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Developmental neurobiology of the anterior areas in amphibians: urodele perspectives

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ABSTRACT Because of its evolutionary grade and its relative simplicity, the Urodele brain provides an excellent archetype for the study of forebrain development. Early experiments on Primary Induction took advantage of the Urodele's manipulatability and ease of use, but due to the fact that its ectoderm was very readily neuralized Anurans (especially *Xenopus*) became the vertebrate of choice for early developmental neurobiology. Recent advances in the molecular biology of neuralization in *Xenopus* may rejuvenate Urodele use in solving the complicated sequence of events during this process of neural induction and to ascertain if separate or a combination of events (*de fault* and inductive) are involved. In the future, the combined use of Urodeles and Anurans will provide much information with regard to the evolutionary conservation of the mechanisms of regional specification, gene expression events, neurulation, neuroblast migration, and axonogenesis during the development of the nervous system. The present review provides some recent examples of this approach of using Urodeles and Anurans in a combinatorial fashion to decipher specific aspects of developmental neurobiology.

KEY WORDS: *forebrain regionalization, neurulation, neuroblast migration, axonogenesis*

Introduction: brief review of early neurogenesis

Development of the nervous system is a complex, epigenetic process. Deciphering this process within higher vertebrates, which contain large numbers of neurons and exhibit overlapping morphogenetic events, has become a very cumbersome, difficult task. Historically, urodele species have served as ideal organisms for the study of neural development. Experimental manipulations can be made on urodeles that are either impossible or much more difficult in other organisms. In addition (especially for axolotls), a number of genetic mutants are available which permit the identification of specific stages for critical developmental events. For studies in developmental neurobiology, urodeles display a number of other advantages. Eggs and embryos are relatively large and develop slowly, allowing for precise temporal isolation of the specific stage of epigenetic interactions. The early embryo has a wide temperature tolerance which is advantageous in embryonic mapping, lineage studies and for the developmental manipulations used to investigate mutant phenotypes. With temperature adjustments stages can be delayed for the purpose of carrying out manipulations which precisely investigate primary or secondary causal events affected by mutations or specific gene expression patterns. As well, individual neurons or neuroendocrine cells are very large, and these cells have a remarkable capacity for regeneration and rejuvenation in urodeles.

Urodeles are also unique in that they provide a comparative model for study at any level; molecular, neurobiological or behavioral. As Herrick (1948) has stated: *"the brains of urodele amphibians have advanced to a grade of organization typical for gnathostome vertebrates....This brain may be used as a standard of reference in the study of all brains, both lower and higher in scale."* Using the urodele to determine the main events in neural development, one can begin to recognize the basic issues to be resolved for almost all of vertebrate developmental neurobiology.

From primary induction onwards, neural development can be conveniently subdivided into a number of major stages. After primary induction, vertebrate embryonic cells that give rise to the nervous system are initially observed as a thickened tissue layer of the dorsal hemispheric neuroectoderm. This thickened sheet of cells is known as the "midline cells of the early embryonic neural plate." During the next stage of neurulation, this layer of plate cells rolls up into a tube and forms the brain and neural tube (Fig. 1; Eagleson and Harris, 1990). At this time, some cells migrate away from the point of tube closure to form a transient mass of cells called the neural crest (Fig. 1). These cells disperse and migrate ventrally and laterally to form ganglia and supporting cells of the future peripheral nervous system (PNS).

Soon after braintube closure the brain bends or undergoes flexures and then the initial proliferation of neuroblasts (during the stage called neurogenesis) occurs along the inner surface of the recently formed neural- and braintube. After proliferation, these neuroblasts migrate into different zones of the central nervous system (CNS) where they aggregate to form nuclei. The neuroblasts then differentiate into neurons with their cell-specific phenotypes.

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Using their specialized growth cones, they send out axons which form specific connections (in stages called *axonogenesis* and *synaptogenesis*). A certain portion of neurons undergo selective cell death (apoptosis) while connections are being eliminated or stabilized.

The present paper describes how the urodele has been used as a model for these various aspects of developmental neurobiology, explains why the urodele went out of favor as a model, and details the advantages urodeles offer as a model system in future studies of neurobiology. Since there are already several good reviews which examine hindbrain and spinal cord developmental neurobiology (e.g., Keynes and Lumsden, 1990; Lumsden, 1990; Stern *et al.*, 1991), this review emphasizes the developmental neurobiology of *forebrain* areas.

Primary induction of the nervous system

Using two urodele species with different pigment characteristics, the classical experiments by Hans Spemann and Hilde Mangold (1924) revealed that transplantation of the dorsal lip of the blastopore of an early gastrula to the ventral hemisphere of a host embryo of the same stage could "induce" a secondary axis which contains organized tissue. The dorsal lip area gives rise to notochordal tissue (Vogt, 1929). Since the organized tissue was of host origin, the specific donor tissue was called the *organizer*. Further heterotransplantation studies employing later stage dorsal lip graft tissue resulted in the absence of forebrain or eye within the secondary embryo. Thus, the results of those early studies utilizing newts revealed that formation of anteroposterior (A-P) axis patterns occurred during gastrulation and that involuting notochordal tissue was responsible for primary induction.

