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Short Report

Proliferation, cell death and reelin expression during development of the retina in a teleost, the common trout (*Salmo trutta fario*)

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ABSTRACT We have analysed the spatiotemporal pattern of cell proliferation and apoptosis during the development of the retina in a teleost. Distribution of proliferating cells in different developmental stages (embryos, alevins, and juveniles) was analysed with an antibody against proliferating cell nuclear antigen (PCNA). After establishment of the retinal lamination, PCNA-immunoreactive (PCNA-ir) cells were frequently observed in all cell layers, with a high majority of proliferating cells located at the marginal (growing) retina. Clusters of PCNA-ir cells were characteristic of the inner nuclear layer (INL). The distribution of apoptotic cells was studied by the TUNEL method, which has revealed significant cell death before completion of the retinal lamination. We have also used an antibody against Reelin (RELN) as a marker for retinal cells that secrete this glycoprotein to the extracellular space. The pattern of RELN expression, as well as that of proliferating and dying cells was very similar to that described in tetrapods, suggesting that this protein regulates the cell migration and synaptogenesis in the different developmental stages of the retina.

Development is a complex process based in a spatiotemporal control of cell proliferation, cells migration to their definitive position and programmed cell death in specific regions. We have analysed the distribution of proliferating and dying cells during the development of the retina in trout to evidence their spatiotemporal patterning. We have correlated these results with the distribution of immunoreactivity to RELN, a glycoprotein that appears to act at short distance as an instructive signal in the regulation of cell distribution.

Heads of embryos (5 to 14 mm in length), alevins (15 to 26 mm in length), and juveniles (without vitelin sac) of trout were used for this study. Paraffin sections of retinas fixed with Bouin's fluid were immunohistochemically treated with anti-PCNA as a marker of cell proliferation (it marks cells in the S phase of the cell cycle). Cryostat sections of retinas fixed with 4% paraformaldehyde were processed with the TUNEL method to detect apoptotic cells, or treated immunohistochemically with an antibody against RELN (gift by Dr. Goffinet, Univ. Namur, Belgium) as a marker of cells that regulate the cell migration.

In small embryos (less than 11 mm in length) the retinal cells were not layered (monolayer stage). In embryos longer than 11 mm (large embryos) and in alevins, the three retinal cell layers (GCL, INL and ONL) were distinguishable, as well as a distinct inner plexiform layer (IPL) separating the ganglion cell layer (GCL) from the inner nuclear layer (INL). Retinal epithelium pigmentation starts in large embryos. An outer plexiform layer (OPL) separating the inner (INL) and outer (ONL) nuclear layers was not clearly observed until the juvenile stage.

Distribution of proliferating cells

In the retinal neuroepithelium of small embryos most cells were proliferating (PCNA-ir). In large embryos and early alevins (14-15 mm length) most proliferating cells occupied the marginal (monolayered) retina (the ring-shaped growing region), while sparse PCNA-ir cells were observed in the INL and practically absent in the GCL and ONL of the central region of the retina (Fig. 1 A,B). In larger alevins (18-20 mm), numerous clusters of PCNA-ir cells were observed in the outer half of the INL and scattered cells were abundant in the ONL, occupying a more central position than INL clusters (Fig. 1C). In alevins, also, a number of PCNA-ir nuclei was observed in the optic nerve head and in the optic fiber layer, being probably of glial cells. A few of these cells could be also observed in the GCL of central regions. These inner PCNA-ir cells probably represent glioblasts or glial cells. In early juveniles the number of PCNA-ir cells was considerably lower, being mostly located in the ring-shaped marginal retina and in the INL, where they were sometimes clustered. The results obtained in young fish are in agreement with those of Julian *et al.* (1998) in young and adult rainbow trout.

The scarcity of proliferating cells observed in the GCL throughout retinal development contrasts with the frequent presence of PCNA-positive cells in the other cell layers, suggesting that new ganglion cells are added to the margin of the GCL from the proliferating marginal ring, while some INL cells and new photoreceptors originate from PCNA-ir cell clusters of the INL, as it was demonstrated in other teleosts (Negishi *et al.,* 1990; Kwan *et al.,* 1996; Julian *et al.,* 1998).

Distribution of apoptotic cells

A significant number of TUNEL-positive (+) elements was observed in retinas of trout embryos. As retinal layering proceeds, the number of apoptotic cells increased: TUNEL+ cells were observed in the inner border of the GCL of large embryos, and also appeared sparsely distributed in the INL and, less frequently, the ONL of alevins (Fig. 2). In alevins about 21 mm in length, apoptotic cells were scarce and sparsely at any retinal cell layer, and they were practically absent in 24-mm alevins.

