination of their retinas. The other four individuals were exposed to RA (as described above), and their spectral sensitivity was measured again 6 weeks later. These four fish were then killed for histological examination of their retinas.

In order to evaluate whether the UV sensitivity points exhibited by large RA-treated fish were generated by an independent cone mechanism, we continued the spectral sensitivity experiments by adding UV illumination to the background [using a 250 W tungsten bulb projected through a UG-11 filter (Corion) and superimposed over the yellow background used in all of the experiments]. Spectral sensitivity at 360, 440, 560 and 640 nm was remeasured for one of the four large RA-treated fish after 1 h of adaptation to the new background conditions.

Histological procedures

As described above, 16 fish were killed for histological examination of the retina: two small pre-treatment fish, three small control fish, three small RA-treated fish, four large control fish and four large RA-treated fish.

All individuals were fully light-adapted when killed by spinal section and the eyes were immediately enucleated and fixed by immersion. Retinas were prepared for histological examination by embedding in Epon. Full details of the protocol used here have been published elsewhere (Browman and Hawryshyn, 1992).

Tissue from the central ventral retina, the area to which stimuli were presented in the spectral sensitivity experiments, was sectioned tangentially (1 μm thick sections) to the base of the cone outer segments. Sections were stained with Richardson's stain for light microscope examination. To ensure that there was no RA-induced displacement of cone cells within the photoreceptor layer, the central ventral retinas of at least one specimen from each experimental treatment were serially sectioned (1 μm thick sections) from the tips of the rod outer segments through to the cone pedicles.

Results

Spectral sensitivity of small fish

All small pre-treatment and control fish exhibited sensitivity peaks at UV and S wavelengths (Fig. 1). M and L mechanisms were also present, although their sensitivity was depressed by the adapting background (Fig. 1). The UV sensitivity points were most effectively fitted with a 360 nm λ_{max} visual pigment absorption curve (Fig. 1). This is consistent with microspectrophotometric estimates of a 365±5 nm λ_{max} for the UV-

