CELL PROLIFERATION IN FIN FISH REGENERATION

Leonor SANTOS RUIZ, Jesús Alberto SANTAMARíA and José BECERRA
Department of Cell Biology and Genetics , Faculty of Sciences, University of Málaga, Campus Universitario de Teatinos, 29071 Málaga, Spain

Teleosts are one of the few vertebrate taxa able to regenerate the whole structure after partial amputation. This make them as good model to study morphogenetic events such as proliferation, growth control, cell-cell and cell-extracellular matrix interactions, and cell differentiation. Teleost fins are composed of a specie-charasteristic number of skeletal elements called ray or lepidotrichium. Each one is composed of two segmented tile-like structures (hemirays), that are bifurcated proximo-distally. The rays end distally in a double palisade of long rigid collagenous macrofibrils called actinotrichia. Inside lepidotrichia, there are vessels and nerves, and the whole structure is covered by a typical fish skin (Becerra *et al*, '83).

After partial amputation, the fins regenerate by means of a process that resembles the ontogenesis (Goss and Stagg, '57: Becerra *et al*, '96). As in other models, regeneration of fin fish comprises wound healing, formation of a blastema, proliferation and differentiation of different cell types (Santamaría *et al* '91). This report deals with proliferative aspects of fin regeneration.

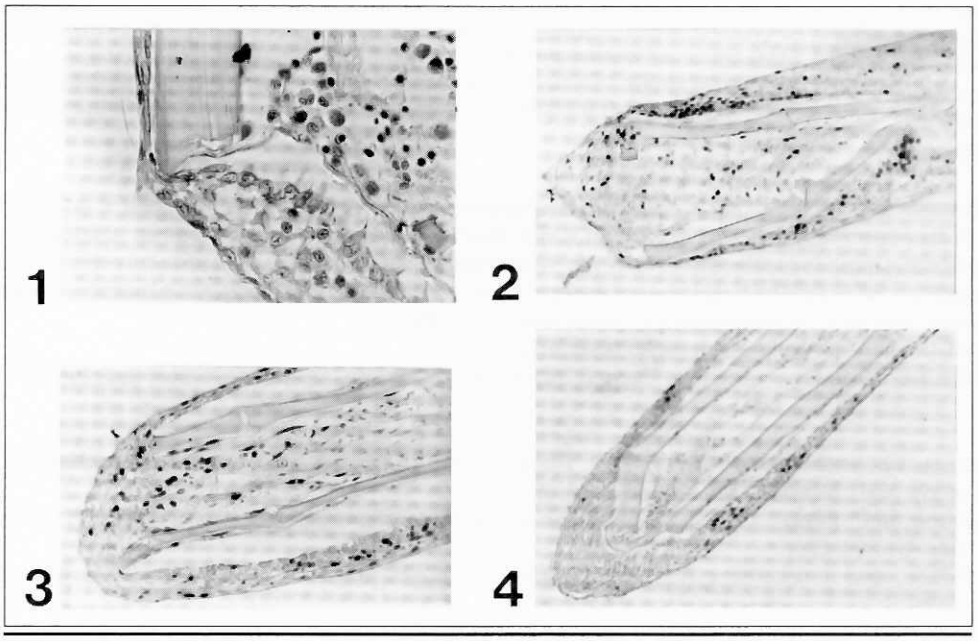

Fig. 1. Longitudinal section of a 12 h regenerated fin ray. The wound is covered by a thin epithelium which basal cells give off projections into the underlaying connective tissue. x400.
Fig. 2. Longitudinal section of a 60 h regenerated fin ray. Observe few proliferating cells in the connective tissue. The apical cap shows smaller proliferating rate than the reste of the epidermis tissue. x90.
Fig. 3. Longitudinal section of a 84 h regenerated fin ray. A small blastema can be distinguished in the distal connective tissue. x150.
Fig. 4. Longitudinal section of a 36 h regenerated fin ray. No proliferating cells can be seen in the connective tissue. Epidermal labelled cells are located far from the cap. x90.

Adult specimens of goldfish *Carassius auratus* (L.) were used. After amputation of the caudal fin at the level of the first bifurcation of the lepidotrichia, samples of the regenerating caudal fin were harvested every 12 hours during the first four days of regeneration, and 12 days after. Twelve hours before sampling, 0,25 mg/g body weigh 5-bromo-2'-deoxy-uridine (BrdU) in Hank's Solution were injected in all cases. Replicating nuclei (cells in S period) were inmunocytochemically detected by the monoclonal antibody anti-BrdU (Boehringer-Mannheim), and tissue sections were counterstained with hematoxilin.

Twelve hours after amputation, the wound was covered by a thin epithelium forming a "cap" that became thicker 12 h late. Since the basal lamina was absent, the cells of the basal layer of the cap protruded into the underlaying connective tissue by means of basal processes (Fig. 1). BrdU incorporation was not detected, neither in the epithelium nor in the connective. First labelled cells appeared 60 h after amputation, around the lepidotrichium stump (Fig. 2). Betwen days 3 and 4, a small population of labeled cells (blastema) could be distinguished (Fig. 3).

First epidermal labelled cells appeared at 36 h, although only in regions far from the cap and more concentrated in the transition ray-interray region (Fig. 4). At 48-60 h all epidermal areas showed high levels of BrdU incorporation (Fig. 2), being the transition ray-interray the highest labelled area (Fig. 5). Distal cap displayed few labelled cells (Fig. 2).

