Int. J. Dev. Biol. 45 (S1): S127-S128 (2001)

Short Report

FGF signalling and blastema growth during amphibian tail regeneration

PATRIZIA FERRETTI*, FANG ZHANG, LEONOR SANTOS-RUIZ and JONATHAN D.W. CLARKE

Developmental Biology Unit, Institute of Child Health, UCL and Anatomy and Developmental Biology, UCL, London, UK

ABSTRACT Urodeles amphibians can regenerate their tails, including spinal cord and ganglia, as adults. The cellular and molecular mechanisms underlying tail regeneration, and particularly recruitment of neural progenitors and the initiation of their division have yet to be fully elucidated. We have studied the role of FGF2 in this process and found that FGF2 is important both for regeneration of the spinal cord and of the mesenchymal tissues of the tail stump, as indicated by its up-regulation in the blastema and in ependymal cells and by the fact that FGF2-soaked beads increase blastema growth as compared to controls. We have also studied the expression of *Pleurodeles Sox1* in regenerating tails, as members of this gene family have been shown to be upstream of FGF signalling in other systems. We show by RT-PCR analysis that this gene is expressed at low levels in normal tail, suggesting that its presence in the adult newt tail may be causally related to its high regenerative capability. Furthermore, this gene is significantly up-regulated following tail amputation indicating that it plays an important role during tail regeneration.

Amputation of the newt tail triggers a complex series of events which lead to faithful regeneration both of the mesenchymal tissues of the tail and of the nervous system within it, namely spinal cord and associated ganglia. These tissues differentiate from the ependymal tube that forms in response to amputation through migration of cells from the spinal cord stump and their subsequent proliferation (Clarke and Ferretti, 1998). The ependymal cells are currently believed to be the main source of cell for spinal cord regeneration, though our preliminary results indicate that neurons migrate into the regenerating tail and may play a role in this process. The mesenchymal progenitor cells of the tail, blastemal cells, are thought to originate through a process of dedifferentiation similar to that described in the limb, and their division is controlled by the nerve and the wound epidermis. Fibroblast growth factors (FGFs) appear to play important roles in this process (Mullen *et al.,* 1996; Poulin *et al.,* 1993). FGFs are small peptide growth factors with multiple biological functions which play significant roles in patterning, growth and differentiation (Szebenyi and Fallon, 1999).

The purpose of this study was to further investigate the molecular mechanisms underlying regeneration of the amphibian tail focusing in particular on FGF2 expression and on the analysis of putative regulators of FGF signalling.

Results

We have recently shown that FGF2 expression is induced in the regenerating spinal cord early after tail amputation, and is expressed in the undifferentiated cells lining the ependymal canal from which the new cord will form (Zhang *et al.,* 2000). Its expression gradually decreases with the onset of differentiation and in the regenerated cord, as in the normal one, it is not detected in the ependymal cells, but is expressed in a subset of neurones. This suggests that FGF2 plays a role both in the early stages of regeneration, possibly in the proliferation of neural progenitors and maintenance of the undifferentiated state. Analysis of the effect of FGF2-soaked beads implanted in the blastema shows that FGF2 has an effect also on blastema growth as exemplified in Fig. 1.

Rapid growth is characteristic of early stages of tail regeneration and in 2 week regenerates BrdU incorporation (Fig. 2A) is observed in blastemal cells, regenerating cartilage, basal epidermal cells and in the ependymal tube. Analysis of FGF2 expression by immunocytochemistry (Fig. 2B-D) shows that FGF2, in addition to being up-regulated in the regenerating spinal cord, is also expressed in a subset of blastemal cells and chondroblasts, and in the basal epidermal layer (Fig. 2B-D). In addition, it is also expressed in differentiating muscle. All together these results indicate that FGF2 plays an important role in tail regeneration and is likely to be involved both in proliferation and differentiation of tail tissues. This is also supported by the finding that expression of the four FGFRs is differently regulated during tail regeneration (Zhang *et al.,* 2001). Analysis of FGFR mRNA in regenerating blastemas by RT-PCR shows no change in the level of expression of FGFR2 and 3, whereas FGFR1 and FGFR4 mRNA are up-regulated following tail amputation. Unlike the other FGF receptors, FGFR4 expression is restricted to the ependymal tube. Work to further elucidate the effects mediated by individual receptors is in progress.