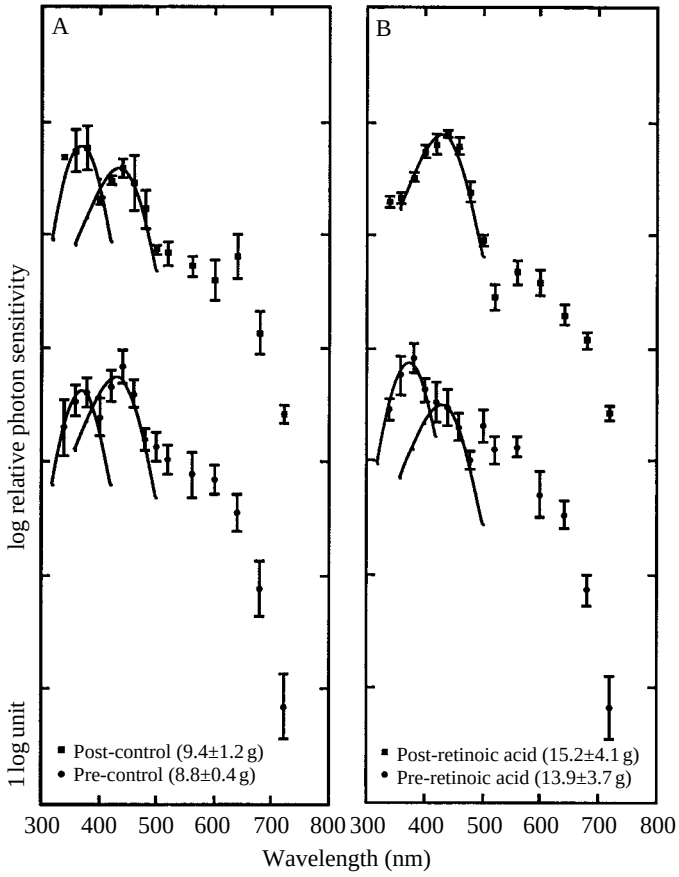

Fig. 1. (A) Mean spectral sensitivity curves for small rainbow trout used as controls obtained from the same individuals before (●, 8.8±0.4 g, *N*=5), and 6 weeks after (■, 9.4±1.2 g, *N*=6), the beginning of the experiment. (B) Mean spectral sensitivity curves for small retinoic acid treated rainbow trout obtained from the same individuals before (●, 13.9±3.7 g, *N*=6), and 6 weeks after (■, 15.2±4.1 g, *N*=5), a single 20 min exposure to 10^{-6} mol l^{-1} retinoic acid. A yellow background was used to 'isolate' the UV-sensitive cone mechanism in all experiments. The 360 and 430 nm λ_{max} visual pigment absorption curves were compared with the appropriate spectral peaks for all fish. Note (i) that visual pigment absorption curves (corrected for ocular media absorption) are represented by solid lines, (ii) that spectral sensitivity curves were arbitrarily arranged on the ordinate, and (iii) that one major division on the ordinate equals 1 log unit. Bars indicate ±1 S.E.M.

sensitive cone pigment in rainbow trout (Hawryshyn and Harosi, 1994) and with estimates for UV-sensitive cones in other fishes (e.g. Hawryshyn and Beauchamp, 1985; Whitmore and Bowmaker, 1989; Hawryshyn and Harosi, 1991). Spectral sensitivity points in the neighbouring S-wavelength region were most effectively fitted with a 430 nm λ_{max} visual pigment absorption curve (Fig. 1). This is consistent with microspectrophotometric estimates of a 434±5 nm λ_{max} for the S-sensitive cone pigment in rainbow trout (Hawryshyn and Harosi, 1994) and with estimates for the S-sensitive cones of brown trout (Bowmaker and Kunz, 1987).

There were no clear changes in the mean or within-individual spectral sensitivity curves of the control fish when measured again 6 weeks later (Fig. 1A). Both UV- and S-wavelength-sensitive cone mechanisms remained as described above. However, 6 weeks after a single exposure to 10^{-6} mol l^{-1} RA, small fish no longer exhibited a UV sensitivity peak (Fig. 1B). The S-sensitive cone mechanism points for these fish were most effectively fitted with a 430 nm λ_{max} visual pigment absorption curve (Fig. 1B).

Since the same pattern was observed for all individual fish within the control and treatment groups reported here (and below), the data are presented as mean spectral sensitivity curves. Before calculating these mean curves, absolute values of log photon sensitivity were normalized for inter-individual variability.

Histology of the ventral retina in small fish

The central ventral retina of the small control rainbow trout used in these experiments (and the same individuals from which the spectral sensitivity curves were obtained) contained a regular square mosaic of cones, consisting of a single cone surrounded by four double cones, with small accessory single cones at each corner (Fig. 2A,C).

ACCs were no longer present, at any level within the photoreceptor cell layer, in the central ventral retinas of small fish exposed to RA (and the same individuals from which the spectral sensitivity curves were obtained) (Fig. 2D). The cone mosaic in these retinas consisted of a single cone surrounded by four double cones. This arrangement is indistinguishable from that observed in larger (approximately 70 g) individuals which have undergone normal growth and development (Fig. 2B).

Spectral sensitivity of large fish

There were no significant changes in the mean or within-individual spectral sensitivity curves of the large control fish during these experiments (Fig. 3A). S-, M- and L-sensitive cone mechanisms were present in all of these individuals, although the sensitivity of the

