Development of the lateral line system in *Xenopus laevis*

I. Normal development and cell movement in the supraorbital system

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SUMMARY

During development of *Xenopus laevis*, the supraorbital lateral line system (i.e. the parietal and supraorbital lines of organs and the anterior auditory group of organs) is all derived from a single primordium located in the ear region of the epidermis. The primordium elongates first by active movement along the dorsal margin of the eye. Individual primary organs are then formed by progressive fragmentation of the streak-like primordium. After fragmentation, passive displacement of the organs due to skin growth seems to play the main role in altering the arrangement of the line system. Transplantation experiments confirmed that non-placodal epidermal cells are not incorporated into the developing system. The active elongation of the primordium is due to cell multiplication, and not due to cell rearrangement or change in cell shape or size. Cell multiplication is not confined to a growth zone, but dividing cells are randomly distributed throughout the primordium. All cells of a primordium have to change position during its elongation.

INTRODUCTION

The lateral line system of amphibians and fishes consists of numerous epidermal sensory organs arranged in several well-defined lines, which extend in a characteristic pattern over the whole body surface (for details in *Xenopus* see Shelton, 1970). The development of this organ system attracted the attention of experimental embryologists in the first half of this century, since it seemed well suited to study some general aspects of embryogenesis such as cell migration, tissue polarity, regeneration and interactions of peripheral nerves with their target organs. Most of the experimental work was carried out with amphibian tadpoles (Harrison, 1904; Stone, 1922, 1923, 1925, 1928a, b, c, 1929, 1931, 1933, 1935, 1936, 1937; Wright, 1947).

The main results of these studies may be summarized as follows: each of the lines, consisting of a number of sensory organs, is generated by an individual primordium. The primordia of the different lines are formed in the head region from specific parts of epidermal placodes (Stone, 1922). The primordia migrate...
within the epidermis along well-defined routes, depositing on their way cell groups which give rise to the individual primary organs (Harrison, 1904; Stone, 1928b, 1933). This movement occurs between the basal membrane and the outer layer of epidermal cells, the inner epidermal cells being displaced (Stone, 1933). The migration pathways are determined within the epidermis, but deviated primordia are able to migrate for some distance on abnormal routes (Harrison, 1904). The direction of the migration is apparently given by an inherent polarity of the primordium and not by specific cues along the trail (Harrison, 1904; Stone, 1928b, c). This polarity seems to be established in the placode at the beginning of migration of the primordium (Stone, 1931, 1936). The number of primary organs eventually formed from one primordium seems to be determined before the onset of migration (Harrison, 1904; Stone, 1928c, 1936).

Cell differentiation takes place in the primary organs, leading to the appearance of sensory cells in the centre surrounded by various supporting cells. Oriented 'budding' of the primary organs eventually leads to the formation of accessory organs, which remain in close proximity. These clusters of organs eventually form the ultimate pattern of the lateral line system (Stone, 1933, 1935, 1936).

During this whole process the system seems to retain a high degree of autonomy. Epidermal cells other than those of placodal origin are not integrated into the organs (Harrison, 1904; Stone, 1933). The outgrowth of nerve fibres supplying the sensory organs seems to have no influence on the morphogenesis of the system (Harrison, 1904; Stone, 1931, 1936; Wright, 1947). More detailed information on the development of the lateral line system is contained in the review by Wright (1951).

The picture that emerged from these early studies demonstrates that the development of this organ system is extraordinarily suited to describe the interplay of different processes in the course of the establishment of a defined morphological pattern. However, it is difficult as yet to integrate these results into modern concepts of developmental biology. One reason for this is the fact that quantitative data regarding the number of cells involved, the kinetics of cell multiplication, the number of organs formed from one primordium, the time dependence of cell migration etc. are entirely lacking.