Later experiments performed by Otto Mangold (1933) demonstrated that the involuted dorsal mesoderm was, indeed, responsible for the induction and anteroposterior patterning of the CNS. Using newt embryos, transplantation of dorsal blastoporal lips into the blastocoel cavity of a host embryo (so called "einsteckung" experiment) also resulted in induced secondary neural structures. The stage of the dorsal lip donor tissue determined the A-P extent of the secondary embryo. Transplantation of *Triturus* early gastrula dorsal lips generated secondary embryos with head structures, while transplanted late dorsal lip areas resulted in mostly posterior neural structures such as the spinal cord and tail (Mangold, 1933). This led to the use of a scoring classification according to the structures induced: *archencephalic*, induced forebrain and anterior placodes; *deuterencephalic*, induced midbrain and hindbrain-like structures; and *spinocaudal*, induced neural tube, somite and tailfin structures.

During the 1930s, 1940s and 1950s using *Triturus* and *Ambystoma*, it was later found that a long list of compounds (many of them irrelevant to neural induction) promoted ectoderm conversion to archencephalic and deuterencephalic neural tissue. In 1941, Barth found that the mere *in vitro* culturing of *Ambystoma* ectodermal fragments from gastrulae would frequently result in neural tissue. As a result it was concluded that neural induction might be completely nonspecific.

During the same period while also working with *Triturus* and *Ambystoma*, various embryologists, including Saxén, Toivonen, Yamada and Nieuwkoop proposed a two-gradient hypothesis to explain A-P axis patterning of the CNS. This hypothesis, proposed

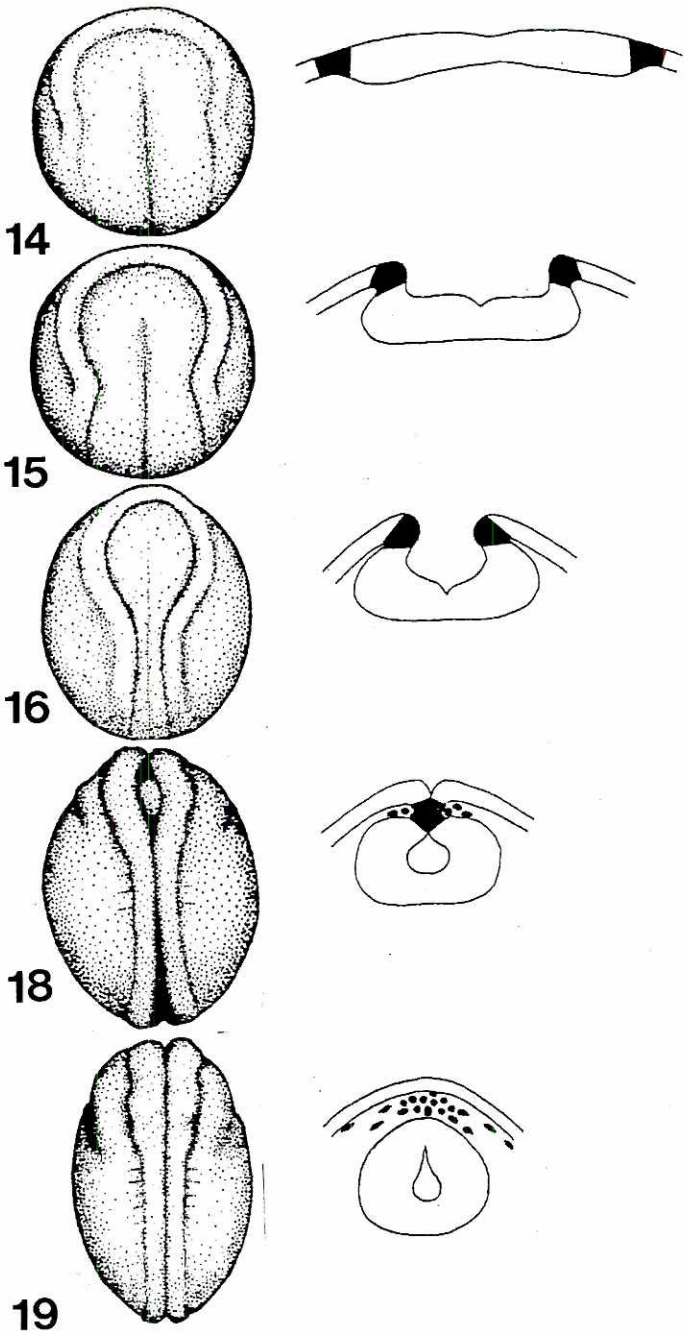

Fig. 1. Diagrammatic representation of the different stages of neural development as represented by neurulation and neural crest migration. *On the left are stages of neurulation in the axolotl (Bordzilovskaya and Detlaff, 1979). On the right are dorsal cross sections at the level of the prospective hindbrain/spinal cord transition (Jacobson, 1959). The blackened portion represents the neural fold (prospective neural crest). Stage 14 (left) is a flattened neural plate stage and a result of prior neural induction. During stage 15 (left), the neural folds begin to uplift a process which continues through stages 16 up to 18 (when the folds fuse; right). During stages 18 and 19 neural crest (darkened areas on right) shear away from between the epidermis and neural tube. Neural crest cells then migrate away, aggregate, differentiate and send out axons (axonogenesis) towards specific targets.*

as a most parsimonious explanation for A-P patterning (Saxén and Toivonen, 1961), suggested that there was an initial "neuralization" by a primary inductive substance that leads to archencephalic structure patterns which is then followed by a second inductor acting to caudalize (spinocaudal structures) the CNS. By mixing two inductors at different ratios, intermediate (deuterencephalic) structures would be predicted to develop (Saxén and Toivonen, 1961). Signaling between different regions within the neural plate or neural tube (called secondary inductions) would be expected to be further required to establish the fine patterning or regional specificity which characterizes the CNS.

