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Re-examining jaw regeneration in urodeles: what have we learnt?

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ABSTRACT Urodele amphibians can regenerate not only their limbs and tails, but also their upper and lower jaws rather faithfully. However, relatively few studies of jaw regeneration in amphibians have been carried out, especially in recent years. It is therefore important to re-examine thoroughly this regenerating system, since the advent of sophisticated morphological techniques and the development of molecular approaches offer the promise of renewed and rapid progress in our understanding of complex developmental problems such as this. This paper briefly reviews some of the early research on jaw regeneration, some of the fundamental questions which have been asked and have yet to be answered, and the work we have carried out in order to understand the molecular mechanisms underlying jaw regeneration in the newt, *Notophthalmus viridescens*. In addition, some aspects of jaw regeneration will be discussed in relation to regeneration of the adult limb.

KEY WORDS: *jaw, limb, regeneration, nerve, retinoic acid*

Introduction

Urodele amphibians represent a valuable model for studying the regeneration of complex body structures. Although much work on limb regeneration has been published, relatively little is known about jaw regeneration in amphibians. Pioneer morphological studies on this system (Vallette, 1929; Goss and Stagg, 1958a,b; Graver, 1973) have not been followed up, either at a more detailed morphological level or at a molecular level. This is not entirely surprising, since the structure of lower and upper jaws is much more complex than the structure of the limb (Figs. 1 and 2). The biological questions posed by jaw regeneration are fundamentally the same as those encountered when trying to unravel any other phenomenon of epimorphic regeneration. Those questions include the following: how faithfully are the missing parts reproduced? What is the extent of the regenerative territory? What is the origin of the progenitor cells which give rise to the regenerate? Which molecular mechanisms underlie regeneration? Are some molecules specific to regeneration in general and others specific to regeneration of a certain organ in particular?

Regenerative capability and regenerative fields

We recently re-examined and extended the morphological analysis of regeneration of upper and lower jaws in the newt, *Notophthalmus viridescens* (Ghosh *et al.*, 1994). In our studies on lower jaw regeneration, the hyoid apparatus, which appears to lack any regenerative ability (Goss and Stagg, 1958a), was not amputated. From our work and previous studies (Vallette, 1929; Goss and Stagg, 1958a) it appears that the more distal jaw amputations produce better regenerates and in a shorter time. In addition, although the original shape of the jaw is largely reproduced in the adult, not all the skeletal structures of the regenerate are identical to the original ones. For example, whereas tooth-bearing bones can regenerate very faithfully, other bones, such as the prearticular and the nasal, have not reformed five months after amputation, and are replaced by cartilage.

Although amputation of larval lower jaws does not result in any apparent skeletal defect (Ghosh *et al.*, 1994), and we have not observed significant abnormalities in larval upper jaw regenerates (Ghosh *et al.*, in preparation), Vallette reported abnormalities in some regenerated upper jaws of newt larvae (Vallette, 1929). However, the fact that the age of the larvae used in those early experiments was not defined, and that urodele species other than *Notophthalmus viridescens* were used, could account for these discrepancies. Furthermore, analysis of the regenerative ability of a variety of soft tissues in adult jaws by scanning electron microscopy has shown that all of the tissues examined, such as epithelia, glands and muscle, appear to be able to regenerate rather efficiently. From our study it has also clearly emerged that

Abbreviations used in this paper: RA, retinoic acid; k8, keratin 8; k18, keratin 18.