It was our aim to establish such data. Xenopus laevis tadpoles were used in these studies. The development of the supraorbital (SO) line system, which turned out to be generated by a single primordium, was investigated. This system proved to be best suited since the individual organs belonging to the system can be unequivocally identified. Furthermore, it rapidly reaches its ultimate organ number, in contrast to the posterior lines which grow continuously as the tail elongates. No detailed description of the development of this organ system in Xenopus laevis was as yet available. Therefore this paper describes the gross morphological features of the developing system. This study allows conclusions to be formed on cell movement and on the passive displacement of the organs.
Cell movement in lateral line system of Xenopus during epidermal growth. The accompanying paper gives a quantification of SO lateral line development with respect to cell number and multiplication.

MATERIALS AND METHODS

Embryos of *X. laevis* and *X. borealis* were obtained by artificially induced spawnings. Human chorionic gonadotropin (Serva) was injected into the dorsal lymph sac (*X. laevis* both sexes 500 units, *X. borealis* males 50 units, 24 h later 75 units, 8 h later 150 units; females 50 units, 24 h later 150 units, 8 h later 300 units). Embryos were kept in tapwater or 1/10-strength Modified Barth's Solution (MBS-H) (Elsdale, Gurdon & Fischberg, 1960) at 22°C. From the 5th day of development larvae were fed on nettle powder. After the 12th day, temperature was raised to 24°C. Under these conditions, most of the embryos and larvae developed exactly in agreement with the time schedule given in the Normal Table of Nieuwkoop & Faber (1967), according to which the animals were staged. When the time difference between two stages was too large for our purposes, an intermediate stage was defined, lying midway between the two and being designated by adding '½' to the earlier of the two stages.

Animals were fixed in 4% formaldehyde and kept there until use. To visualize nuclei in the epidermis, the fixed animals were placed for 10 min in an aqueous solution containing 1 µg/ml 4,6-diamidino-2-phenylindoledihydrochloride (DAPI, Boehringer Mannheim), a DNA-binding fluorescent dye. The head of a larva was then cut in the median plane, and the skin of each half was peeled off with watchmaker forceps under the dissecting microscope and mounted in distilled water between two coverslips. Photographs were taken with a Zeiss ICM 35 microscope, equipped with epifluorescence optics, on Ilford HP5 black and white film. Drawings and measurements were made from these photographs.

For epidermis transplantations, stage-22 *X. laevis* and *X. borealis* embryos were decapsulated manually and transferred to full-strength MBS-H. A fine tungsten needle was used to cut out a piece of epidermis with some underlying material attached to it from a *X. laevis* host. A corresponding piece of *X. borealis* epidermis of the same age was implanted into the wound.

For the labelling of DNA-synthesizing nuclei, an incision was made along the ventral margin of the eye, and the animal was then placed for 30 min in 1/10-strength MBS-H containing (methyl-³H)-thymidine (400 µCi/ml, 47·5 Ci/mmOL). Subsequently, animals werefixed in 4% formaldehyde for 24 h, and then washed several times. Skin preparations were transferred in 15% ethanol to a gelatine-covered slide, dried at 40°C to fix the specimen to the slide, and processed for autoradiography. Ilford L4 dipping film emulsion and Kodak D19 developer were used. Exposure was 18 days for stages 35/36, 39 and 39½ and 9 days for later stages. After staining with DAPI, preparations were evaluated with the aid of fluorescence microscopy. Silver grains were readily detectable above the brightly fluorescing nuclei.
RESULTS

Morphology of SO lateral line development

The cells of placodes, of the primordia and of the lateral line organs have spherical or proximodistally elongated nuclei, in contrast to the surrounding epidermis whose laterally extended, flattened cells contain large flattened nuclei. The primordia are located between the basal lamina of the epidermis and the outer epidermal cell layer, but only very few epidermal nuclei overlay the primordia (our unpublished data, also Stone, 1933). In skin preparations stained with the fluorescent DNA-binding dye DAPI, epidermal nuclei exhibit only a weak fluorescence which contrasts with the bright and condensed appearance of the lateral line nuclei. The technique allows an easy identification of the lateral line cells within the epidermis.