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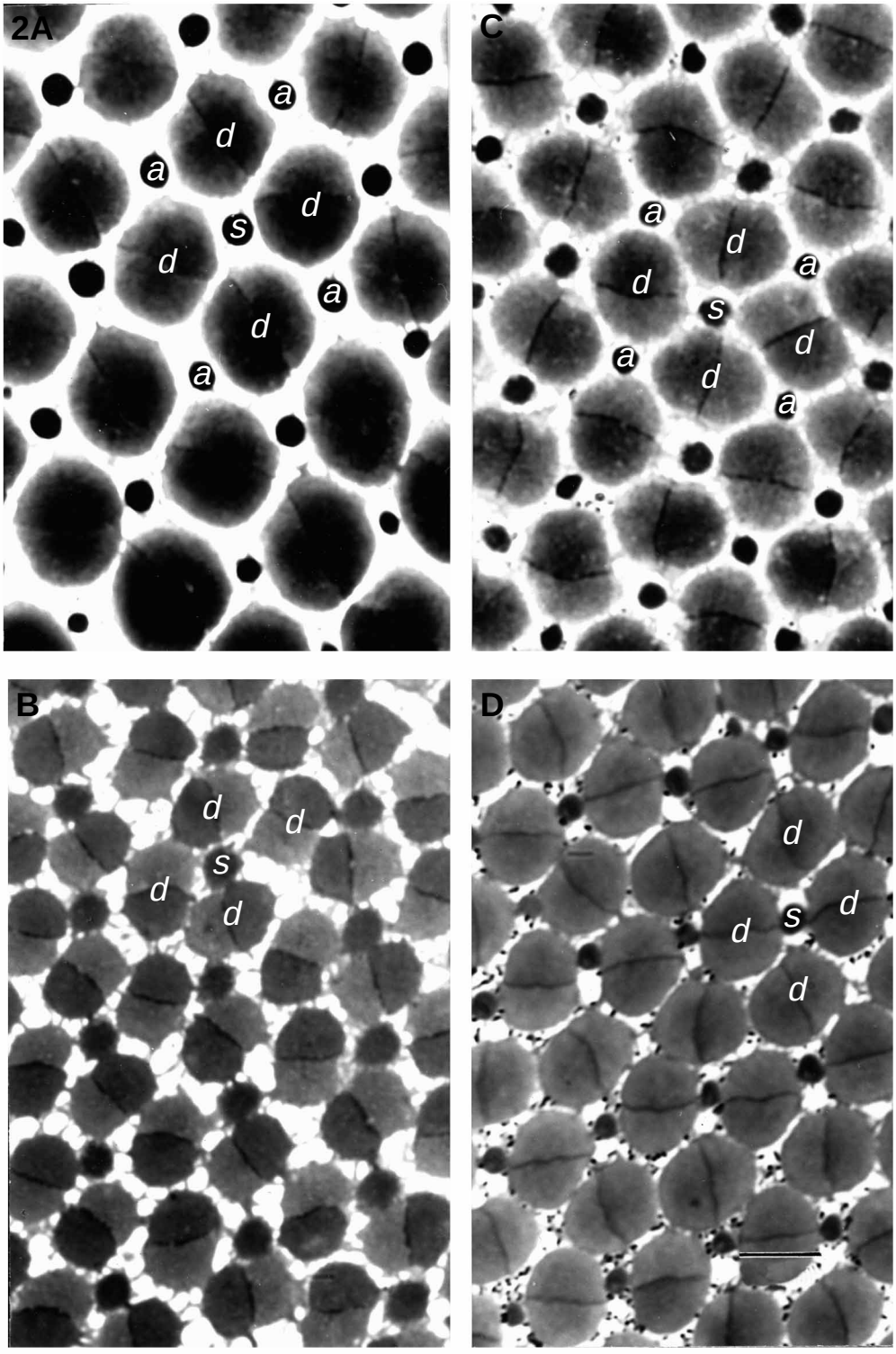
Fig. 2. (A) Cone photoreceptor cell mosaic in the central ventral retina of a small rainbow trout (approximately 10 g). (B) Cone photoreceptor cell mosaic in the central ventral retina of a large rainbow trout (approximately 100 g). (C) Cone photoreceptor cell mosaic in the central ventral retina of a small rainbow trout from the control group of the RA spectral sensitivity experiments. (D) Cone photoreceptor cell mosaic in the central ventral retina of a small rainbow trout 6 weeks after a single 20 min exposure to 10^{-6} mol l^{-1} retinoic acid. All micrographs are from tangential sections cut through the base of the cone cell outer segments. *a*, accessory corner cone (=small single cone); *d*, double cone; *s*, central single cone. Scale bar, 10 μm.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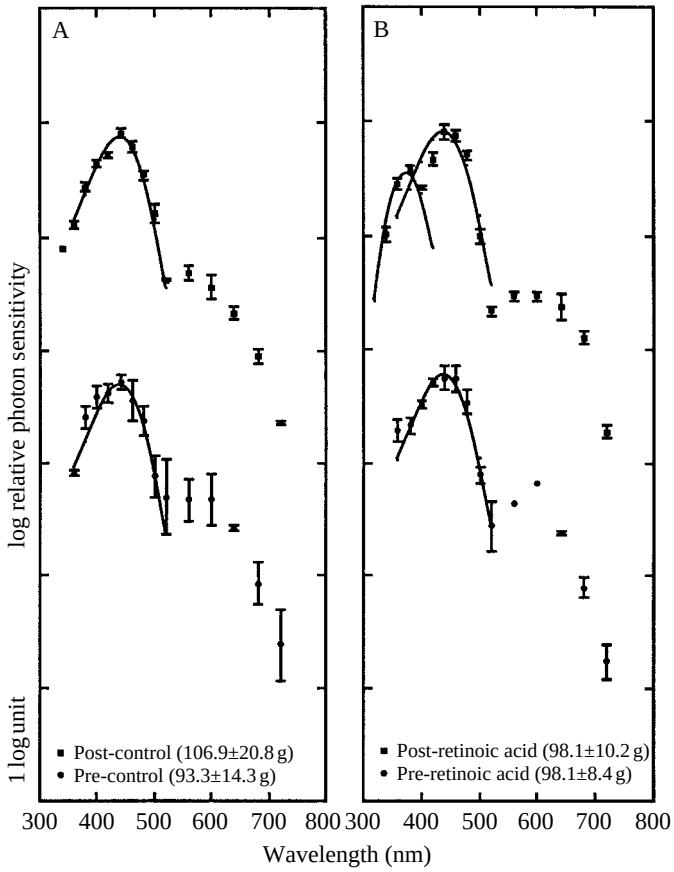

