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How does the urodele ear develop?

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ABSTRACT An overview is provided of the structural and molecular events causing the transformation of undifferentiated epidermal cells together with the underlying mesenchyme to become the complex, three-dimensional ear. While tremendous progress has been achieved in a few model systems, enough is not yet known about the comparative embryology of ear development to provide causal explanations of the adult structural differences among species. It is hoped that the changes in selector and/or structural genes, as well as changes in the spatio-temporal induction of structural gene activation, and possible changes in the interactions between the various embryonic sources which contribute to the ear, will soon be understood. The most promising new avenue for research appears to be studies which combine classical transplantation tissue experiments with modern gene expression analyses and modern *in vitro* assays of the role of putative morphogens or trophic factors. It is emphasized that it is not understood what is missing in the developmental program of those salamanders which have lost a basilar papilla. Direct comparison of gene expression patterns and xenoplastic transplantations in salamanders of comparable stages which either do or do not develop this organ should help to clarify the molecular events that have led to this major evolutionary novel feature of the vertebrate ear.

KEY WORDS: *salamanders, otocyst, induction, brainstem, development of the ear, otic efferents*

Introduction

The amphibian inner ear shows more variation with respect to its form and the presence or absence of certain sensory epithelia then all other tetrapod ears together (Lewis *et al.*, 1985; Fritzsch and Wake, 1988). In addition, the path taken by the perilymphatic (periotic) canal around the ear is unique to amphibians but also shows an extensive variation (Lewis and Lombard, 1988). Among amphibians, the ear of salamanders is particularly diverse.

This review outlines adult diversity, followed by an explanation as to why the development of the salamander ear is one of the best understood examples of amphibian and vertebrate ear development. In order to bring the relevant information into the context of evolutionary diversification of the developmental program of the ear and to stress the importance of a comparative embryological understanding of otic development, salamander ear development will be compared with *Xenopus*, chicken and mouse ear development. It is hoped that this review will reinvigorate research into the salamander ear, which in some aspects appears to be closer to mammals than to other amphibian or avian model systems.

Evolution of the salamander ear

Like other tetrapod ears the salamander ear has three semicircular canals to detect rapid movement in space, two sensory receptors for gravistatic information (the utricle and the lagena), the saccule for low frequency sound and gravistatic information, and two exclusively auditory endorgans (the basilar papilla and the amphibian papilla; Fig. 1). Comparison with other vertebrates and other amphibian orders suggest that the basilar papilla, an organ unique to tetrapods and the coelacanth *Latimeria* (Fritzsch, 1987), may have evolved in the aquatic ancestor of tetrapods when a separate lagenar recess formed as a caudal extension of the saccule (for review see Fritzsch, 1992). The evolutionary history of the amphibian papilla, a hearing organ unique to amphibians, suggests that it may be derived from the papilla neglecta (Fritzsch and Wake, 1988). Transformation of the papilla neglecta into an amphibian papilla may have been selectively stabilized by the amphibian-specific sound pathway that is channeled through the unique amphibian periotic canal through the ear (Lewis and Lombard, 1988). The inner ear of some salamanders like the axolotl, while unique in some respects, may nevertheless represent a reasonable approximation of an ancestral tetrapod ear. In contrast, parts of the hearing system of other salamanders and caecilians appear to have regressed. Frogs represent a unique tetrapod hearing adaptation (Fritzsch and Wake, 1988; Fritzsch, 1992).

The ear forms from several embryonic sources: the inner ear derives from the otic placode in combination with neural crest, whereas the otic capsule and periotic mesenchyme form from neural crest, somitic mesoderm, and paraxial cephalic mesoderm

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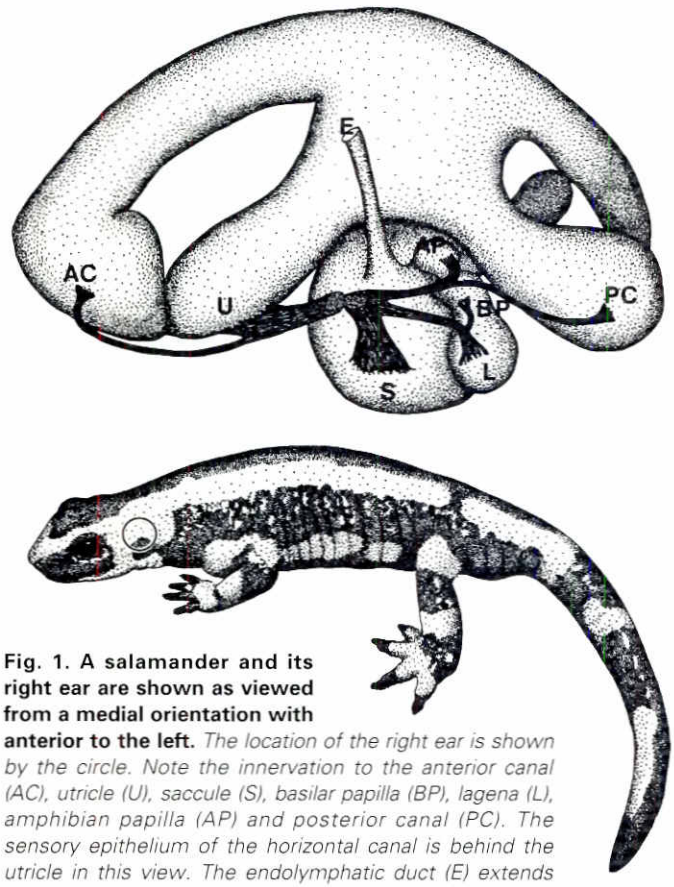

