SPATIAL-TEMPORAL EXPRESSION OF CALBINDIN D-28K DURING EMBRYONIC AND POSTNATAL DEVELOPMENT OF THE FISH RETINA

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In contrast to the situation in mammals and birds, fish continue to grow throughout life, although in fish there are unique features about this growth. In particular, the eyes and brain grow by two processes: hyperplasia and cell addition. The retina in particular grows by adding rings of new cells at the margin (Raymond, 1977). Despite the enormous growth of these organs, there are no overt consequences detectable in the behavior of the animal (Bernald, 1989). Fish have a retinal structure characteristic of all vertebrates: gelatinous, thin, and transparent. Moreover, many aspects of the retinal structure and visual function in fish are typical of all vertebrates.

To study some aspects of the development and differentiation of the retina and the optic tectum of the trout we have used calbindin D-28K (CB) as a marker since CB is a calcium-binding protein that has been shown to be well conserved across species and has been shown to appear in postmitotic neurons during migration to their final position and during dendritic and axonal outgrowth. Thus, the study of CB expression in the fish visual system offers a novel opportunity to study the timing of the maturation of neurons in the fish visual system.

The experimental animals used in the present study were trout (Salmo fario L.). The technique performed was the indirect peroxidase-antiperoxidase (PAP) method described in Vecino et al., (1993). The specificity of this CB antiserum has been reported previously (Ellis et al., 1991).

The developmental stages of the trout are given in day-degrees; i.e. the factor of water temperature times the number of days until hatching. According to this, hatching takes place at 440ºC (around 30 days after fertilization).

In a previous study we have shown that the time course of expression of CB in the trout retina conform generally with the vitreal to scleral progression of differentiation. However, CB immunoreactivity was expressed earlier in amacrine cells than in ganglion cells. Thus we can say that at least for ganglion cells, CB was not present for several days after commitment to a definitive cell phenotype. Moreover, ganglion, amacrine and bipolar cells undergo further maturation after beginning CB expression (Vecino et al., 1993).

In the present study we show how the pattern of expression of CB during the development of the trout retina can be reflected in the juvenil and adult retina. Thus, the margin ring of the adult retina, where new cells are adding, shows a pattern of CB immunoreactivity similar to embryos at stage 227ºC. As we look more centrally to the retina the pattern of CB expression resembles embryos at stage 440ºC and only in the center of the retina we can see the distribution of cells immunoreactive to CB as the mature retina.

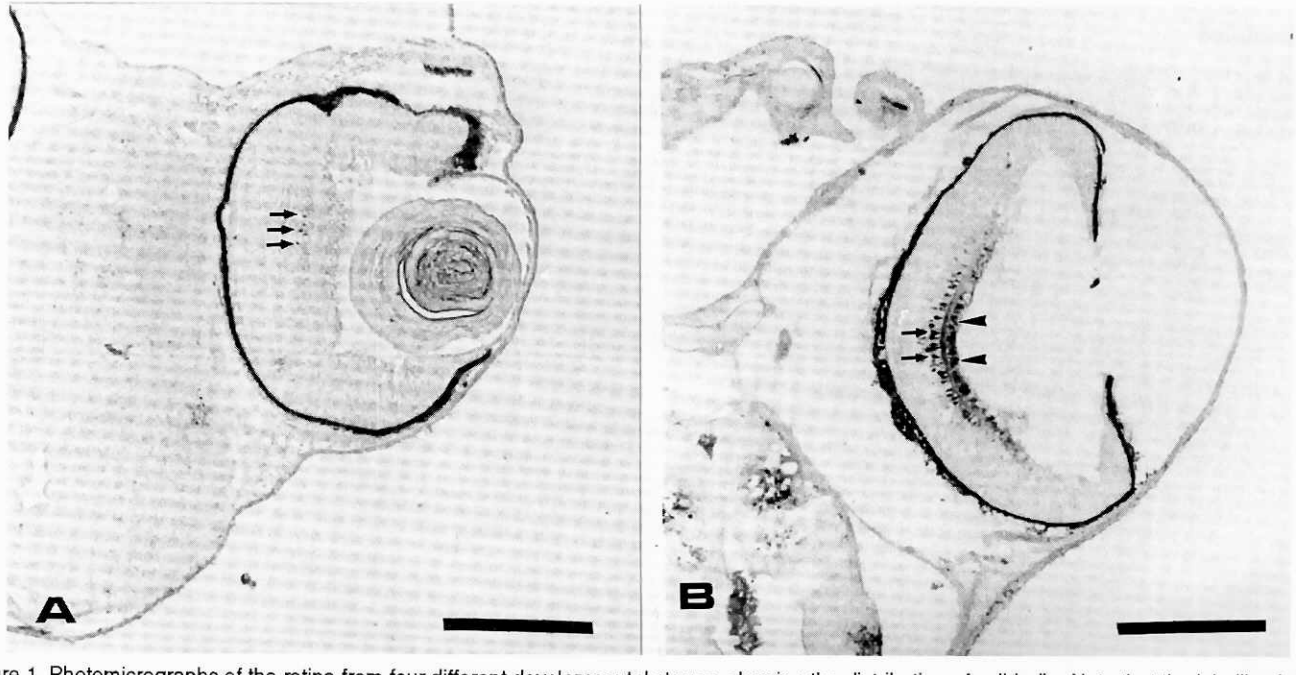

Figure 1. Photomicrographs of the retina from four different developmental stages, showing the distribution of calbindin. Note that the labelling in the central retina is stronger and in more retinal layers versus the peripheral retina. Thus, the following description refers only to the distribution of calbindin immunoreactivity in the central retina. A: retina from embryos at stage 227ºC. The only calbindin immunoreactive cells are located in the inner nuclear layer (arrows). B: retina section from embryos at stage 440ºC. Strongly labelled immunoreactive cells are located within the inner nuclear layer (arrows) and the ganglion cell layer (arrowheads).

The higher expression of CB in the ganglion cells were seen at the hatching time 440ºC. Thus we can conclude that the pattern of expression of CB in the fish retina follows the spatial-temporal sequence of maturation of the cells.

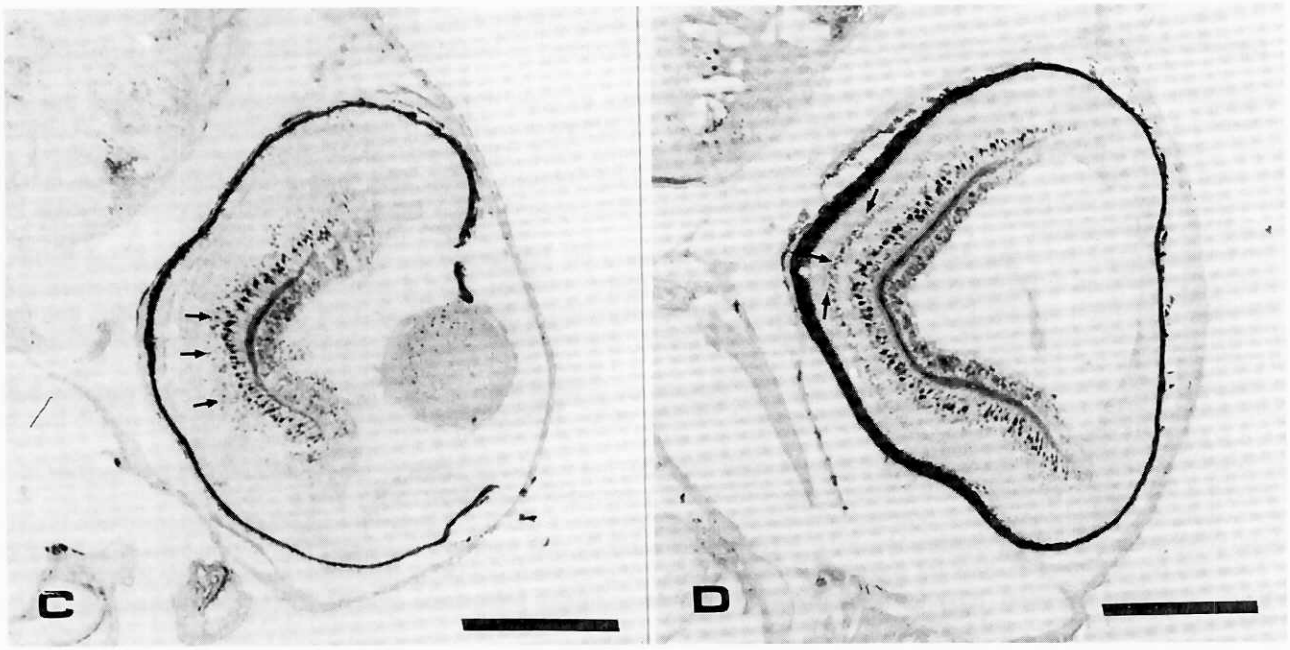

Figure 1. C: Retina from alevins15 days after hatching. The pattern of immunoreactivity is similar to B. Note however the presence of faint immunoreactive cells located at the bipolar cell position close to the outer plexiform layer (arrows). D: retina from 1 month old fish. The bipolar cells are located in their correct layer position in the central retina (arrow). As we look to the peripheral retina, the pattern of immunoreactivity resembles the anterior stages, younger patterns being observed in more peripheral regions. Scale Bar=250 µm.

References

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