

## Ray and inter-ray blastemas interact to control bifurcations of *Danio rerio* fin rays

CARMEN MURCIANO, JOSEFA RUIZ, DAMIÁN MASEDA, TAHÍA D. FERNÁNDEZ, IVÁN DURÁN, FERNANDO MARÍN-GIRÓN, JOSÉ BECERRA and MANUEL MARÍ-BECCA\*

Department of Cell Biology and Genetics, Faculty of Science, University of Málaga, Spain

**ABSTRACT** Teleost fin rays bifurcations are characteristic of each fin ray of the fishes. These structures are formed of two parenthesis-like dermal bones (hemi-rays) and surrounded by a loose connective tissue and a multistratified planar epidermis. Each ray is joined to their neighbour rays by the interray. The interray is composed of a loose connective tissue surrounded by the epidermis (Becerra *et al.*, 1983). In previous works we have shown the necessity of tissues neighbour to the ray blastema for a proper bifurcation (Marí-Becerra *et al.*, 1996, 1999). In this communication we present evidences in favour of the hypothesis that the interray blastema is specifically necessary for a proper differentiation of the bifurcation processes in each ray during regeneration in *Danio rerio* (Hamilton-Buchanan) (Cyprinidae, Teleostei).

### Materials and Methods

We have performed partial ablations and heterotopical grafts in order to obtain recombinants between rays and interrays from different positions within the fin. The operations carried out were as follows:

Single ray ablations (the regeneration occurring after this operation may lead to a non-bifurcating ray depending on the outcome of a re-epithelization process which may occur previous to the ray regeneration).

Heterotopical ray graftings. Ray fragments were grafted into the base of the fin interray in anaesthetised fishes (Birnie, 1947) (after this operation a cut was made ablating the fin at the level of the graft so that a ray with a new ray-interray recombinant was made) (The ray graft was labeled with either Dil or white cells (light pigmented cells at the tip of the long rays in the tail fin).

In previous work we demonstrated the necessity of the presence of tissues neighbour to the ray blastema for a proper bifurcation to occur (Marí-Becerra *et al.*, 1996, 1999). Lateral rays in the tail fin are unbifurcated and partially lack this neighbour tissue. Ectopic interray neighbour to the first unbifurcated ray was also obtained carrying out a small hole at its base which was abnormally regenerated as an ectopic ray. Cuts made at this level lead to first unbifurcated ray with two neighbour interrays (one normal and one ectopic).

### Results

Partial ablations, heterotopical graftings and small holes have been used to obtain lateral rays, which do not normally bifurcate, surrounded with ectopic interrays from different positions within the tail fin.

Previous studies showed that the isolation of rays during regeneration led to absence of interrays and ray bifurcations leading us to propose that the neighbour tissue are necessary for a proper bifurcation to occur. We have compared the regenerates obtained after single ray ablations in which a previous re-epithelization may occur. Depending on this process, a quick regeneration of the epidermis with a thin loose connective tissue in between, the ray renegegate bifurcates. When the re-epithelization occurs the ray does not bifurcate or does it at a very distal level, whereas its absence leads to a normal bifurcation. Expression of several genes at the distal blastema and epidermis during normal regeneration have been studied. There is a number of genes candidate to be involved in epidermis-blastema interactions (*msxC*, *bmp4* and its receptors (Géraudie *et al.*, 1998). In the first case (the presence of a previous re-epithelization) the expression of these genes are restricted to the ray blastema and absent in the neighbour interray (Marí-Becerra and Akimenko, unpublished results). These results suggest that exclusively the interray blastema, and not the neighbour ray blastemas, are necessary for a positioned bifurcation.

It is possible that the absence of neighbour tissue in lateral rays is the cause of their not being bifurcated. In this second instance the ray blastema is neighbour to two interray blastemas. In Figure 1 we show the result regenerate of a small hole at the base of a first unbifurcated ray which grows outside the fin. This fin was cut at the level of the ectopic ray (asterisk) and neither the regenerate of the original rays nor the ectopic rays bifurcated or did it (aborting after two or three segments distal to the bifurcation, and not differentiating a central interray) at a very low frequency. These results suggest the first unbifurcated might be incapable of bifurcating normally, in normal position and with a central interray, even in the case two neighbour interrays are present. In order to test this we further grafted heterotopically a proximal fragment of this ray into the central interray normally neighbour to bifurcating rays. We have observed that, in some instances, the grafted ray bifurcated in a similar way to their neighbour short rays. We could recognize the grafted tissue by the white cells (light pigments shown at the distal apex of this first long ray) (Fig. 2).

Grafts have also been labeled by Dil staining. This method was first used to analyze blastema formation (Akimenko *et al.*, in preparation). Cell mixing between ray and interray blastemas is not significant but white cells may migrate away the grafted ray. Autonomous vs. non-autonomous behaviour have been analyzed by these methods. Grafted rays may bifurcate in the presence of neighbour interrays which show normal pigmentation.