Fig. 1. A salamander and its right ear are shown as viewed from a medial orientation with anterior to the left. *The location of the right ear is shown by the circle. Note the innervation to the anterior canal (AC), utricle (U), saccule (S), basilar papilla (BP), lagena (L), amphibian papilla (AP) and posterior canal (PC). The sensory epithelium of the horizontal canal is behind the utricle in this view. The endolymphatic duct (E) extends from the saccule into the cranial cavity.*

(Couly *et al.*, 1993). A functionally essential evolutionary change involves the transformation of the periotic tissue into perilymphatic, sound conducting pathways (de Burlet, 1934) to allow sound pressure perception (van Bergeijk, 1967). Apparently, this transformation of perilymphatic tissue and its coupling to a sound pressure receiver has happened independently three times among bony fish and a fourth time in *Latimeria* and presumably the aquatic ancestors of tetrapods (Fritzsch, 1992). In all these cases the perilymphatic specialization becomes associated with a modified sensory epithelium. In amphibians these sensory epithelia are the saccule, the basilar papilla and the amphibian papilla. In addition, there are modifications of the otic capsule to form windows through which the sound pressure induced particle motion can enter and leave the inner ear: the stapes foot plate is inserted in the otic capsule and the round window functions as a pressure relief opening (de Burlet, 1934; Fritzsch, 1992).

In summary, the salamander ear, while in many aspects as unique as other amphibian ears (periotic canal, amphibian papilla), may nevertheless be regarded as a reasonable approximation to the ancestral tetrapod condition. Salamander ears differ with respect to the absence or presence of the basilar papilla. If that component is studied in a comparative developmental approach, understanding evolution in terms of divergence of developmental pathways might be facilitated. The origin of the basilar papilla or cochlea (a unique sensory organ devoted to terrestrial hearing), will thus be comprehended. Moreover, this knowledge may ultimately facilitate the analysis of structural and/or regulatory gene mutations that govern the developmental reorganizations associated with the basilar papilla.

That scenario also contends that the evolution of a sound pressure receiving inner ear requires modifications in a number of topologically adjacent tissues of *four* different embryonic origins. Only in the concerted modification of those diverse tissues is the morphological substrate for sound pressure reception achieved. Therefore, in order to understand the evolution of this system, we need to understand how mutations of structural and regulatory genes have modified tissue interactions in this most complex area of the head. Those mutations presumably induced and stabilized the various modifications for sound pressure reception observed in amphibian ears.

Of placodes and their diversification

Placodes are a major embryonic source that contributes to sensory formation of the vertebrate head (von Kupffer, 1895). Thus, the placode of the ear (the otic placode) is not unique in the way it forms sensory cells or ganglion cells but shares this developmental activities with other placodes. For example invagination, skeletogenic interactions with mesoderm (Webb and Noden, 1993) are carried out by other epidermal thickenings in the head. Even migration of the otic placode may occur under certain conditions (Gutknecht and Fritzsch, 1990)

Details of the spatial configurations of otic placode interactions such as with the notochordal mesoderm, paraxial mesoderm, neural crest and rhombencephalon, all of which have been variously implied or shown to play a role in otic placode induction (Yntema, 1955; van de Water, 1983; Jacobson and Sater, 1988), have only partially been analyzed in some vertebrates. In salamanders the otic placode maintains a rather stable topographic relationship to the various lateral line placodes and the eye (Northcutt *et al.*, 1994).

Most recently several molecular markers have been identified that are exclusively expressed in the otic placode (Ekker *et al.*, 1992; Akimenko *et al.*, 1994). Expression of these markers allows, for example, in a fish an unequivocal identification of the two sets of dorsolateral placodes, the otic and the lateral line placodes, at the earliest possible stages of their formation. *In situ* hybridization for these genes or immunocytochemistry to detect their proteins would likely help to achieve this unequivocal identification of lateral line and otic placodes in various vertebrates. Comparing the earliest stage of placodal formation in direct developing salamanders, which lack formation of lateral line organs (Fritzsch, 1990), and salamanders with a full complement of these organs could elucidate whether the lateral line placodes are selectively suppressed at the earliest possible stage of their development, as previously suggested (Fritzsch, 1990).

