

## The development of the posterior body in zebrafish

John P. Kanki\* and Robert K. Ho

Department of Molecular Biology, Moffett Laboratory, Princeton University, Princeton, NJ 08544, USA

\*Author for correspondence (E-mail: jkanki@watson.princeton.edu)

### SUMMARY

In order to understand the developmental mechanisms of posterior body formation in the zebrafish, a fate map of the zebrafish tailbud was generated along with a detailed analysis of tailbud cell movements. The fate map of the zebrafish tailbud shows that it contains tissue-restricted domains and is not a homogeneous blastema. Furthermore, time-lapse analysis shows that some cell movements and behaviors in the tailbud are similar to those seen during gastrulation, while others are unique to the posterior body. The extension of axial mesoderm and the continuation of ingression throughout zebrafish tail development suggests the continuation of processes initiated during gastrulation. Unique properties of zebrafish posterior body development include the bilateral distribution of tailbud cell progeny and the exhibition of different forms of ingression within specific tailbud domains. The ingression of cells in the anterior tailbud only gives rise to paraxial mesoderm, at the exclusion of axial mesoderm. Cells of the posterior

tailbud undergo subduction, a novel form of ingression resulting in the restriction of this tailbud domain to paraxial mesodermal fates. The intermixing of spinal cord and muscle precursor cells, as well as evidence for pluripotent cells within the tailbud, suggest that complex inductive mechanisms accompany these cell movements throughout tail elongation. Rates of cell proliferation in the tailbud were examined and found to be relatively low at the tip of the tail indicating that tail elongation is not due to growth at its posterior end. However, higher rates of cell proliferation in the dorsomedial region of the tail may contribute to the preferential posterior movement of cells in this tailbud region and to the general extension of the tail. Understanding the cellular movements, cell fates and gene expression patterns in the tailbud will help to determine the nature of this important aspect of vertebrate development.

Key words: zebrafish, posterior body, tailbud, fate map

### INTRODUCTION

The mechanism of posterior body formation and the development of the embryonic tail in vertebrates has been an area of controversy for over 70 years. In the 1920s Holmdahl proposed that the vertebrate body plan followed two separate and distinct programs of development (Holmdahl, 1925). The first program, called primary body development, was responsible for the formation of the head and trunk regions of the embryo while a separate process, termed secondary body development, was responsible for the formation of more caudal regions. The early phases of primary body development were characterized by the stereotyped morphogenetic movements of gastrulation, which resulted in the formation of three morphologically distinct germ layers of endoderm, mesoderm and ectoderm. Primary body development was referred to as 'indirect' because subsequent tissues and organs were formed from these three germ layers. However, Holmdahl and others believed that, during secondary body development, the tissues of the caudal body were derived from a homogeneous proliferating blastema at the distal end of the embryo called the 'tailbud'. The posterior neural tube, vertebral column and somitic musculature arose from mesenchymal cells of the tailbud without apparently going through either the morphogenetic cell movements characteristic of gastrulation or the formation of recognizable germ layers. Thus secondary body formation was

referred to as 'direct' development. Despite these differences, identical tissue types, which are anatomically contiguous with each other, were formed by these two processes (Griffith, 1992).

Other investigators of that time, such as Pasteels, thought that the formation of the tail region represented a continuation of gastrulation and that posterior body development did not differ qualitatively from the development of head and trunk regions (Pasteels, 1943). In more recent years, both ideas have received support from research on posterior body development in a variety of animal systems. Fate mapping and extirpation studies indicate that distinct domains of the tailbud are restricted to specific cell fates (Bijtel, 1931; Spofford, 1945; Smithberg, 1954; Tucker and Slack, 1995; Schoenwolf, 1977, 1978; Nakao and Ishizawa, 1984; Gont et al., 1993; Catala et al., 1995). In addition, the use of molecular techniques for the detection of gene expression has demonstrated that the tailbud consists of discrete regions of gene expression providing further evidence that the tailbud is not a homogeneous blastema, as proposed by Holmdahl (1925; see Joly et al., 1992; Schulte-Merker et al., 1992; Gont et al., 1993; Joly et al., 1993; Thisse et al., 1993; Hammerschmidt and Nusslein-Volhard, 1993; Talbot et al., 1995).

However, there is evidence suggesting that the posterior body does not develop exclusively by the continuation of developmental mechanisms seen in the anterior. In mammalian and

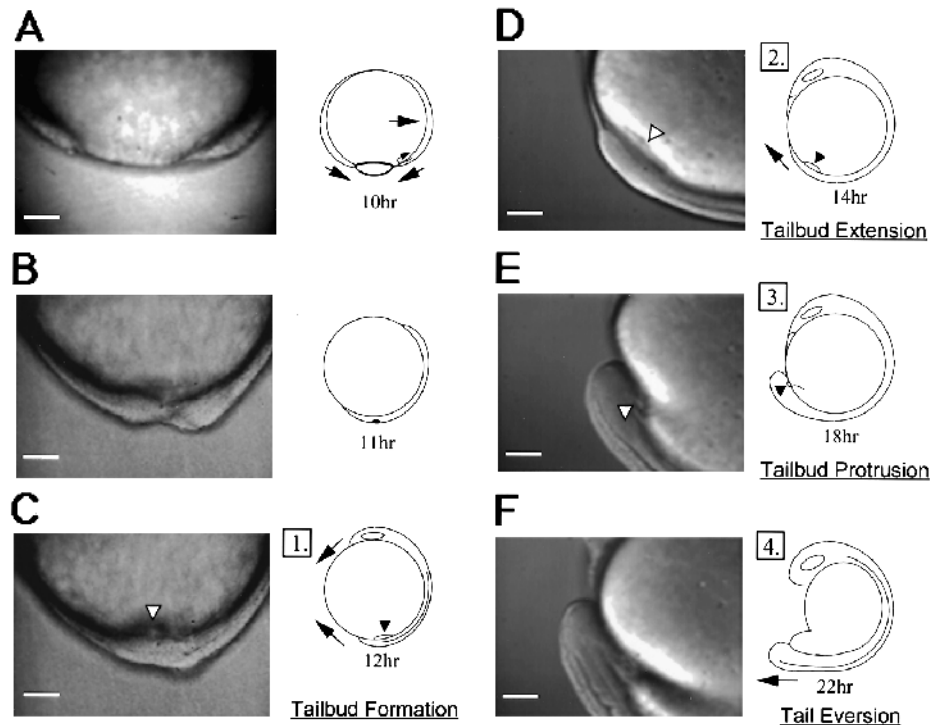
avian embryos, the posterior neural tube has been shown to develop through a distinct process called secondary neurulation (Criley, 1969; Schoenwolf and Delongo 1980; Schoenwolf, 1984; Griffith et al., 1992). In the anterior spinal cord, the neurocoel forms by the dorsal fusion of the neural folds while, in the posterior, the neurocoel forms by a very different process called cavitation. In the lumbosacral region of the chick, the anterior- and posterior-derived neurocoels are transiently distinct. The anterior neurocoel is positioned dorsally over the posterior neurocoel prior to their fusion and formation of a single continuous spinal canal (Criley, 1969; Schoenwolf and Delongo, 1980). In addition, certain genes, such as the vertebrate homolog of the *Drosophila evenskipped* gene, are exclusively expressed in the posterior indicating that some genes may be specifically involved in posterior development (Joly et al., 1993; Barro et al., 1995). A number of mutant mouse and zebrafish lines have been described exhibiting severe posterior defects but relatively normal head and trunk regions (Chesley, 1935; Halpern et al., 1993; Neumann et al., 1994). The dramatic posterior defects caused by the loss of specific genes, such as *wnt3a* in the mouse (Takada et al., 1994) or the *T* gene in both mouse (Hermann et al., 1990) and zebrafish (Schulte-Merker et al., 1992) mutants, suggest that the developmental processes of the tail are distinct from those of the anterior, and therefore selectively affected by the loss of these genes.