Although there is increasing evidence that FGF signalling is involved in development of several tissues, including neural tissue, it is not clear how FGF signalling is initiated and regulated. It has been recently shown that expression of another member of the FGF family, FGF4, is controlled by *Sox2*, and that *Sox2* and FGF work synergistically to initiate neural induction (Fraidenraich *et al*., 1998; Mizuseki *et al*., 1998). *Sox* genes are transcription factors containing a DNA binding domain, the HMG-box, and several of them are expressed in a spatio-temporally restricted pattern in the developing nervous system. In order to establish whether *Sox* gene expression is regulated during tail regeneration in a similar fashion to FGF2, we have studied changes in the expression of a *Pleurodeles* gene named "*Sox1*" (Chardard *et al.,* 1993) by RT-PCR. The available sequence of this gene is within the HGM-box and has a high percentage of nucleotide identity with *Sox* genes expressed in the nervous system in other species.

***Address correspondence to:** Patrizia Ferretti. Developmental Biology Unit, Institute of Child Health, UCL and Anatomy and Developmental Biology, UCL, London, UK. e-mail: ferretti@ich.ucl.ac.uk

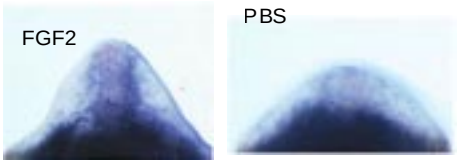

Fig 1. Effect of FGF2-soaked beads on tail regeneration. *Beads soaked either in 0.2 mg/ml FGF2 or in PBS were implanted in the tail tip 3 and 9 days after tail amputation and the blastemas collected at 12 days of regeneration. Note the larger size of FGF2-treated blastema as compared to the control.*

As shown in Fig. 3A *Pleurodeles Sox* is up-regulated in response to amputation in the regenerating tail blastema. Furthermore, it is expressed at low levels in normal spinal cord and up-regulated in the regenerating ependymal tube (Fig. 3B). Up-regulation is observed also in the blastema without ependymal tube and may be due to the presence in this tissue of DRG and Schwann cell precursors. Given the high conservation of the HMG-box motif, particularly among *Sox* genes within the same group, it is not clear at present whether our primers amplify only one *Sox* transcript in *Pleurodeles*.

Conclusions

All together our data show that there is significant up-regulation of *Sox* during tail regeneration, and suggest that this gene(s) is important for spinal cord regeneration. Furthermore, changes in *Sox* expression during regeneration parallel that of FGF2, with the highest level of expression being observed in 2 week blastemas. This expression pattern is consistent with the possibility that *Sox* genes may control FGF2 expression in the regenerating cord and

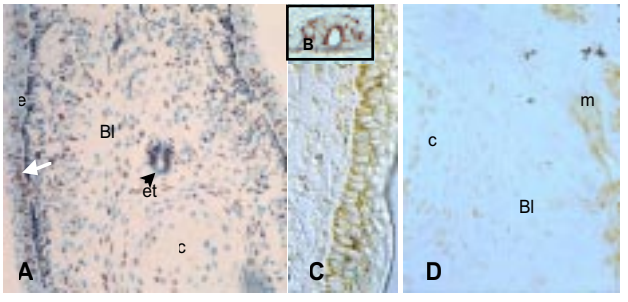

Fig. 2. Cell proliferation and FGF2 expression in 2 week tail blastemas. (A) *BrdU-positive nuclei (brown) are observed in basal epidermal cells (white arrow) and in some cells of the ependymal tube (et), cartilage (c) and blastema (Bl).* **(B-D)** *FGF2 protein expression (brown) displays a similar pattern of expression to that observed with BrdU. e, epidermis; m, differentiating muscle.*