In order to follow the development of the supraorbital lateral line, skin preparations were taken from the head region of tadpoles of different ages. In Fig. 1A (stage 33/34) the position of the eye, the ear and nasal pit can readily be recognized. They may serve as landmarks to locate the individual primordia (Fig. 2B). Ventral to the ear the primordia which give rise to the occipital and aortic lines are located. The primordium of the infraorbital line is an elongated group of cells between ear vesicle and eye extending ventrad along the posterior margin of the eye. Adjacent to this primordium but more dorsally lies a circular group of cells, the primordium of the supraorbital line system. It is this primordium whose fate will be followed. The drawings of Fig. 2 depict the whole developmental sequence.

From stage 33/34 to 39 the SO primordium elongates and stretches for some distance along the dorsal margin of the eye. At stage 39 it exhibits a club-shaped appearance, being broadest at the anterior tip, which has now reached a point dorsal to the centre of the eye. Sometimes a transitory interruption of the primordium is seen near the posterior end, where it is usually thinnest.

At stage 39½ the primordium has further advanced along the dorsal margin of the eye (Fig. 1B, Fig. 2B). The club shape has disappeared and the first signs of segmentation are visible. The separation of the primordium into different fragments can clearly be recognized at stage 40. The number and size of the fragments is variable. Some of the large fragments show constrictions. A large anterior fragment typically bends slightly dorsad. The constriction and breaking of the fragments into smaller units continues until stage 43, when the individual primary organs are recognized unequivocally although many of them are still joined together. Only at stage 47 are all the organs separate.

Between stage 40 and 41 the anteriormost part of the primordium becomes bent in a dorsal direction. This part of the developing line system becomes separated from the main group of organs between stage 42 and 43. It remains in a dorsal position and gives rise to the parietal lateral line (Fig. 2). The anterior part of the main row of organs is dislocated ventrad relative to the ear–nasal pit.
Fig. 1. Skin preparations of stage 33/34 (A) and stage 39½ (B) embryos. The skin was stained with the fluorescent dye, DAPI, to visualize cell nuclei. In each specimen, the nasal pit (n), the supraorbital (so), infraorbital (io), aortic (a) and occipital (oc) lateral line primordia can be recognized. In (A), the area of contact between skin and ear vesicle (E), the rest of the lens (l), which is still attached to the epidermis at that stage, and the dorsal margin of the eye (em) serve as landmarks. In (B), the centre of the eye (ce) is indicated (small cell condensation).
reference line used in Fig. 2A. At the same time a few organs at the posterior end of the SO line begin to turn ventrad. Eventually they occupy a position at the anterior margin of the ear vesicle, as seen from wholemount preparations (not shown).

In about 50% of all cases studied, the anteriormost organ of the SO line elongates, constricts, and buds off an additional organ in an anterior direction between stage 47 and 49, thus extending the line by one unit. After stage 47½ (Fig. 3) the primary organs of the SO line system elongate as they grow larger (Fig. 4). Elongation is dorsoventral in the SO line and in an anteroposterior direction in the parietal line (Fig. 5). At stage 49 the former primary organs can be seen each divided up into a number of organs lying close together in an orderly array forming an elongate ‘plaque’ (Murray, 1955). Plaque size and number of organs per plaque increase continuously. At stage 54 a large plaque may consist of up to 11 organs. A similar process of accessory organ formation has been described for other amphibians as well (Stone, 1937). The development of the lateral line system in *X. laevis* from stage 54 onwards is described in some detail by Shelton (1970).

**Exclusive contribution of primordial cells to the lateral line system**

As mentioned in the Introduction, the primordium is assumed to move actively in the epidermis, the organ system being established from the primordium alone, and epidermal cells not being integrated. This mechanism has not yet been established for *Xenopus* and not for the SO line primordium. To study this question we made use of the fact that *X. borealis* cells can be distinguished from *X. laevis* cells in the fluorescent DNA stainings. *X. borealis* nuclei have numerous heterochromatic granules, whereas *X. laevis* nuclei exhibit a smooth appearance (Thiebaud, 1983). At stage 22 a piece of *X. borealis* epidermis was transplanted to *X. laevis* tadpoles into the prospective path of the primordium. If the *X. laevis* primordium integrates epidermal cells on its way through the *X. borealis* area, these should be recognized in the developed organs at stage 47½ as *X. borealis* cells.