Since most urodeles were easily neuralized (especially the forebrain structures), they fell out of favor for studying these processes of primary and secondary induction. During the 1960s and 1970s more developmental neurobiology studies employed the anuran *Xenopus* as a test organism, primarily because *Xenopus* embryos are less prone to inadvertent neural induction. *Xenopus* also became very popular for its more rapid developmental rate and the convenience of "hormone induced" breeding which this species permits. *Xenopus* embryos are therefore available for experimentation at all times of the year.

Exploiting the technical breakthroughs in genetic engineering and the development of other molecular techniques which occurred during the 1970s and 1980s, a number of studies using *Xenopus* showed that peptide growth factors (activin and fibroblast growth factor) are potent inducers of dorsal and ventral mesoderm (e.g., Asashima *et al.,* 1990). Activin also induces explanted ectoderm (animal caps) to become neural tissue, but this occurs indirectly through an initial induction of dorsal mesoderm.

Hemmati-Brivanlou and Melton (1992) injected *Xenopus* embryos with RNA which encodes a truncated form of an activin receptor that lacks the intracellular kinase domain. This transcript acted as a dominant negative mutation by blocking activin signaling. A lack of activin response was observed in these embryos, and this presumably resulted in the failure of mesoderm formation. However, an additional and somewhat surprising observation was the finding that neural differentiation occurred in the ectoderm of injected embryos (Hemmati-Brivanlou and Melton, 1992). This was interpreted in terms of activin being the inducer of mesoderm and that a weak activin induction promotes an epidermal fate for ectoderm. These investigators proposed that neuroectoderm is the default state when activin signal reception is abolished. Such a "neural default state" explains earlier studies by Grunz and Tacke (1989) and Green and Smith (1990) in which cell dissociation of *Xenopus* blastula and early gastrula ectoderm resulted in a neural fate. The neural fate can be explained as a default state due to the removal of epidermal-inducing signals during dissociation.

The interpretation of those studies has been complicated by the fact that yet another neural inducer has also been discovered. Lamb *et al.* (1993) demonstrated that the secreted protein *noggin* has all the characteristics (e.g., rescue from ultraviolet-induced axial defects, expression at the right time and area; neuralizing ectoderm in the absence of mesoderm) required of an endogenous neural inducer. Both the default and noggin studies are involved in initial neuralization, but not in patterning of the nervous system. A posteriorizing or caudalizing factor would therefore also be required.

From these studies with *Xenopus*, a rejuvenation of interest in urodeles as a model of neural induction should reoccur. *Why are urodeles more readily neuralized? Do they have fewer activin receptors?* An understanding of these issues might settle the question of "default" neuralization (activin) vs. instructive induction *(noggin),* or might reveal why two neural inducers could be necessary. Using recombinant DNA techniques to screen for these genes in urodeles as well as examining the possible effects of follistatin (an activin inhibitor) upon urodele neurulation might help clarify the causal aspects of vertebrate neural induction. Such studies using distantly related amphibians (e.g., *Xenopus* vs axolotl) could appraise the possibility that basic mechanisms for neural induction are shared among vertebrates.

Regional specification within the CNS

After Otto Mangold's studies (1933) on A-P patterning of the CNS, further experiments indicated that the detailed patterning within the CNS was not strictly induced by chordamesoderm. These experiments indicated that broadly delineated regions which have morphogenetic field characteristics were induced, and that these fields progressively undergo a process of stepwise determination. Gradually, during neurulation, the ectopic transplantation of small pieces of neural plate reveals that the grafts retain the information characteristic of their normal (prospective) A-P location, irrespective of its grafted location. Also, the extirpation studies with *Xenopus* of Corner (1961) indicated that such morphogenetic specification may proceed from an anterior to posterior direction. Early graft rotation studies by C.O. Jacobson (1964) supported this concept of initial anterior then posterior specification of subregions in the neural plate. More recent studies by Model (1982) using the axolotl also tested progressive regionalization of the CNS within the urodele neural plate. She transplanted prospective forebrain plate tissue from stage 14 (early neural fold) embryos to the posterior prospective hindbrain regions and found that the prospective forebrain tissue was still capable of forming the giant Mauthner neurons of the hindbrain. This result contradicts Corner's (1961) results with *Xenopus.* But *Xenopus* embryos develop much more rapidly than the urodeles and undergo a number of specification or determinative events at an earlier stage then urodeles (Cornel and Holt, 1992). One must, therefore, be very careful when applying "basic concepts" to different vertebrate species. With the increased use of gene expression events as markers in various developmental stages, further studies of regionalization in both urodeles and anurans should permit accurate interpretations concerning the genes which are involved in determinative events.