Fig. 3. (A) Mean spectral sensitivity curves for large rainbow trout used as controls obtained from the same individuals before (●, 93.3±14.3 g, *N*=4), and 6 weeks after (■, 106.9±20.8 g, *N*=4), the beginning of the experiment. (B) Mean spectral sensitivity curves for large retinoic acid-treated rainbow trout obtained from the same individuals before (●, 98.1±8.4 g, *N*=4), and 6 weeks after (■, 98.1±10.2 g, *N*=4), a single 20 min exposure to 10^{-6} mol l^{-1} retinoic acid. A yellow background was used to 'isolate' the UV-sensitive cone mechanism in all experiments. The 360 and 430 nm λ_{max} visual pigment absorption curves were compared with the appropriate spectral peaks for all fish. Note (i) that visual pigment absorption curves (corrected for ocular media absorption) are represented by solid lines, (ii) that spectral sensitivity curves were arbitrarily arranged on the ordinate, and (iii) that one major division on the ordinate equals 1 log unit. Bars indicate ±1 S.E.M.

M and L mechanisms was depressed by the adapting background (Fig. 3). There was no evidence of a sensitivity peak in the UV (Fig. 3A). Spectral sensitivity points in the S-wavelength region were most effectively fitted with a 430 nm λ_{max} visual pigment absorption curve (Fig. 3).

Six weeks after a single exposure to 10^{-6} mol l^{-1} RA, large fish exhibited spectral sensitivity peaks at UV and S wavelengths (Fig. 3B). The UV sensitivity points for these individuals were most effectively fitted with a 360 nm λ_{max} visual pigment

absorption curve (Fig. 3B). The S sensitivity points were most effectively fitted with a 430 nm λ_{max} visual pigment absorption curve (Fig. 3B). M and L mechanisms were also present although, as before, their sensitivity was depressed by the adapting background (Fig. 3).

For one large RA-treated fish, spectral sensitivity at 360, 440, 540 and 640 nm was remeasured after the addition of UV illumination to the background. For this individual, the sensitivity of the 360 nm point was depressed by 1.0 log unit. There was little or no change in the sensitivity of the 440 nm (0.1 log unit), 560 nm (0 log unit), and 640 nm (0 log unit) points.