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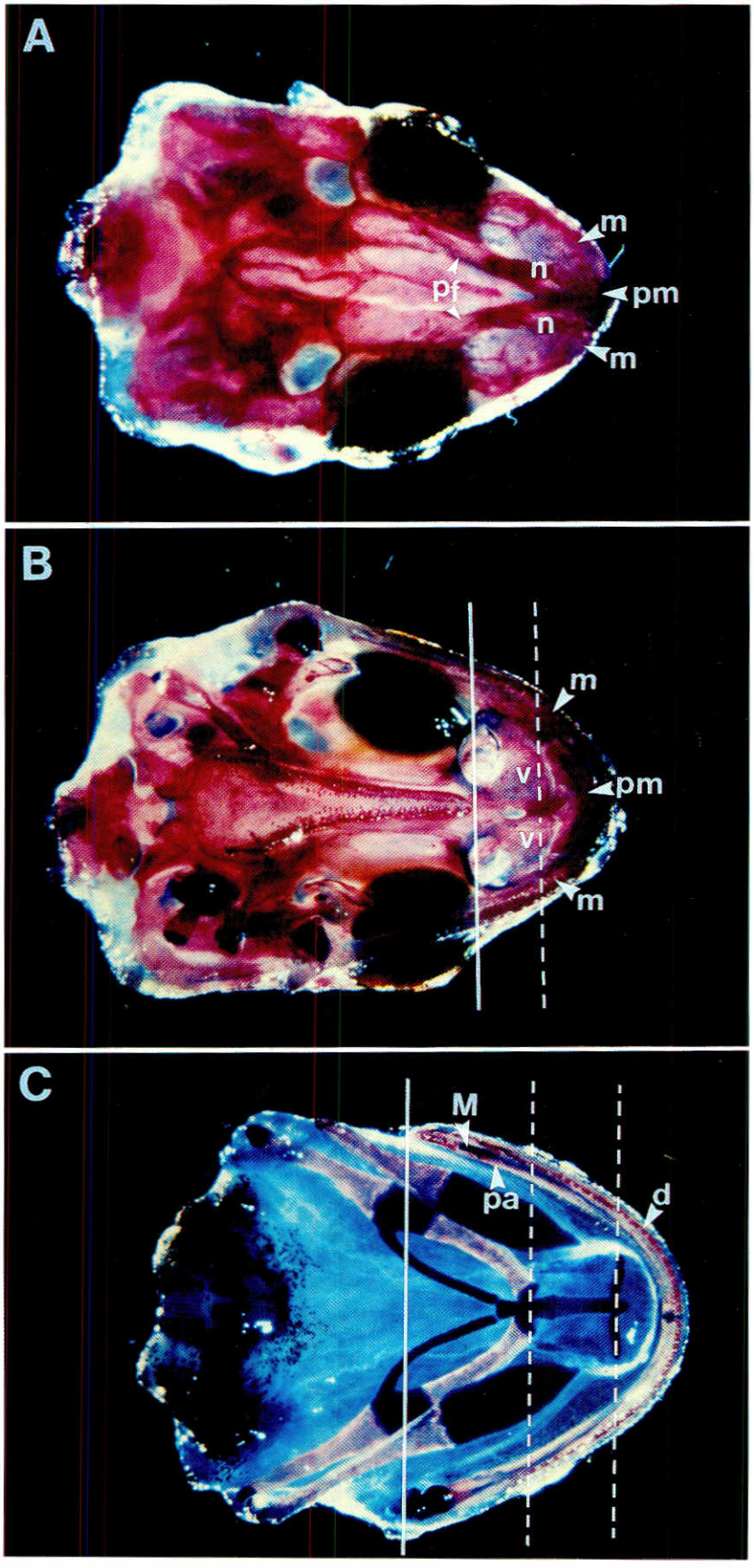

regeneration of the jaw is not a simple recapitulation of development, since the regenerated teeth are bicuspid like the normal adult teeth, rather than monocuspid as the larval teeth. Correspondingly, amputated larval jaws regenerate monocuspid teeth. The capability of larval and adult jaws to regenerate a different type of dentition according to the age of the animal may be due to a different hormonal milieu in larva and adult that, either directly or indirectly, affects morphogenesis of the tooth.

