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Development of the mechanoreceptive lateral-line system in the axolotl: placode specification, guidance of migration, and the origin of neuromast polarity

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Summary. The mechanosensory lateral-line system offers a unique opportunity to study a wide variety of developmental phenomena, including cell migration, the origin of polarity, and pattern formation. In this study, we use a series of transplantation experiments to examine some of the factors affecting the origin of the lateral-line placodes, the establishment of sensory organ polarity and placement, and the guidance of cell migration in the Mexican axolotl (*Ambystoma mexicanum*). We find that placode-forming ectoderm is at least partially specified as early as the beginning of neurulation, and we suggest that this may be a result of early processes involved in neural induction. Furthermore, we find that the migration of the primordia on the body depends on the presence of both the ectoderm and the subjacent mesoderm for guidance. Sensory organ polarity on the body appears to be the result of an interaction between the primordia, which deposit organs of set polarity relative to the direction of migration, and the substrate, which determines the direction of migration. Spacing of the organs is independent of the substrate, and may be due to an intrinsic property of either the primordia or the emerging organs themselves. Finally, we suggest that the lateral-line primordia are guided, as they migrate, by a contact guidance mechanism.

Key words: Lateral-line system – *Ambystoma mexicanum* – Pattern formation – Cell migration – Contact guidance

Introduction

The lateral-line system is a unique sensory system found in fish and aquatic amphibians (see Wright 1951; Sand 1984; Blaxter 1987; Coombs et al. 1988, 1989; Lannoo and Smith 1989; Webb 1989, for reviews). In the generalized condition, the lateral-line system contains two types of sensory organs: mechanoreceptive neuromast and electroreceptive ampullary organs. Unlike ampullary organs, the neuromasts within each line possess a distinct morphological polarity (see below) and are formed by a single, highly visible migratory primordium[1]. Adjacent lines may contain neuromasts with different polarity. The question then becomes: what determines the polarity of the neuromast organs, the primordia or the substrate through which they migrate?

The neuromast receptor organ is the functional unit of the mechanoreceptive lateral-line system. Each neuromast is composed of numerous hair cells covered with a gelatinous cupula. Each hair cell is morphologically and physiologically polarized, being maximally sensitive to movement along one axis. All the hair cells within each neuromast are aligned so that the organ is maximally sensitive to movement along its long axis (see Flock 1965; Shelton 1971; Russell 1976). We have previously shown, in the axolotl, that this polarity is evident as soon as the organ is formed (Smith et al. 1988).

Neuromasts are usually arranged in lines which lie in definite patterns on the head, body, and tail. The neuromasts within each line are uniformly polarized either parallel or perpendicular to the axis of the line, depending on the species and the line (Lannoo 1987a, b; Coombs et al. 1988).

In the axolotl, as in other aquatic urodeles, neuromasts are arranged in a definite topography; they form three lines on the body and three major lines on the head (e.g. Lannoo 1987b and references therein; Lannoo and Smith 1989; Smith et al. 1988). On the body, neuromasts in the dorsal body line (DL) are polarized dorsoventrally, while those in both the middle (ML) and ventral (VL) body lines are oriented rostrocaudally (see Fig. 1 for abbreviations and locations of the lines).

Neuromasts on the head are arranged into multiple rows within each line. In both the supraorbital (SO) and infraorbital (IO) lines, neuromasts are arranged in

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[1] In this paper we have used the term "placode" only to refer to the thickened ectodermal areas prior to migration. Migratory anlagen, which are beneath the ectoderm, are referred to as "primordia".

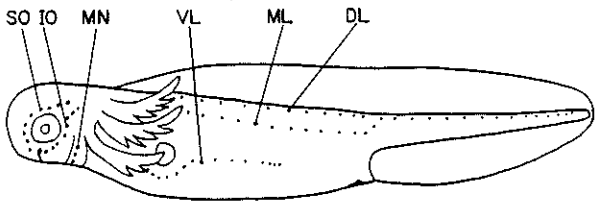


Fig. 1. Locations of the major neuromast lines on an axolotl hatchling. No attempt has been made to indicate either polarity or the correct number of neuromasts. The lines consist of the supraorbital (*SO*), infraorbital (*IO*), and mandibular (*MN*), lines on the head, and the ventral (*VL*), middle (*ML*), and dorsal (*DL*) body lines. After Smith et al. (1988)

single rows circumorbitally, and are polarized tangentially to the margin of the eye. On the snout, each line forms several rows of neuromasts. Within each line, the adjacent neuromasts in the outside rows are oriented orthogonal to one another. This orthogonal arrangement on the snout, and the dorsoventral-rostrocaudal polarization of adjacent neuromasts in separate lines on the body may be functionally significant, since it constitutes the minimum arrangement necessary to detect water displacements through 360° in a plane tangential to the body (Dijkgraaf 1963; Sand 1984; Görner and Mohr 1989).

Lines of neuromasts are formed by a series of pre- and post-otic ectodermal placodes that undergo extensive migration over the head and body, depositing organs at regular intervals and laying down the cranial nerves which supply them (Harrison 1904; Landacre 1921; Stone 1922; Metcalfe 1985; Lannoo and Smith 1989). We have previously examined the timing of development of these lines, and some features of neuromast formation in the Mexican axolotl (Smith et al. 1988).