Histology of the ventral retina in large fish

The central ventral retinas of all large control fish in these experiments (and the same individuals from which the spectral sensitivity curves were obtained) contained a regular square mosaic of cones, consisting of a single cone surrounded by four double cones (Fig. 4A). No ACCs were found at any level within the photoreceptor cell layer of the central ventral retina. However, a small population of ACCs appears to be present near the optic nerve head and along the embryonic fissure, even in large (80–914 g) fish (Beaudet *et al.* 1993).

The central ventral retinas of large RA-treated fish possessed ACCs (Fig. 4B), and all of these individuals were UV-photosensitive (Fig. 3B). There appeared to be a multiplication of ACCs in some areas (Fig. 4B). However, ACCs were not found in all areas examined. Further, several of the areas examined exhibited a modified mosaic consisting of a single cone surrounded by six double cones (Fig. 4B). Since we are currently able to identify ACCs only by their relative position within the square photoreceptor cell mosaic, their exact position in these areas is uncertain and, therefore, they have not been labelled on the photomicrograph (Fig. 4B).

Discussion

Our results demonstrate the following. (1) RA induces a precocial loss of UV photosensitivity, and a loss of ACCs, in small juvenile rainbow trout. (2) RA induces a return of UV photosensitivity in large juvenile rainbow trout that have lost their UV cone mechanism during normal development. This implies that the developmental loss of UV photosensitivity is reversible (also see Browman and Hawryshyn, 1994). (3) It follows that, ACCs can reappear in areas of the retina from which they had previously been lost and away from the circumferential growth zone and/or embryonic fissure (also see Browman and Hawryshyn, 1994).

Although our experiments cannot elucidate the mechanism by which RA acts to induce a loss and reappearance of UV photosensitivity and ACCs, the evidence currently available supports the contention that RA is involved. RA, nuclear retinoic acid receptors (RARs) and cellular retinoic acid binding proteins (CRABPs) are found throughout the embryonic and adult vertebrate retina (Wiggert *et al.* 1978; Eisenfeld *et al.* 1985; Milam *et al.* 1990; Stumpf *et al.* 1991; McCaffery *et al.* 1992). Further, RARs and CRABPs are differentially expressed, both spatially and temporally, and regulate different target genes

