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Postembryonic development of the posterior lateral line in zebrafish

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SUMMARY

We examine how the posterior lateral line of the zebrafish grows and evolves from the simple midbody line present at the end of embryogenesis into the complex adult pattern. Our results suggest that secondary neuromasts do not form through budding from the embryonic line, but rather new waves of neuromasts are added anteroposteriorly. We propose that the developmental module that builds the embryonic pattern of neuromasts is used repeatedly during postembryonic development and that additional (secondary) primordia generate the additional neuromasts. We show that differentiated neuromasts migrate ventrally, and eventually generate 'stitches' by successive bisections. We also examine the repatterning of the terminal neuromasts, which anticipates the up-bending of the tail leading to the highly asymmetrical caudal fin of the adult (which develops exclusively from the ventral part of the tail). Because terminal repatterning affects all aspects of tail formation, including its sensory development, we speculate that terminal axis bending may have become intimately associated with the terminal Hox genes before the appearance of the tetrapod lineage.

Key words: Neuromast, Primordium, Stitch, Migration, Pattern, Axis bending

INTRODUCTION

Much has been understood about the genetic mechanisms that underlie embryogenesis in model systems such as *Drosophila* and *Caenorhabditis*. By contrast, the mechanisms involved in postembryonic growth have remained largely untouched so far. We have decided to investigate this process in the case of the lateral line of the zebrafish, *Danio rerio*. The lateral line is a sensory system made of discrete sense organs arranged in a reproducible pattern, where growth can easily be defined and followed.

The individual sense organs of the lateral line, the neuromasts, are mechanosensory organs that comprise hair cells and support cells. The neuromasts of the head form the anterior lateral line system (ALL); those on the body and tail form the posterior lateral line system (PLL). In most fish species, a major component of the PLL is the conspicuous line of neuromasts that extends from ear to tail along the flanks of the animal and gave the system its name.

In zebrafish embryos, the PLL comprises a line of seven to eight neuromasts that extends from head to tail along the horizontal myoseptum. This set of neuromasts is formed by a primordium, which originates from a head placode and moves along the horizontal myoseptum to the posterior end of the body. During its migration, the primordium deposits clusters of cells at regular intervals of five to six somites. Each cluster will become a neuromast, thereby generating the embryonic pattern of evenly spaced neuromasts (Metcalfe et al., 1985).

The adult PLL is much more complex (Metcalfe, 1989). It comprises more than a thousand neuromasts clustered in dorsoventral stitches, i.e. vertical rows of up to 20 closely apposed neuromasts. The stitches are themselves loosely arranged in lines that extend from head to tail along the sides of the body.

In this paper we examine how the simple embryonic pattern grows and evolves into the more complex adult pattern. We show that five processes are involved in this repatterning: formation of additional neuromasts along existing lines, ventral migration of differentiated neuromasts, formation of additional lines, rearrangement of the tail neuromasts and formation of dorsoventral stitches. We propose that the developmental module that builds the embryonic pattern is used repeatedly during postembryonic development.

MATERIALS AND METHODS

Wild-type zebrafish were obtained from a local pet shop and kept in a 60 l tank at 28°C. Larvae were raised in cages made with nylon gaze and held on the sides of the tank. Neuromast hair cells were labelled by incubating live individuals for 30 minutes in DiAsp (Sigma D-3418) (Collazo et al., 1994) 5 μM in tank water. The individuals were then anaesthetised in 0.5 mM tricaine and transferred to methylcellulose for examination (Westerfield, 1994). Most observations were done with a Nikon SMZ-2B dissection microscope fitted with an orange filter, under illumination provided by a Panasonic blue-light-emitting diode P930-ND supplied with a 4V DC current source.

Axonal pathways were labelled by exposing the fish for 60 minutes to DiAsp at a concentration of 125 mM and returning them to normal (tank) water for 5 hours. Trans-synaptic labelling of the sensory neurones can be observed after 5 hours with a compound microscope, and is still detectable the next day. Alternatively, incubation in 250

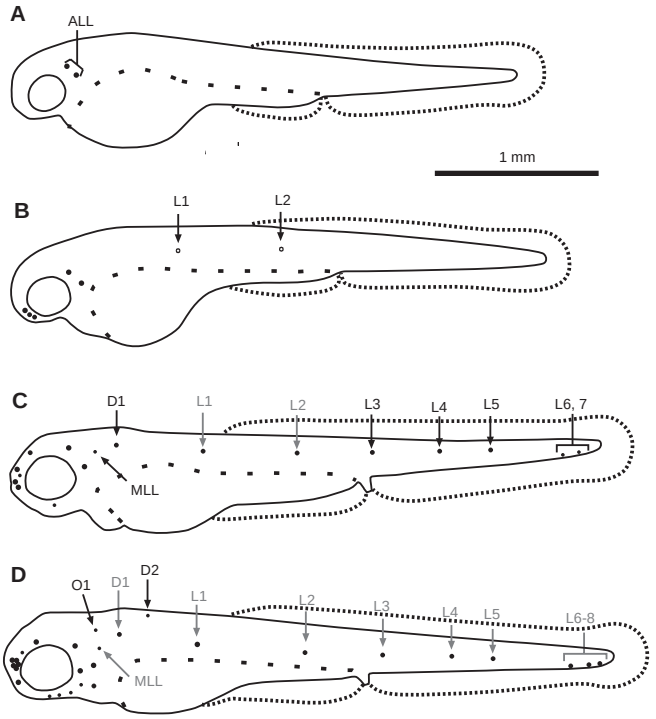

Fig. 1. Establishment of the primary PLL (posterior lateral line) system during zebrafish embryogenesis, at 48 (A), 55 (B) and 72 (C,D) hours after fertilisation. The earliest neuromasts belong to the anterior lateral line system (A). The embryonic PLL develops progressively between 55 (B) and 72 hours (C,D). Newly formed neuromasts are arrowed and labelled in black, neuromasts formed previously are marked in grey. ALL, anterior lateral line neuromasts; D1-2, dorsal neuromasts; L1-8, lateral neuromasts; O1, occipital neuromast; MLL, middle lateral line system. Anterior is towards the left and dorsal is upwards.