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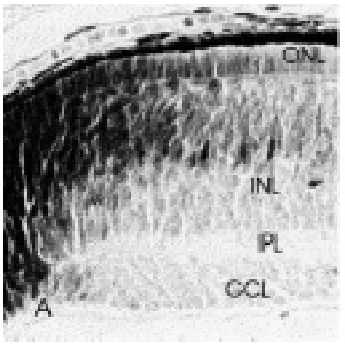

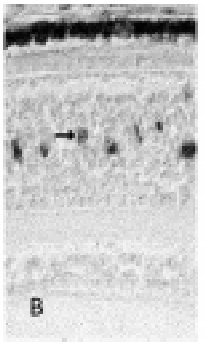

Fig. 1. PCNA-immunoreactive cells in the retina of a 14,5 mm (A: marginal retina; B) central retina) and 20 mm (C) alevins. *Arrows indicate clusters of PCNA-ir cells.*

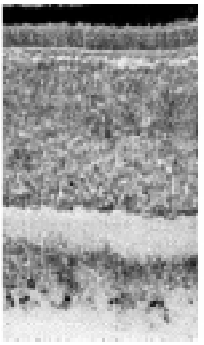

Fig. 2. TUNEL+ cells in the retina of a 19 mm alevin.

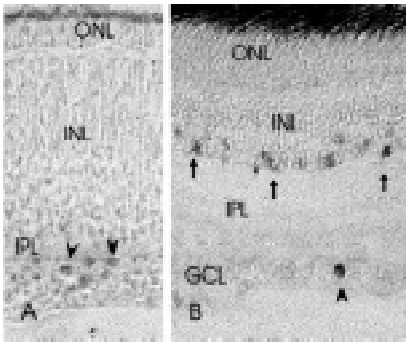

Fig. 3.- RELN-immunoreactivity in retina of a 12 mm embryos (A) and a 21mm alevin (B). *Faint RELN-ir perikarya in the GCL are indicated with arrow heads and those of the INL with arrows.*

These results indicate that during the morphogenesis of the trout retina programmed cell death probably eliminates ganglion cells that have not made the appropriate synaptic contacts in the optic tectum, as well as some INL cells, as it has been suggested in chick (Cook *et al.,* 1998).

Distribution of RELN immunoreactivity

In 12-mm embryos, faint RELN-ir perikarya were observed in the most central region of the GCL, and a diffuse RELN-ir band was also observed in the thin IPL of this region (Fig. 3A). In embryos more than 12 mm in length and in early alevins, RELN-ir perikarya were observed in GCL and, specially, in the INL, both separated by the diffuse immunoreactive band in the IPL (Fig. 3B). In the INL, these cells occupied different levels of the inner half of the layer and exhibited a pear-shaped perikaryon and a inwardly directed process. Probably they represent amacrine cells. RELN-ir cells of the GCL were very faintly stained, and whether they represent ganglion cells or other type of cell cannot be assessed. In late alevins RELN immunoreactivity appeared in the IPL and in a few perikarya of the internal border of the INL. In juveniles, low RELN immunoreactivity appeared in scattered cells at the innermost level of the INL.

These observations in trout suggest that during retinal development RELN is early secreted to the IPL to form a kind of filter to cell migration, contributing to the establishment of the intraretinal circuitry. In mouse, expression of disabled-1 (an adapter protein mediating RELN action) in amacrine cells indicates a role for RELN in retinal differentiation (Rice and Curran, 2000). As development proceeds, immunoreactivity to RELN diminishes but the RELN-ir cells of the retina could be responsible of the maintenance of certain circuits in the IPL.

Conclusions

In conclusion, although no apoptotic nuclei were found in the ONL of quail retina (Marín-Teva *et al.,* 1999), the pattern of distribution of proliferating and apoptotic cells in all retinal layers observed during trout development is similar to that described in tetrapods (Young, 1984; Beazley *et al.,* 1999), revealing that birth and dead of retinal cells are part of morphogenetic processes well conserved along the evolution. Besides, the number of cells during the retina development in vertebrates appears to be adjusted following the same spatiotemporal gradients (Kwan *et al.,* 1996; Marín-Teva *et al.,* 1999; Dyer and Cepko, 2001).

The RELN-ir band appears to contribute to the separation of two different compartments, one containing ganglion cells and other containing intrinsic cells. The pattern of RELN expression in the developing retina of trout is similar to that described in amniotes (see Bernier *et al.,* 2000), indicating that developmental mechanisms involving RELN in the retina are probably conserved. The function of RELN in the retina differentiation does not appear to be related with laminar development since no differences in laminar organisation were observed between the reeler mutant and the wild mice. We suggest that the abundant RELN observed in the IPL of trout could act as a filter avoiding that the new cells generated in the IPL clusters migrate to the GCL, and/or in the differentiation of some amacrine cell circuits.

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