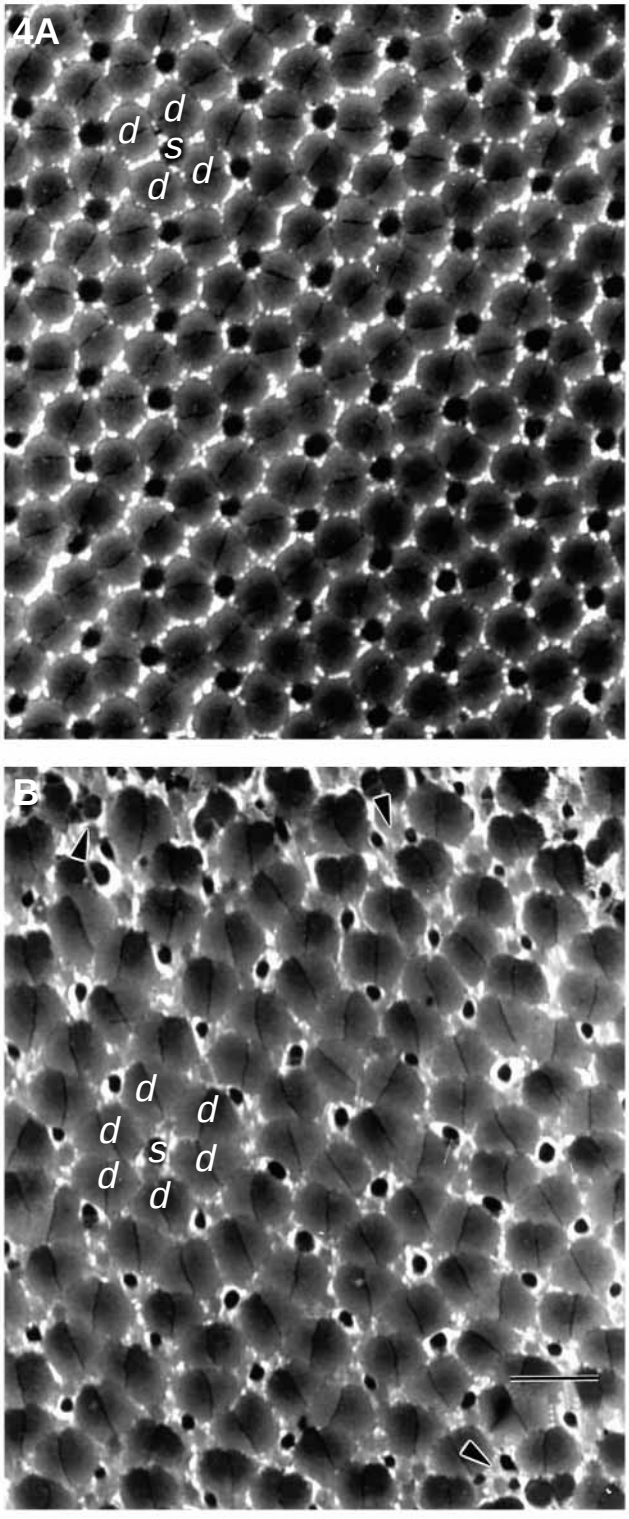
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Fig. 4. (A) Cone photoreceptor cell mosaic in the central ventral retina of a large rainbow trout (approximately 100 g) from the control group of the large retinoic acid spectral sensitivity experiments. (B) Cone photoreceptor cell mosaic in the central ventral retina of a large rainbow trout (approximately 100 g) 6 weeks after a single 20 min exposure to 10^{-6} mol l^{-1} retinoic acid. All micrographs are from tangential sections cut through the base of the cone cell outer segments. *d*, double cone; *s*, central single cone. Arrowheads highlight areas in which there appear to be multiple single cones. Scale bar, 10 μm.

during embryonic and adult life, as well as in specific cell types at different stages of differentiation (Saurat, 1991; Petkovich, 1992). These observations suggest that RA is involved in neuronal differentiation in the inner retina, both during development and in juveniles and adults (De Leeuw *et al.* 1990; Stumpf *et al.* 1991; Hyatt *et al.* 1992; Macaione *et al*. 1992).

Loss of the UV cone in RA-treated rainbow trout

The timing and extent of ACC loss from the retinas of small RA-treated rainbow trout, of the same size and age, are variable. This is true for normally developing fishes as well (Lyall, 1957*a*,*b*; Ahlbert, 1976; Wahl, 1992; Kunz *et al.* 1994). There is also topographic variability; ACCs are not lost simultaneously throughout the retina (Lyall, 1957*a*,*b*; Ahlbert, 1976; Wahl, 1992; Kunz *et al.* 1994). The issue of spatial and temporal variability in the developmental loss of ACCs from the fish retina requires further investigation.

The fate of the ACCs that disappear from the retina of rainbow trout is still uncertain. It has been suggested that, at least in Atlantic salmon (*Salmo salar*), they die, degenerate and are removed from the photoreceptor cell layer by phagocytosis over a period of hours or days (Kunz *et al.* 1994). However, in adult brown trout, Atlantic salmon and rainbow trout, ACCs remain at the optic nerve head, embryonic fissure and peripheral growth zones (Kunz, 1987; Bowmaker and Kunz, 1987; Beaudet *et al.* 1993). It is not known whether these cells contain a UV photopigment.

Reappearance of the UV cone in RA-treated rainbow trout

The timing and extent of ACC reappearance in the retinas of large RA-treated fish are also variable. Although these retinas appeared to possess ACCs in the central ventral quadrant, some areas were incompletely repopulated. In addition, multiple single cones were occasionally observed in the corners of the mosaic (Fig. 4B). There also appeared to be a coincident multiplication of the mosaic's double cone elements: in several locations we observed six double cones surrounding a single cone, a pattern that we have never observed in normal individuals (Fig. 4B). We interpret these unusual observations as a possible artefact of the RA exposure, i.e. an overproduction of new cone elements, including double cones, induced by RA. However, multiple ACCs have been observed in the square photoreceptor cell mosaics of two freshwater fishes near the time when these cones are lost from the retina during normal development (Wahl, 1992; C. M. Wahl, personal communication). Thus, the effects of RA reported here may mimic transitory events that occur during normal retinal development.