The formation of the zebrafish tailbud immediately follows gastrulation, which is characterized by a series of stereotypic morphogenetic cell movements. Epiboly causes the margin of the blastoderm to move vegetally over the yolk cell, while ingression/involution movements take place at the margin (Warga and Kimmel, 1990; Shih and Fraser, 1995). Cells undergoing ingression/involution movements form the deeper hypoblast layer, which is the precursor of mesodermal tissues. The superficial cells remaining in the epiblast layer will

give rise to ectodermal and neurectodermal derivatives (Kimmel et al., 1990; Shih and Fraser, 1995). Cells of the epiblast and hypoblast converge towards the future dorsal side of the embryo resulting in the extension of the embryo along the anterior-posterior axis. The tailbud forms as the cells of the blastoderm margin meet and fuse over the ventral yolk plug representing the beginning of the 'tailbud' or 'bud' stage (Westerfield, 1993; Kimmel et al., 1995). The cells of the tailbud will contribute largely to the formation of the zebrafish posterior body.

We have conducted a detailed analysis of cell fate and movement in the tailbud of the developing zebrafish to better understand the process of posterior body formation. The zebrafish embryo is optically clear and amenable to microinjection and cell labeling techniques making it particularly attractive for single cell lineage analysis (Kimmel, 1989; Kimmel et al., 1990). Our fate map of the zebrafish tailbud showed that it contains distinct tissue-restricted domains with respect to cell fate and is not a homogeneous blastema. Furthermore, time-lapse analysis shows that some cell movements and behaviors in the tailbud are similar to those seen during gastrulation, while other cell movements are unique to the tailbud and may reflect discrete developmental mechanisms during posterior development.

Rates of cell proliferation in the tailbud were also examined to address potential mechanisms of tail elongation. Although the rate of cell division at the tip of the tail was found to be relatively low, cell proliferation in the presumptive spinal cord, within the dorsal medial region of the tail, may contribute to the cell movements observed in the tailbud in addition to the general extension of the tail. Understanding the tissue lineages, cellular movements and gene expression patterns in the tailbud will help to determine the processes responsible for the development of the vertebrate posterior body.



**Fig. 1.** Zebrafish tail formation. (A-F) The developmental sequence of a zebrafish embryo before and during different stages of tail formation. The right side of each panel shows a drawing of the entire embryo at the indicated developmental stages while the left side shows a closer view of the corresponding tail forming regions in developing embryos (bar=100  $\mu$ m). The cell movements at the end of gastrulation are shown in A while the fusion of the blastoderm margin is shown in B. Numbers in boxes indicate specific stages of zebrafish tail development (C-F). Arrows indicate the direction of cell movements, while arrowheads indicate the position of Kupffer's vesicle.

## MATERIALS AND METHODS

### Fate map of the zebrafish tailbud

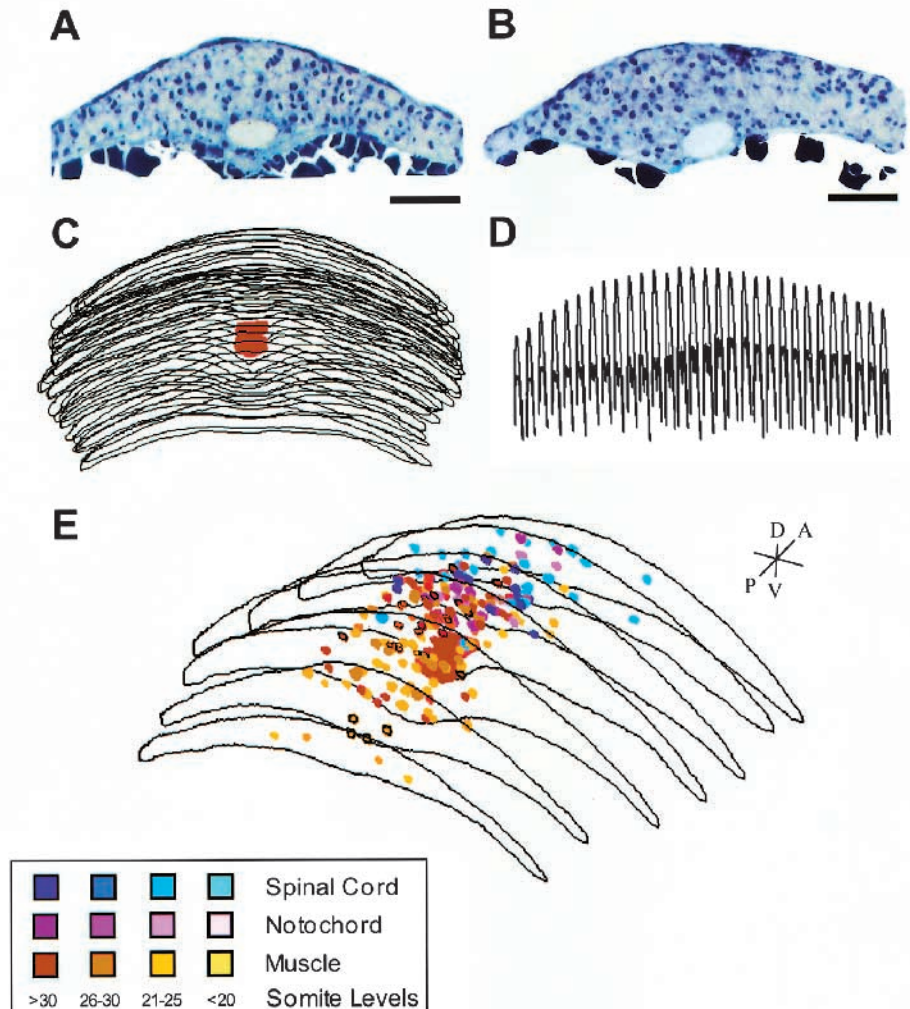
Zebrafish embryos were collected from a laboratory breeding colony, maintained at 28.5°C and developmentally staged according to procedures described (Westerfield, 1993; Kimmel et al., 1995).