The lateral-line system offers unique opportunities for studying several developmental phenomena. These include: 1) the control of morphogenetic movements (in the form of extensive cell migration along known routes), 2) pattern formation (of a repetitive series of organs along the length of the animal), and 3) the origin of polarity in both the placodes (to determine the direction of migration) and the neuromasts themselves. The system is accessible, and migrating primordia can be easily observed in living embryos (Lannoo and Smith 1989). Furthermore, the primordia migrate as discrete units (Landacre 1928; Stone 1933; Winklbauer and Hausen 1983a, b, 1985a, b; Metcalfe 1985; Smith et al. 1988; Lannoo and Smith 1989) and not as part of an amorphous sheet of cells.

Despite these advantages, little work has been done on the embryogenesis of this remarkable system since the classical studies of Stone (1922, 1928a, b, 1929, 1933, 1938; see also Wright 1951; Blaxter 1987; Lannoo and Smith 1989, for reviews). In this study, we examine the origin of the placodal ectoderm, and some of the factors responsible for the guidance of cell migration and the origin of polarity in the neuromasts of the lateral-line system of the Mexican axolotl (*Ambystoma mexicanum*).

Materials and methods

Animals. We used albino and wild-type embryos of the Mexican axolotl (*Ambystoma mexicanum*) from spawnings between animals raised in the University of Ottawa axolotl colony. Embryos were maintained at 18° C in 25% Holtfreter's solution (865 mg NaCl, 12.5 mg KCl, 25 mg $CaCl_2$, 50 mg $MgSO_4$, 50 mg $NaHCO_3$ per liter, pH 7.4; see Asashima et al. 1989) supplemented with 0.1 μg/ml each penicillin and streptomycin. To obtain the appropriate developmental stages, embryos were occasionally maintained at 4° or 10° C for short periods of time to slow development. Developmental stages were determined using the normal tables of Bordzilovskaya and Dettlaff (1979), see also Bordzilovskaya et al. (1989).

Surgical manipulation of embryos. The procedures we followed in surgically manipulating axolotl embryos are described by Asashima et al. (1989). Briefly, embryos were demembranated using sharpened watchmaker's forceps, passed through two rinses of sterile calcium-free Steinberg's medium (3.4 g NaCl, 50 mg KCl, 205 mg $MgSO_4$, 560 mg Tris per liter, pH 7.7, supplemented with 50 mg/l gentamicin sulphate) and placed in operating dishes, lined with a Permoplast Paraplast mixture and containing Ca^{++}-free medium. Embryos older than stage 33 were anesthetized by adding 0.005% benzocaine to the operating medium (Vanable 1985).

Transplantations were performed using hair loops and electrolytically-sharpened tungsten needles. Transplanted tissues were held in place with glass bridges. When healing had begun (usually 30–60 min post-operatively), part of the medium was replaced with 100% Steinberg's medium (as above plus 50 mg/l $CaCl_2$). After an additional 30–60 min, the glass bridges were removed, and the embryos were transferred to 24-well tissue culture plates (Corning Plastics, Corning, NY, USA) lined with 1% Noble agar and containing 100% Steinberg's medium. After 1–2 days, the medium was changed to 25% Holtfreter's solution supplemented with 50 mg/l gentamicin. Surgically manipulated embryos were periodically examined with a dissecting microscope to check the progress of the lateral-line primordia. Each series of operations was performed at least in triplicate.

Scanning electron microscopy. Surgically manipulated embryos were allowed to develop to the open mouth stage (stage 44) before processing. This ensured that all neuromasts had erupted and would be visible (see Smith et al. 1988). The embryos were anesthetized with benzocaine, fixed overnight in 2.5% glutaraldehyde in PIPES buffer containing 5 mM $CaCl_2$ (pH 7.4), rinsed in buffer, dehydrated in a graded ethanol series, and critical point dried from CO_2. They were then mounted on stubs with silver paste, sputter-coated with Au:Pd (60:40), and examined with a Philips 505 scanning electron microscope (SEM).

To observe the migrating primordia, unoperated stage 36–38 embryos were fixed (as above) and dissected in buffer. Incisions were made dorsal, rostral, and caudal to the migrating primordia, and the ectoderm was carefully peeled from the underlying mesoderm. The flaps of ectoderm were left in place during processing and, when the embryos were mounted, were pulled back and held in place with a small amount of silver paste.

Results

Timing of placode determination

In the axolotl, the DL, ML, SO, and IO lines originate from placodes in the otic area, dorsal to the gill bulge, and begin their migration at about stage 35 (Smith et al. 1988). Prior to migration, these ectodermal thickenings cannot be distinguished from the surrounding ectoderm in living embryos. Therefore, we have previously deter-

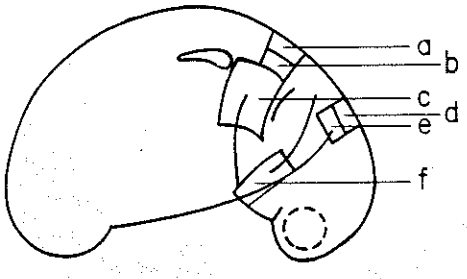


Fig. 2. Locations of the lateral-line placodes on a stage 26 axolotl embryo, as determined by the mapping experiments of Smith et al. (1988). Locations of the DL (*a*), ML (*b*), VL (*c*), SO (*d*), IO (*e*), and MN (*f*) placodes are shown. From Smith et al. (1989)