In both upper and lower jaws, the ability to regenerate the missing part depends on the level of amputation. Vallette demonstrated that the regenerative ability of the upper jaw is impaired when the amputation is performed immediately distal to the eyes, resulting in complete removal of maxilla, premaxilla and nasal bones, and the olfactory apparatus (see Figs. 1 and 2). However, faithfulness in patterning of the regenerate appears to correlate with the level of amputation. The incidence of defective regenerates increases with shifting the level of amputation proximally (Vallette, 1929) . As reported by Goss and Stagg (1958a), a progressive decrease in the size of regenerated lower jaws was observed when amputations were carried out closer and closer to the mandible articulation. Complete regenerative failure was reported following exarticulation of the mandible, even when the soft tissues of the floor of the mouth were left in place at the time of mandible removal and amputated at a later time. It therefore appears that these tissues not only cannot undergo metaplasia and produce cartilage and bone, but that their regenerative ability is impaired in the absence of the mandible. Furthermore, Goss and Stagg (1958b) have shown that when the intermandibular region is amputated, leaving the mandibles intact, regeneration does occur. This observation suggests that amputation of the mandible is not a prerequisite for regeneration of the tissues of the floor of the mouth to proceed, and that the presence of either the intact mandible or a mandibular stump is indeed necessary either as a source of inductive factor(s), or of progenitor cells. Together with the hypothesis that the chondrocranium

Fig. 1. Whole-mount preparations of normal upper (A, B) and lower jaws (C) stained for cartilage (blue) and bone (red) of adult *Notophthalmus viridescens*. (A) *Dorsal and* **(B)** *ventral view of the skull. The bones of the amputated part of the upper jaw are indicated (m, maxilla; n, nasal; pf, prefrontal; pm, pre-maxilla; v, vomer).* **(C)** *Dorsal view of the lower jaw, which consists of the mandible, the soft tissues forming the floor of the mouth and the hyoid apparatus including the tongue. The bones of the mandible are indicated (d, dentary bone; pa, prearticular bone; M, Meckel's cartilage). Different levels of amputation are indicated by vertical lines. Continuous lines indicate the plane of amputation at which regeneration is inhibited, while dotted lines indicate levels of amputation which are within the regenerative territory.*

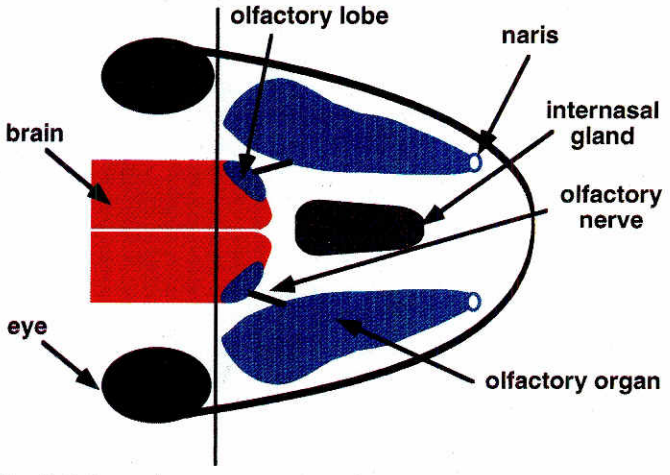

Fig. 2. Schematic representation of the urodele olfactory system (based on Vallette, 1929). *The proximal level of amputation at which regeneration does not occur is indicated by the vertical line. Note that the olfactory lobes are removed by this operation.*

acts as "a structural template" during development (Thorogood, 1988), this observation raises many questions about the inductive capability of the craniofacial skeleton which are beyond the scope of this review.