Around 12 days after amputation, a conspicuous proliferating cell population was located in the intraray space and flanked by the actinotrichial palisades (Fig. 6). Epidermal proliferation was prominent but restricted to the subterminal epidermis, being scarce at the distal cap. By this time, the basal epidermal layer was not labelled at the level of the blastema (Fig. 6), but strongly labelled more proximally where lepidotrichia became apparent. Conversely, the interray regions showed a higher number of labelled cells at every levels of the epidermis, including the basal layer, whereas connective tissue presented proliferating cells closely related with the lepidotrichial dorsal and ventral edges.

According to these results, it is likely that re-epithelization of the wound, that ends few hours after the injury, occurs by migration of cells from the edges of the cut surfaces, and then, few hours later, cell proliferation begin at the remaining epidermis, but not at the cap covering the distal area. Narrowing of the epidermis near the section, was according to this hypothesis (Becerra *et al*, '96).

In spite of that the two tissues involved in fin regeneration (epithelium and connective) begin to proliferate at different times and with different rates, they present evident relationships. Firstly, the epithelium lined the damaged connective tissue by migration of epithelial cells. Then it proliferates, probably to compensate the loss of cell layers. Only when the distal cap is well stablished, the underlying connective tissue begin to proliferate (Géraudie, '77 and '80). This suggests the presence of certain factors released from the epidermal cap, that could trigger proliferation in the connective tissue cells. Scleroblasts covering the lepidotrichial stump and fibroblasts of the intraray mesenchymatic tissue proliferate and form the blastema within the first four days after amputation. Since no muscle cells are present in fin fish and changes in epithelial mesenchyma are not evident, it is likely that the blastema proceeds only scleroblasts and fibroblasts.

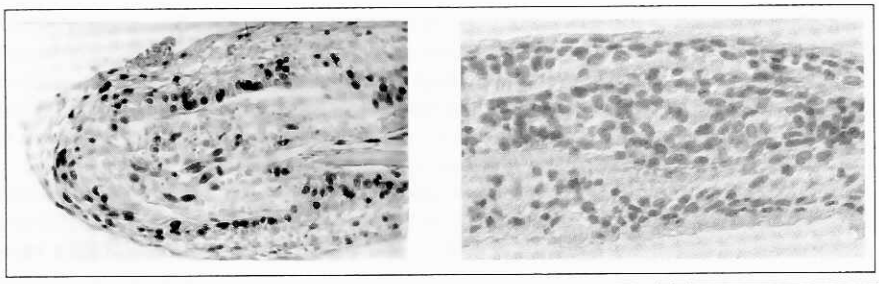

Fig. 5. Longitudinal section of a 60 h regenerated fin at the level of the ray-interray transitional region. Every epidermal regions are labelled. x140.

Fig. 6. Transversal section of a 12 days regenerated fin ray. A consolidated blastema can be seen betwen the palisades of actinotrichia. Epidermal cell proliferation is aboundant, except at the basal layer. x340.

In fin fish regeneration, epidermal basal layer proliferates in matured ray regions and in interray areas, however it does not proliferate in immature ray regions. It has been reported that epidermal basal layer of immature rays synthesizes actinotrichia (Geraudié, '77; Santamaría *et al*, '96), and this impairs proliferation. However, intralepidotrichial connective cells arround actinotrichia are positive to an anti-actinotrichia antiserum, whereas the basal layer of the epidermis are negative, thus suggesting that connective cells, and not epidermal cells, synthesize actinotrichia (umpublished results from the authors).

This work was supported by DGICYT (PB95/1134) and Junta de Andalucía, Spain.

The authors are indebted to Pilar Sánchez Palomeque and Luis Escassi, students of Sciences, for their technical assistance.

References

Becerra, J., Junqueira, L.C.U., Bechara, I.J.; and Montes, G.S. (1996). Regeneration of fin rays in teleosts: a histochemical, radioautographic, and ultrastructural study. Arch. Histol. Cytol. Vol. 59. 1:15-35.

Becerra, J., Marí-Beffa, M., and Carmona, M.C. (1987). Cytological changes during the earlier stages of differentiation in tail fin regeneration of teleosts. Verh. Anat. Ges. 81:689-690.

Becerra, J., Montes, G.S., Bexiga, S.R.R., and Junqueira, L.C.U. (1983).Structure of ghe tail fin in teleosts. Cell Tissue Res. 230:127-137.

Géraudie, J. (1977). Initiation of the actinotrichial development of the early fin bud of the fish *Salmo*. J. Morphol. 151:353-362.

Géraudie, J. (1980). Mitotic activity in the pseudoapical ridge of the trout pelvic fin bud, *Salmo gairdnieri*. J. Exp. Zool. 214:311-316.

Goss, R.J., and Stagg, M.W. (1957). The regeneration of fins and fin rays in *Fundulus heteroclitus*. J. Exp. Zool. 136:487-508.

Marí-Beffa, M., Santamaría, J.A., Fernández-Llebrez, P., and Becerra, J. (1996). Histochemically defined cell states during tail fin regeneration in teleosts fishes. Differentiation. 60:139-149.

Santamaría, J.A. and Becerra, J. (1991). Tail fin regeneration in teleosts: cell-extracellular matrix interaction in blastemal differentiation. J.Anat. 176:9-21.

Santamaría, J.A., Marí-Beffa, M., Santos-Ruiz, L., and Becerra, J. (1996). Incorporation of Bromodeoxyuridine in regenerating fin tissue of the Goldfish *Carassius auratus*. J. Exp. Zool. 275:300-307.