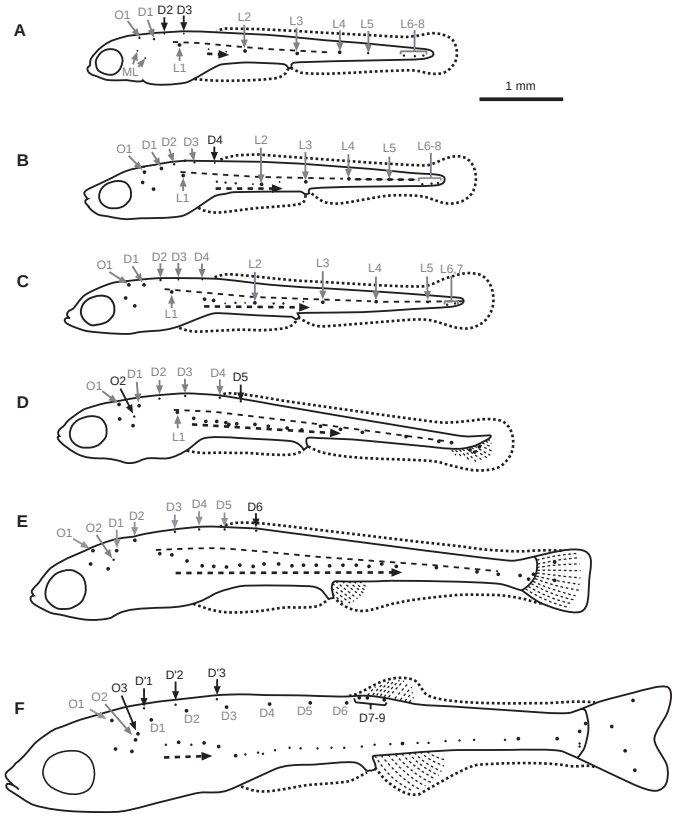

Fig. 2. The pattern of PLL neuromasts in larvae up to 8 mm. The dashed arrow indicates the progressive completion of the L-PLL by secondary neuromasts. D1-9, dorsal neuromasts; D′1-3, neuromasts of the new dorsal line; L1-8, lateral neuromasts; O1-3, occipital neuromasts; ML, middle lateral line system. Anterior is towards the left and dorsal is upwards.

mM DiAsp results in a more intense labelling of the neurones but with a decrease in contrast. The micrographs that illustrate this paper were taken with a DAGE CCD camera on a Zeiss Axioplan microscope fitted with Nomarski optics and epifluorescence illumination.

Postembryonic growth was measured by the size of the fish rather than by its age, because sib fish raised in the same conditions may grow at very different rates, and because we have observed that the development of the lateral line is much more closely related to fish size than to fish age.

RESULTS

Formation of the embryonic PLL

The first neuromasts to differentiate appear at about 48 hours after fertilisation, just anterior to the otic vesicle. They belong to the ALL (Fig. 1A). Shortly thereafter, the neuromasts of the embryonic or primary PLL appear progressively. First are the neuromasts of the lateral branch of the PLL (L-PLL), also called midbody line or main trunk line, which differentiate in a head-to-tail sequence (Fig. 1B,C) (Metcalfe et al., 1985; Raible and Kruse, 2000).

By 3 days, the primary L-PLL is complete and comprises seven or eight neuromasts, the first five (L1-L5) of which are regularly spaced along the lateral myoseptum (Fig. 1C). The last two or three neuromasts of the L-PLL (L6-8) are clustered near the tip of the tail, in a more ventral location (Fig. 1C,D).

At this time, two additional neuromasts have appeared. One forms at the level of somite 2, slightly dorsal to the horizontal myoseptum (D1 in Fig. 1C). This is the first neuromast of the dorsal branch of the PLL (D-PLL). The other additional neuromast (MLL in Fig. 1C) appears at the level of the otic vesicle and is the first of the so-called middle line. This line is considered to be independent of the anterior and posterior lateral line systems (Northcutt, 1989). Shortly thereafter, a third additional neuromast (O1 in Fig. 1D) appears dorsal to the otic vesicle and initiates the supratemporal line, which has been named occipital in zebrafish (Metcalfe et al., 1985). At this time the D-PLL may already comprise a second neuromast (D2 in Fig. 1D).

Thus by the end of embryogenesis (Fig. 1D) the three canonical branches of the PLL (lateral, dorsal and occipital) are present. During subsequent growth, only the lateral and dorsal branches undergo a massive increase and repatterning, and we have focused our attention on these. Five different processes are involved in this repatterning: formation of additional neuromasts along existing lines, ventral migration of differentiated neuromasts, formation of additional lines, rearrangement of the tail of the fish and formation of stitches. Although the first three processes overlap in time, we will consider them separately.