Oscar Schotte and E.M Edds (1940) investigated regionalization and secondary induction in anterior sensory areas of amphibians. They did reciprocal transplants of ventral, prospective mouth ectoderm between frog gastrulae and salamander gastrulae. They obtained salamander larvae with frog-type horny jaws and adhesive organs, and frog tadpoles with salamander balancers and dentine teeth. Such reciprocal transplantations between distantly related embryos are called xenoplastic grafts. The ectoderm inducers were later identified as head mesoderm and endoderm. Considering that anurans and urodeles probably diverged in the Paleozoic about 400 million years ago, it was quite unexpected that the anterior inductive capacity of the host tissue remained similar enough to evoke donor tissue towards an anterior donor sensory organ in such a region-specific fashion. Such xenoplastic trans-

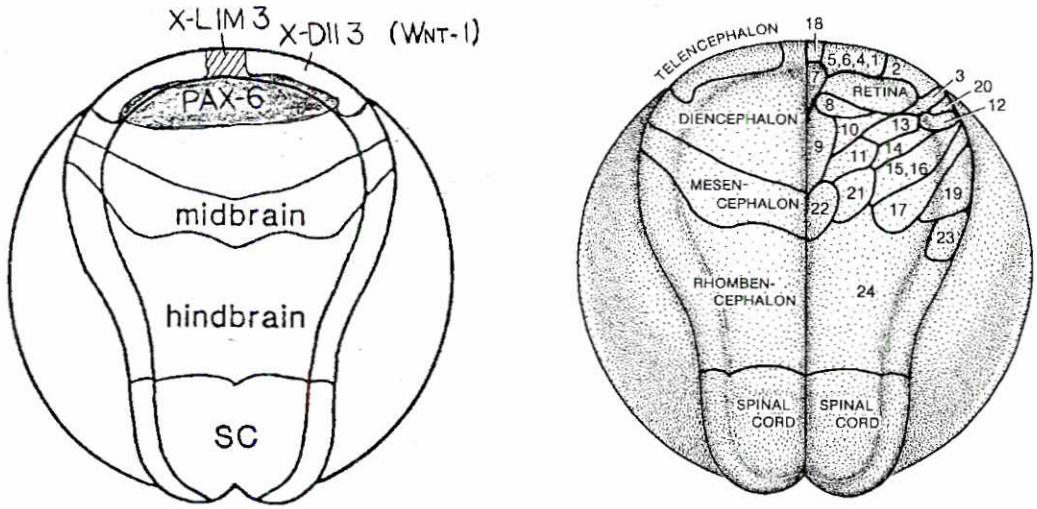

Fig. 2. Schematic summary of neural plate gene expression events (left) and prospective neural plate map (right) *(from Eagleson and Harris, 1990). Distal-less (X-dll-3) expression precedes and overlaps Wnt-1 expression. See text for explanations. Presumptive areas of expression include the following: 1. area olfactoria primitiva; 2. primordium piriforme; 3. primordium hippocampi; 4. lamina terminalis/nervus terminalis; 5. anterior preoptic area; 6. magnocellular preoptic nucleus; 7. supra chiasmatic nucleus; 8. chiasmatic ridge; 9. ventral hypothalamic nucleus/infundibulum; 18. hypophysis (anterior pituitary).*

plantations which examine gene expression events during regional specification of the CNS should provide an interesting evolutionary approach to formulating basic concepts in developmental neurobiology.

Gene expression during regionalization events of the neural plate

A number of genes have been recognized that are initially expressed during various stages of amphibian neural plate regionalization. This review section will emphasize studies dealing with regionalization and gene expression in anterior (brain) areas of amphibians. Other more extensive reviews (McGinnis and Krumlauf, 1992; Ruiz i Altaba, 1994) deal with patterns of homeobox gene expression and A-P patterning of hindbrain and spinal cord. Few of these gene expression studies have, however, utilized urodeles.

A number of studies have implicated localized A-P differences in (secondary) inductive capacities along the anterior neural plate. Since neural plate tissue lacks both *noggin* and *follistatin*, it has been suggested that such regional specification within areas of the CNS involves a multistep process of secondary inductions-sometimes called "homeogenetic neural induction." Since neural tissue inducing neural tissue signals can interact only over short distances, cascades of secondary inductions must play an important role in brain A-P regional specification. A number of molecular markers, most related to homeobox genes, have been used to study the establishment of local A-P character (Dekker *et al.*, 1992; Papalopulu and Kintner, 1993). These studies also utilize exogastrulae or "Keller sandwiches," in which mesoderm does not underlie or appose ectoderm, thus further indicating that A-P patterning may depend upon signals from within neuroectoderm and not from underlying mesoderm (Ruiz i Altaba, 1992, 1994). A number of genes are primarily (and initially) expressed in anterior brain areas during the regional specification events of neurulation. These genes include *distal-less* (Papalopulu and Kintner, 1993), *wnt*-like genes (Busse and Seguin, 1992; Tannahill *et al.*, 1992; Wolda *et al.*, 1993), POU-like genes (Agarwal and Sato, 1991), *pax 6*-like genes (Li *et al.*, 1994) and a LIM class homeobox gene (Taira *et al.*, 1993). To this point only the *wnt*-like gene has been isolated and its spatial-temporal patterns of expression investigated in urodeles (Busse and Seguin, 1992). Though functional correlations for the different genes may exist for urodeles when compared to *Xenopus*, it seems that the timing of these gene expression and specification events may differ.