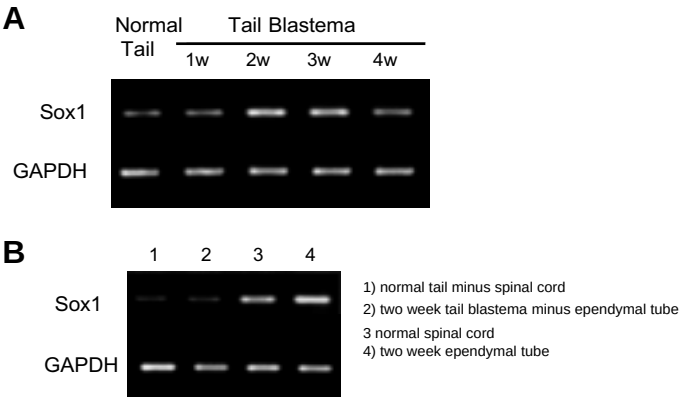

Fig. 3. Expression of Pleurodeles *Sox1* mRNA assessed by RT-PCR. (A) *Sox1 is up-regulated in response to amputation and its levels are particularly increased in 2 and 3 week regenerates.* **(B)** *Sox1 is up-regulated both in the ependymal tube and in the blastema. The nucleotide sequence of the 5' primer used is "aatggcacaggagaacc" and of the 3' primer is "aatggcacaggagaacc".*

it will be important to perform functional studies both *in vivo* and *in vitro* to establish whether this is the case.

References

CHARDARD, D., CHESNEL, A., GOZE, C., DOURNON, C., and BERTA, P. (1993). Pw Sox-1: the first member of the Sox gene family in Urodeles. *Nucleic Acids Res* 21: 3576.

CLARKE, J. D. W., and FERRETTI, P. (1998). CNS regeneration in lower vertebrates. *In* "Cellular and Molecular Basis of Regeneration: From Invertebrates to Humans" (Ferretti, P. and Géraudie, J. Eds.), pp. 255-269. John Wiley and Sons, Ltd, Chichester, England.

FRAIDENRAICH, D., LANG, R. and BASILICO, C. (1998). Distinct regulatory elements govern Fgf4 gene expression in the mouse blastocyst, myotomes, and developing limb. *Dev Biol* 204: 197-209.

MIZUSEKI, K., KISHI, M., MATSUI, M., NAKANISHI, S., and SASAI, Y. (1998). Xenopus Zic-related-1 and Sox-2, two factors induced by chordin, have distinct activities in the initiation of neural induction. *Development* 125**:** 579-87.

MULLEN, L. M., BRYANT, S. V., TOROK, M. A., BLUMBERG, B., and GARDINER, D. M. (1996). Nerve dependency of regeneration: the role of *Distal-less* and FGF signaling in amphibian limb regeneration. *Development* 122**:** 3487-3497.

POULIN, M. L., PATRIE, K. M., BOTELHO, M. J., TASSAVA, R. A., and CHIU, I. M. (1993). Heterogeneity in the expression of fibroblast growth factor receptors during limb regeneration in newts *(Notophthalmus viridescens). Development* 119: 353-61.

SZEBENYI, G., and FALLON, J. F. (1999). Fibroblast growth factors as multifunctional signaling factors. *Int Rev Cytol* 185: 45-106.

ZHANG, F., CLARKE, J. D. W., and FERRETTI, P. (2000). FGF-2 up-regulation and proliferation of neural progenitors in the regenerating amphibian spinal cord. *Dev. Biol.* 225: 381-391.

ZHANG, F., CLARKE, J. D. W., SANTOS, L., and FERRETTI, P. (2001). Differential regulation of FGFRs in the regenerating amphibian spinal cord *in vivo. Submitted.*