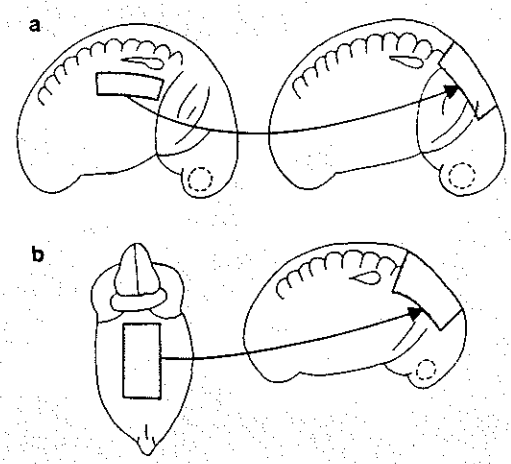


Fig. 3a, b. Stage 28 **a** and 26 **b** embryos showing the areas of ectoderm transplanted to determine the timing of specification of the placode-area ectoderm. Non-placode-forming ectoderm from the flank **a** or belly **b** was used

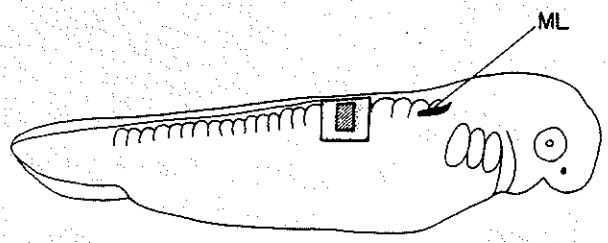


Fig. 4. Stage 35 embryo showing the locations of the ectoderm lifted for ectodermal rotations (*stippled area*), and the somite which was extirpated (*hatched area*) as described in the text. The teardrop-shaped area labelled *ML* shows the approximate location of that primordium at this stage

mined their locations by transplanting pigmented wild-type ectoderm into albino embryos and observing which areas give rise to pigmented lateral-lines (see Fig. 2). Below, these areas and the immediately adjacent ectoderm will be termed "placode-area" (see Fig. 3).

We then sought to determine at what developmental stage the placodes are specified. To approach this, at stages 14, 20, 24, 26, or 28 we replaced placode-area ectoderm with non-placodal flank or belly ectoderm from donor embryos of the same stages (Fig. 3). If normal lateral lines later arose from the transplanted tissue, we might reasonably conclude that the operation had been performed at a stage before which inductive signals specifying the placodes were present (or at least before they ceased to be present).

However, even at the earliest stage, normal lines never formed. In particular, when pigmented ectoderm was placed on albino hosts, pigmented primordia were never seen migrating from the graft area. Therefore, although a few neuromasts were occasionally observed under SEM, they were not from the grafted tissue and must have arisen from incompletely removed host placodes. Thus, though we cannot say when determination of the placodes occurs, we can say that the specification of placode-area ectoderm differs from that of other ectoderm as early as the beginning of neurulation (stage 14).

Manipulation of the substrate

Although previous workers have stated that the environment through which the primordia migrate does not affect the placement of the neuromasts, we have recently questioned the validity of this conclusion (Lannoo and Smith 1989). Therefore, we designed two tests to clarify the role of the substrate. In the first test, square pieces of ectoderm covering somites 8 to 10 were removed from stage 35 embryos (Fig. 4). Some grafts were immediately replaced and allowed to heal in the same orientation (sham operations), while others were rotated either 90° (with the original rostral edge either dorsal or ventral) or 180° before being replaced.

When embryos were examined during and after the migration of the primordia, it was found that the middle body line (ML) primordia migrated normally along the flanks of sham-operated embryos (Fig. 5b). Middle body line formation was also normal through ectoderm which had been rotated 180°, although in one case the ML appeared to deviate slightly in the graft area (Fig. 5c).

In embryos where the ectoderm was rotated 90°, the ML primordia either stopped at the rostral edge of the grafted area, or were diverted and followed an abnormal, irregular course over the body (Fig. 6). This occurred regardless of the direction of the 90° rotation.

Abnormal epidermal protuberances sometimes formed at the edges of the ectodermal grafts (see Figs. 5c, 6a, b). However, these appeared to be neither formed by the primordia nor involved in their arrest or diversion for several reasons. First, the primordia appeared to be able to migrate around them without being diverted (see VL in Fig. 5c). Second, neuromasts were never observed on the protuberances, which were usually covered with ciliated ectodermal cells. Finally, migratory arrest or diversion of the primordia was observed whether or not these protuberances formed in the vicinity. It remains unclear why these growths form, but it may be due to ectodermal puckering during healing of the grafts.

Flank ectoderm in the path of the ML primordium was also rotated 90° (such that the rostral edge of the

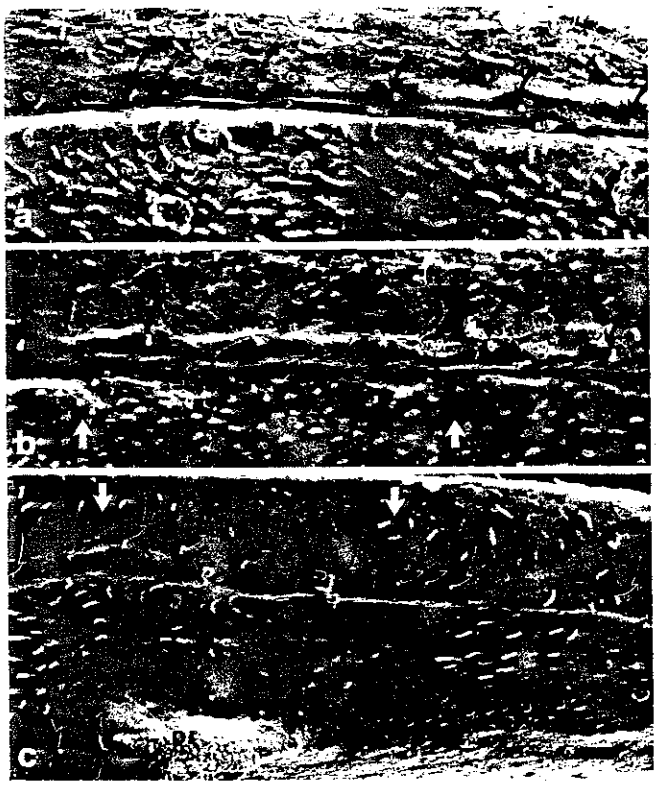
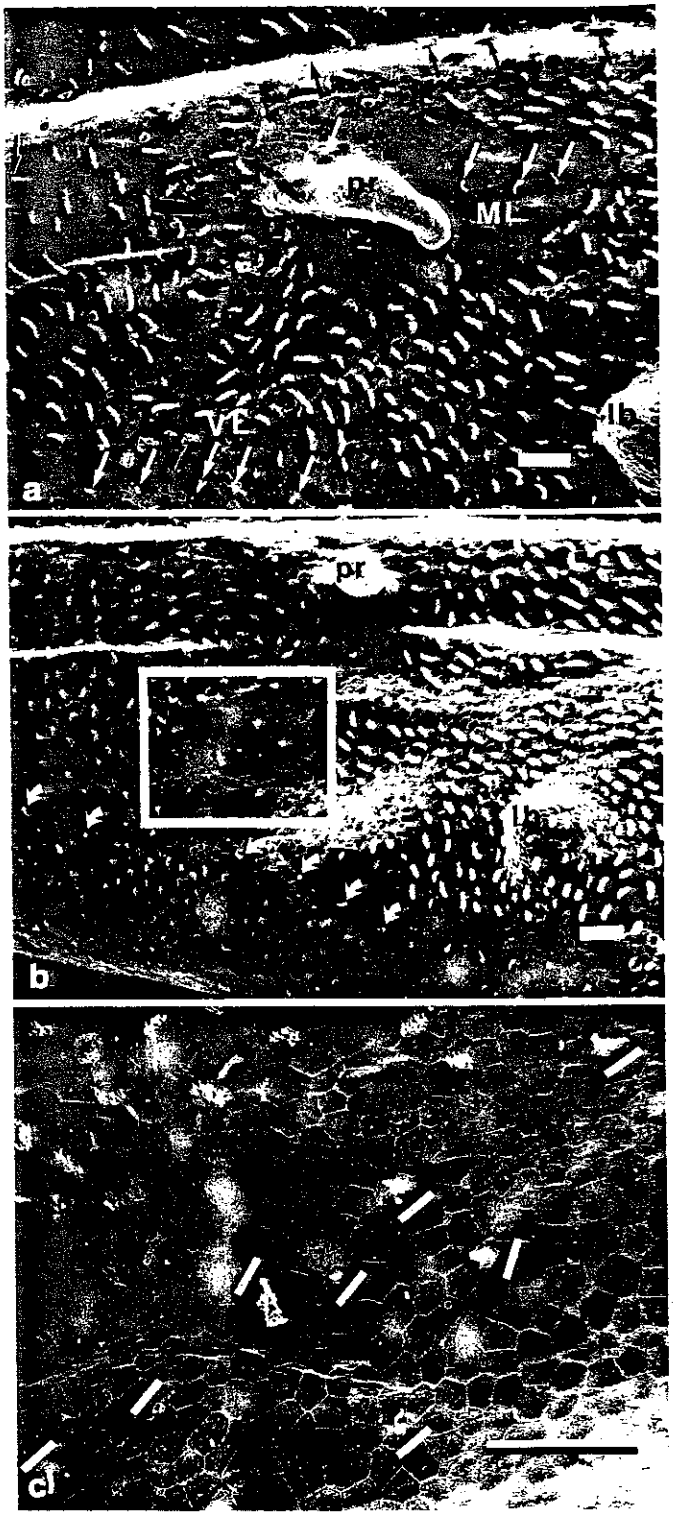


Fig. 5a–c. Middle body line neuromasts (*curved arrows*) just posterior to the forelimb bud on an unoperated control larva **a**, and those on which the ectoderm in this area was either lifted and replaced in the same orientation (sham; **b**) or rotated 180°, **c**. Migration was unaffected, and MLs which ran the length of the body were formed in all cases, although the line deviates slightly in the graft area of **c**. The VL (*short arrows*) was also deviated dorsally around an epidermal protuberance (*pr*) near the edge of the graft in **c**. Note that ciliated epithelial cells (*ci*) form regular arrays everywhere except in the immediate vicinity of the neuromasts. Dorsal is at the top of all plates, and rostral is at right in **a** and at left in **b** and **c**. *Large white arrows* show the anterior and posterior graft boundaries. Bars 100 μm

graft area was ventral) at stages 14, 20, 26, and 28. Rotating the ectoderm at any of these earlier stages did not affect the migration of the ML primordium, and normal middle body lines formed.

In the second series of experiments, the ectoderm covering somites 8 to 10 of stage 35 embryos was lifted as described above, except that the ventral incision was not made. Somite 9 was extirpated, and the ectoderm was replaced in its normal orientation and allowed to heal (see Fig. 4). Removal of somites in this manner caused the MLs to either stop or be diverted at the ros-



arrows in **b**) neuromasts form approximately normal lines. Note, in both cases, that ciliated epidermal cells remain present along the normal ML pathway, but are absent around the deviated neuromasts in (**b**). Epidermal protuberances (*pr*) are present at the graft edge in both cases (see text). **c** Enlargement of the area indicated in **b**. The lines indicate the long axis (polarity) of the neuromast organs. Note that they are roughly aligned along the direction of migration rather than in the rostrocaudal direction typical of ML and VL neuromasts. (In these and subsequent SEM preparations, the cupulae are sometimes twisted and do not reflect the orientation of the sensory epithelium beneath.) Bars 100 μm

Fig. 6a–c. Area of the right side just caudal to the forelimb bud (*lb*) on two larvae with ectodermal patches rotated 90° (**a** and **b**). **a** The ML neuromasts proceed as far as the graft area and stop. **b** ML neuromasts form an abnormal pattern after the primordium was deflected at the edge of the graft. Although deviated slightly, both the DL and VL (*labelled arrows* in **a**. *VL, curved*

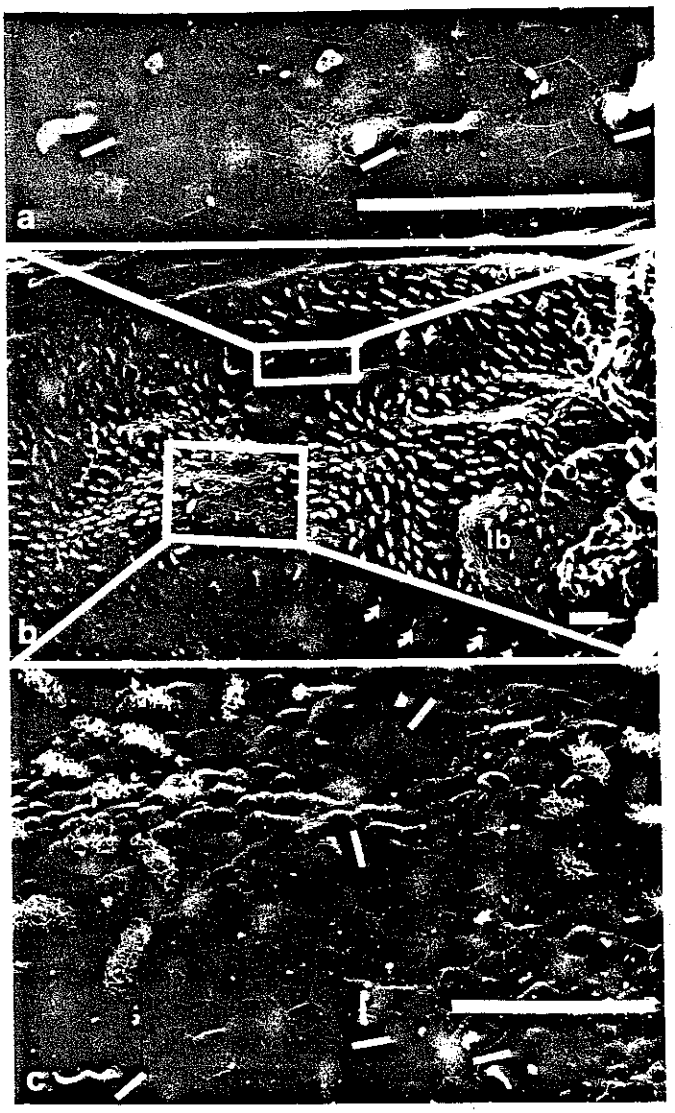


Fig. 7a–c. Area of the right side, just caudal to the forelimb bud (*lb*) of a hatchling with an extirpated somite **b**. Observations of the living embryo showed that the ML primordium migrated to the rostral edge of the resulting hole, then ventrally to the VL path which it followed to beneath the gills, and then ventrally onto the belly. *Arrows* show the locations of some of these neuromasts. **a** and **c** are enlargements of the areas indicated in **b**, showing the polarity of the neuromasts, which changes from rostrocaudal to dorsoventral and back to rostrocaudal. Note again that ciliated epidermal cells are abnormally absent around the ectopic neuromasts, but present along the normal ML path. Bars 100 μm

tral edge of the holes in the somitic mesoderm (rather than at the edge of the ectodermal grafts). This indicates that the migrating ML primordia are not merely adhering to (and being guided by) the overlying ectoderm, but require the presence of the subjacent (in this case somitic) mesoderm as well.

In one notable case, the ML primordium was observed (in the living embryo) to migrate ventrally to the VL pathway, follow this path rostrally to a point ventral to the gills, and turn ventrally onto the belly, forming a U-shaped line (Fig. 7). The DL primordium was also stopped or diverted in all cases. The migration of the

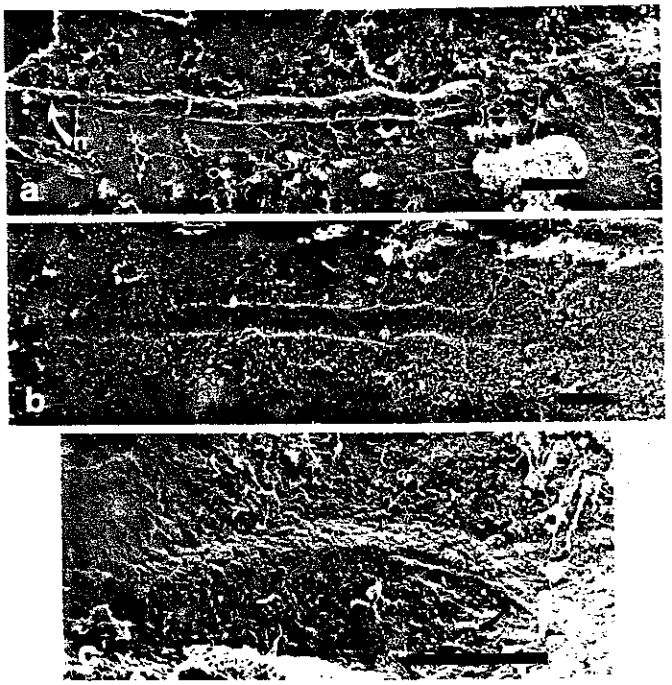


Fig. 8a–c. Migrating middle body line (ML) primordium viewed on the underside of the ectoderm of a stage 36 embryo **a**, and the groove left in the subjacent mesoderm **b** by the same primordium. **c** Supraorbital (*SO*) primordium on the underside of the head ectoderm of a stage 37 embryo as it courses around the optic vesicle. No obvious structures are visible preceding the primordia. Note that the SO primordium **c** appears to be more closely apposed to the ectoderm than the ML one. *n*, presumptive nerve inserted in the proximal end of the primordia. Bars 100 μm

VL primordium was not affected, except when the ML primordium blocked its path.

When the polarity of the neuromasts in the diverted MLs was examined, it was found that the neuromasts generally retained their normal polarity with respect to the direction of migration rather than the rostro-caudal axis of the embryo (see Figs. 6 and 7). So, for example, if a ML primordium migrated caudally, ventrally, and then rostrally, the neuromasts in the line were oriented rostrocaudally, dorsoventrally, and rostrocaudally, respectively.

Direct observation of migrating primordia

Having determined that the substrate could affect migration, the tissues immediately adjacent to migrating primordia were examined. In this way, we hoped to determine whether any visible structures (on either the basal surface of the flank ectoderm or the underlying mesoderm) were present and could be responsible for directing the migration of the primordia. The ectoderm immediately surrounding migrating ML, VL, and SO primordia of fixed stage 36–38 embryos was lifted. The body primordia left clearly visible depressions in both ectoderm and mesoderm, but no obvious morphological structures were seen distal to any of the primordia (Fig. 8). The body primordia (Fig. 8a, b) consisted of

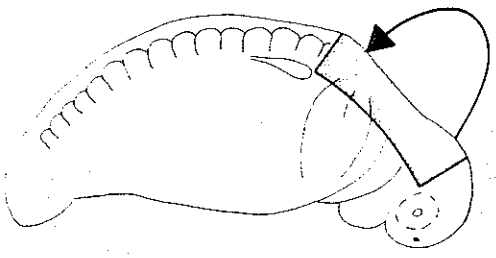


Fig. 9. Right side view of a stage 29 embryo showing the area of ectoderm lifted and rotated 180° to reverse the placodes

long, cylindrical masses of tightly apposed cells. The presumptive nerves which would supply the mature neuromasts appeared to be co-migrating with the primordia (supporting the observations of Metcalfe 1985).

Unlike the body primordia, each SO primordium (Fig. 8c) appeared to spread laterally as it progressed around the eye and seemed to be flatter and more tightly adherent to the overlying ectoderm. This widening effect may correlate with the formation of multiple neuromast rows. No grooves could be seen in the subjacent tissues. The presumptive nerves, as with the body primordia, were inserted into the proximal (caudal) ends of the primordia. Interestingly, as the path of a SO primordium curved around the eye, its attendant presumptive nerve appeared to follow a slightly more direct route, suggesting that the nerve may be drawn by the migrating primordium.

Determination of neuromast polarity

To determine whether the substrate directly specifies the polarity of the neuromasts in each line, head (SO and IO) and body (DL and ML) placodes were reversed on the same embryo (Fig. 9). Placode reversals were performed on stage 26, 29, and 34 embryos. We hoped, in this way, to force head primordia to migrate along body pathways and vice versa. In many cases lines did not form, or were shorter than normal, presumably due to surgical damage to the placodes or the substrate, and/or a mismatch between the placode and the substrate. In the cases where lines did form, the placement of lines and polarity of the constituent neuromasts was always the same as in sham-operated and unoperated controls. Orthogonal couplets were never seen on the body, and were always seen (when lines were present) on the snout.

Discussion

Origin of placodes

The manner in which placodal ectoderm becomes distinguished from other ectoderm, and the timing of placode specification, are unknown. Hörstadius (1950) speculated that the lateral-line placodes arise as the result of an induction by the neural crest cells as they migrate beneath the ectoderm (see Lannoo and Smith 1989, for review). However, on the basis of the differing characteristics of neural crest cells and placodal primordia, Northcutt and Gans (1983) and Gans and Northcutt (1983) have argued that neither tissue is causally related to the formation of the other.