According to the grafting studies previously discussed, anterior neural plate area fates are determined first (Corner, 1961; Eagleson and Harris, 1990) then hindbrain area fates are determined, and lastly midbrain areas are specified (Eagleson and Harris, 1990). Thus, it is conceivable that forebrain areas may induce midbrain regionalization through interactions with the anterior hindbrain (Nieuwkoop and Albers, 1990). Some recent studies that examined regionalized gene expression events in neural plate stage embryos tend to support this possibility of forebrain then hindbrain then midbrain regionalization. I will attempt to correlate gene expression spatio-temporal patterns with the earlier and recent grafting studies that implicate regionalization events. I will then propose heteroplastic experiments using urodeles that might clarify some of these concepts concerning regionalization within the vertebrate anterior CNS.

In urodeles Busse and Seguin (1992) isolated two *wnt*-like genes from the axolotl and found that one of these (*Awnt*-5B) was restricted to neural tissue. *Awnt*-5B was first detected during axolotl gastrulation and persisted during neurulation. A majority of the *Awnt*-5B transcripts were localized within the neural plate. Timing of the expression of this gene indicated a response to neural induction (Busse and Seguin, 1992), but a more localized expression pattern within a presumptive brain region would be more indicative of a regional specification role for this gene.

Due to the greater number of studies with Anurans, the rest of this discussion will deal with early neural plate gene expression within *Xenopus laevis*. Wolda *et al.* (1993) investigated the expression patterns of *Xwnt*-1 and *Xwnt*-3 during *Xenopus* development. These genes are first expressed in stage 16 *Xenopus* embryos within the most anterior region of the neurula stage embryo. Later (stage 22) *Xwnt*-1 and *Xwnt*-3 expression is observed along the

dorsal aspect of the A-P axis. Therefore, early expression of these genes is restricted to anterior sensory and alar (sensory) plate tissue.

Investigations of *Pax*-6 expression reveal that it is expressed during early neural plate stages. It is expressed in the anterior neural plate within the presumptive hypothalamic and eye or retinal regions (Papalopulu and Kintner; Harris and Hirsch, personal communications). Papalopulu and Kintner (1993) have found that *distalless (X-dll3)* is expressed in the anterior extent of the *Xenopus* neural plate embryo. This expression is first observed in stage 14 embryos and is localized within the lateral portions of the most anterior transverse neural ridge tissue. This tissue is fated to become telencephalon, ventral hypothalamus, cement gland and olfactory areas (Eagleson and Harris, 1990; Eagleson *et al.*, 1995). The cement gland is homologous to the urodele balancer organs. These both are ephemeral larval organs derived from ectoderm lying just outside and anterior to the neural plate. Later expression patterns supported these anterior prospective fates. Papalopulu and Kintner (1993) observed specific expression patterns for *X-dll3* within the placodal olfactory, ventral hypothalamus and telencephalon tissues within stage 25 embryos.

Taira *et al.* (1993) investigated the expression of a LIM class homeobox gene and observed early neural plate expression patterns for *Xlim-3*. This gene is initially expressed in stage 14 embryos within the medial anterior neural ridge tissue which is fated to become ventral hypothalamic and pituitary tissue (Eagleson *et al.*, 1986; Eagleson and Harris, 1990). Taira *et al.* (1993) concluded that this *Xlim-3* gene might be involved in specification of distinct (but related) neuronal and neuroendocrine tissues.

Since many of these genes appear in specific spatial expression patterns during early neurulation when grafting studies indicate regionalization events, initial specification may occur relatively early- perhaps before neural tube formation (Fig. 2). Since these genes are found to be important during early regionalization of the *Xenopus* nervous system, it would be interesting to search for the urodele homolog(s) and study sequence relationships and patterns of expression during urodele neurulation. For example, the eyeless mutant axolotl (e/e), which does not form optic vesicles, would be a prime candidate for studies which examine axolotl-like *Pax-6* and/or *lim-3* gene expression, since the neural plate areas that express these genes are the specific areas affected by the mutation (Cuny and Malacinski, 1986). Heteroplastic experiments could be performed between *Xenopus* and axolotl to determine whether either normal or eyeless axolotl neural plates are capable of expressing *Pax-6*-like genes when tissue is grafted in neurulating *Xenopus* embryos. In addition *Xenopus* tissue could be grafted to axolotl neural plates to see if the eyeless and normal axolotl can induce Pax-6 expression in a *Xenopus* graft. Such heteroplastic studies could reveal whether regionalization events are highly conserved, and would better localize the specific tissue (responding or inducer) affected by the eyeless mutation. Similar heteroplastic studies which examined *Xwnt-2* gene expression (Tannahill *et al.*, 1992) indicated that axolotl embryonic tissue can induce *Xwnt-2* expression in *Xenopus* neural plate patches. Future studies could therefore take advantage of the more slowly developing urodele embryo and thereby more easily identify intermediate regionalization events during development of the anterior CNS. Such neural tissue interactions cannot be as precisely delineated using the faster developing *Xenopus* embryo.