Individual cells at the tailbud stage, between 10.5 and 11.5 hours postfertilization (h.p.f.), were labeled using fluorescent lineage tracing molecules in order to follow their development within the growing embryo. The 'tailbud' was designated as the mound of tissue extending 300 µm anterior to its leading posterior edge. A cell injection apparatus, consisting of an electrophysiology stimulator and amplifier built around a fixed-stage Zeiss Axioscope microscope with epifluorescence and differential interference contrast (DIC) optics, allowed individual cells to be identified under high magnification. Glass microelectrodes were filled with Rhodamine-dextran or Fluorescein-dextran conjugates ( $10 \times 10^3 M_r$ ; Molecular Probes), mounted onto a Leitz micromanipulator and used to intracellularly label single cells (Warga and Kimmel, 1990). Labeled cells were observed under epifluorescent and white-light DIC optics using a Zeiss Axioplan microscope equipped with a low light level silicon-intensified camera. The embryos were positioned in three orientations to record the spatial locations of labeled cells, with respect to anteroposterior (A-P), mediolateral (M-L) and dorsoventral (D-V) axes within the tailbud. These images were recorded digitally and stored on a computer (Macintosh). Embryos were allowed to develop

an additional 24 hours and the subsequent fates of the labeled cells were determined and correlated with their initial positions in the tailbud to create the tailbud fate map.

A grid on acetate was laid over the images of the tailbud in different orientations to ascribe three-dimensional coordinates to the labeled cells to determine their position in the tailbud. The characteristic tailbud morphology and landmarks, such as the location of Kupffer's vesicle (see below), were used to align the tailbud images of different embryos in order to consolidate these data. A three-dimensional representation of the tailbud was created to summarize and analyze the fate map data from a large number of different embryos. Images of serial plastic sections through the tailbud were taken every 10 µm and imported into a Silicon Graphics workstation (Iris Crimson VGX) where they were compiled using VoxelView Ultra 2.2 (Vital Images) software. Outlines of the tailbud at each plane of section were separated by an appropriate number of blank images in order to recreate the 10 µm spacing of the sections and to recapitulate an accurate three-dimensional representation of the tailbud framework. Representations of the labeled cells were positioned into the model with respect to all three axes. These cell representations were colored to indicate their eventual cell fates and final positions along the A-P axis of the developed tail. This model could be rotated in three dimensions in order to facilitate the analysis of spatial relationships of the labeled cells with respect to their final tissue fates and also allowed the analysis of subgroups of cells.

**Fig. 2.** Tailbud fatemap model. (A) A 10 µm transverse section through the newly formed tailbud at the level of Kupffer's vesicle; (B) a sagittal section through the midline of the tailbud (bar, 50 µm). In B, posterior is to the left and dorsal is upwards. (C) A dorsoposterior view of the three-dimensional model of the tailbud with the location of Kupffer's vesicle colored red. Outlines of 30 transverse sections (300 µm total) were compiled to create the tailbud model with accurate proportions. The tailbud model was rotated to show a slightly oblique lateral view in D. (E) Color coded representations of labeled cells embedded into this model according to the axial coordinates determined at the time of cell labeling. The orientation of the model is indicated by the axes shown in the upper right corner and only some of the tailbud outlines are shown in order to better visualize the cells within the tailbud framework. The box shows the color coding used to designate tissue fates of the tailbud cells and their A-P position within the developed tail. Final tissue types were classified as either neural, blue; axial mesoderm, purple or paraxial mesoderm, yellow/orange/red. The intensity of the color indicates the final A-P level such that darker shades of colors indicate more posterior positions along the A-P axis. The A-P axis was divided into four regions according to somite levels. From anterior to posterior these regions represent: (1) A-P levels anterior to somite 20; (2) somite level 21-25; (3) somite level 26-30 and (4) somite level 31-35.



### Determination of cell movements in the tailbud

The transparency of the zebrafish embryo allows cell movements and differentiation of even very deep cells to be observed. Time-lapse microscopy was used to continuously observe and record the developmental behaviors of labeled cells within living embryos. Single cells were labeled intracellularly as described above or small groups of cells were labeled with a lipophilic dye, PKH2-GL (Sigma). Embryos were embedded in agarose (0.1%) under a bridged coverslip or suspended in methylcellulose (3%) and monitored on a Zeiss Axioplan microscope attached to a time-lapse work station consisting of computer-controlled shutters over separate white light and fluorescent light sources and a motorized focus-controller. The 'AxoVideo' (Cyto) computer program was used to digitize and record multiple focal planes through the embryo at 10 minute intervals over the course of 6-10 hours. At each focal plane both white light and epifluorescent images of the embryo and the labeled cells were recorded. Taking images through a series of focal planes at each time point allowed the detection of changes in relative depth of the labeled cells within the immobilized embryo. Fluorescent and white light images were merged using Adobe Photoshop 3.0 allowing the movements and differentiation of the labeled cells to be followed within the context of the developing tailbud.

### Histology

Histological sections were taken through the tailbud of embryos that were fixed and embedded at varying stages of posterior development. Briefly, the embryos were dehydrated in an ethanol series and were incubated three times for 30 minutes in 100% acetone. Embryos were placed in a 50:50 acetone:Araldite (Polysciences, Inc.) solution for 16 hours, followed by two 3 hour incubations in 100% Araldite. Embryos were placed into blocks and baked at 60°C for 16 hours. After trimming the blocks, 5 µm serial sections were cut using a Dupont diamond knife on an LKB ultramicrotome. Sections were floated onto water, dried onto slides and counterstained with either Toluidine blue (1%) or Eosin Y (2%) in 0.5% sodium borate. The slides were then coated with Araldite and baked at 60°C for 16 hours.

### Mitotic indices in the tailbud

Embryos at tailbud stages were fixed in 4% paraformaldehyde-PBS. They were then rinsed 15 minutes in dH<sub>2</sub>O followed by 8 minutes at 60°C in 0.1 M HCl. Embryos were rinsed three times for 5 minutes in dH<sub>2</sub>O, incubated in Schiff's reagent 5-10 minutes at room temperature, rinsed in PBS and mounted dorsal side upwards in 80% glycerol (Kiernan, 1981). Mitotic figures were recorded in different regions of the tailbud of 4 embryos at 14 h.p.f. and 6 embryos at 18 h.p.f. Total cell counts (stained nuclei) were determined for each region and the ratios of mitotic/total cell number (mitotic indices) were calculated and averaged.

## RESULTS

### Tailbud formation and development

We have divided the early formation of the posterior body into four distinct stages immediately following gastrulation: tailbud formation, extension, protrusion and tail eversion. At the end of gastrulation, the cells of the blastoderm margin come together, fusing over the ventral yolk plug (Fig. 1A,B). During the first stage of tailbud formation this aggregation of marginal cells establishes the initial tailbud at 11-12 h.p.f. (Fig. 1C). Cells originating from the ventral side of the embryo make up the posterior half of the tailbud, while the dorsal-derived cells constitute the anterior half. During the second stage, the tailbud extends along the ventral side of the yolk cell (12-17 h.p.f.), stopping once it reaches the midpoint along the ventral side (Fig. 1D). During the

third stage (17-18 h.p.f.), cells accumulate in the tailbud forming a larger protruding aggregate (Fig. 1E). Once the tailbud undergoes the fourth stage of 'eversion' away from the yolk cell (>18 h.p.f.), the extending appendage is designated as the developing 'tail' (Fig. 1F).