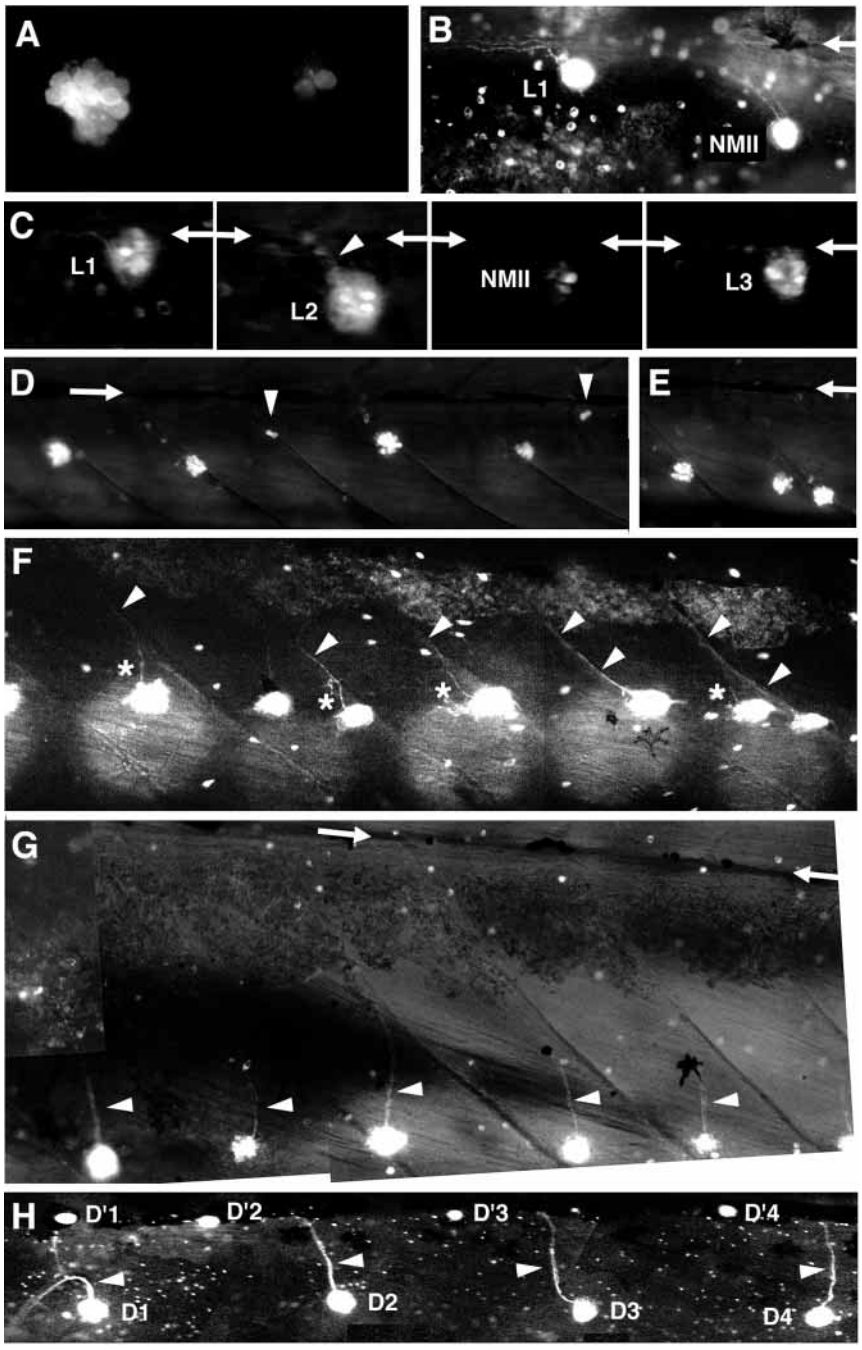

Fig. 3. Ventral migration of neuromasts during postembryonic growth. The position of the horizontal myoseptum is indicated by the white arrows. (A) Size difference between a primary (left) and a secondary (right) neuromast shortly after the latter has begun to differentiate. (B) Neuromast migration varies along the anteroposterior axis. L1 has not moved, yet the secondary neuromast (NMII) that has formed on the next somitic boundary has migrated a long distance. (C) Onset of neuromast migration: L2 has begun to migrate (and to a lesser extent NMII) but L1 and L3 are still close to the myoseptum. The two axons innervating the neuromast L2 are indicated by an arrowhead. (D) At later stages younger secondary neuromasts (arrowheads) begin to migrate as soon as they differentiate. (E) Two neuromasts are occasionally observed at the same position, and migrate together. (F) The axons of sensory neurones follow the neuromasts in their migration. The tracks of the axons presumably reflect the migratory pathway of the neuromasts. In general the migration follows the intersomitic grooves (arrowheads), but may migrate straight ventralwards (asterisks). (G) As migration proceeds, the neuromasts tend to follow less and less the somitic boundaries (arrowheads). (H) The neuromasts of the dorsal line (D1-4) also migrate ventrally. In this case there is no indication that migration follows intersegmental limits. The neuromasts of the D′ line have already formed. Their axons, and the dorsal course of the D axons, are obscured by a stream of melanocytes along the dorsal midline of the fish. Anterior is towards the left and dorsal is upwards.

Formation of secondary neuromasts

Starting 3-4 days after fertilisation, soon after the completion of the embryonic L-PLL line, new neuromasts differentiate along the myoseptum, interspersed between the primary neuromasts. We never detected any indication of budding or fragmentation of the primary neuromasts, however. The earliest secondary neuromasts (NMII) always form between L1 and L2 (Fig. 2A) and are progressively followed by more NMIIs in a head-to-tail sequence, with a spacing of usually two somites between consecutive NMIIs (Fig. 2A,B). At that stage, the size and the number of hair cells of the primary neuromasts have greatly increased, and the NMIIs are easily distinguished by their smaller size (Fig. 3A). By 6 days, the NMIIs have reached a size similar to that of the primary neuromasts. Occasionally, however, the latter can still be distinguished from the NMIIs by their larger size (Fig. 2C). In such cases it can be seen that no NMII has yet differentiated posterior to L3, indicating that the formation of NMIIs is much slower than that of the primary neuromasts.