Neurulation and neural tube formation

The main forces which establish the shape of the embryonic amphibian brain and spinal cord involve cellular movements, cellular rearrangements and cell shape changes (Jacobson, 1994; Eagleson *et al.*, 1995). Urodele brain and neural tube formation proceeds in the absence of cell division (Jacobson, 1994), and if cell division is inhibited in *Xenopus*, early brain and neural tube formation is little affected (Harris and Hartenstein, 1991). Cell division is thus not a major factor in early neural tube formation (Eagleson *et al.*, 1995).

In *Xenopus*, convergence and extension movements squeeze the fates of neural progenitors from a wide, short anterior area (stage 11.5) into a narrow keyhole shape by the neural plate stage 16 (Keller *et al.*, 1992). Since upfolding and fusion of the lateral anterior neural ridge occurs simultaneously with a downward (rostral) rotation of medial neural ridge areas, the neurulation movements of the prospective forebrain areas are quite complicated compared to the rest of the CNS. An end result of these morphogenetic movements is the dispersion of forebrain structures that appear to originate from the same area of the neural ridge and plate (Eagleson and Harris, 1990; Eagleson *et al.*, 1995). Due to these bending and convergence and extension movements during braintube closure the anterior neural ridge ultimately gives rise to olfactory areas, telencephalon, preoptic hypothalamus, diencephalon floor and hypophysis (Eagleson and Harris, 1990; Huang and Moody, 1992; Kawamura and Kikuyama, 1992). Stage 15 neural ridge tissue located at the lateral anterior portions rise and fuse as well as rotate approximately 90° ventrally to become the most anterior aspect of the future brain (at stage 22). Meanwhile, the most anterior region of the medial neural ridge of the stage 15 embryo becomes tucked rostrocaudally and rotated ventrally with respect to the rest of the forebrain. These movements, along with extension and convergence in the A-P direction, result in the anterior medial plate becoming rostral in the larval *Xenopus* brain.

Neural tube formation in *Xenopus* differs when compared to urodeles in at least three aspects. First, at the neural ridge stage, anterior brain structures in urodeles are *not* derived from the anterior ridge tissue as in *Xenopus*, but are derived from anterior plate areas (Jacobson, 1959). Second, the notoplate and notochord seem to play a more important mechanical role in neurulation in urodeles (Jacobson, 1994) compared to *Xenopus* (Youn and Malacinski, 1981). Finally, *Xenopus* optic vesicles begin to bulge ventrolaterally prior to braintube closure (Eagleson *et al.*, 1995), whereas urodele optic vesicle evagination begins after neural tube closure. Recent studies with zebrafish by Macdonald *et al.* (1994) indicate that *Pax 6* and *rtk 1* expression may maintain cells in a proliferative state after brain tube closure. Our studies with BrdU uptake in *Xenopus* (Eagleson *et al.*, 1995) indicate that stage 15 embryos undergo a wave of mitosis at the ventrolateral aspects where *Pax 6* expression is initially observed (Fig. 2). Again, the timing of *Pax 6* expression may be an important aspect of neurulation and subsequent optic vesicle evagination. Initial regionalization of the optic areas in *Xenopus* seems to precede neural tube formation and optic vesicle evagination, whereas urodeles may exhibit a later regionalization pattern. This would be predicted due to their slower development. Again, the eyeless mutant axolotl *(e/e)* may be useful for revealing important mecha-