The jaw blastema

From previously published studies and our own work (Goss and Stagg, 1958a; Ghosh, *et al.,* 1994), it is apparent that jaw regeneration, like limb regeneration, proceeds by formation of a blastema, a growth zone of undifferentiated mesenchymal cells (blastemal cells) which, after a proliferative phase, differentiate into the tissues of the regenerate. The issue of the origin of blastemal cells in regenerating limbs has been addressed by many groups and by means of a variety of techniques for decades (see Wallace, 1981, and Ferretti and Brockes, 1991 for reviews; Pecorino *et al.,* 1994). Although the mechanisms underlying formation of limb blastemal cells have not been fully elucidated, the majority of the published studies suggest that the mesodermal tissues of the stump and the Schwann cells contribute to blastema formation following a process of dedifferentiation. A similar process of dedifferentiation is also likely to occur during regeneration of the jaw, as supported by the observation that the first occurrence of chondrogenic differentiation in lower jaws is observed in the mesenchyme adjacent to the stump of the prearticular bone, and not as a continuation of the Meckel's cartilage (Goss and Stagg, 1958a; Ghosh *et al.,* 1994). The extent to which this cartilage contributes to blastema formation is still unclear, but its role in regeneration does not appear to be a major one. In contrast, the contribution to the regenerative process of stump cartilages seems to be more important in regenerating upper jaws. After accumulation of blastemal cells at the cut surface of the stump, chondrogenesis appears to occur primarily around the edge of the nasal cartilage, suggesting that this cartilage contributes significantly to blastema formation (Vallette, 1929; Ghosh *et al.,* 1994). Nonetheless, our understanding of the role played by the different stump tissues in regenerating jaws is still very fragmentary. Lineage studies will have to be carried out in order to elucidate the contribution of the various tissues of the stump to blastema formation.

A clear demonstration of different cell populations in the apparently homogeneous limb blastema has come from the use of monoclonal antibodies against blastemal and tissue-specific antigens (Kintner and Brockes, 1985; reviewed by Ferretti and Brockes, 1991). Most of those antibodies recognize intermediate filament proteins (Ferretti *et al.,* 1989; Ferretti and Brockes, 1990). We have applied a similar approach to begin to characterize regenerating jaws at a molecular level. Analysis of regenerating jaws by mean of monoclonal antibodies has indicated that, notwithstanding possible differences in the principal tissues of origin of blastemal cells in lower and upper jaws, the cells recruited to form the blastema lose markers of the differentiated state and begin to express a different set of molecules (Ghosh *et al.,* 1994). This observation further supports the view that dedifferentiation of stump tissues occurs in jaws as well as in limbs following amputation.

Particularly interesting patterns of reactivity were observed by staining regenerating limbs with monoclonal antibodies against the human keratin pair 8 (type II) and 18 (type I) and with the monoclonal antibody 22/18 (see below). In fact, limb blastemal cells, regenerating nerves, and myogenic cells *in vitro* are strongly stained by these anti-keratin antibodies (Ferretti *et al.,* 1989), and 22/18 recognizes an important subset of blastemal cells whose division is nerve dependant (Kintner and Brockes, 1985; Fekete and Brockes, 1987). Recently, we have confirmed that keratins are indeed induced in the limb blastema by isolating the newt homologues of K8 and 18 and analysing the distribution of their transcripts by *in situ* hybridization (Ferretti *et al.,* 1993; Corcoran and Ferretti, in preparation). This pattern of expression is striking, since keratin intermediate filaments are characteristically

TABLE 1

SUMMARY OF THE EXPRESSION OF BLASTEMAL ANTIGENS, ROLE OF INNERVATION AND EFFECTS OF RA TREATMENT DURING LIMB AND JAW REGENERATION

	blastemal markers keratin 8 and 18	blastema and nerve dependency marker 22/18	nerve	RA
limb	+	+	dependent	dysmorphogenesis (extra elements)
lower jaw	+	+	?	no defects
upper jaw	+	+	?	dysmorphogenesis (truncations)

expressed by epithelial but not by mesenchymal cells, and it is generally agreed that in the regenerating limb neither the epidermis nor the subepidermal glands contribute cells to the blastema. The expression of simple epithelia keratins in the newt limb blastema and in the regenerating nerve raises the question of whether there is a relationship between keratin expression and the extraordinary regenerative capacity of urodeles. Interestingly, also the glial cells of the goldfish optic nerve, a tissue that grows throughout life and is capable of functional regeneration (Giordano *et al.*, 1989), contain keratin 8. Analysis of expression and distribution of these transcripts in a variety of normal and regenerating tissues of the newt (Table 1) has shown that the newt homologues of keratin 8 and 18 are also expressed in jaw and tail blastemas. Their expression therefore appears to be related to the undifferentiated and pluripotent state of the blastemal cell, rather than to a specific regenerating organ (Ferretti and Ghosh, in preparation). This may also indicate that the capability of adult urodeles to establish a population of progenitor cells from the differentiated tissues of the stump following amputation is controlled through the same mechanisms in different organs.