From then on, the developmental stages are expressed in terms of fish size (see scale bar on the figure), as age is not a reliable indicator of growth (see Materials and Methods). When the larvae reach 5 mm, NMIIs appear on the tail (Fig. 2D,E), and still later in the caudal fin (Fig. 2E,F). During this part of the formation of the L-PLL, the dorsal line (D-PLL) is extending even more slowly, from two to three neuromasts

when the first NMII appear (Fig. 2A) to five to six neuromasts in a 7 mm fish (Fig. 2E) and eight to nine in the adult.

Neuromast migration and formation of new lines

The neuromasts of the L-PLL migrate ventralwards during postembryonic growth, such that most of them end up much closer to the ventral midline than to their original lateral position. This movement does not affect all neuromasts in the same manner (Fig. 3B). Migration first affects L2, the second neuromast of the primary L-PLL, shortly after completion of this line. The four panels of Fig. 3C illustrate the incipient migration at the time the earliest NMIIs are forming; the white arrows show the level of the myoseptum along which the primary line originated. In this fish, neuromast L2 has already moved ventrally, whereas L1 and L3 are still close to the myoseptum. The NMII, which is barely differentiated, has also begun to move ventrally (third panel, Fig. 3C). In general, younger neuromasts (as judged by their size) tend to lag slightly behind older ones in their ventral displacement (arrowheads in Fig. 3D); more posterior neuromasts begin to migrate later, and do so to a lesser extent, in proportion to the tapering of body size (Fig. 2D,E). The clear exception to this progressive displacement of the entire line are the anteriormost neuromasts, which migrate very little (Fig. 2F; Fig. 3B; Fig. 4).

The sensory neurones of the PLL have their cell bodies located in a cranial ganglion just posterior to the ear. Their axons follow the neuromasts in their migration (Fig. 3), and their tracks presumably reflect the migratory pathways of the neuromasts. In general the L-PLL neuromasts follow the intersomitic grooves (Fig. 3F, arrowheads), but neuromasts may also migrate straight ventralwards (Fig. 3G, arrowheads). Occasionally two neuromasts are formed at approximately the same position (Fig. 3E,F); we could not decide whether this was due to the accidental splitting of an originally unique neuromast, or to the independent formation of two neuromasts at the same position.

As the ventral migration of the neuromasts of the L-PLL proceeds, the neuromasts of the dorsal line also undergo a ventral migration that progressively brings them in a dorsolateral position (Fig. 2F; Fig. 3H).

Contrary to the L-PLL case, the D-PLL axonal tracks show no indication that the dorsal neuromasts follow intersomitic boundaries in their migration (Fig. 3H). The two to three dorsal neuromasts formed at the base of the dorsal fin do not seem to migrate at all (Fig. 4B).

After the dorsal neuromasts have begun their ventral migration, a new line of dorsal neuromasts, D′-PLL, forms at the same dorsal position where the original line developed (Fig. 2F; Fig. 3H). The D′ line comprises about seven neuromasts when complete (Fig. 4B). When the fish reaches about 1 cm, a new line (L′-PLL) forms at the same lateral position where the original L-PLL developed (Fig. 4B). This line ends up comprising about ten neuromasts. The development of these new lines parallels exactly that of the initial lines, except that the process is much slower. The spacing between consecutive neuromasts is usually two somites.

Rearrangement of the terminal part of the L-PLL

The caudal fin displays dramatic morphological changes during postembryonic growth. The embryonic fin fold (Fig. 5A) is symmetrical around the tail (protocercal fin). In the adult, the caudal fin also appears symmetrical, yet it is entirely derived from the ventral fin fold (Fig. 5G). The disposition of the terminal neuromasts of the L-PLL also differs between the embryo and the adult. In the embryo, the primary L-PLL runs along the horizontal myoseptum, except for the posteriormost cluster of two to three neuromasts, which lies in a more ventral position (Gompel et al., 2001) (Fig. 1C; Fig. 5A). In the juvenile (8 mm) fish these neuromasts are aligned dorsoventrally, i.e. orthogonal to their original arrangement (Fig. 2F). We have examined the transition from one pattern to the other.

The first indication of a dorsoventral asymmetry in the tail fin is the formation of a mesenchymatic swelling ventral to the tip of the notochord (Fig. 5C). As soon as this swelling becomes detectable, melanocytes migrate to and delineate its edge (Fig. 5B-E, arrows) and the penultimate neuromast migrates ventrally and posteriorly (Fig. 5C-F, arrowheads), thereby initiating the rearrangement of the posterior cluster. A second factor in this rearrangement is the upbending of the notochord (Fig. 5F), which occurs later, and results in a dorsalisation of the terminal neuromast. This bending accompanies (or is accompanied by) the formation of fin rays (Fig. 5F-G). At this stage the terminal cluster of neuromasts has assumed a nearly dorsoventral orientation relative to the major body axis (Fig. 5F). The fact that the axis itself is bent at the posterior end of the body makes the meaning of dorsal and ventral somewhat confusing, therefore it is best to refer to