The hindbrain has been suggested to play a role in the induction and development of the ear because of its juxtaposition with the otic placode and apparent inductive effects (Harrison, 1936; Yntema, 1955). The work of Meier (1978; Jacobson, 1988) shows that the ear appears to be stable with respect to certain mesodermic landmarks in the surrounding tissue. However, boundaries in somitomeres are different between amphibia and amniotes (Jacobson, 1988). As a consequence, the ear forms adjacent to the anterior half of somitomere 4 of salamanders and

adjacent to somitomere 6/7 in amniotes. This difference may relate to the formation of the amphibian papilla of all amphibians (Fritzsch and Wake, 1988). Alternatively, it may be without any apparent consequence. Similar divergencies are known such as the different formation of notochord in salamanders and frogs (Hall, 1987) or the absence (mouse) or presence (salamanders) of cellular contact between the invaginating otic cup and the hindbrain (van de Water, 1983).

In summary, no large scale topologic differences in otic placode formation are known among salamanders. Nevertheless, xenoplastic transplantations between salamanders which either do or do not form a basilar papilla would be one way to evaluate the conservation of the morphogenetic field of otic induction and ear formation. That is the following question could be answered: *Are the modifiers for the development of the ear intrinsic to the otocyst or a consequence of a different interaction between the otic placode and the surrounding mesenchyme?* This approach may be particularly useful for characterizing interaction(s) between the otic placode and the periotic mesenchyme that may be responsible for the enormous variety of periotic systems observed among salamanders (Lombard, 1977).

Induction of the otic placode

As explained above, there is a rather stable position between the otic placode and the adjacent mesoderm and neuroectoderm. This appears to play a role in the inductive interactions occurring among them (Harrison, 1945; Yntema, 1950, 1955; van de Water, 1983; van de Water *et al.*, 1992; Fig. 2). Extirpation of the otic placode in salamanders will result in the induction of a new ear in the grafted foreign ectoderm. This observation suggests that both hindbrain and mesoderm are involved in establishing the morphogenetic field for otic induction. Consistent with this suggestion is that both hindbrain and mesoderm are alone able to induce otic placodes which lead to imperfect ears in salamanders (Harrison, 1945). Only when hindbrain and mesoderm act together, will a complete ear form. Yntema (1950) showed a temporal effect of induction through a series of transplantations of variously aged donor ectoderm on hosts. Harrison (1945), Yntema (1950, 1955) and later Jacobson (1963, 1966) provided further evidence that these inductive actions of mesoderm and neural tissue are overlapping and to some extent redundant.

Jacobson (1963) showed that the proximity to the hindbrain is crucial for invagination of the otocyst in a salamander. He rotated the neural plate alone or together either with the adjacent neural fold and/or the placodal epidermis. Ear formation was obtained along the rostro-caudal axis of the graft in rotations involving all ectoderm. No ears were obtained when the neural plate was rotated with neural fold alone. However, ears formed in the appropriate position in rotations of neural plate without neural fold. These data show that information about where to form an ear is present in the mesoderm/endoderm. This information can be overruled only if the entire placodal/neural crest/neural plate tissue is rotated. Whatever the nature of these inductive signals, xenoplastic transplantations between salamanders and frogs can clearly lead to donor specific ears under the inductive influence of the host (Andres, 1949), thus indicating a high degree of conservation of the inductive process, at least between amphibians.

Nevertheless, the actual sequence of inductive steps apparently differs among amphibians. For example, in some ranid frogs the otic placode is able to undergo morphogenesis when transplanted into the postero-ventral flank at the slit blastopore/early neural plate stage (Zwilling, 1941). In contrast, the otic placode of a salamander does not achieve autonomy for self-differentiating until the late neurula stage (Yntema, 1939). These data indicate a substantial heterochronic shift in the timing of independence of the otic placode for further differentiation. *In summary, although the molecular mechanism of mesodermal and neuronal induction in the otic placode and otocyst formation are not yet understood, it appears that different factors are acting in succession to create the otic placode and subsequently the otocyst.*

There is a need to study these early inductive events in more salamanders and in greater detail to elucidate the relative importance of mesoderm and neuroectoderm in this induction as well as the timing events of induction. It would not be surprising to find various degrees of epithelial autonomy and even changes in the inductive competence of mesoderm and neuroectoderm, as has already been well established for another placodally derived tissue, the lens (Grainger *et al.*, 1988).

Invagination to form the otocyst

Invagination as a morphogenetic process existed in chordates prior to the evolution of craniates, the only chordates with an ear, in the form of gastrulation and neurulation. Invagination occurs in other placodes as well such as the olfactory placode (Webb and Noden, 1993). Depending on the evolutionary history of appearance of various placodes, the invagination process displayed by the otic placode may not represent a unique

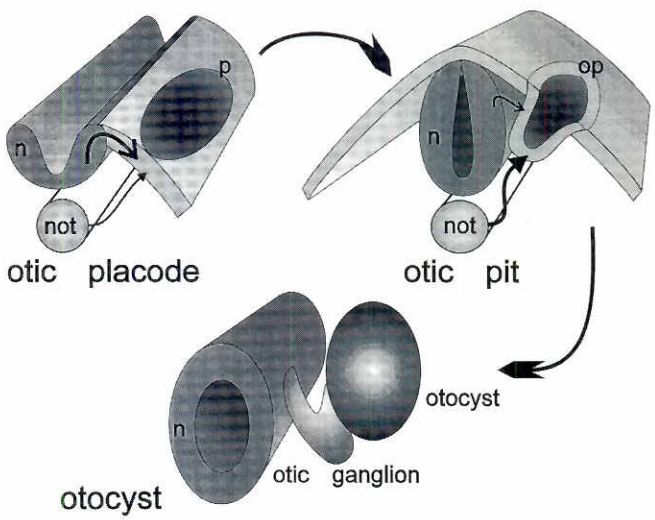

Fig. 2. This drawing shows the presumed inductive interactions between the neuroectoderm (n) and the mesoderm (represented by the notochord, not) which induce an otic placode (p) which will invaginate into an otic pit (op) that will separate from the ectoderm (light grey) to become the otocyst. *Experimental evidence indicates that there is variation in the relative importance of the neuroectodermal and mesodermal inductive actions (arrows) probably over time (as shown here) and also between species.*

evolutionary achievement. Rather, the invagination mechanism may have been 'borrowed' from other existing invaginations. Although superficially similar, it is unclear whether otocyst invagination follows the same principles known for gastrulation (Keller *et al.*, 1992) and neurulation (Jacobson, 1991) such as forceful intercalation to produce convergent extension. A gene that may play a role for invagination of the otocyst has been analyzed in the chicken (*int-2*; Represa *et al.*, 1991). Clearly, *int-2*, a member of the fibroblast growth factor family (FGF), is expressed at the right time and the right place in mice (Wilkinson *et al.*, 1988), frogs (Tannahill *et al.*, 1992) and to some extend in the chicken (Mason and Mahmood, 1993). However, a recent targeted gene disruption of *int-2* in mice (Mansour *et al.*, 1993) showed formation of an otocyst but also reported some ear defects which were highly variable and sometimes different on the left and the right side. Although all this evidence argues against a direct role played by *int-2* in otocyst invagination, it clearly underscores the potential role of *int-2* for otocyst differentiation. Neither the distribution nor a possible role for *int-2* has yet been analyzed in salamanders.

In summary, the detailed mechanisms of otocyst invagination and its molecular governance is not understood. Despite the reservations mentioned above, a good candidate appears to be the exploration of trophic factors such as FGF-3 (the *int-2* gene product). A major difference between the otic placode and the adjacent lateral line placodes, based on their spatio-temporal differences of appearance, could be the potential access to these factors. In this context the demonstration of Yntema (1950) that grafted ectoderm may still be induced to form lateral line organs after the ability to form ear has ceased needs to be re-examined. The outcome of such heterochronic transplantation experiments could show that only a restricted area of placodal tissue will invaginate owing to the spatio-temporal restricted influence of certain rhombomeres of the hindbrain and/or specific areas of cephalic mesoderm. It is possible that the developmental sequence of otic and lateral line placode formation (in *Xenopus* at st. 21 and stage 31, respectively; Nieuwkoop and Faber, 1967; Winklbauer and Hausen, 1983; in axolotl at st. 21 and st. 23-29, respectively; Northcutt *et al.*, 1994) may indeed reflect the order in which they evolved. Perhaps the lateral line placode is nothing else but an otic placode that has failed to invaginate because it was induced too late and too far away form the otic region to complete its invagination process.

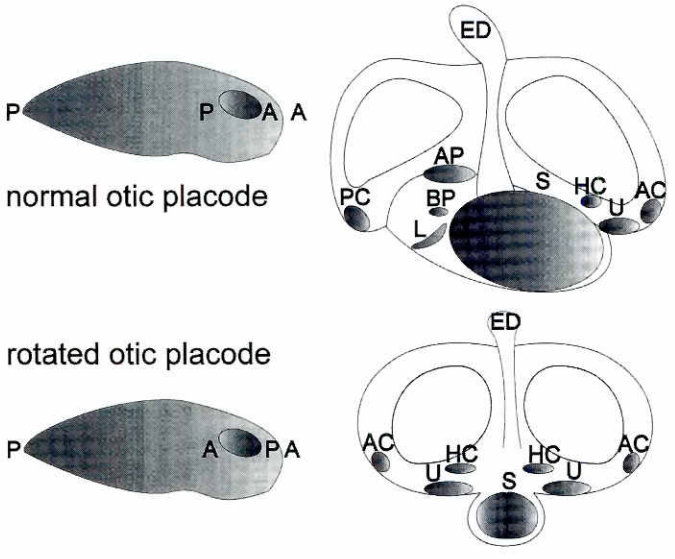

Fig. 3. At the level of otic pit formation the polarity of the ear becomes fixed and leads to the normal differentiation of an asymmetric ear. *Rotation of the otic placode with a reversal of the anterior-posterior axis (A,P) leads to the formation of an enantiomorphic ear which consists of either two anterior halves (as shown) or two posterior halves. Modified from Harrison (1945).*

Fixation of polarity in the otocyst

Numerous experiments in amphibians have demonstrated that the axis of polarity of the otocyst is fixed at early stages of otic pit formation (Harrison, 1945). This fixation of polarity provides topologic specificity and precedes differentiation of the otocyst in amphibians. The amphibian is the only vertebrate mapped in this regard in appropriate detail. In contrast to the otocyst, the placode is still an equipotential system and can regulate to form a whole ear from parts or from two placodes in salamanders (Harrison, 1945).

Transplantation of rotated placodes leads, in many instances, to enantiomorphic twins. That is, mirror duplicates form across a transverse plane that may consist of two anterior or two posterior halves (Fig. 3; Harrison, 1945; Yntema, 1955). This reversal in polarity will happen along the anterior-posterior axis alone until the dorso-ventral axis becomes fixed somewhat later during otic cup formation. Yntema (1955) suggested that the fixation of polarity in the ear rudiment may be a local expression of a general polarity of the body (Fig. 3). Clearly, this formation of enantiomorphic twins in transplanted otic placodes shows that its polarity follows largely the pattern of comparable duplication phenomena in developing and regenerating limbs (Harrison, 1945; Tickle *et al.*, 1982). As in the developing eye and the developing limb, the anterior-posterior axis is specified prior to formation of the dorso-ventral axis. More detailed transplantation experiments will be necessary to establish whether an area equivalent to the zone of polarizing activity (Gilbert, 1991) exists at the posterior margin of the otic placode that will form a gradient of a putative morphogen. Whether retinoic acid plays any role in this specification, as suggested for the developing limb (Thaller *et al.*, 1993), remains to be shown. At any rate, the invaginating otic cup is no longer a harmonic and equipotent system. Rather it is at the level of the otic placode that the isotropy, that is the possibility to change freely according to external influence, is lost. In amphibians, polarization becomes first fixed along the anterior-posterior axis and soon afterwards at the dorso-ventral axis.

Most recently a number of genes have been found to be expressed in a spatio-temporal restricted pattern in the developing ear of zebra fish (Ekker *et al.*, 1992; Akimenko *et al.*, 1994), frogs and chicken (Ramirez and Solursh, 1993). While causality has not yet been established between these genes and identified morphogenetic steps, their spatio-temporal distribution pattern makes them good candidates to specify dorso-ventral, anterior-posterior and medio-lateral axes. Whether their gene products are responsible for axis determination or whether these genes are expressed as a consequence of a process that specifies the

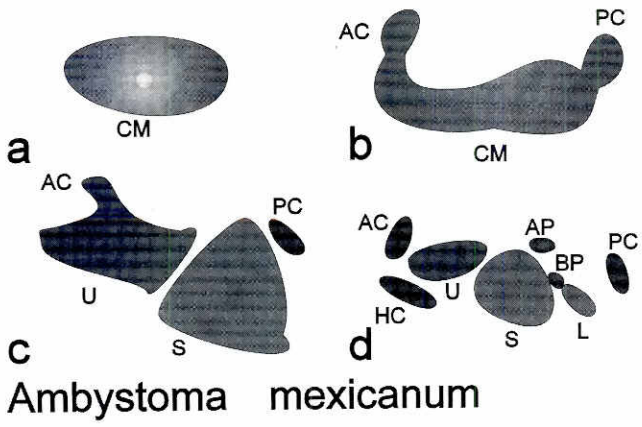

Fig. 4. The segregation of sensory epithelia in *Ambystoma* is shown. *Note that the common macula (CM; A) first gives rise to the anterior and posterior canal cristae (AC, PC; B). At later stages the utricle and saccule (U, S; C) and even later the horizontal canal (HC), the amphibian papilla (AP), the lagena (L), and the basilar papilla (BP) segregate. Before the final segregation they may already be committed to form specific epithelia from specific regions of the otocyst. Modified from Norris (1892).*

polarity needs to be examined with appropriate transplantation and correlated by means of *in situ* hybridization experiments.

In this context analysis of mutant mice with various deformations of the ear as well as with targeted disruptions of specific genes has helped to elucidate some of the hindbrain/otocyst interactions. For example, in the *Kreisler* mutant the placode is induced at a distance from the hindbrain and the otocyst invaginates away from the brain. The brain is abnormal and lacks formation of rhombomere boundaries caudal to rhombomere 3. In addition, the hindbrain shows differences in the expression patterns of some genes (Frohman *et al.*, 1993). The *Kreisler* gene has been isolated and shown to act upstream of these genes (Cordes and Barsh, 1994). This discovery provides the first molecular correlate for the requirement of a rhombomere/otocyst interaction. It will be important to identify the homologue of the *Kreisler* gene in a salamander and establish whether it is expressed in a similar fashion.

