CELL PROLIFERATION IN FIN FISH REGENERATION

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Teleosts are one of the few vertebrate taxa able to regenerate the whole structure after partial amputation. This makes them a 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 species-characteristic 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 (Santamaria et al '91). This report deals with proliferative aspects of fin regeneration.

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 weight 5-bromo-2'-deoxy-uridine (BrdU) in Hank’s Solution were injected in all cases. Replicating nuclei (cells in S period) were immunocytochemically 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 later. 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). Between 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 established, 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 epiderial mesenchyma are not evident, it is likely that the blastema proceeds only scleroblasts and fibroblasts.

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 (Géraudie, '77; Santamaría et al, '96), and this impairs proliferation. However, intralepidotrichial connective cells around actinotrichia are positive to an anti-actinotrichia antisera, whereas the basal layer of the epidermis are negative, thus suggesting that connective cells, and not epidermal cells, synthesize actinotrichia (unpublished results from the authors).

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References