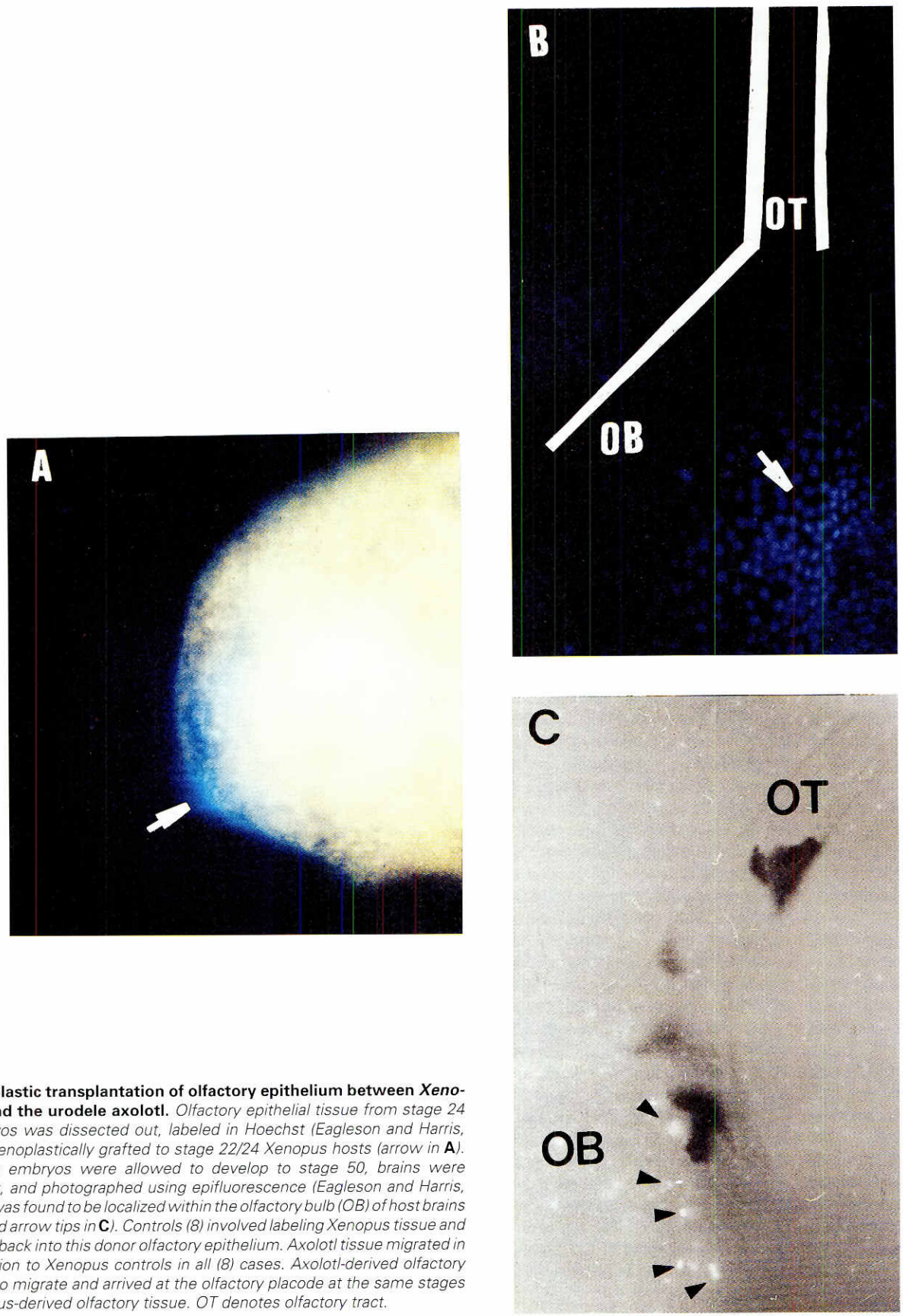

Fig. 3. Xenoplastic transplantation of olfactory epithelium between *Xenopus laevis* and the urodele axolotl. *Olfactory epithelial tissue from stage 24 axolotl embryos was dissected out, labeled in Hoechst (Eagleson and Harris, 1990), then xenoplastically grafted to stage 22/24 Xenopus hosts (arrow in* **A***). The Xenopus embryos were allowed to develop to stage 50, brains were dissected out, and photographed using epifluorescence (Eagleson and Harris, 1990). Label was found to be localized within the olfactory bulb (OB) of host brains (arrow in* **B** *and arrow tips in* **C***). Controls (8) involved labeling Xenopus tissue and re-inserting it back into this donor olfactory epithelium. Axolotl tissue migrated in a similar fashion to Xenopus controls in all (8) cases. Axolotl-derived olfactory tissue begin to migrate and arrived at the olfactory placode at the same stages as the Xenopus-derived olfactory tissue. OT denotes olfactory tract.*

nisms involved in optic vesicle evagination. Is the eyeless condition due to a lack of early regionalization events controlled by *Pax 6* genes? From time to time, animals heterozygous for the eyeless gene will produce a number of larvae that will have very small eyes or be "microeyes". These microeye larvae are usually sterile and when added to the genotypic ratio of the heterozygous eyeless mating , it indicates that they are probably genetically eyeless. Could "microeyes" be due to a lack of continued *Pax 6* expression after neural tube formation? Perhaps this is a mutation whereby the timing of *Pax 6 expression* is altered? Further studies comparing urodeles with *Xenopus* may reveal or clarify spatial and temporal mechanisms which guide optic vesicle evagination.

Braintube neurogenesis, gene expression and axonogenesis

Soon after vertebrate braintube closure there are several waves of mitosis along the inner surface of the neural tube, and a number of genes exhibit restricted patterns of gene expression. Soon after, neuroblasts differentiate and send out neurite processes. The formation of specific neuronal connections requires that an axonal process of one neuron come into contact with its specific target. A variety of extrinsic factors influence the course of growing axons. *In vitro* studies indicate a large variety of extrinsic factors that can influence axonal pathfinding, but whether these operate in the developing organism is not certain. *The tailbud urodele embryo, because of its large size and resilience to surgical manipulation, provides an ideal model for the study of factors that might influence axonal pathfinding. In vivo* studies with *Xenopus* embryos indicate that gene expression events after neural tube closure may influence axonal pathfinding in the forebrain. For example, *X-dll3* is expressed in a restricted region of the stage 25 prosencephalon (Papalopulu and Kintner, 1993) within a region of the developing tract of the post-optic commissure (Cornel and Holt, 1992). Thus, gene expression boundaries (Wilson *et al.*, 1993) may be responsible for determining the position of axonal tracts *in vivo*. To test causality, *in vivo* axonal pathfinding must be assessed in conditions where these boundaries are ablated (mutants) or aberrantly located (surgical manipulation).