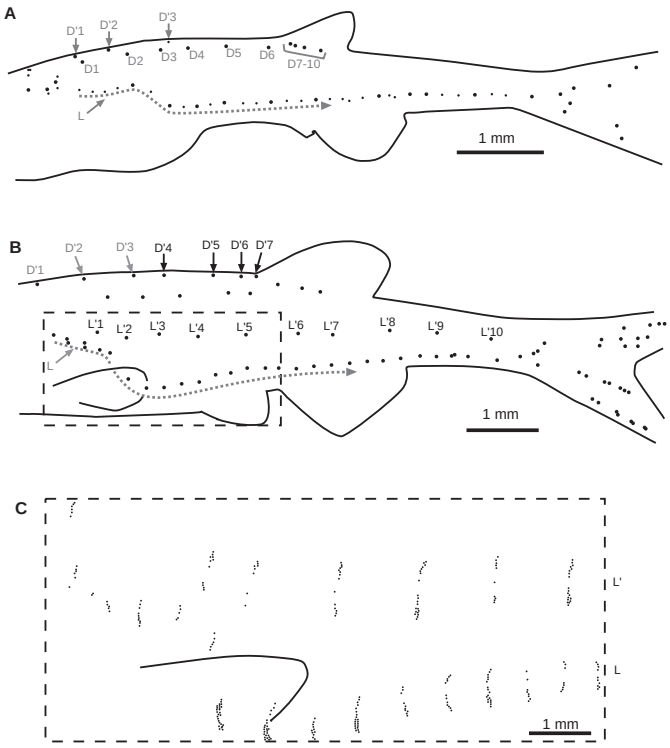

Fig. 4. Formation of the L′-PLL line. (A,B) The pattern of PLL neuromasts just before and after the L′ line has formed. The neuromasts of the L line are not numbered; the dotted arrow (in grey) outlines the distorted geometry of this line due to unequal migration. (C) Each neuromast of the D, D′, L, and L′ lines later forms a vertical 'stitch'. The frame corresponds to the boxed area in B, at an older stage. Anterior is towards the left and dorsal is upwards.

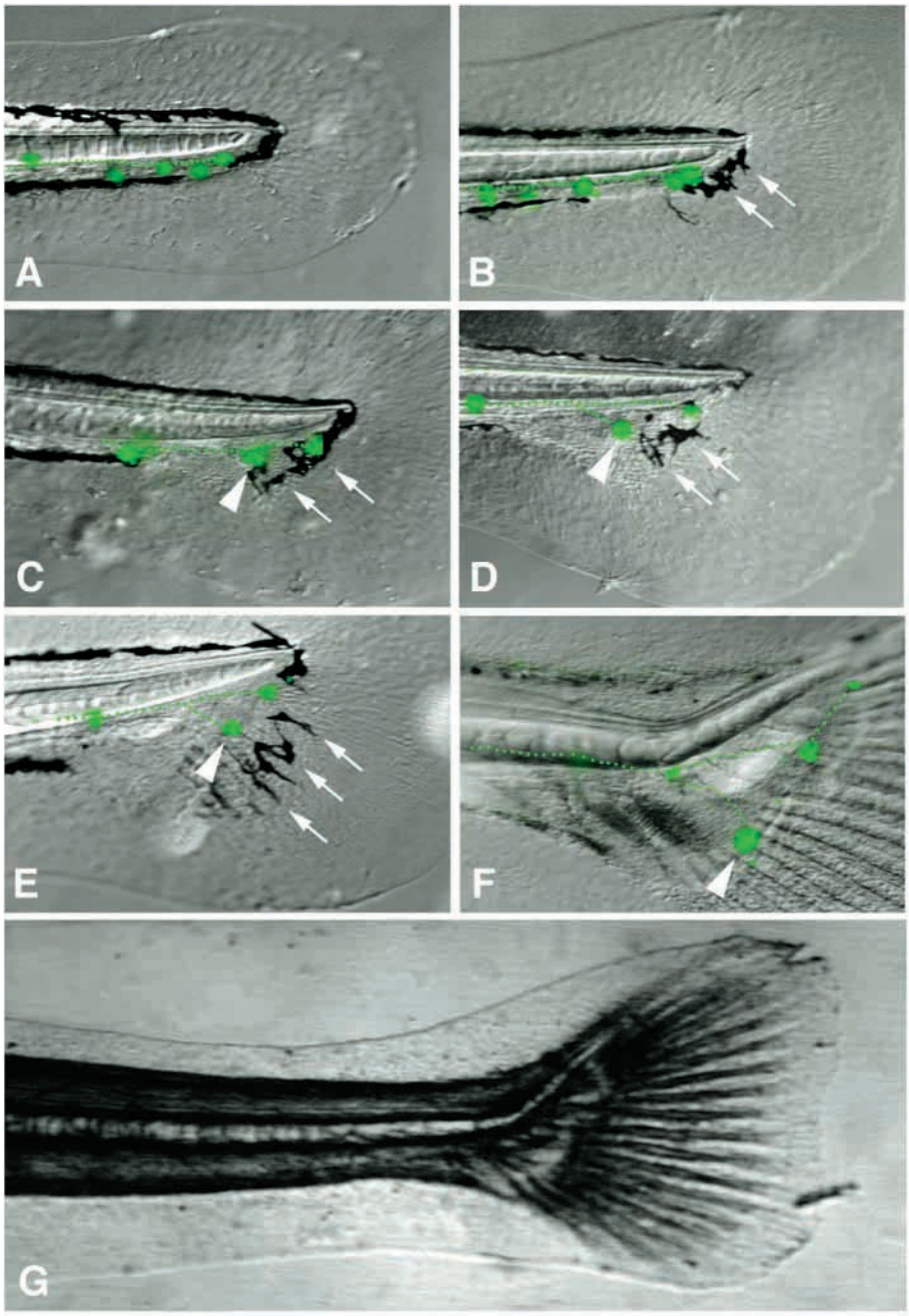

Fig. 5. Rearrangement of the terminal part of the L-PLL and structure of the tail. The neuromasts and their axons (green) are visualised in fluorescence and the image is superimposed on a Nomarski view of the same field. The axonal paths are indicated with a dotted line because the fluorescence is too weak to show when superimposed to the diascopic view. Given the thinness of the tail, the neuromasts on the other side of the fish are also seen. Each panel represents a different fish. (A) Terminal neuromasts along the ventral edge of the tail before the onset of the rearrangement. (B) Elongation of the melanocytes (white arrows). (C, D) Formation of a ventral mesenchyme and beginning of the migration of the penultimate neuromast (white arrowhead). (E) Appearance of the caudal fin rays. (F) Rearrangement nearly complete. (G) At a slightly later stage, lower-scale view to illustrate how the homocercal, symmetrical caudal fin of the zebrafish is entirely derived from the ventral fin fold of an intrinsically asymmetrical, heterocercal tail. Anterior is towards the left and dorsal is upwards.

the panels of the figure, which illustrate the actual process explicitly.