Therefore, we began our study by directly examining when placode-forming ectoderm becomes distinct from other ectoderm. Replacing presumptive placodal ectoderm with other (non-placodal) ectoderm as early as stage 14 (early neurula) prevented the formation of the appropriate lateral line(s). Neither migrating primordia nor neuromasts were ever observed arising from wild-type (pigmented) ectoderm grafted onto albino hosts, although such primordia are frequently observed when pigmented placode-forming ectoderm is grafted onto such hosts (Smith et al. 1988; Lannoo and Smith 1989).

This may indicate that the lateral-line placodal ectoderm is completely specified at or prior to the beginning of neurulation. Alternatively, non-placodal ectoderm may not be competent to respond to a signal which occurs later in development. In either case, it is clear that a functional difference exists between placode-forming tissue and other ectoderm as early as the onset of neurulation. This is much earlier than had previously been suspected (i.e., Hörstadius 1950), and well before neural crest cell migration, which begins at stages 23–24 in the branchial area of the axolotl (see Löfberg et al. 1989). However, we cannot eliminate the possibility that the neural crest cells supply a later signal (to begin migration, for example).

Gans and Northcutt (1983) have suggested that placodal ectoderm and cranial neural crest arise ontogenetically (and possibly also phylogenetically; Northcutt and Gans 1983) from a common precursor tissue. This explanation is particularly appealing for three reasons. First, as pointed out by Gans and Northcutt (1983), both the lateral-line placodes and the neural crest become migratory, and all of the major head placodes (except the lens) and the neural crest form similar neural/sensory tissues. Second, the lateral-line and other major sensory placodes (ear, lens, nose) all arise from a band of ectoderm which is immediately adjacent to the cranial neural folds (Northcutt and Gans 1983). Finally, there is our finding that this ectoderm is distinct from other ectoderm (at least in its ability to form lateral-line placodes) as early as the beginning of neurulation.

These functional, spatial, and temporal similarities make it tempting to speculate that the placode-forming ectoderm arises not only from the same precursor tissue as cranial neural crest (ectoderm), but also as a result of some variation of the same neuralization and/or regionalization (head *vs* trunk and/or plate *vs* fold) signals which occur during gastrulation. Indeed, such a possibility has been suggested by Albers (1987).

Guidance of migration

Harrison (1904) and Stone (1928b) concluded that the tissues through which the lateral-line primordia migrate were not responsible for determining where the neu-

romast organs are deposited. Nevertheless, many of their own data seem to suggest that the substrate guides the migrating primordia (Lannoo and Smith 1989).

To test whether manipulation of the substrate could affect the migration of (specifically) the middle body line (ML), we rotated the flank ectoderm in the path of the ML at stage 35, immediately prior to the passage of the primordium (Smith et al. 1988). Sham operations and 180° rotations did not affect the migration of the primordia, but the ML primordia were always blocked when the ectoderm was rotated 90°. Sometimes the primordia were stopped completely, and sometimes they were diverted and wandered (apparently aimlessly), unless they encountered the pathway of another line, which they could follow in either direction. Similarly, the removal of a somite stopped or diverted the ML primordia at the anterior edge of the hole in the mesoderm. However, when flank ectoderm was rotated 90° at earlier stages, migration was unaffected.

These results strongly suggest that there are distinct pathways, or "tracks", which guide migrating primordia. These tracks require the presence of both the ectoderm (in the proper orientation) and (at least on the body) the subjacent mesoderm. Thus, the primordia are not likely to adhere to only one of these tissues, nor depend solely on one for guidance information.

Since rotating flank ectoderm 180° does not affect the migration, and since diverted ML primordia can migrate in the opposite direction on the VL track, the tracks must not be polarized. This also indicates that the tracks are interchangeable, at least on the body.

The finding that rotation of the ectoderm at early stages does not affect migration may suggest that the ectoderm can adjust to new orientations. Alternatively, the tracks may be formed just prior to the onset of migration. In light of Stone's (1928a, 1929) findings that placodes transplanted into the path of the host primordia do not begin to migrate until the latter reach them, this result suggests that the substrate changes immediately prior to the onset of migration, allowing migration to occur. It is tempting to speculate that such a change may occur in the form of a morphogenetic wave immediately preceding the primordium (see Lannoo and Smith 1989, for discussion) similar to those which have been postulated for somite formation (Armstrong and Graveson 1988) and pronephric duct migration (Gillespie and Armstrong 1986; see Armstrong 1989, for review).

Origin of neuromast polarity

The polarity of the neuromasts is different for each line (Lannoo 1987a, b; Lannoo and Smith 1989). Is the substrate responsible for determining this polarity as well as the location of each line? If so, it alone would be responsible for determining the type of line formed.

To examine whether the substrate played a role in determining neuromast polarity, we lifted and rotated the otic area ectoderm so that head (SO and IO) placodes faced caudally and body (DL and ML) placodes faced rostrally. Lines formed which contained neuromasts with polarities which were normal for their new locations, rather than for the placodes which should have formed them. This may indicate that the substrate is responsible for determining the polarity of the neuromasts. Alternatively, the reversed placodes may be "respecified" according to their new location. This would imply that the general signal conferring the ability to form placodes (discussed previously) is quite distinct from a later signal responsible for specifying the type of line which will form. A third alternative is that the reversed placodes merely turn around, bypass each other, and migrate along their normal routes. At present we cannot distinguish between these alternatives.

We can glean some additional information from the experiments where the ML was diverted. The polarity of the neuromasts in these lines was maintained relative to the direction if migration, rather than to the rostrocaudal axis of the embryo (see Figs. 6, 7). This implies that the ability of each primordium to deposit neuromasts with a set polarity is determined as early as the beginning of migration.