In many teleosts, a transient structure called Kupffer's vesicle can be observed during this period of posterior body formation (Laale, 1985). Although its function and origin are not well understood, Kupffer's vesicle is a good morphological landmark for the tailbud and its development. After the marginal cells fuse over the blastopore, Kupffer's vesicle forms between the yolk cell and the mound of tailbud cells and migrates with the tailbud until the stage of tail eversion (Fig. 1C-E; arrowheads), at which time it moves into the tail tissue. Once Kupffer's vesicle is within the tailbud tissue, it gradually shrinks and disappears as the tail undergoes eversion. During stages one and two, the posterior edge of Kupffer's vesicle approximates the boundary between the ventral- and dorsal-derived tissues.

### Fate map of the zebrafish tailbud

Fig. 2 shows histological sections through the tailbud and the three-dimensional model that was developed in order to create and analyze the tailbud fate map. Fig. 2E shows the tailbud fate map with representations of the 105 cells that were labeled within the hour following tailbud formation (stage 1). Bilaterally symmetric representations of each cell are shown to provide a more coherent picture of lineage-specific domains within the tailbud. By 36 hours of development, most of the progeny of these labeled cells exhibited distinct cellular morphologies (Kimmel et al., 1995) and were categorized as either neurectodermal, axial mesodermal or paraxial mesodermal derivatives. For example, neurectodermal derivatives exhibited neuronal characteristics, such as axons, while paraxial mesodermal derivatives exhibited typical muscle tissue phenotypes. The cells have been colored to reflect their eventual cell fate, while the intensity of the color indicates the final positions of these cells with respect to the anteroposterior axis. Cells outlined in black gave rise to progeny bilaterally, on both sides of the animal. Clones of cells that were distributed bilaterally always gave rise to the same tissue type and were generally within 1- to 2-somite levels of each other.

The anterior and posterior domains of the tailbud contributed to distinct cell lineages. Fig. 3A shows the tissue fates of the subset of cells residing in the posterior half of the tailbud, initially originating from the ventral side of the gastrula-stage embryo. Fig. 3B shows the fates of cells in the anterior half of the tailbud, which was initially derived from the dorsal side of the gastrula-stage embryo. Ventral-derived cells only gave rise to paraxial mesoderm derivatives, mostly somitic muscle, while dorsal-derived cells gave rise to all tissue types. This indicates that the posterior tailbud domain, composed of ventral-derived cells, is restricted to paraxial mesodermal fates. Furthermore, the spinal cord and notochord are exclusively derived from the anterior half of the tailbud. A closer examination of the distribution of lineage-related cells within the anterior domain of the tailbud (Fig. 3C-F) shows further evidence of tissue-restriction. Fig. 3C and E shows the distribution of cells that became neurons (in blue) and notochord (in purple). The cells fated to become neurons were all derived



from dorsal layers of the tailbud, within 25  $\mu\text{m}$  of the dorsal surface. Cells fated to become notochord were located in the ventromedial region of the tailbud. In general, notochord and spinal cord precursors were localized to specific tissue-restricted domains within the anterior tailbud that were contiguous with these respective domains established further anterior in the trunk.

Fig. 3D and F shows the distribution of cells in the anterior tailbud that became paraxial mesoderm. These cells were found in mediolateral regions of the tailbud but, in addition, were found to colocalize with the dorsal tailbud cells giving rise to the spinal cord. Despite their colocalization, individual cells within this region were never observed to produce clones of mixed fates. Labeled cells gave rise to either neurectodermal or mesodermal progeny but not both. In addition, the cells fated to paraxial mesoderm in both the anterior and posterior regions of the tailbud gave rise to muscle tissue distributed along the entire length of the posterior body (Fig. 3A,D,F), indicating that the movement of cells into the hypoblast occurs continuously throughout the elongation of the tail. The cells that undergo this transition from superficial dorsal regions into the hypoblast were restricted to paraxial mesoderm lineages and did not give rise to axial mesoderm.

Examination of the anteroposterior distribution of cells giving rise to muscle and spinal cord within the tail (indicated by color intensity) showed that cells generally located in dorsomedial regions of the tailbud tended to move further caudal than cells in more ventral or lateral positions. This was particularly evident in cells giving rise to paraxial mesoderm (Fig. 3F). Cells in dorsomedial positions tended to be distributed in the posterior tail (somite levels  $>25$ ), as indicated by their dark orange or red colors, while cells in ventrolateral positions, indicated by yellow or light orange colors, tended to be distributed in the anterior tail (somite levels  $<25$ ). The correlation of cell depth with anteroposterior levels within the posterior body (Fig. 4) also indicates that dorsal cells generally travel the furthest posterior in the tail. These data suggest that there are distinct domains of posterior cell movement in the tailbud and that the strongest posterior movement of cells occurs in dorsomedial regions.

### Cell movements within the developing tailbud

The movements of labelled cells in the developing tail were followed by time-lapse microscopy. Single cells were labeled intracellularly while small groups of cells were labeled with a lipophilic dye. An example showing the movements of clones of three single tailbud cells is shown in Fig. 5A-D and summarized in Fig. 5E-G. Each labeled cell and its progeny have been colored in order to distinguish these clones from one another. With respect to their relative spatial positions within the tailbud, the furthest anterior labeled cells were colored yellow and were located dorsomedially, the more lateral and posterior labeled cells were colored green, while the furthest lateral and posterior cells were colored blue (Fig. 5A,E). Relative changes in mediolateral positions were followed in time lapse by recording the labeled cells at different focal planes (see Methods). The yellow and green cells originated from the anterior half of the tailbud, while the blue cells came from the posterior half of the tailbud. During tailbud extension, the blue cells moved anteriorly relative to the green and yellow cells which moved posteriorly past them