\*Address correspondence to: Dr. M. Marí-Becerra. Department of Cell Biology and Genetics. Faculty of Science, University of Málaga, 29071 Málaga. Spain. e-mail: becca@uma.es

tion (Fig. 2). These rays also showed a slight overgrowth as compared to the small neighbour rays. This suggests a double behaviour of the graft: some characters seem to display a *herkunftsgemäss* (origin dependence) behaviour whereas others show an *ortsgemäss* behaviour (new position dependence). Our results stress the role of the interray in inducing positioned bifurcations in the ray blastema.

We also propose that the inducing signals may be heterogeneously distributed along the fin. In order to support the existence of heterogeneously distributed signals along the dorso-ventral axis of the tail fin we also obtained immunocytochemical evidence of the heterogeneous distribution of FGF-2 in the epidermis of a developing and regenerating tail fin of *Danio rerio* and *Carassius auratus* L. (Cyprinidae, Teleostei). These epitopes are co-expressed with the absence of bifurcation inductive potential of the tail fin of *Danio rerio*. Whereas signals expressed in the ray blastema have been proposed to control ray bifurcations, such as *Sonic hedgehog* (Laforest et al., 1998), no non-autonomous signal has been studied as involved in this process.

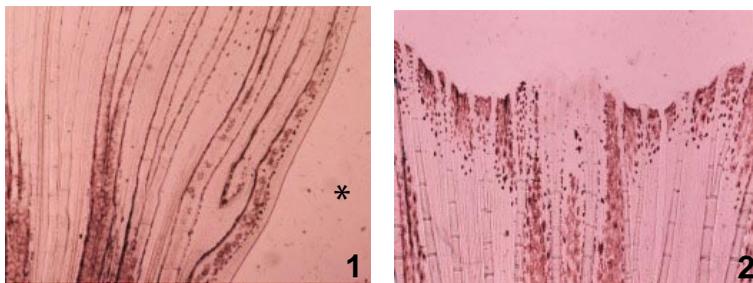
## Conclusions

In the present communication evidences are shown on the necessity of interrays blastema for a proper differentiation of the ray bifurcations. We show that the interray blastema may exert an induction on the ray blastema. This heterogeneous inductive potential from the interray blastema could be involved in the control of the different bifurcation patterns and asymmetries in the rays of *Danio rerio*.

Supported by a grant from DIGESYC PB98-1409.

## References

BECERRA, J., MONTES, G.S., BEXIGA, S.R.R. and JUNQUEIRA, L.C.U. (1983) Structure of the tail fin in teleosts. *Cell Tissue Res.* 230: 127-137.



**Fig. 1.** After a hole in proximal regions of the lateral non-bifurcating ray, this may regenerate outside the fin forming a new ectopic first ray. When these original and ectopic rays are cut, neither of them show any sign of bifurcation. When single rays regenerate separated from their neighbour rays, the interray is never formed and they never bifurcate. However, in some instances, aborted incomplete bifurcations may occur at a low frequency in the first ray irrespective of the presence of ectopic interray. This suggest that the first non-bifurcating ray occurs due to a process different to the absence of one of the neighbour interrays. X17.

**Fig. 2.** A proximal fragment of a non-bifurcating ray grafted to an interray, normally neighbour to a bifurcating ray, may regenerate showing bifurcations along their structure. A non-autonomous behaviour, probably dependent on the interray blastema, may be deduced according to the white cell pattern (clear pigmentation in the distal region of the grafted ray (central region)) shown by the graft derivatives. X17.

BIRNIE, J.H. (1947) Regeneration and Transplantation of fin rays in the goldfish. *Anat. Rec.* 99: 648.

GÉRAUDIE, J., AKIMENKO, M.A. and SMITH, M.M. (1998) The dermal skeleton. In "Cellular and Molecular Basis of Regeneration" (Géraudie, J. and Ferreti, P. eds.) pp.: 167-185.

LAFORREST, L., BROWN, C.W., POLEO, G., GÉRAUDIE, J., TADA, M, EKKER, M. and AKIMENKO, M.A. (1998) Involvement of the *Sonic hedgehog*, *patched 1* and *bmp2* genes in patterning of the zebrafish dermal fin rays. *Development.* 125: 4175-4184.

MARÍ-BEFFA, M., MATEOS, I., PALMQVIST, P. and BECERRA, J. (1996) Cell to cell interactions during teleosts fin regeneration. *Int. J. Dev. Biol. Supp. R.* 179S-180S.

MARÍ-BEFFA, M., PALMQVIST, P., MARÍN-GIRÓN, F., MONTES, G.S. and BECERRA, J. (1999) Morphometrical study of the regeneration of individual rays in teleosts tail fins. *J. Anat.* 195: 393-405.