Thus, the type of body line formed, including the polarity of the constituent neuromasts, appears to be the result of a co-ordinated effort by the migrating primordia and the substrate tracks guiding them along carefully restricted routes. The head lines, with their complex placement of neuromasts in multiple rows (which probably occurs after the initial migration: Winklbauer and Hausen, 1985b; Smith et al. 1988) may well require a more elaborate mechanism.

Determination of neuromast spacing

Very little can be determined about the mechanism(s) governing the spacing of the neuromasts within each line. Since neuromasts are deposited by diverted ML primordia moving ventrally (perpendicular to the usual direction of migration), we may infer that the deposition of neuromasts is not due to any regularly repeated feature of the substrate, such as somites (see Detwiler 1934; Lannoo and Smith 1989), or of the tracks which the primordia normally follow.

It is important to note that neuromasts in the DL and ML usually line up along the body to form orthogonal couplets, despite the fact that the two primordia migrate at different times (Smith et al. 1988). Since it appears unlikely that repeated features of the substrate are responsible for organ placement, something else must be responsible for co-ordinating organ placement in the different lines. A timing mechanism may be involved, whereby organs are deposited at regular intervals following the beginning of migration, but such a model is not without flaws (see Lannoo and Smith 1989).

One important observation is that ciliated ectodermal cells disappear from around diverted lateral-lines in areas where they would normally persist, and persist where they would normally disappear, around where the lines should have been (see Figs. 6 and 7). This lends support to our previously published hypothesis that emerging hair cells of the neuromasts actively inhibit

the ciliated ectodermal cells they superficially resemble (Smith et al. 1988). As we speculated in this previous study, such an inhibitor might also play a role in determining the spacing of the neuromasts within a line by inhibiting the formation of other neuromasts within a certain radius. Such a mechanism could form the coordinated pattern of the DL and ML (described above) independent of the time and rate of migration of the two primordia, if the first organ in each line were always deposited at the same rostrocaudal level.

Nature of the guidance mechanism

Clearly, some form of substrate track is responsible for guiding the migration of the primordia. The question naturally arises: what is the nature of these tracks? A variety of mechanisms has been proposed to explain the guidance of cell migration (see Trinkaus 1976, 1984; Erickson 1985a). These include chemo- and galvanotaxis, contact inhibition, adhesive gradients (haptotaxis), and contact guidance.

Both chemotaxis and galvanotaxis (guidance by electric fields, see Cooper and Keller 1984) imply a distant source of guidance information, and thus predict that only unidirectional migration, toward (or away from) the source, would occur. Our findings that ML primordia can migrate randomly if diverted and, more importantly, can migrate in the opposite direction, effectively eliminates these models. Similarly, haptotaxis (Carter 1967) and the differential adhesion hypothesis (Steinberg 1970; see also Poole and Steinberg 1982; Zackson and Steinberg 1986) predict unidirectional migration (along an adhesion gradient) and can therefore also be eliminated.

Contact inhibition (see Erickson 1985b), and a similar model based on population pressure (Thiery et al. 1984), predict that cells would always move from densely to less densely populated areas, and would thus tend to disperse. However, the cells of the lateral-line primordia move as discrete, compact masses, and all the cells within each primordium travel in the same direction, making these unlikely mechanisms.

Most of the evidence presented in this report is consistent with contact guidance as the mechanism responsible for guiding the lateral-line primordia. This model, proposed by Weiss (1947; 1961 for review) has also been suggested as the guidance mechanism responsible for directing neural crest cell migration (see Ebendal 1977; Löfberg et al. 1980; Newgreen 1989) and axonal outgrowth (Letourneau 1975), among others.

Contact guidance is caused by physical or biochemical variations in the substrate. A key prediction of this mechanism is that the pathways formed in the substrate would permit bidirectional migration, which we have observed. In addition, these pathways would probably be relatively narrow (as our tracks appear to be) in order to prevent retrograde migration of the cells. This mechanism does not explain the actual movement of the cells (as the various gradient mechanisms tend to), but only the guidance of that movement. It predicts that movement could still occur in the absence of the guidance mechanism. Since primordia can continue to migrate (albeit randomly) once diverted from their tracks, the actual movement of the cells must be an inherent property of the primordia, independent of the guidance mechanism.

Two major ways in which contact guidance could function have been described (Newgreen 1989; for reviews see Dunn 1982; Trinkaus 1982). The first is guidance by physical structures, either of the substrate cells themselves, or the extracellular matrix (ECM) produced by them. The second way is through adhesive guidance, in which pathways of higher (but uniform) adhesiveness are surrounded by areas of relatively low adhesiveness.

Direct SEM examination of migrating ML, VL, and SO primordia and the substrate surrounding them proved inconclusive. While body primordia appeared to be capable of pushing both mesoderm and ectoderm out of the way (leaving distinct grooves in their wake), no obvious structures were seen preceding them. This would seem to indicate that the morphology of the substrate does not play a role in guidance. However, we cannot exclude the possibility of physical guidance by some component(s) of the ECM, which may have been destroyed during our preparation for SEM. Similarly, we cannot exclude the possibility of adhesive guidance.

Therefore, the lateral-line primordia appear to be guided by some form of contact guidance. Though we cannot determine the exact nature of this guidance information, it is obvious that the guidance mechanism is no stronger than the inherent force driving the migration, since the primordia are quite capable of jumping their tracks when they are blocked.

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