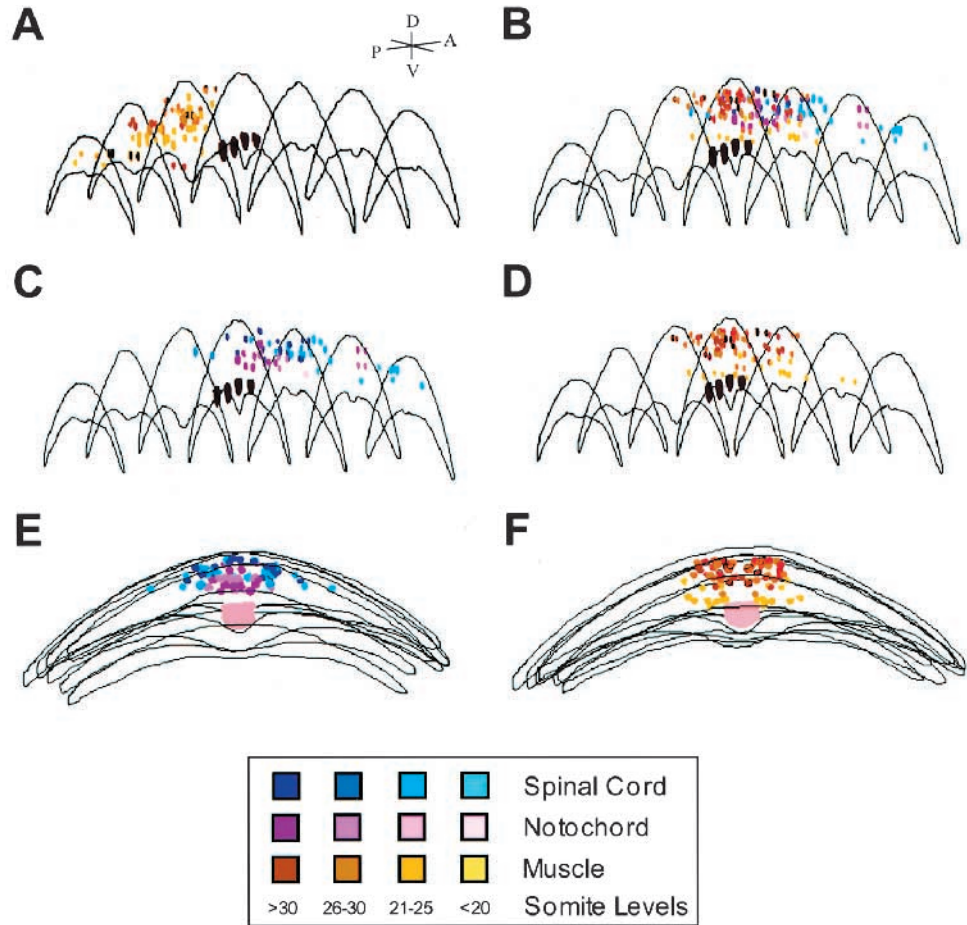
(Fig. 5B,F). At tailbud protrusion the green cells moved further laterally and eventually stopped advancing, while the yellow cells continued to the tip of the everting tail (Fig. 5C,D,F,G). Thus the initial anteroposterior relationship of these cells was reversed during development (see below) and maintained within the mature tail. These cells all subsequently differentiated into muscle cells.

This example demonstrates the different types of cell movements that were observed within the tailbud. Dorsal-derived cells in the anterior tailbud moved posteriorly as the tail elongated. Although the yellow cells were initially located most anterior, they traveled the furthest into the posterior tail. The movements of the green cells demonstrate the ability of cells to move for a variable amount of time within the dorsomedial posterior flow of cells before exiting from it (Fig. 5C,F). The relative A-P positions of these cells in the developed tail was consistent with their relative positions with respect to the stronger dorsomedial flow of cells in the tailbud. The blue cells, which were the furthest from the dorsomedial region, remained in anterior regions of the tail, while the yellow cells, in the middle of the dorsomedial region moved to the furthest posterior tip of the tail. The green cells, initially in off-center positions of the dorsomedial region, moved further laterally and became situated between the blue and yellow cells in the developed tail (Fig. 5E-G). Thus the direct observation of relative cell movements within the tailbud confirmed the fate map data suggesting that posterior cell movement within the dorsomedial region of the tailbud was the strongest.

### Unique cell movements are observed in the posterior tailbud

The final positions of the blue cells in relatively anterior tail regions was consistent with their relative distance from the dorsomedial region, however, these cells also exhibited cell movements unique to the ventral-derived cells in the posterior half of the tailbud which plays an important role in their restriction to paraxial mesodermal fates. Fig. 6 shows a group of cells within the posterior tailbud, labeled with a lipophilic dye, moving through the tailbud during tailbud extension. The distinct movements of these cells were evident as the tailbud was analyzed in three different orientations at three different time points following tailbud formation. The posterior tailbud cells moved laterally and anteriorly, circumventing the cells of the extending anterior tailbud (Fig. 6C,F,I). The lateral (Fig. 6A,D,G) and posterior views (Fig. 6B,E,H,J) show that these labeled cells not only moved laterally, but also ventrally beneath the epiblast layer of the advancing cells of the anterior tailbud. 'Subduction', a geological term describing tectonic plates moving under one another, describes these movements very well (Fig. 7A,B) and suggests how all of the posterior tailbud cells, including those in superficial regions, become placed within the deeper hypoblast. This lateral and ventral movement places these cells into domains destined for paraxial mesoderm development and may explain their restricted cell fate. Furthermore, the relative lack of cell mixing between posterior tailbud cells and the epiblast layer of the anterior tailbud demonstrates the distinction between these two domains within the tailbud.

A summary of cell movements in the tailbud is seen in Fig. 7C. Once the tailbud formed and begun extension, the majority of cells in the posterior tailbud moved laterally and ventrally



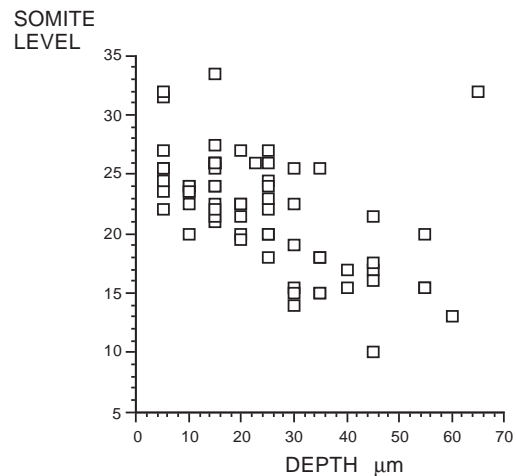
**Fig. 3.** Cell fates within subregions of the tailbud. (A) The positions of ventral-derived cells that were labeled within the posterior half of the tailbud; (B) the positions of dorsal-derived cells that were labeled within the anterior half of the tailbud. (C-F) Subgroups of cells that were labeled in the anterior tailbud. (A-D) Slightly oblique lateral views of the tailbud model with posterior to the left and dorsal upwards as indicated by the axes in the upper right corner of A. Kupffer's vesicle is shown in dark red as a positional reference. (E,F) Posterior views of the tailbud with dorsal upwards and Kupffer's vesicle shown in light pink. Cells in C and E gave rise to spinal cord (blue), or notochord (purple) while cells in D and F gave rise to muscle tissue. The box indicates the same color coding of the tailbud cells as in Fig. 2.

around the extending cells of the anterior tailbud (Fig. 7C; red arrows), however, cells in dorsomedial positions of the posterior tailbud continued to move posteriorly, but eventually moved ventrolaterally into the segmental plate (data not shown). These observations are consistent with the fate map data indicating that cells in dorsomedial regions of both the anterior and posterior tailbud generally move further posterior in the tail. The anterior tailbud cells moved posteriorly while some superficial cells continued to move ventrolaterally into the deeper hypoblast throughout tail extension (Fig. 7C; blue arrows). As these cells moved into the hypoblast, they joined the posterior tailbud cells and together contributed to paraxial mesoderm along the entire extent of the developed tail. Thus any given somite within the tail could contain cells originating from both anterior and posterior halves of the tailbud (Fig. 3A,D).