In summary, classical embryological manipulations and modern studies of gene expressions are both compatible with the idea that the polarity of the developing ear is fixed at the otic cup stage through hindbrain/mesoderm interactions with the otic placode. Unfortunately, the embryological and molecular data were obtained in rather different model systems. It will be useful to combine both in the same animal to establish whether embryonic manipulation leads to modification of the expression patterns of genes which are supposedly involved in axis specification. Two possible factors involved in polarity formation (retinoic acid and *int-2*) should be tested in paradigms similar to those established for limb pattern formation (Riley *et al.*, 1993).

Formation and segregation of sensory epithelia

The ontogeny of sensory epithelia apparently closely follows the known and inferred evolutionary history of the cognate organism. For example, the utricle/ anterior vertical/ horizontal canal epithelia segregate together from the sacullar/ lagenar/ papilla amphibiorum/ papilla basilaris epithelia to form the anterior (dorsal, superior) as compared to the posterior (ventral, inferior) subdivision of the ear of most vertebrates (Fig. 4; Norris, 1892; Fritzsch and Wake, 1988). Apparently this differentiation and segregation of sensory epithelia can take place if otocysts are transplanted to ectopic locations or *in vitro* and even when the otocyst is reopened (Harrison, 1945; van de Water, 1983; Swanson *et al.*, 1990; Sokolowski *et al.*, 1993). What is unclear in most of the studies which deal with transplantation is the degree of axis fixation of the otocyst at the time of transplantation, as well as the required time schedule of continuous influence of hindbrain and mesoderm interaction for complete differentiation.

The single *in vitro* study of salamander ears (Jacobson, 1963) revealed that normal differentiation of the ear requires the synergistic action of mesoderm and neural plate at least until neurulation is complete. Unfortunately, that study did not show the details of morphological differentiation that can be obtained *in vitro*. New experiments testing for windows in inductive competence for mesoderm and neuroectoderm action in otocyst formation, polarization and differentiation will therefore be needed. Such experiments should combine salamander tissue in ways analogous to experiments which have been performed in mammals (van de Water *et al.*, 1992).

Xenoplastic transplantations of an otic placode have shown that differentiation can proceed in a grafted tissue without major disruption in distantly related host amphibians (Andres, 1949). This approach, in combination with detailed anatomical and neuroanatomical evaluation may be used to test the interaction of the otocyst of the axolotl in other salamanders, including those which do not form all the sensory epithelia of the ear. Apparently, these xenoplastic transplantations have not been attempted. They may, however, potentially help to dissect the spatio-temporal pattern of species-specific gene activation in the induction and differentiation phases of the otocyst.

In summary, the descriptive and experimental evidence on the formation of subdivisions and segregation of sensory epithelia in salamanders has shown that the basilar papilla/cochlea is an outgrowth of the ventral part of the otocyst that may become fixed as a latent pattern soon after the formation of the otocyst is complete. The segregation of the sensory anlage and its morphogenesis and histogenesis is critically dependent on interaction between the otocyst and the surrounding periotic mesenchyme.

Formation of the perilymphatic ducts

After otocyst formation is completed, subdivision of the epithelia and morphogenesis of the ear proceeds along the previously induced pathways. While formation of the epithelia may have reached a large degree of autonomy at this stage, morphogenesis of the ear requires further interaction with the periotic mesenchyme to form the cochlea and the semicircular canals in mice (Yntema, 1955; van de Water, 1983). These data show that morphogenesis of the ear is largely eliminated in the absence of periotic mesenchyme and cytodifferentiation is reduced to the vestibular type of hair cells in mice (van de Water, 1983). However, cartilage may develop in the absence of an otocyst in some species but will also be induced by the otocyst in foreign mesoderm, thus providing double security for ear capsule