Parallel to the migration of the penultimate neuromast, chondrogenic foci form within the ventral mesenchymatic swelling. This process has been carefully documented in medaka embryos (Ishikawa, 1990). At the earliest stages of neuromast migration, however, we have not detected the presence of foci within the swelling, making it unlikely that the displacement of the neuromasts depends on such foci. The alternative possibility, that the formation of chondrogenic foci depends on the migration of the penultimate neuromast, cannot be excluded, because a role for neuromasts in the induction of dermal bones has been documented (Pehrson, 1922; Pehrson, 1958; Devilliers, 1947). We favour, however, a third possibility, that the reorganisation of the terminal neuromasts and the organisation of chondrogenic foci are independent responses to the same asymmetrical signal. Interestingly, in the dorsalised medaka mutant *Da* (double anal fin), an additional set of hypural plates forms dorsal to the notochord, which does not bend anymore, and the terminal neuromast is displaced to a more ventral position (Ishikawa, 1990).

Formation of stitches

After the basic pattern of four lines stretching from head to tail is complete, each neuromast is somehow converted in a 'stitch' of up to 20 or more neuromasts extending dorsoventrally (Fig. 4C; Fig. 6A). This process begins, when the fish reaches 10-12 mm, with the neuromasts of the L-PLL, which have by now assumed a ventral position. Stitching occurs progressively in anteroposterior waves, such that at 13 mm, the anterior stitches may comprise three to four neuromasts, whereas in the tail the neuromasts are still single or in pairs; at 16 mm the anterior stitches comprise five to seven neuromasts, whereas the posterior ones now count two to four neuromasts. At this stage, stitching has also begun in the other three lines of the PLL (D, D′, L′). The number of neuromasts in a stitch increases progressively up to 30 neuromasts in the largest (anterior) stitches and six to eight neuromasts in the posterior stitches, in a 30 mm fish. We have not followed the process further.

The first step in the formation of a stitch (Fig. 6B) is the appearance of a very small neuromast at some distance from the founder neuromast, and connected to it by two axons (Fig. 6B, inset). The process is then repeated to produce stitches with three (Fig. 6C), four (Fig. 6D) and more neuromasts. In each

