AN ANTISERUM AGAINST CERATOTRICHIA (SELACHIAN) RECOGNIZES ACTINOTRICHIA IN TELEOST REGENERATING FINS

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The regenerative capacity of animals is an extended phenomenon that has concerned developmental biologists since the begining of this century. Several experimental models, of vertebrates and invertebrates, are presently under study. Although there is no homology between the tetrapod limb and the fin of fishes, the latter is becoming an interesting structurally simple model [2, 7]. As in other systems, partial amputation is followed by wound healing, blastema formation, cell proliferation and differentiation until the whole repair of the structure through an epimorphic process.

Teleost fins are composed of segmented and bifurcated skeletal elements, the rays or lepidotrichia, which are made of two tile-like dermal bone hemirays or hemisegments. These structures contain connective tissue with nerves and blood vessels and are sourounded by the skin. Each ray ends in a double palisade of long rigid rods called actinotrichia (Figs. 1 and 2), which have been reported to play a morphogenetic role [5].

From the distal blastema some cells differentiate into lepidotrichia-forming cells or scleroblasts which restore the hemisegments on both sides of the rays; other cells give rise to fibroblasts that regenerate the intraray connective tissue [1, 6]. Five days after amputation, actinotrichia are visible beneath the subepidermal basement membrane and they remain in this distal area of the regenerating fin until the end of the regenerative process by means of polymerization/depolymerization [4].

Blastemal cells contacting the new-formed actinotrichia, likely differentiate into scleroblasts that synthesize and release materials that form lepidotrichial hemisegments. Blastemal cells that do not contact actinotrichia, probably differentiate into fibroblasts that restore the intraray connective tissue.

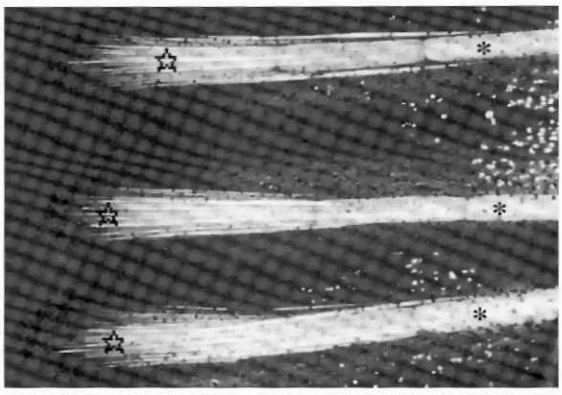

Fig. 1. Micrography of a in toto distal portion of a caudal fin of teleost showing actinotrichia at the end of each ray. Polarization light observation. Lepidotrichia (asterisks); actinotrichia bundle (stars). X 200.

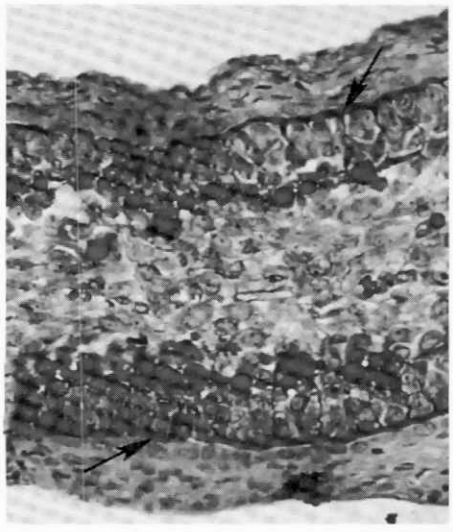

Fig. 2. Transverse section through a regenerated caudal fin stained with picrosirius-hematoxilin. Note the dark (red) profiles of actinotrichia. Scleroblasts form the new hemisegment (arrows). X 580.

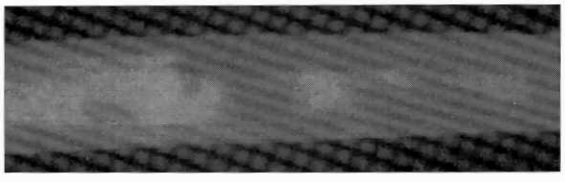

Fig. 3. Micrography of a in toto ceratotrichia stained with picrosirius and observed under polarization light. X 200.

In selachian fishes, ceratotrichia (Fig. 3) are the only skeletal structure of fins. Ceratotrichia have a chemical composition very similar to teleost actinotrichia. In both, the main proteinaceus component is a collagenous protein called elastoidine [3]. The likely role of actinotrichia in fin morphogenesis, led us to their detailed study.

In this report we present preliminary results on the use of an serum against ceratotrichia to study teleost fin regeneration. Immunological *in vivo* blockade by injection of the antiserum in the regenerating blastema was also performed.

Preliminary electrophoretic studies showed that actinotrichia from *Carassius auratus* and *Sparus aurata*, and ceratotrichia from *Scyliorhinus canicula*, had the same main protein of $\sim$ 22 KDa. We used extracts of ceratotrichia for raising antisera in rats. 0.05 mg of ceratotrichia in 1 ml of PBS 0.1 M at pH 7.3 conntaining Freund coadyuvant was injected subcutaneously. After a second dose, the third dose was injected intraperitoneally together with incomplete Freund coadyuvant. After a week animals were bled.

The characterization of the antiserum was made by "western-blot" and immunocytochemistry in tissue sections of control and regenerating fins.

Immunocytochemistry on tissue sections showed that the antiserum (1:1000 dilution) selectively bound to actinotrichia (Fig. 4). Westenr-blot studies using different extracts (ceratotrichia, actinotrichia from several species of teleosts and fins without actinotrichia) revealed several bands in ceratotrichia extracts, one band in actinotrichia extracts and no band for the extracts from fin teleosts lacking actinotrichia (Fig. 5).

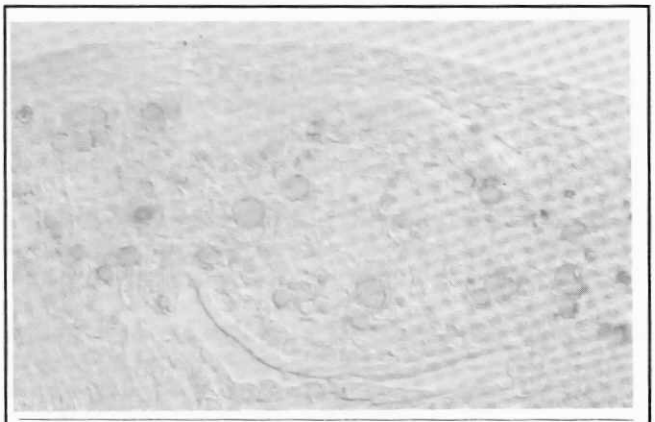

Fig. 4. Transverse section of a regenerating ray of *C. auratus* immunostained with anti-ceratotrichia serum. Note immunoreactivity actinotrichia. Nomarski optic. X 600.

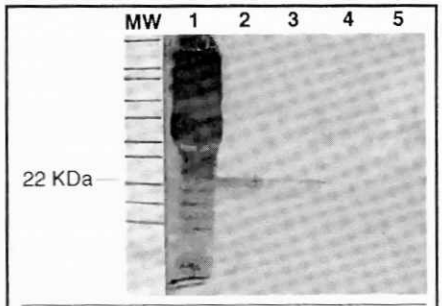

Fig. 5. Western blot with anti-ceratotrichia serum. Lanes shows different extracts: 1, ceratotrichia; 2, distal part of fins from *S. aurata*; 3, from *C. auratus*; 4 and 5, extract from caudal fin lacking actinotrichia from *S. aurata* and *C. auratus*, respectively. MW: standard molecular weights Mark 12 from Novel, stained with amido black. Extracts with actinotrichia react in a main single band of ~22 KDa.

Thus, this antiserum specifically recognizes actinotrichia in tissue sections and in immunobolts, showing a main band of ~22 KDa.

In order to block actinotrichia formation we injected the anti-ceratotrichia serum , using a pico-injector, in three days regenerating tail fins. Microinjections was carried out *in vivo* in specimens of *C. auratus* and the antiserum was administered in the 5th ray of the dorsal hemitail of the caudal fin. For control, preimmune serum was injected in the 5th ray of the ventral hemitail of the caudal fins from the sames specimens injected with the ceratotrichia antiserum.

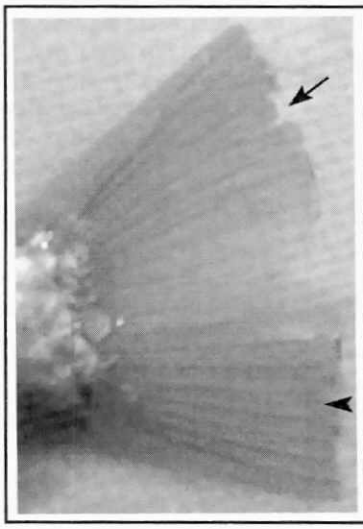

Fig. 6. Fresh and *in toto* caudal fin of *C. auratus* five days after the injection of the antiserum in the 5th ray of the dorsal hemitail (arrow). Note inhibition of growth and the normal pattern in the control injected with preimmune serum (arrowhead). X1.

Five days after injections the regenerative processes was checked by direct observation of specimens. In all cases the regeneration was homogeneous excepting in the rays were the antiserum was administered (Fig. 6).

This interesting results confirm a role of actinotrichia in regeneration and probably also on ontogenesis of teleosts fin.

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