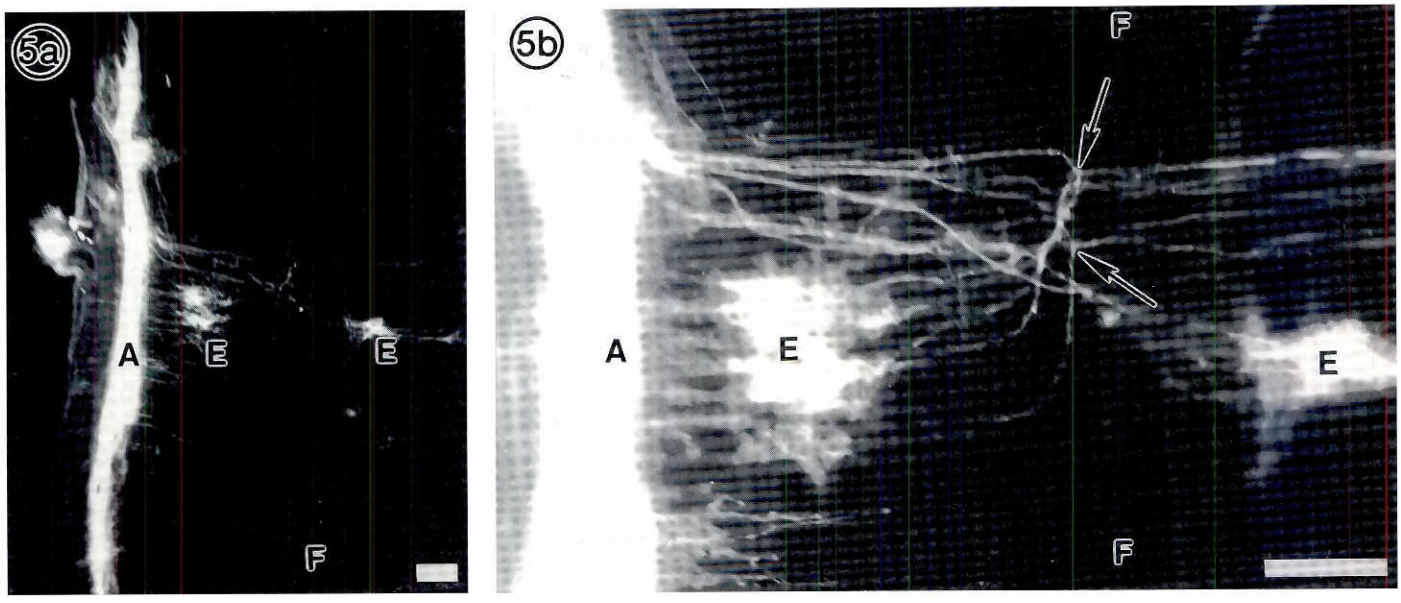

Fig. 5. These micrographs show the central projection of the VIII nerve afferents (A) and efferents (E) in a juvenile axolotl as revealed with diffusion of the lipophylic dye, DiI, in a whole-mounted brain stem. *Note that the efferent perikarya (E) are bilaterally distributed and tall efferent axons show bifurcations near the floor plate (F) which cross to the contralateral side (arrows, b). Bar indicates 100 µm in both cases.*

formation (Yntema, 1955). This chondrogenic ability of the otocyst could be stage dependent and may be fixed at different stages in different species. It appears that certain growth factors like TGF-ß are the mediators of this chondrogenic signal and will also act as a suppresser at later stages (van de Water *et al.*, 1992).

The extracellular matrix molecule hyaluronan acts as a propellant for the formation of the horizontal canal in a frog, *Xenopus* (Haddon and Lewis, 1991). If hyaluronidase is injected adjacent to the leading edge of the growing horizontal canal, its formation will be blocked. Nevertheless, the sensory cells of the horizontal canal segregate, thus revealing that both events are clearly distinct. It would be important to know whether similar defects can be achieved in the growing vertical canals or whether the hyaluronidase effect is specific to the horizontal canal, which is absent in jawless vertebrates.

In summary, the placodally derived otocyst and the mesodermally and neural crest derived periotic mesenchyme need to interact to promote complete morphogenesis of the ear. It is unclear at which time in development complete autonomy of either tissue for its specific differentiation is achieved and at least for morphogenesis of the semicircular canal and for cytodifferentiation of the cochlea there appears to be a need for continuous, probably reciprocal interaction of these tissues to assure complete differentiation.

Innervating the ear: origin of otic ganglion cells

Formation of the otic ganglion (statoacoustic or VIIIth nerve ganglion) and the role of innervation for trophic support of hair cells and otic differentiation is controversial. Earlier descriptive data supported the idea of a neural crest contribution to otic ganglia (Adelmann, 1925; Szepsenwol, 1933). Later experimental data suggest an exclusive formation of otic ganglia from placodal tissue (Yntema, 1955; D'Amico-Martel and Noden, 1983). In fact, the otic ganglia seem to be no different from other placodally derived ganglia which form as a first step in placodal development (von Kupffer, 1895; Noden and van de Water, 1986).

The migration of otic ganglia away from the invaginating placode can be as readily observed as the delamination of the neural crest (von Kupffer, 1895; Webb and Noden, 1993). This delamination of ganglia starts in chicken before otocyst formation is completed (Hemond and Morest, 1991). Extirpation of various parts of the otocyst in mice revealed that the ganglion is predominantly derived from the antero-ventral aspect of the otocyst (Li *et al.*, 1978). Comparable extirpations need to be performed in salamanders in order to elucidate how early and at which position of the otocyst ganglion cell formation takes place. The formation of ganglion cells at a specific site offers also some insight into the timing of polarity fixation of the otocyst of which the formation of ganglion cells is an integral part.

In conclusion, both comparative and experimental data overwhelmingly demonstrate the placodal origin of the otic ganglion cells, whereas the majority of glial cells are contributed by the neural crest. Unclear is whether the ganglion cells derive in various species from the same area of the otocyst and show a comparable time course of delamination. That is, is the otic induction evolutionary conserved with respect to the earliest mosaic of otocyst subdivision?