Using zebrafish, Macdonald *et al.* (1994) showed that the earliest neurons in the forebrain differentiated along boundaries of expression of different regulatory genes. The pathways that these neurons pioneer also develop along such boundaries. In experimentally manipulated brains such as *cyclops*, the mutation alters *Pax 6* expression such that it lacks a ventral boundary and is expressed throughout the dorsal-ventral aspect of the diencephalon. This mutation results in a disruption of neural patterning in this area, indicating that expression boundaries play an *in vivo* role in growth cone guidance (Macdonald *et al.*, 1994).

Again, another urodele that might prove interesting for the study of axonal pathfinding would be the eyeless mutant axolotl. How does this mutation alter diencephalon axonal pathfinding? It has been shown that the immunoreactive-LHRH (irLHRH) axons do not properly connect to the median eminence through the hypothalamo-hypohyseal tract (Eagleson and Malacinski, 1986; Maccagnan and Muske, 1992). This explains the sterile condition for this mutant (Van Deusen, 1973). Could this defect be due to a lack of early gene expression *(Pax 6)* cues needed by pioneer axons? Could it lead to the formation of a defective scaffold which is needed for later developing axons? In zebrafish, the postoptic commissure (TPOC) forms along the *Pax 6* expression boundary, and its ventral character extends into the dorsal diencephalon. It may provide an early scaffold for later fasciculation (Macdonald *et al.*, 1994).

In order to test if the tract of the TPOC is essential for proper retinal ganglion outgrowth, Cornel and Holt (1992) tested the pathfinding characteristics of the retinal ganglion cells in *Xenopus*. These authors delayed TPOC outgrowth to a later stage by inhibiting development and performing heterochronic optic vesicle grafts. Since the TPOC develops later in the axolotl, they also performed heteroplastic grafts between *Xenopus* and the axolotl. They found that the optic axons navigate properly in the absence of preformed TPOC tracts, but TPOC tracts may still be important scaffolds for setting up the hypothalamo-hypophyseal tracts. Further studies using urodeles, especially mutant axolotls with forebrain defects, could provide interesting models for axonal pathfinding within the forebrain. *Thus urodeles, due to their delayed development, can be utilized to investigate the various in vivo cues required for axonal pathfinding.*

Neuroblast mixing and migration

Cell intercalation and migration occurs within the forebrain (Eagleson and Harris, 1990). During brain morphogenesis, originally contiguous cell clusters break up and become dispersed along the ventral hypothalamus and pituitary (Eagleson and Harris, 1990; Kawamura and Kikuyama, 1992). Another example of migrating neuroblasts within the forebrain is the ir-LHRH cell. In urodeles, these cells originate in the olfactory placodal epithelium, migrate in and settle into the olfactory bulb, the preoptic area and the hypothalamus (Murakami *et al.*, 1992). Mutations in cell adhesion molecules may alter or stop such neuroblast migration phases, for example, in Kallman's syndrome, which has the attributes of hypogonadism and anosmia (see Schwanzel-Fukuda *et al.*, 1992).

I have recently performed xenoplastic experiments to determine how evolutionarily conserved these migratory patterns might be (Fig. 3). Fluorescently labeled placodal tissue from axolotl stage 24 embryos was transplanted to the olfactory region of stage 23/24 *Xenopus* embryos. Preliminary results (Fig. 3) indicate that this tissue is capable of migrating into host olfactory bulb areas. Further studies will investigate whether such xenoplastic tissue can settle into preoptic and hypothalamic areas. These studies indicate that the forebrain cues for migration pathways might be highly conserved.

Another example of defective neuroblast migration is observed in the spastic mutant in the axolotl. This is a cerebellar mutant that is behaviorally observed to coil and swim on its back. The cause is not certain, but Ide *et al.* (1977) suggested that faulty cell-cell interactions during migration are probably the primary sites of this mutation's action. If presumptive tissue could be labeled, these cells could also be followed within mutant and normal animals to determine if the gene expression defect is within the migrating neuroblast cells or the substratum on which they migrate. In addition, xenoplastic transplantations could be attempted. Again, by using heterotransplantations between normal and mutant embryos as well as xenoplastic transplantations between urodele and anuran embryos, evolutionary and developmental information can be gained concerning cues for migratory patterns within the CNS.

Conclusions

Urodeles played a dominant role in early studies of developmental neurobiology. As studies now become more focused on specific tissue-tissue interactions that evoke regionalization and subsequent specification of organs and tissues, the more slowly developing urodele embryo will become more practical as a model system than faster developing anuran (e.g., *Xenopus*) model systems. Regionalization (or setting up of morphological fields) takes place early during neural plate stages. After neural tube closure, more specified gene expression boundaries appear. After neuroblasts leave mitotic cycles, migrate and then send out neurites, the more sharpened gene expression boundaries provide important guidance cues for axons. As molecular probes for urodeles become available, these various aspects of developmental neurobiology will be studied more frequently with urodeles. Due to their surgical resilience, temperature tolerance, and slower developmental rates, stage-specific interactions can be more precisely isolated in urodeles. In addition, xenoplastic transplantation studies between anurans and urodeles will further contribute to a greater understanding of both evolutionary and developmental aspects of neurobiology.

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