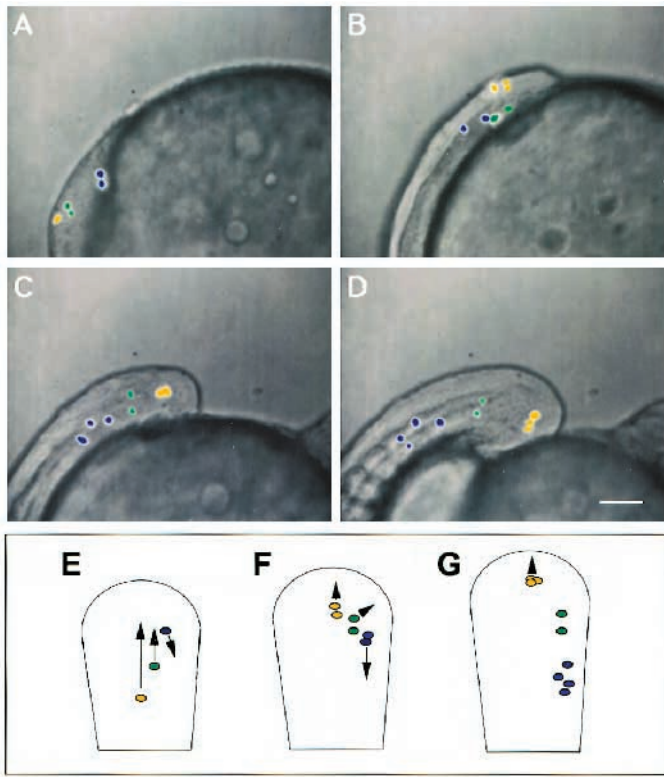
#### The tailbud undergoes cell rearrangements prior to tail eversion

During the tailbud protrusion stage at 17-18 h.p.f., the most posterior edge of the tailbud stopped migrating along the yolk cell and became relatively fixed (Fig. 1E). As cells accumulated at this point, causing the tailbud to enlarge and protrude, a major shift in the relative positions of tailbud cells occurred. The region at the posterior edge of the tailbud gave rise to the future proctodeum anlage, the future anus at the end of the hind yolk and, as the tailbud cells continued to move caudally, they were observed to 'roll over' this fixed point on the yolk cell

causing the tailbud to protrude posteriorly forming a recumbent fold. The formation of this recumbent fold was characterized by cells of the anterior tailbud moving dorsoposteriorly relative

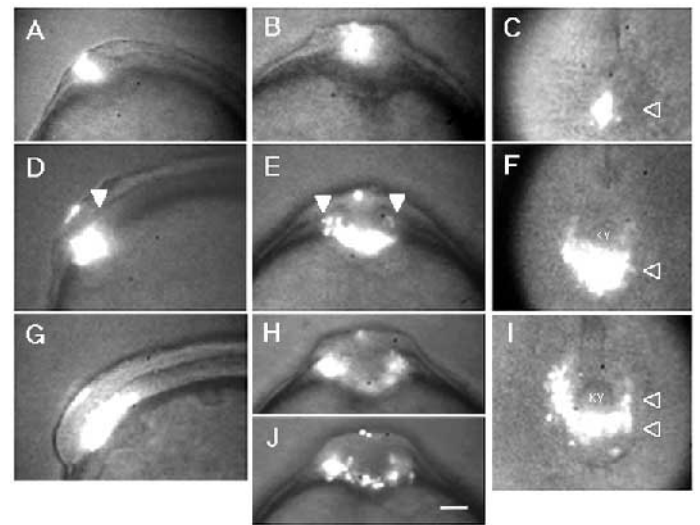


**Fig. 4.** Correlation of tailbud cell depth with A-P levels in the tail. The depth of the starting position of labeled cells in the tailbud was correlated to their final A-P position in the developed tail. All points represent cells that gave rise to muscle tissue. Depth of the cells is shown by their distance, in microns, from the dorsal surface of the tailbud. The final A-P level of the cells is indicated by the somite level within tail.

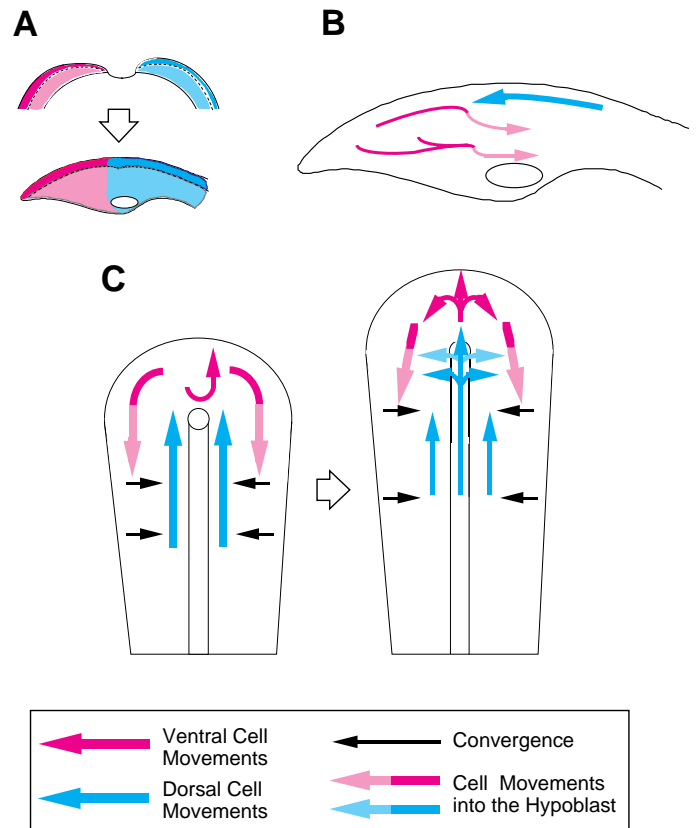


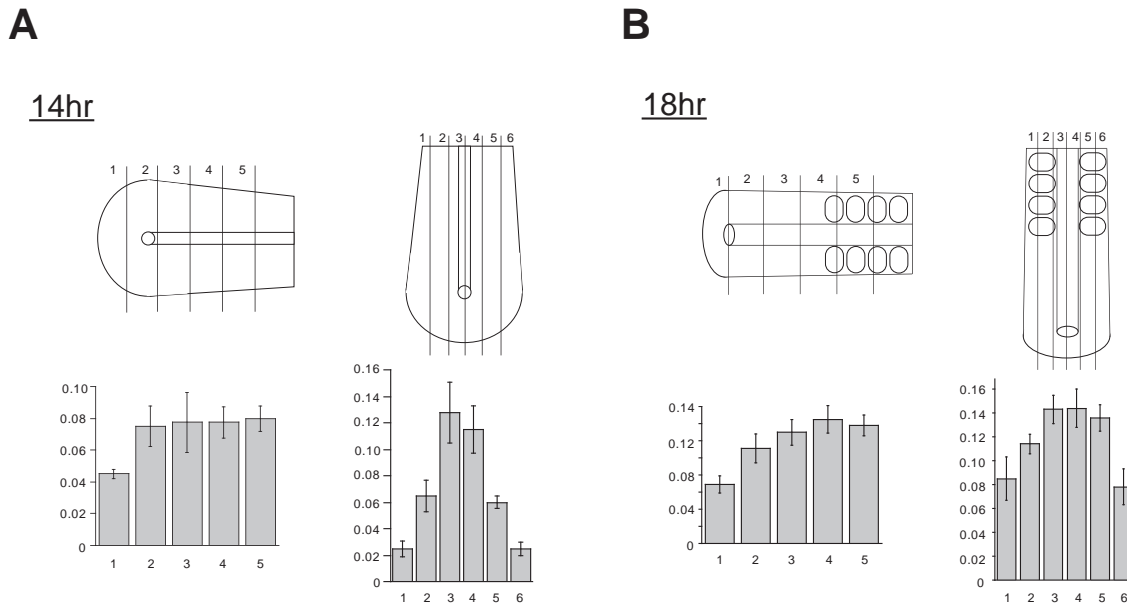
**Fig. 5.** Movements of single cells in the developing tail. (A-D) Images from a time-lapse sequence where the movements of three labeled cells were recorded as the tail developed. The tail is viewed laterally as it extends posteriorly along the yolk cell towards the upper right. Dorsal is towards the upper left. Clones of these cells were colored (yellow, green or blue), in order to distinguish them from each other in this figure. These images show tail development from approximately 15.5 h.p.f. until 20 h.p.f. with about 1.5 hours between each image (bar, 50  $\mu$ m). (E-G) Dorsal views of the tailbud summarizing the relative positions and movements of these clones. The colors of these cells are the same as in A-D. The direction of cell movements are indicated by the arrows.