What is the source of new ACCs?

Our observation of ACC reappearance in the central areas of the retina in large RA-treated rainbow trout is unusual, but not without precedent. The fish retina continues to grow throughout life and new cone cells are normally added at the circumferential germinal zone, and perhaps at the embryonic fissure (Raymond, 1985; Fernald, 1991; Kunz *et al.* 1994). Further, rod cells are constantly added to the fish retina as it grows (Raymond, 1985; Fernald, 1991). These new rods are inserted into the photoreceptor mosaic interstitially, throughout the retina, and establish synaptic connections with pre-existing retinal neurones (Raymond, 1985; Fernald, 1991). The source of new rods is a specialized population of undifferentiated neuroepithelial cells termed 'rod precursors' (Raymond, 1985). Rod precursors are scattered throughout the differentiated retina and are normally located in the outer nuclear layer (ONL) at the time of their differentiation into rods (Raymond and Rivlin, 1987). Recent evidence indicates that rod precursors, and other retinal precursor cells, are pluripotent and are not restricted to producing new rods: under some conditions they differentiate into cones (Raymond *et al.* 1988; Braisted and Raymond, 1990; Raymond, 1991; Hitchcock and Raymond, 1992; Adler, 1993). On the basis of these observations, we suggest that rod precursors are a likely source of new ACCs and that they are added to the photoreceptor cell mosaic in a manner analogous to that described for rods (Raymond, 1985; Fernald, 1991). It is possible that RA, alone or in conjunction with other gene transcription agents (e.g. thyroid hormone), induces rod precursor cells to produce pluripotent neuroepithelial cells which would possibly go on to become cones rather than differentiating into rods (*sensu* Raymond, 1991). This form of RA-induced sensory cell regeneration has recently been reported for auditory hair cells in ototoxically poisoned adult rat organ of Corti (Lefebvre *et al.* 1993). Further, in rats, RA apparently triggers retinal progenitor cells to differentiate into photoreceptor cells (Kelley and Reh, 1993).

Comparison of the effects of RA and thyroxine on the rainbow trout visual system

In fishes and amphibians, thyroid hormones are associated with significant morphological, physiological and biochemical changes in the visual system (Evans and Fernald, 1990; Hoskins, 1990). In an earlier study, we reported that treating small rainbow trout with thyroid hormone induces a precocial loss of the UV photoreceptor mechanism (Browman and Hawryshyn, 1992). Further, T_4 also induces a reappearance of UV photosensitivity in large rainbow trout that have lost their UV cone mechanism during normal development (Browman and Hawryshyn, 1994). As described above, we have been able to reproduce these results using RA.

The effects of T_4 and RA were similar, with the following exceptions. (1) RA induced a more rapid loss of UV cones than did T_4; the effects of RA on spectral sensitivity in the UV were discernible approximately 2 weeks after exposure, while it was 5–6 weeks before animals exposed to T_4 exhibited the same level of response. (2) Fish exposed to T_4 for 6 weeks no longer possessed the vertical pigmented bars (i.e. 'parr marks') typical of juvenile rainbow trout, but had become silvered. RA-treated fish did not exhibit this change in external appearance. This latter observation indicates that the effect of RA is

more specific than that of thyroxine. (3) The method of treatment, a 6 week exposure for T_4 compared with a single 20 min exposure for RA, also indicates that the pathway of action for RA is more direct than that for T_4.

Why do thyroid hormone and RA induce similar developmental processes in the retina of these fishes? In fact, both of these agents produce similar developmental abnormalities in other systems (e.g. Manns and Fritzsch, 1991; Old *et al.* 1992; Juriloff and Harris, 1993). Further, like RARs, thyroid hormone receptors (TRs) have been located in several brain areas of fishes (White *et al.* 1990) and in the retinas of metamorphosing *Xenopus* (Kawahara *et al.* 1991). These similarities between the spatial distribution of TRs and RARs, and of the effects of RA and thyroid hormones on developmental processes, are probably more than coincidental.

TRs and RARs, upon interaction with specific DNA hormone response elements (HREs), form heterodimers resulting in dual responsiveness to RA and thyroid hormone (Forman *et al.* 1989; Glass and Rosenfeld, 1991). Heterodimers of this type elicit functions that are distinct from those induced by TRs or RARs alone (Marks *et al.* 1992). In addition, RXRα (whose structure is very similar to that of the TRs: Lazar, 1991; Laudet *et al.* 1992) is a promiscuous partner of both RARs and TRs and may thereby underlie developmental pathways triggered by RA or thyroid hormone, alone or in combination (Graupner *et al.* 1989; Bugge *et al.* 1992; Kliewer *et al.* 1992; Marks *et al.* 1992). It is now clear that, for several specific cases of gene expression, thyroid hormone and RA do indeed interact (e.g. Umeseno *et al.* 1988; Davis and Lazar, 1992; Glass and Rosenfeld, 1991; Juriloff and Harris, 1993).

With all of these recent observations in mind, we postulate that the changes in the developmental trajectory of the UV cone mechanism induced by T_4 and RA may result from an interaction of their nuclear receptors and HREs. The gene locus regulating ACCs might possess an enhancer region containing a string of homologous HREs for T_4 and RA, or an HRE that recognizes both hormone/receptor complexes or their combinations in heterodimers. The combinational nature of this interaction may differ for the loss *versus* the reappearance of the UV cone mechanism, perhaps explaining how the same agent can induce a loss of ACCs at one point during the life history and their reappearance later.

Functional significance of a life-history-related loss and reappearance of UV photosensitivity

In rainbow trout, the UV cone mechanism is involved in the detection of, and orientation to, the **e**-vector of the polarized light field (Hawryshyn, 1992; Parkyn and Hawryshyn, 1993). In addition, the UV cone mechanism is directly involved in colour vision and extends the range of wavelengths and intensities over which colour discriminations can be made (Neumeyer, 1992; Coughlin and Hawryshyn, 1994). The UV cone mechanism also contributes to visually guided foraging behaviour, perhaps as a contrast enhancer (Browman *et al.* 1994; Loew *et al.* 1993). It would not be surprising to find that the UV cone mechanism, like the other cone mechanisms, makes a multi-faceted contribution to the visual abilities of these animals and that the nature of this contribution changes during an individual's life history.

Despite the studies cited above, the adaptive significance of a developmental loss of UV photosensitivity, and of its possible return at a later time during the life history of the same individual, is unclear. In all of the species for which a developmental loss of UV photoreception has been documented, the UV mechanism disappears near the time when these fishes move from shallow to deeper waters and when they change from feeding upon small crustacean zooplankton to larger food items. Similar changes occur in the retinas of other fishes when they move to deeper waters (e.g. Boehlert, 1979; Kitamura, 1990). On the basis of these observations, we suggest that the loss of the UV cone mechanism in small juvenile rainbow trout, and perhaps in other species, is associated with a habitat shift to deeper waters (in which a UV photoreceptor would be useless), and with a change from a diet of small zooplanktonic crustaceans (the location of which would be improved by a contrast-enhancing UV receptor, see Beaudet *et al.* 1993; Browman *et al.* 1994) to larger crustaceans and small fishes. Further, regeneration of the UV mechanism may be associated with its role in orientation to the **e**-vector of polarized light fields and, therefore, with long-distance migration (Hawryshyn, 1992; Parkyn and Hawryshyn, 1993). In this regard, it is noteworthy that elevated levels of thyroid hormone are associated with the initiation of both the seaward migration (during which the UV cone mechanism is lost) and the freshwater migration (during which the UV cone mechanism may reappear) in salmonid fishes (Woodhead, 1975; Youngson, 1989; Youngson and Webb, 1993). It remains to be seen whether the same is true for RA.

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