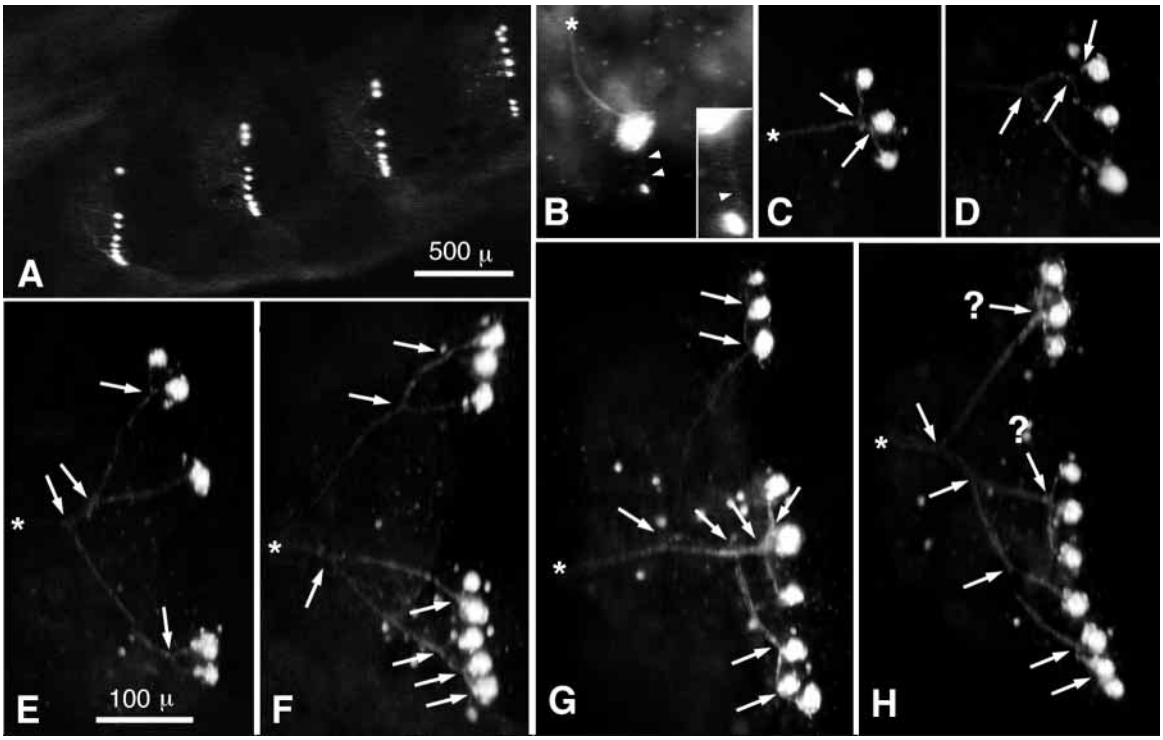

Fig. 6. Formation of stitches. (A) Four consecutive stitches of the L-PLL line. The axons innervating the different neuromasts of one stitch are most likely collaterals of the same axons that innervated the founder neuromast. (B) The first step in the formation of a stitch: a small neuromast comprising only one differentiated hair cell has formed about 50 μm away from the founder neuromast, to which it remains connected by axons (arrowheads). Inset shows a magnified, contrast-enhanced view of the axons connecting the two neuromasts (arrowhead). (C-H) A series of stitches of increasing sizes. As judged from this series, and from the branching pattern of the collaterals, stitching occurs by a succession of budding events, each of which generates one (or at most two) additional neuromast. The arrows point to axonal bifurcations that presumably reflect budding events. The asterisk marks the common root of the innervating fibres. All panels represent stitches at the caudal level of the L-PLL except A,B, which illustrate stitches at the pectoral level; thus, the asterisked axons in B are directed dorsalwards, whereas in C-H they are directed anteriorwards. Scale bar for B-H is shown in E. Anterior is towards the left and dorsal is upwards.

case the axons innervating the new neuromasts appear as branches that elongate passively as the new neuromast moves away from the 'founder' neuromast (Fig. 6E-H). Thus, each stitch is almost certainly the result of a budding process, as documented in the case of neuromast regeneration after tail amputation in the salamander (Stone, 1937). Indeed, it is known that support cells are capable of forming new hair and support cells in amphibian neuromasts (Jones and Corwin, 1996) and in zebrafish (Williams and Holder, 2000).

The details of the branching pattern of the axons suggest that stitching results from rounds of single neuromast budding. This is consistent with our observation that at the beginning of the process, stitches comprise only two neuromasts, and their size increases progressively over a long period. Only rarely are axonal trifurcations observed (Fig. 6G, question marks).

DISCUSSION

Growth of the lateral line through the iteration of a modular process

The first step in the formation of the PLL system occurs during embryogenesis and results in the formation of a line of 7±1 neuromasts along the horizontal myoseptum, the lateral branch of the PLL (L-PLL), and of two additional neuromasts that prefigure the dorsal and occipital branches. We have examined how this embryonic pattern progressively extends during larval growth.

Our data are most easily accounted for by assuming that during larval growth, new primordia migrate from head to tail and deposit successive waves of neuromasts that progressively fill in the gaps in the primary line. We propose, therefore, that the postembryonic growth of this system occurs by a reiteration of the same elementary process first observed during embryogenesis, and that additional (secondary) primordia generate the secondary neuromasts. Recent data of Sapède et al. (Sapède et al., 2002) have demonstrated the existence of at least one such secondary primordium.

How many times does the elementary process unfold? In the case of the lateral PLL (L and L′-PLL), the process unfolds at least three times: one for the embryonic line, one for the postembryonic completion of this line, and one for the L′-PLL line, which also forms along the horizontal myoseptum. This number is likely to be an underestimate, however. The neuromasts of the first postembryonic wave appear at two-somite intervals, yet the L-PLL of older fish has one neuromast per somite. This cannot be explained by asynchrony in neuromast differentiation, because it has been shown that cell deposition itself takes place at two-somite intervals (Sapède et al., 2002). One has to assume, therefore, that the L-PLL needs

at least one additional primordium to reach completion (unless presumptive neuromasts are deposited on each somite, but some of them differentiate much later than others). Furthermore, the first wave of secondary neuromasts extends from L1 posteriorwards, yet in older fish, neuromasts are present anterior to L1, indicating that at least one additional primordium is needed to fill in the region anterior to L1.

It appears that, whenever the elementary process can be observed in isolation, it seems to generate no more than eight to ten neuromasts as in the embryonic L-PLL, the D-PLL or the L′ and D′-PLL. This suggests the existence of some intrinsic limitation. One possibility is that the mechanism involved in the establishment of a somatotopic projection (Gompel et al., 2001) can operate for a limited number of neuromasts at a time. This would predict that similar elementary processes should be found in fishes with different lateral line patterns, as has indeed been documented by Sapède et al. (Sapède et al., 2002). Assuming that the elementary process is indeed limited to the generation of about eight neuromasts, the first postembryonic iteration would extend along the body and lead to the pattern illustrated Fig. 2D. A second and third iteration would fill the gaps left by the first wave and begin to extend along the tail (Fig. 2E). At least two additional iterations would be needed to form the neuromasts anterior to L1, and to fill in the pattern on the tail (Fig. 2F). The final L-PLL, by far the most extensive of the body, would therefore require at least six iterations of the elementary process.

Lateral line stem cells

The hypothesis of reiteration implies the existence in the postotic region of a capability to generate new primordia responsible for the generation of the new neuromasts. This could be due to the induction of new (secondary) placodes or, alternatively, it could be due to the persistence in the postotic region of lateral line stem cells capable of generating new primordia as well as additional neurones. The existence of neural stem cells in adult vertebrates is now well established (Blau et al., 2001). In *Xenopus*, part of the eye (the ciliary marginal zone) remains undifferentiated and acts as a growth zone, which produces, during its whole life, the different cell types that constitutes the retina (Harris and Perron, 1998). Within this growth zone occurs a recapitulation of the different steps that lead to the formation of the embryonic eye, with the same cascade of gene activities leading to the progressive formation of additional retina. Similar growth zones are also found in fish and mammalian eyes (Fisher and Reh, 2000; Tropepe et al., 2000).