The epidermis of a *Xenopus* embryo and tadpole consists of an inner, sensorial and an outer, epithelial cell layer. For unknown reasons, these two layers of the
Fig. 3. Skin preparation of stage 47½ embryo. The nasal pit (n), the anterior auditory group of organs (aa), which occupies a position above the anterior margin of the ear vesicle (as seen from whole mount preparations, not shown), the supraorbital line (so) and, dorsally, the parietal line (p) of primary lateral line sense organs can be recognized. Bar = 100 μm.
Cell movement in lateral line system of Xenopus

Fig. 4. Transformation of primary organs into organ plaques. (A) Three elongating primary organs at stage 48 (10 days of development), (B) three elongating primary organs already showing constrictions (stage 49, 12 days of development), (C) a plaque derived from a single primary organ (stage 52, 21 days of development), and (D) a large plaque of a stage 54 tadpole (26 days of development). Bar = 100 μm.

X. borealis transplants were found to be not congruent at the end of the experiment. In Fig. 6, the X. borealis regions are outlined for the two layers of the epidermis separately. In one case (Fig. 6A), some X. laevis-derived organs are seen to lie completely within a X. borealis environment with regard to the outer epidermal layer but not the inner one. In the other case (Fig. 6B), some X. laevis-derived organs are surrounded completely by X. borealis cells of the inner epidermal layer. In both of these cases, and in all other cases examined so far, all cells of organs surrounded by X. borealis epidermal cells are of X. laevis origin, and no X. borealis cells could be detected in such organs (Fig. 7). This finding is in agreement with the notions of Harrison (1904) and Stone (1933), that epidermal cells other than primordial ones are not integrated into the developing lateral line system.

Active extension and passive stretching of the primordium

The total length of the supraorbital primordium greatly increases during development. In principle two processes may contribute to this elongation: active elongation of the primordium relative to the epidermis, and an overall growth of the epidermis leading to a passive stretching of the primordium. In Fig. 2A the distance between ear and nasal pit is indicated as a landmark. This line may be taken to roughly measure the growth of the epidermis in the direction of the primordial extension. In Fig. 8 growth of the epidermis is compared with the elongation of the primordium. Between stages 33/34 and 39½ the length of the primordium is increased by a factor of seven. This linear elongation proceeds at a rate of 20–30 μm/h. In the same interval the epidermis is expanded by only a factor of 1-2. This shows that the primordium moves actively in relation to the epidermis during this period. Between stage 39½ and 40½, the rate of epidermal
Fig. 5. Skin preparation of a stage-53 embryo. The nasal pit (N), part of the infraorbital line (io), the anterior auditory group of organs (aa, not clearly separated from so line), the supraorbital line (so) and, dorsally, the parietal line (p) of lateral line organs can be discerned. Bar = 250 μm.
Cell movement in lateral line system of Xenopus

Fig. 6. Results of transplanting at stage 22 a piece of *X. borealis* epidermis into the prospective path of a *X. laevis* SO primordium. The *X. borealis* area within the *X. laevis* epidermis is indicated separately for the outer layer (o) and the inner layer (i) of the epidermis. *X. laevis* primordial material has invaded the *X. borealis* area and formed primary organs there. Posterior margin of nasal pit indicated. Fixation at stage 47½.

Fig. 7. Example of a supraorbital lateral line organ derived from a *X. laevis* primordium which has developed in implanted *X. borealis* epidermis up to stage 47½ (skin preparation stained with DAPI). *X. borealis* nuclei are characterized by the occurrence of small, brightly stained spots. The organ contains no *X. borealis* cells. Top left: *X. laevis* epidermal cells, bottom left: *X. borealis* so organ, for comparison. A total of 12 *X. laevis*-derived so organs which developed in implanted *X. borealis* epidermis were examined so far. None contained *X. borealis* cells. Bar = 50 μm.

growth in this region suddenly increases more than fourfold. It is this period when the fragmentation of the primordium is initiated.