Role of the wound epithelium in jaw regeneration

Epithelial-mesenchymal interactions are of fundamental importance in controlling gene expression in both the face and limb during development (Summerbell *et al.*, 1973; Wedden *et al.*, 1988; Tickle, 1991; Richman and Tickle, 1992; Brown *et al.*, 1993). The specialized wound epithelium of the regenerating limb is also believed to play a role in patterning equivalent to that played by the apical ectodermal ridge of the developing limb (Stocum and Dearlove, 1972). A thorough characterization and analysis of the role of the wound epithelium in jaw regeneration has not yet been carried out, but morphological analysis of regenerating jaws demonstrates that 2 to 3 weeks after jaw amputation a thickened epidermis resembling that of the regenerating limb is indeed present at the tip of the jaw blastema (Goss and Stagg, 1958a; Ghosh *et al.*, 1994). In order to compare the wound epithelium from regenerating limbs and jaws, we have studied the expression of the regeneration-associated keratin NvKII in jaw blastemas. As previously shown, this newt keratin is induced in the wound epithelium of the limb blastema and down-regulated at the mRNA level by a morphogenetic dose of RA (Ferretti *et al.*, 1991, 1993). Significantly, NvKII is not expressed in the jaw wound epithelium (Ferretti and Ghosh, in preparation), and this may reflect both different inductive abilities of these epithelia and differences in their ability to respond to the underlying mesenchyme, as suggested during development of the chick limb bud and facial primordia (Richman and Tickle, 1992; Brown *et al.*, 1993). The factors controlling the organ-specific pattern of NvKII expression in the wound epithelium are not known, but it is conceivable that the underlying mesenchyme may control this different phenotype in both limb and jaw wound epithelia.

Role of the nerve in jaw regeneration

Limb blastemal cells proliferate under the influence of the nerve and of the wound epithelium, but it is not known how proliferation of blastemal cells is regulated in the jaw. The issue of whether regeneration of the upper jaw is nerve dependent was much debated at the beginning of the century (von Szutz, 1914; Guyenot and Vallette, 1925; Vallette, 1929). The emerging view was that the apparent nerve dependency observed after proximal amputation of the upper jaw, which included all of the olfactory apparatus (Fig. 2), really reflected the limits of the regenerative territory (Fig. 2) rather than a neural requirement (Vallette, 1929). The role of innervation during regeneration of the lower jaw has been more recently examined by Finch (1975), who also concluded that this process is nerve independent. Yet, none of the published studies incontrovertibly proves that complete denervation was obtained, and this is not surprising due to the technical difficulties of denervating upper and lower jaws, and the lack of neuronal molecular markers at the time when the work was carried out.

As mentioned before, in regenerating limbs the monoclonal antibody 22/18 reacts with an antigen expressed in cells whose division depends on the presence of the nerve (Fekete and Brockes, 1988). Lack of its expression in regenerating jaws may further support the view that regeneration is nerve independent. However, 22/18 reactivity has been recently detected in both lower and upper jaw blastemas (Ferretti and Ghosh, in preparation). This observation has interesting implications for the debate on the role of innervation during jaw regeneration. The prediction is that, if 22/18 indeed reflects nerve dependency, jaw regeneration is nerve-dependent (Table 1). Careful denervation experiments in which the extent of denervation and axonal regrowth is monitored by monoclonal antibodies need to be carried out in order to finally resolve this important issue.