**Fig. 7.** Summary of tailbud cell movements. (A) The fusion of the blastoderm margin over the yolkplug during tailbud formation. The blue color indicates cells from the dorsal side of the embryo (right), while the pink color indicates the ventral-derived cells (left). The dark colors represent superficial cell regions while the light colors represent the hypoblast. The top drawing shows a lateral view of the late gastrula embryo with the vegetal pole upwards. The lower drawing shows the tailbud after blastopore closure and the respective contribution of dorsal- and ventral-derived cells to the anterior (right) and posterior (left) halves of the tailbud. (B) The ensuing subductive movements of cells within the newly formed tailbud. The red and blue arrows indicate the direction of cell movements from their respective halves of the tailbud. The two-color arrows indicate the transition of cells from superficial regions (dark color) into the deeper hypoblast (light color). (C) The general movements of cells in dorsal views of the tailbud during its formation (left) and extension (right). Posterior is upwards. Movements of cells are represented by colored arrows as described above. Black arrows indicate convergent cell movements.



**Fig. 6.** Movements of posterior tailbud cells. Optical sections through the developing tailbud show the movements of a group of labeled cells originating in the posterior tailbud. Images were taken at (A-C) 13 h.p.f.; (D-F) 14 h.p.f. and (G-J) 16.5 h.p.f.. (A,D,G) Lateral views of the tailbud with posterior to the bottom left and dorsal towards the upper left. (B,E,H,J) Posterior views with dorsal upwards. (H) An optical section at the same time point as J but slightly anterior at the level of Kupfer's vesicle. (C,F,I) Dorsal views with posterior downwards. The solid arrowheads in D, E indicate the boundary between the hypoblast and the overlying epiblast layers. The open arrowheads in C, F and I indicate the approximate levels where transverse optical sections (B,E,H,J) were taken. Kupfer's vesicle (KV) is indicated and the bar, 50  $\mu$ m.





**Fig. 8.** Mitotic indices within subregions of the tailbud. (A,B) The illustrations at the top show dorsal views of the tailbud divided into subregions along the A-P axis (left) with posterior to the left, and along the mediolateral axis (right) with posterior downwards. Below are graphs showing the mitotic indices calculated for the corresponding regions shown above them. The mitotic index is the ratio of the number of cells undergoing mitosis to the total number of cells in a given region. The mitotic indices represent data averaged from 4 embryos at 14 h.p.f. in A, and 6 embryos at 18 h. p. f. in B. Standard error bars are indicated.

to the cells of the posterior tailbud. The cells of the posterior tailbud became displaced ventrolaterally, perhaps due to the arrested movement of cells at its posterior edge (see Fig. 9). As the eversion of the tail continued, the boundary between these two domains was shifted further posterior causing the anterior tailbud cells to eventually comprise more dorsal regions of the extending tail while the posterior tailbud cells were shifted into more ventral regions of the everting tail (data not shown). At this stage, Kupffer's vesicle was prominent as it entered the tailbud (Fig. 1E) contributing to the tailbud's transient enlargement as the posterior end of the tailbud protruded off of the yolk cell. In addition, furrows along the yolk cell could be detected extending anterolaterally from the proctodeal region indicating hindyolk tube formation.

#### Mitotic domains within the tailbud

In order to determine the role of cell proliferation in tail formation, the mitotic indices were determined for regions within the tailbud (Fig. 8A,B). The tip of the tail exhibited relatively low rates of mitosis and therefore the extension of the tail is not due to the addition of cells at its posterior end. A region of relatively high proliferation was identified in more anteromedial regions of the tailbud during both migration (14 h. p. f.) and eversion (18 h. p. f.). Mitotic rates were 2-4 times higher in the medial region of the tailbud than at its lateral edges.

#### DISCUSSION

The controversy over vertebrate posterior body formation has centered on whether it forms by a developmental mechanism that is distinct from gastrulation, which is responsible for the

formation of the anterior body (Holmdahl, 1925; Pasteels, 1943; Griffith et al., 1992; Gont et al., 1993; Catala et al., 1995). As in many such arguments, the point of view is subjective depending upon whether one is searching for similarities versus differences, and on whether characteristics are compared in general terms or in specific detail. The methods of analysis also play an important role in these arguments; for example, the early comparisons of anterior versus posterior development were limited to morphological criteria (Holmdahl, 1925; Pasteels, 1943) without the benefit of molecular probes (Gont et al., 1993) or cell imaging technology (Kimmel and Warga, 1988; Schoenwolf and Sheard, 1990). Advances in both of these areas have contributed much to our understanding of both anterior and posterior development; however, the distinction of these two processes can still be debated. The formation of the zebrafish posterior body exhibits developmental processes in support of both sides of this argument in that it shows the continuation of some gastrulation processes but also displays several distinct properties that are unique to this region.