It is difficult to assess the further elongation of the primordium due to the splitting off of the parietal organ anlagen which occurs at stage 41 (Fig. 2). As a rough approximation, the proportion of the primordium giving rise to the parietal organs may be dismissed from consideration and only the elongation of the rest of the primordium moving anteriorly calculated in relation to epidermal growth (Fig. 8, triangles). This estimate suggests that after fragmentation of the
primordium at stage 40, the overall elongation of the line is primarily produced by epidermal growth. It seems quite possible that the separation of the parietal part of the primordium from the main mass is also produced by differential epidermal growth in this region.

**On the mechanism of active primordial extension**

In the above calculation the total length of the primordium including the interjacent parts of the epidermis was measured. In Fig. 9 the length of the primordium proper, that is after omission of the epidermal spaces between the fragments, is taken into consideration. The elongation of the primordium or,
after stage 39\frac{1}{2}, of the sum of its fragments in direction of its movement is compared with the total area covered by the primordium. The parallel increase of both parameters shows that the gross longitudinal extension of the primordium is determined by its growth and not by the definite and characteristic changes of form during elongation.

The area occupied per cell (70 μm², area of primordium divided by cell number) remains constant over the whole period. As the primordium retains the thickness of one cell layer, the increase in area covered by the primordium and thus the rate of elongation is given by the rate of increase in cell number and not by an enlargement or change in shape of the primordial cells.

**The topology of cell multiplication**

Since the transplantation experiments have indicated that there is no integration of epidermal cells into the SO lateral line primordium during development, the increase in cell number must be exclusively due to cell multiplication within the primordium. The question then arises whether cell multiplication occurs in a definite growth zone, e.g. at the anterior tip of the primordium, or whether cells of the whole primordium divide.

![Graph](image)
Embryos at different ages were labelled for 30 min with $^{3}$H-thymidine, and skin preparations were autoradiographed as described above. In the drawings of Fig. 10 the position of labelled cells in selected primordia at stages 35/36, 39 and 39½ are indicated. The labelled cells are scattered randomly within the primordium.

**DISCUSSION**

*Description of normal development*

The development of the whole lateral line system of *Xenopus laevis* is briefly described in Nieuwkoop & Faber (1967). Changes in the organ system during metamorphosis are described by Shelton (1970). For our purposes, a more detailed description, especially of the supraorbital lateral line system up to metamorphosis, was desirable. Some as yet unknown features emerged from our observations.

In the more dorsal part of the head anterior to the ear vesicle, Shelton (1970) discerns a supraorbital complex of lateral line rows, with a parietal and a supraorbital line, and an anterior auditory group of organs. Our results indicate that all these rows or groups of organs arise from a single common primordium.

In the early work on the development of the lateral line system, most attention was focused on the formation of the body lines extending into the trunk and tail of the embryo. It is reported that a primordium, in the course of its migration, is continually segregating and leaving behind the primary organs, until the
material of the primordium is exhausted (Harrison, 1904; Stone, 1933). We confirmed this mode of organ formation in the body lines of *X. laevis* by transplanting a vitally stained placode and following the fate of the outgrowing primordium (unpublished observations).

For the supraorbital primordium, such simultaneous migration and organ formation is not prominent; instead the phase of primordial migration is more or less separate from organ formation. If a wave of fragmentation and organ segregation progresses in the primordium from posterior to anterior, it has to do so very rapidly. At stage 39½, after considerable primordial elongation, the first slight signs of organ segregation may be recognized. Only 5 h later, at stage 40, most of the primordium is already in the process of fragmentation. However, there is still a movement of the unfragmented, most anterior portion of the line, in a dorsal direction. This is the only feature that reminds us of the mode of organ formation in the body lines.

When the tail of a growing amphibian tadpole increases in length, there is concomittantly an increase in the number of lateral line organs in the body lines. Stone (1933) showed this to be due to a budding process whereby the posteriormost organ of a line delivers an organ in a posterior direction, which in turn buds off another organ still more caudal, etc. This process of secondary elongation of a line was also observed in the supraorbital line, although being much less prominent. Between stages 47 and 49, the average organ number in this line increases only by about one half organ (see accompanying communication).

**Elongation of the primordium and cell movement**

Earlier authors (Harrison, 1904; Stone, 1933; Wright, 1947) claimed that, in amphibians, epidermal cells never contribute to the formation of the lateral line system. Instead, all cells of the system are assumed to be derived from primordia of placodal origin. Our results from transplantation experiments are in agreement with this notion. They show that cells of the SO primordium move actively to new locations within the epidermis, and that they are the only source for the formation of primary organs.

To assess the importance of the active extension of the primordium relative to its passive stretching due to epidermal growth, the actual increase in length of the primordium was compared with an approximation of skin growth in the anterior–posterior direction. Between stages 33/34 and 39½ active expansion of the primordium must be the main process leading to elongation. After stage 40, with the beginning of fragmentation, active expansion of the SO line ceases.

Fragmentation itself may be brought about, at least in part, by passive stretching of the primordium. Between stages 39 and 41, the length of the primordium increases linearly, as measured without the gaps between the arising fragments (Fig. 9). For these gaps to appear, the total length of the primordium has to increase faster between these stages. It is just at this time
interval when the growth of the skin in the direction of the primordium is accelerated (Fig. 8).

Likewise, further changes in the arrangement of the SO lateral line material are probably due to passive translocation of its parts. One may consider, for example, the appearance of the gap between the parietal and the anterior supraorbital organs after stage 42. From Fig. 2 it is obvious that this is due to a ventrad translocation of the SO organs relative to the reference line. The parietal organs remain in place. If active migration were involved, the individual organs would have to move in a highly coordinated manner, parallel to each other with their velocity adjusted so as to maintain during movement the smooth and regular appearance of the line. This seems less likely than the assumption that differential growth of the skin in the head region accounts for the translocation of the SO organs. Such skin growth must occur at this time to allow for the pronounced broadening of the tadpole's head at the level of its eyes. Lack of appropriate position markers in that area makes it difficult, however, to follow in detail rearrangements within the epidermis during growth.

Several possibilities to form an elongated multicellular structure out of a compact one may be envisaged. Cell rearrangement, changes in cell size, cell shape or cell number, could alone or in combination serve to make elongation possible. For example, the rearrangement of a nearly constant number of cells accompanies the elongation of the pronephric duct in the axolotl (Poole & Steinberg, 1981). Alterations in cell size and shape contribute to the elongation of the urodele notochord (Mookerjee, Deuchar & Waddington, 1953). In the case of the *Xenopus* SO primordium, growth of the primordium by cell multiplication plays the main role.

The primordium remains a one-cell-thick layer throughout development (unpublished observations). The increase in length of the primordium is paralleled by an increase in the area occupied. Since the area occupied per cell remains constant, cell multiplication must occur, and expansion of the primordium due to changes in cell shape or size can be excluded. Cell rearrangement may occur during the shape changes of the whole primordium (Fig. 2A), but this seems not to contribute substantially to final elongation.

Autoradiographic analysis has shown that there is no growth zone which could, for example, give off nearly immobile cells posteriorly when situated most anterior in the primordium. Obviously all primordial cells have to change position throughout elongation, with the exception of the most posterior ones. Speed will thereby depend on position, being largest, 20–30 μm/h, at the anterior end. This maximal value lies well within the wide range of velocities known for amphibian cells in vivo, e.g. 2 μm/h for *Triturus alpestris* melanocytes (Epperlein & Claviez, 1982), 700 μm/h for *X. laevis* larval epidermis cells during wound closure (Radice, 1980). Whether all cells migrate individually, or whether a coherent cell sheet is stretched by the activity of its anterior locomotory margin, cannot be deduced from our observations.
REFERENCES


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