Effects of retinoic acid on jaw regeneration

Retinoic acid (RA) induces formation of duplicate structures in the regenerating newt limb (Maden, 1982; Stocum, 1991) and can induce a number of craniofacial abnormalities in many vertebrates including man (Sulik *et al.*, 1988; Wedden *et al.*, 1988). We have therefore addressed the issue of whether and how this powerful teratogen, which is also a putative endogenous morphogen believed to play multiple roles during development, affects jaw regeneration (Table 1). We have found that a dose of RA which affects morphogenesis in the regenerating limb induces formation of truncated upper jaw regenerates, while lower jaw regenerates appear normal (Ghosh *et al.*, in preparation). Therefore, under these experimental conditions, RA does not produce duplications, as in regenerating limbs, but instead deletions of structures which parallel those induced in developing avian jaws (Wedden *et al.*, 1988). This observation is interesting, since it indicates that very similar developmental mechanisms may operate in these two systems, and that the regenerating upper jaw could provide a parallel model for the study of teratogenic effects of RA.

Agenda for the future

The molecular characterization of regenerating jaws is still in its infancy. On the basis of the studies so far carried out on regeneration of the jaw and our current understanding of limb regeneration, it appears likely that some of the mechanisms underlying regeneration in these two systems, such as formation of blastemal cells and possibly the control of their proliferation, are

similar. In contrast, the events that lead to the establishment of jaw and limb identity must be somehow different. Such events could either be controlled by jaw- or limb-specific genes, or by activation of the same genes in a different spatio-temporal combination that would result in different growth patterns and cell-cell interactions. Interestingly, many of the genes considered to play an important role in patterning during development, such as homeotic genes and segment polarity genes, have been detected both in developing limbs and facial primordia (Hill *et al.,* 1989; Gavin *et al.,* 1990; Dollé *et al.,* 1992). Nothing is however known about their expression in regenerating jaws. In order to start tackling the important issues of control of growth and identity in regenerating jaws, it will be necessary to further assess the pattern of expression of already identified molecules which may be induced during regeneration, and to identify new ones which may participate in the cascade of events leading to regeneration of a jaw rather than a limb. Identification of some of the molecular events relevant to the regenerative process which are either common to limbs and jaws, or specific to jaws, will be valuable in order to gain better insight into the molecular events underlying pattern specification. A better understanding of the developmental potential of progenitor cells from different organs may have important implications for tissue grafting in surgical practice.

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References

BROWN, J.M., WEDDEN, S.E., MILLBURN, G.H., ROBSON, L.G., HILL, R.E., DAVIDSON, D.R. and TICKLE, C. (1993). Experimental analysis of the control of expression of the homeobox-gene *Msx-1* in the developing limb and face. *Development 119:* 41-48.

DOLLÉ, P., PRICE, M. and DUBOULE, D. (1992). Expression of the murine *Dlx-1* homeobox gene during facial, ocular and limb development. *Differentiation 49:* 93-99.

FEKETE, D.M. and BROCKES, J.P. (1987). A monoclonal antibody detects a difference in the cellular composition of developing and regenerating limbs of newts. *Development 99:* 589-6029.

FEKETE, D.M. and BROCKES, J.P. (1988). Evidence that the nerve controls molecular identity of progenitor cells for limb regeneration. *Development 103:* 567-73.

FERRETTI, P. and BROCKES, J.P. (1990). The monoclonal antibody 22/18 recognizes a conformational change in an intermediate filament of the newt, *Notophthalmus viridescens*, during limb regeneration. *Cell Tissue Res. 259:* 483-93.

FERRETTI, P. and BROCKES, J.P. (1991). Cell origin and identity in limb regeneration and development. *Glia 4:* 214-24.

FERRETTI, P., BROCKES, J.P. and BROWN, R. (1991). A newt type II keratin restricted to normal and regenerating limbs and tails is responsive to retinoic acid. *Development 111:* 497-507.

FERRETTI, P., CORCORAN, J.P. and GHOSH, S. (1993). Expression and regulation of keratins in the wound epithelium and mesenchyme of the regenerating newt limb. *Prog. Clin. Biol. Res. 383A:* 261-269.

FERRETTI, P., FEKETE, D.M., PATTERSON, M. and LANE, E.B. (1989). Transient expression of simple epithelial keratins by mesenchymal cells of regenerating newt limb. *Dev. Biol. 133:* 415-424.

FINCH, R. (1975). The influence of the nerve on lower jaw regeneration in the adult newt, *Triturus viridescens. J. Morphol. 129:* 401-414.