The ear and neurotrophic substances

There is a large body of evidence demonstrating the arrival of afferent fibers in the developing ear before onset of hair cell maturation (Fritzsch *et al.*, 1993). This phenomenon was suggested to play a role in hair cell maturation (Pirvola *et al.*, 1991). However, numerous *in vitro* studies in mice (van de Water, 1983) and in chicken (Sokolowski *et al.*, 1993) as well as graftings with or without adjacent statoacoustic ganglia (Corwin and

Cotanche, 1989) indicate that innervation is not necessary for histogenesis of the cochlea. More recent experimental data strongly support this conclusion also for the lateral line (Kelley and Corwin, 1992) and electroreceptors (Fritzsch *et al.*, 1990). It, however, appears that some hair cells require innervation for long term maintenance. In addition, the detailed electrophysiological maturation of some hair cells may depend on neurotrophic interactions (Sokolowski *et al.*, 1993).

In contrast to the rather limited effect of ganglion cells on the differentiating ear, the developing cochlear ganglion cells show better survival rates *in vivo* when co-cultured with the otocyst or the rhombencephalon (Ard *et al.*, 1985; van de Water *et al.*, 1992). Possible mediators of this neurotrophic influence may be nerve growth factors such as brain derived neurotrophic factor (BDNF) and neurotrophin 3 (NT-3) which are known to be present in the developing otocyst of rats and chicken around the critical time (Pirvola *et al.*, 1992; Hallböök *et al.*, 1993).

Indeed, tissue culture experiments indicate that distal processes of ganglia navigate to innervate sensory areas (van de Water, 1983; Bianchi and Cohan, 1993). These studies suggest that the developing otocyst releases a tropic factor that attracts the growing ganglionic processes towards the differentiating sensory epithelia (Hemond and Morest, 1992; Bianchi and Cohan, 1993). The nature of this molecule(s) is currently unknown.

Direct proof for the role of any of these factors by *in vivo* experiments is still missing. For example, injection into the otocyst followed by a reduced rate of natural cell death in ganglion cells and/or blockade of the receptors and/or the putative trophic factors followed by an enhanced rate of ganglion cell death has not yet been demonstrated. However, transgenic mice with disrupted neurotrophins (BDNF, NT-3; Ernfors *et al.*, 1994; Farinas *et al.*, 1994) or their receptors (TrkB, TrkC; Fritzsch *et al.*, 1995) show various defects in otic innervation. Indeed, a double knockout mutant for both TrkB and TrkC lacks any innervation of the ear while showing a normal development of all sensory epithelia (Fritzsch *et al.*, 1995). These data suggest that these two receptors and their neurotrophins are both necessary and sufficient to maintain the otic innervation. In addition, the data show that there is complete autonomy of the ear in many aspects of its differentiation once the otic ganglion cells are formed. Comparable data are needed in the axolotl.

The efferent system to the ear

Traditionally the efferent system of the ear (Roberts and Meredith, 1992; Warr, 1992) was suggested to arrive after the afferents (Cohen and Cotanche, 1993). However, it was pointed out recently that early stages of afferent and efferent synaptogenesis cannot be distinguished and thus may lead to false negative results with respect to the presence of efferents (Sobkowicz, 1992). Re-examination of arrival of efferent fibers showed that they enter the developing otocyst much earlier then previously suggested and at about the same time as afferents in mice and chicken (Fritzsch and Nichols, 1993; Fritzsch *et al.*, 1993). There is the intriguing possibility that these fibers, being derived from a sub-population of facial motoneurons (Fritzsch and Northcutt, 1993), may respond to the BDNF present at that time in the developing ear (Hallböök *et al.*, 1993) and known to be important for motor neuron survival (Oppenheim *et al.*, 1992). Unfortunately these molecular data are not known for the axolotl.

Another important aspect of efferent development is the acquisition of bilateral distribution of cells and axons. This happens in chicken through migration of neurons across the floor plate to the contralateral side (Fritzsch *et al.*, 1993) but through extension of axons in mice (Nichols and Fritzsch, 1994). Preliminary data in the axolotl suggest that efferents send axon collaterals across the floor plate (Fig. 5) thus creating the bilateral distribution of cells and fibers (Fritzsch and Wahnschaffe, 1987) through a process comparable to mice. Given that in *Xenopus* and other frogs the inner ear efferents are exclusively ipsilateral (Fritzsch and de Caprona, 1984) it appears that the only available model system to study the mammalian efferent development is the axolotl. In addition, axolotls share to some extent their fiber pathway with mammals: some of the efferent fibers enter the octaval nerve root (Fritzsch and Wahnschaffe, 1987). In contrast, in chicken and *Xenopus* efferent axons enter the facial root and reroute at the periphery to reach the ear (Fritzsch *et al.*, 1993; Hellmann and Fritzsch, 1996). Thus axolotls can be used to study molecular aspects of pathfinding in the octaval efferent system in a mammal-like model system.

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