We expect that the postembryonic growth of the PLL involves the same genes that are expressed during the formation of the primary PLL during embryogenesis, such as *zath1*, *notch3*, *delta A* and *delta B* (Itoh and Chitnis, 2001) and *eya1* (Sahly et al., 1999), as well as the markers CB701 and CB403 (Gompel et al., 2001). This expectation has been confirmed for at least two of these markers, *eya1* (Sahly et al., 1999) and CB701 (Sapède et al., 2002). The idea that postembryonic growth is modular, in the sense that it relies on successive reiterations of the embryonic processes, could be of general relevance for the development of organs with a high degree of internal organisation (such as sensory systems).

Neuromast migration and formation of stitches

The ventral migration of the neuromasts of the L-PLL begins soon after their differentiation, before the completion of the primary line. The final pattern appears therefore to result from the combination of two orthogonal processes: the migration of the primordium in the anteroposterior direction and the migration of the neuromasts in the dorsoventral direction.

Control of the dorsoventral migration of neuromasts according to their position along the anteroposterior axis could lead to a great flexibility in the final pattern of the PLL. For example, in the zebrafish, the lack of migration of neuromast L1 and those anterior to it will keep the anterior L-PLL away from the pectoral fins. In general, the adult shape of the L-PLL can vary widely from one fish species to another, and appears remarkably adapted to their morphology and habits. The dual control suggested by our observations would certainly facilitate such a fine control of pattern.

The formation of stitches almost certainly results from the budding of founder neuromasts. The stitches are oriented dorsoventrally, consistent with the idea that neuromasts can only migrate along the dorsoventral axis, at least in zebrafish. The different neuromasts of each stitch seem to be innervated by collaterals of the same axons that innervated the founder neuromast. The formation of stitches appears therefore to lead to an amplification of the system, rather than to add a new functionality to it.

Head-to-tail sequence of maturation

From the formation of the primary line to the formation of stitches, all steps of PLL development unfold in a head-to-tail sequence. This sequence is readily explained for the formation of the primary neuromasts, as they are deposited in the same sequence by the migrating primordium. The reason why a similar sequence should be observed for subsequent steps, such as ventral migration or stitching, is less obvious, as several weeks separate the formation of a given neuromast from its stitching. For example, hormonal signals would be expected to trigger the formation of stitches in a more synchronous manner. For the moment, we have no definite cues to explain how this progressive maturation can reproducibly unfold on such a large scale, both in time and in space. One possibility is that stitching occurs when some threshold in dorsoventral size is reached. Given the general tapering of the body from head to tail, this could indeed result in a progressive increase in stitch formation and size from head to tail. Alternatively, the orderly spread of stitching from head to tail may reflect a more basic tendency to organise growth along this axis, as illustrated, for example, in the anteroposterior sequence of somite formation, or Hox gene expression.

Postembryonic tail patterning

The tail of teleost fishes shows a dramatic repatterning during postembryonic growth. The terminal part of the tail undergoes a dorsal bending such that the symmetrical fin fold of the embryo becomes transformed in the highly asymmetrical caudal fin of the adult, which develops exclusively from the ventral part of the tail. We observed that the terminal part of the PLL shows concomitant changes, i.e. a displacement of the terminal neuromasts such that the most posterior one becomes dorsal, whereas the more anterior one becomes ventral. The transformation of the terminal L-PLL does not follow passively

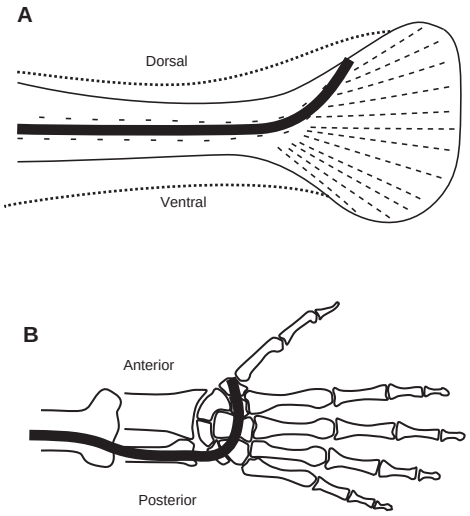

Fig. 7. Distal bent of the fish caudal fin (A) and of the tetrapod limb (B). The main axis of limb and fin are represented by the thick line.

the bending of the tail, however, but rather anticipates it and accompanies the very first signs of asymmetry. This observation suggests that terminal repatterning is a major feature of tail morphogenesis and affects directly all aspects of tail formation, including its sensory development.

The transition from symmetrical to asymmetrical tail observed in zebrafish development recapitulates a major evolutionary transition. The ancestral tail fin was protocercal, i.e. symmetrical and continuous with the dorsal and ventral fin folds, as is still the case in the zebrafish embryo (Fig. 1). In most extant fishes, however, the tail has become heterocercal, that is, the tail is bent towards its dorsal ridge, and the caudal fin extends form its ventral side, as is particularly obvious in sharks. As a final twist, most ray-finned fishes develop a homocercal tail, which appears symmetrical although it is intrinsically asymmetrical (Fig. 5G; Fig. 7A).

It is known that terminal axis bending also takes place during the formation of the tetrapod limbs (Fig. 7B) (Duboule 1994; Coates and Cohn, 1998), such that the digits derive from the posterior edge of the limb tip. This similarity between limbs and tail could be fortuitous. Our results show, however, that axis bending pervades all aspects of tail development in present-day fishes (even those that have a seemingly symmetrical tail fin). We propose, therefore, that terminal axis bending became intimately associated with the terminal Hox genes before the appearance of the tetrapod lineage. When the Hox system was exploited to specify proximodistal identities in the tetrapod limbs (but not in the paired appendages of fish or arthropods), terminal axis bending may have been imposed upon limb development as a consequence of this recruitment.

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