GAVIN, B.J., MCMAHON, J.A. and MCMAHON, A.P. (1990). Expression of multiple novel *Wnt-1/int*-related genes during fetal and adult mouse development. *Genes Dev. 4:* 2319-2332.

GHOSH, S., THOROGOOD, P. and FERRETTI, P. (1994). Regenerative capability of upper and lower jaws in urodele amphibians. *Int. J. Dev. Biol. 38:* 479-490.

GIORDANO, S., GLASGOW, E., TESSER, P. and SCHECHTER, N. (1989). A type II keratin is expressed in glial cells of the goldfish visual system. *Neuron 2:* 1507-1516.

GOSS, R.J. and STAGG, M.W. (1958a). Regeneration of lower jaws In adult newts. *J. Morphol. 102:* 284-309.

GOSS, R.J. and STAGG, M.W. (1958b). Regeneration in lower jaws of newts after excision of the intermandibular region. *J. Exp. Zool. 137:* 1-11.

GRAVER, H.T. (1973). The polarity of the dental lamina in the regenerating salamander jaw. *J. Embryol. Exp. Morphol. 30:* 635-646.

GUYENOT, E. and VALLETTE, M. (1925). Régénération du néz et de la mâchoire supériore chez les Urodéles. *C.R. Soc. Biol. 93:* 1276-1278.

HILL, R.E., JONES, P.F., REES, A.R., SIME, C.M., JUSTICE, M.J., COPELAND, N.G., JENKINS, N.A., GRAHAM, E. and DAVIDSON, D.R. (1989). A new family of mouse homeobox containing genes: molecular structure, chromosomal location and developmental expression of Hox-7.1. *Genes Dev. 3:* 26-37.

KINTNER, C.R. and BROCKES, J.P. (1985). Monoclonal antibodies to the cells of a regenerating limb. *J. Embryol. Exp. Morphol. 89:* 37-51.

MADEN, M. (1982). Vitamin A and pattern formation in the regenerating limb. *Nature 295:* 672-675.

PECORINO, L.T., LO, C.D. and BROCKES, J.P. (1994). Isoform-specific induction of a retinoid-responsive antigen after biolistic transfection of chimaeric retinoic acid/thyroid hormone receptors into a regenerating limb. *Development 120:* 325-333.

RICHMAN, J.M. and TICKLE, C. (1992). Epithelial-mesenchymal interactions in the outgrowth of limb buds and facial primordia in chick embryos. *Dev. Biol. 154:* 299-308.

STOCUM, D.L. (1991). Retinoic acid and limb regeneration. *Semin. Dev. Biol. 2:* 199-210.

STOCUM, D.L. and DEARLOVE, G.E. (1972). Epidermal-mesodermal interaction during morphogenesis of the limb regeneration blastema in larval salamanders. *J. Exp. Zool. 181:* 49-62.

SULIK, K.K., COOK, C.S. and WEBSTER, W.S. (1988). Teratogens and cranial malformations: relationship to cell death. *Development 103 (Suppl.):* 213-232.

SUMMERBELL, D., LEWIS, J. and WOLPERT, L. (1973). Positional information in chick limb morphogenesis. *Nature 244:* 492-496.

THOROGOOD, P. (1988). The developmental specification of the vertebrate skull. *Development 103 (Suppl.):* 141-153.

TICKLE, C. (1991). Retinoic acid and chick limb development. *Development (Suppl.):* 113-121.

VALLETTE, M. (1929). Régénération du museau et territoires de régénération chez les Urodéles. *Bull. Biol. Fr. Belg. 63:* 95-148.

von SZUTZ, A. (1914). Beiträge zur Kenntniss der Abhängigkeit der Regeneration vom Zentralnervensystem. *Arch. Entw.Mech. Org. 38:* 540-545.

WALLACE, H. (1981). *Vertebrate Limb Regeneration.* Chichester, John Wiley and Sons.

WEDDEN, S.E., RALPHS, J.R. and TICKLE, C. (1988). Pattern formation in the facial primordia. *Development 103 (Suppl.):* 31-40.