#### A comparison of the vertebrate 'tailbud'

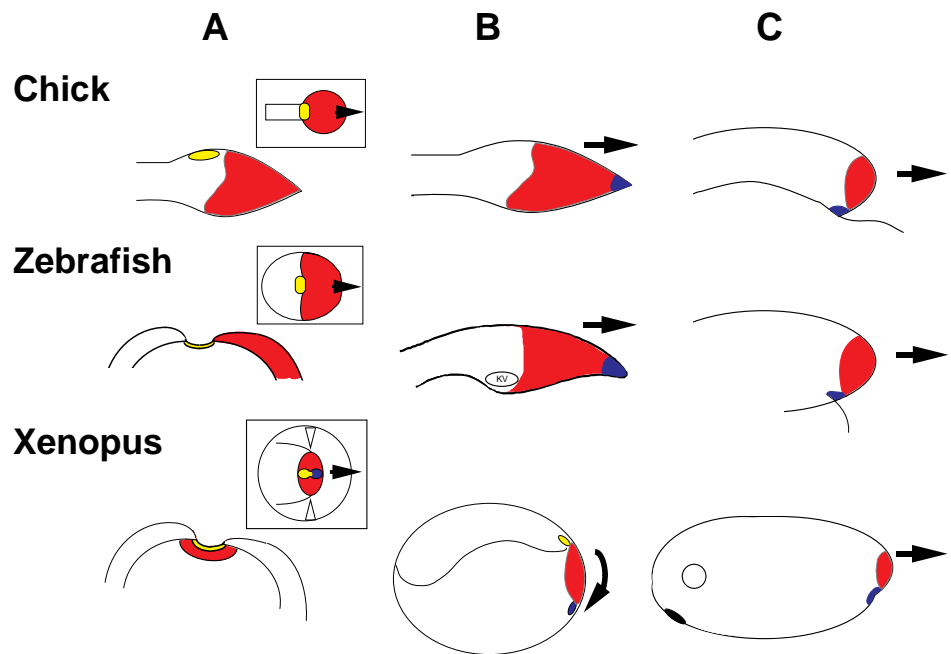
The term 'tailbud', which is defined morphologically as a protruding aggregate of cells at the posterior end of the embryo, may serve as a source of confusion as one compares this structure across vertebrate species. 'Tailbud' implies that this structure gives rise to tail tissues whose anterior limit is generally defined by the proctodeum anlage, the future anus (Schoenwolf, 1977, 1978; Tam, 1984). In *Xenopus*, the 'tail-forming region' and the 'tailbud' are distinct, since the proctodeum moves relatively anterior as the tail extends. Since the relative position of the proctodeum changes through development, the region considered the 'tail' also changes. This causes



cells anterior to the 'tailbud', initially in regions of the posterior trunk, to be considered part of the 'tail' at later stages (Tucker and Slack, 1995). In zebrafish the 'tailbud' not only gives rise to tail tissues but also to posterior trunk tissue anterior to the anus. The anus in the zebrafish is located at the level of somite 17; however the tailbud contributes to posterior trunk tissues as far anterior as somite 11-12. Therefore a more appropriate term for the mound of tissue formed over the blastopore may be 'endbud' rather than 'tailbud' due to its contribution to posterior trunk structures (Spratt, 1947; Schoenwolf, 1977). This 'endbud' resembles the aggregate of cells observed in both the chick (HH stage 11-12: Hamburger and Hamilton, 1951) and the mouse (8.5 days postcoitum; d.p.c.) that forms at the end of node regression when it reaches the posterior end of the primitive streak (Spratt, 1947; Tam, 1984). In these animals, as in the zebrafish, this 'endbud' is composed of cells derived from regions both anterior and posterior to the node (comparable to the zebrafish blastopore closure) and contributes to tissues of the posterior trunk as this cell aggregate extends further caudad (Fig. 9A,B). The 'tailbud' stage in the chick and the mouse (HH stage 15 and 10-10.5 d.p.c. respectively) is characterized by the fusion of ectoderm and mesendoderm at the posterior tip forming the future cloacal membrane/proctodeum anlage, the formation of the posterior or tail folds, and the protrusion of the embryo's posterior end (Hamburger and Hamilton, 1951; Hogan et al., 1994). The 'tailbud' stage in these animals correlates best with the stage of tailbud protrusion and the beginning of tail eversion in the zebrafish (Fig. 1D,E). During these stages, the posterior end of the endbud stops extending along the yolk cell, demarcating the future proctodeal region, the lateral furrows of the hindyolk begin to form and the cells within this posterior aggregate generally give rise to postanal tissues. Therefore the 'tailbud' stage described in the chick and the mouse may be more comparable to the stages of tailbud protrusion and tail eversion in the zebrafish (Fig. 9C).

In amphibians, a morphologically distinct 'tailbud' forms at relatively late stages (stage 27) following the end of neurulation rather than gastrulation (Nieuwkoop and Faber, 1967; Elsdale and Davidson, 1983; Tucker and Slack, 1995). The inability to distinguish this structure earlier may be due to the relatively spherical shape of the embryo versus other vertebrates that have much flatter configurations. In chick, mouse and

zebrafish, the proctodeum anlage forms at the posterior tip of the tailbud. At tail eversion, the tailbud cells form a recumbent fold over this ventroposterior region and begin to protrude posteriorly from the embryo. In amphibians, the proctodeum anlage must move a relatively great distance around the posterior end of the embryo in order to reach its final ventral location, which may account for the longer times necessary to distinguish an actual protruding 'tailbud'. In amphibians, the 'tailbud' is derived from tissue exclusively anterior and/or lateral to the blastopore (stage 12-13), seemingly without any contributions from cells further posterior (Spofford, 1945; Smithberg, 1954; Gont et al., 1993; Tucker and Slack, 1995). However, in *Rana* and *Xenopus*, the lateral walls of the circumblastoporal collar, or slit, come together over the blastopore forming the neurenteric canal with openings at both its anterior and posterior ends (Pasteels, 1943; Gont et al., 1993). The anterior opening represents the position of the posterior neuropore while the posterior opening will give rise to the proctodeum anlage (Fig. 9A). The ventral movement of the proctodeum anlage results in the shifting of these lateral walls to the posterior end of the embryo forming the posterior wall.



**Fig. 9.** Tail development across vertebrate species. (A-C) Comparable periods of tail development are shown for three vertebrate species showing relative cell movements (arrows) and posterior domains that contribute to paraxial mesoderm (see text). Lateral views of the end of gastrulation are shown in A where the blastoderm margin closes over the yolk plug in zebrafish (10-11 h.p.f.) and *Xenopus* (stage 12-13), and the node ends its regression in the chick (HH 11-12). The blastopore closure and chick node are shown in yellow. The red regions indicate domains that will contribute cells to posterior somites. The inset illustrations show smaller dorsal views of these regions with the solid arrows indicating the direction of posterior extension towards the right. In *Xenopus*, the lateral walls of the blastopore fold upwards (open arrowheads) and fuse leaving openings at its anterior (yellow) and posterior end (blue). The blue indicates regions that will give rise to the proctodeum anlage. In B this region moves ventrally around the end of the *Xenopus* embryo (stage 13-27), while in chick (HH 12-15) and zebrafish (12-17 h.p.f.) this region extends posteriorly at the tip of the 'endbud'. In all cases, posterior extension is to the right and dorsal is upwards. In C all three species exhibit tail eversion. Due to the arrested movement and relatively fixed ventral position of the proctodeal precursors, cells extend posteriorly over this region of the future anus causing the tail to evert. Tail eversion corresponds to developmental stages following: stage HH15 in the chick, 17 h.p.f. for zebrafish, and stage 27 for *Xenopus*.

At this stage, the amphibian tail-forming region has a configuration with its tissues composed of cells both anterior and posterior to the posterior neuropore, with the proctodeum anlage at its posterior end (Fig. 9C). If the amphibian embryo were flattened out at this stage, the tailbud would resemble that of other vertebrates such as lamprey, zebrafish, chick and, probably, mouse (Spofford, 1945; Romer, 1970; Nakao and Ishizawa, 1984; Catala et al., 1995).

The zebrafish tailbud is not a homogeneous blastema, but contains domains that are restricted with respect to cell fate as well as gene expression (Joly et al., 1992; Schulte-Merker et al., 1992; Gont et al., 1993; Joly et al., 1993; Thisse et al., 1993; Hammerschmidt and Nusslein-Volhard, 1993; