CELL TO CELL INTERACTIONS DURING TELEOSTS FIN REGENERATION

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Cell to cell interactions operative during fin regeneration in both species *Carassius auratus* and *Brachydanio rerio* were analyzed in an experiment (Model I) of total and partial ablations.

Non-randomized groups of 10 to 15 specimens of *Carassius auratus* were subjected to the following experimental treatments: a.- total ablation through varying proximo-distal levels, b.- partial ablation of individual rays, c.- partial ablation of individual hemirays and d.- total ablation and daily surgycal separation of selected rays by scalpel. The following morphometric variables were computed: width, length, area, bifurcation distance (BD), segmentation phase shift, width relation during bifurcation (Mandelbrot index) and total regenerate length. These data were analyzed by multivariate statistics which include principal components test in order to study under a multidimensional perspective the existing relationships among these variables in order to statistically characterize regeneration. Conditions a, b and c have also been studied in *Brachydanio rerio* obtaining comparable results to those from *Carassius auratus.* These results morphometrically analyze experiments formerly carried out by Morgan (1902), Nabrit (1929) and Goss and Stagg (1957).

The main results are summarized in the following figures. During regeneration the dimension and form of the LFCs (lepidotrichia forming cells) layer, the first sign of differentiation in proximal blastema, (asterisks in figure 1 a) change in relation to some morphometric variables: length, width or BD of the ray segment (figure 1 a). Regenerates following treatment b are thinner, occasionally of 4 to 5 cells wide, and show a segment length reduction of 20 to 30 % (Figure 1 b). Lepidotrichia segmentation and bifurcation is lacking in extremelly large segments and ray polarity vary dependent on existing interray tissue, probably due to cell tractions being affected, following treatment d (Figure 1 c). These results suggest an autonomous partially size-invariant blastemal mechanism acting on fin ray segmentation control and other non-autonomous cellular mechanisms operating in right-left or anterior-posterior (in paired-fins) axes partially responsible of segmentation and bifurcation at long distance. These results were postulated by theoretical models of cell tractions (Oster et al., 1983; Oster et al., 1985)in which condensation, bifurcation and segmentation are controlled by form-dependent cell to cell interaction mechanisms. Furthermore, local interactions may affect both segmentation and bifurcation within hemiblastema at their distal 400 µm (Figure 2 a). Coincidence in both hemiray segmentations desappears in the initially regenerated segments following treatment b (Figure 2 b) and in most of the hemiblastemal regenerate following treatment c (Figure 2 c). In these treatments abnormal, partial segmentation processes have also been observed (Marí-Beffa and Becerra, 1996).

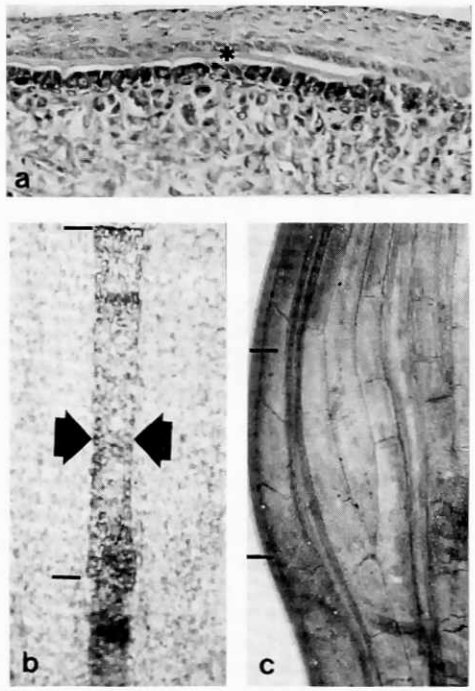

Figure 1 a. Transversal section of a regenerate of Tilapia melanopleura following total ablation stained with hematoxilin-eosin. Observe the homogeneous distribution of LFCs (asterisk) in a hemiblastema x 250. 1 b. Segment of a fin ray regenerate in group b. Observe both its small width (distance between arrows), almost 4 to 5 cells wide, and normal length (bars) x 170. 1 c. Regenerate following treatment d. Fin ray polarity changes depending on interray tissue, absent during marked distance (bars) x 20.

Histological studies of treatment b and c suggest a quick and complete reepitelization in 3 to 4 days by which apical epidermis looses its contact with the blastema-forming stump. Moreover, connective tissue regeneration is much slower but quicker than regenerates in group a. Both the dimensional reduction and the lack of actinotrichia (apical rods of a hyperpolimerized collagen-like molecule) in their first half, observed in these regenerates, suggest that blastemal proliferating cells recruitment can be experimentally affected. Both the quick distalization process that occur in treatments b and c regenerates and the homogeneous cell proliferation in blastema (Santamaría y col., 1996) are also indicative of partial appearance of cell movements (Figure 3 b). During these processes a group of proliferating and probably migrating blastemal cells condensate in LFCs (asterisks in Figure

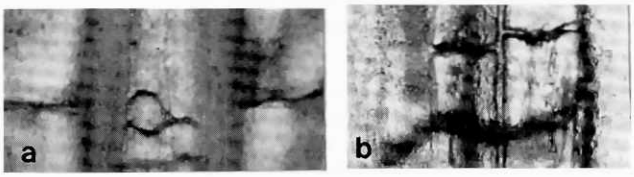

Figure 2 a. Detail of a regenerate in group b. Observe lack of coincidence in hemilepidotrichia segmentation x 50. 2 b. Detail of hemisegmental regenerate in group c. Observe independence both in segmentation and bifurcation x 125.

3 a) inducing changes in cell shape in the basal epidermis layer. This is suggestive of secondary intertissue local-range interactions through basal lamina independently operative in each hemiblastema probably implicated in the establishment of the final pattern.

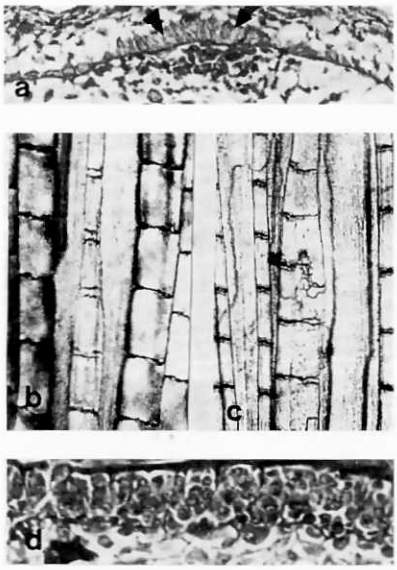

Figure 3a. Transversal section of a regeneratein group b stained with hematoxilin-eosin. Basal epidermal layer cells are prismatic as they adjoin condensing LFCs (arrowheads). x 300. 3 b. Detail of a fin ray regenerate in group b. Observe reduction in segmental width as compared with intact neighbour rays. x20. 3c. Lateral regeneration of a distal fin ray in group b. Observe caotic processes of segmentation and/or bifurcation x20. 3d. Successive fin ablations may produce abnormal differentiation of LFCs sheet in *Carassius auratus*. x 350.

Segmentation coincidence measured in fishes of treatments a, b and c indicate that controlling mechanisms must be probably occuring distal to LFCs, in a 3 to 4 cells wide range, where the transcripts of the genes *zf-msx a-d* are expressed (Akimenko at el., 1995). Finally, some experimental animals were studied following succesive amputations and regenerations. In these experiments and in other conditions of uncontrolled physiological affections, segmentation and bifurcation processes are mixed in complicated segmental morphologies (Figure 3 c). The histological study of the regenerates display anomalous LFCs differentation in a variable number of small groups of condensed cells synthezising the extracellular matrix of the lepidotrichia (Figure 3 d). This cell disorganization may be due to perturbations in epidermal-connective tissue interaction signals.

In summary, five different interaction processes have been studied in our work : a.- apical intertissue interaction between wound epidermis and stump recruiting cells in blastemal formation; b.- short-range interactions among LFCs controlling lepidotrichia segmentation (Marí-Beffa and Becerra, 1996); c.- long-range interactions between hemiblastema controlling coincidence in lepidotrichia segmentation; d.- long-range lateral interactions between blastema of differente neighbour fin rays controlling segmentation and bifurcation (see Marí-Beffa and Becerra, 1996) in which cell tractions may also be operative; and e.- short-range intertissue inductions of cell shape changes in both basal epidermal layer cells and condensing LFCs (Figure 4). All these cel interaction mechanisms of morphogenesis control must be coordinately acting in the apical blastema if fin ray regenerates of teleosts and probably are also operative during its development.

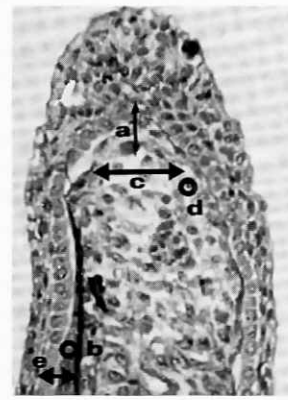

Figure 4. Arrows indicate signals referenced in text. a-e are different types of morphogenetic interactions

Mutational analysis of *Brachydanio rerio* have provided a huge number of mutations affecting fin development (Mullins et al., 1994) and regeneration (Johnson et al., 1995). However, very few mutations showing phenotypes in fin ray segmentation and bifurcation have been published yet. LFCs differentiation is affected in the temperature-sensitive mutation *rg6* (Johnson et al., 1995). Experiments must be carried out in order to study its involvement in epidermal-connective tissue induction during fin regeneration. We have also analyzed *zf-msx b* and *zf-msx d* transcripts expressions in blastemal cell recruitment and segmentation processes. These results as well as other developmental genes expression studies will be published elsewhere.

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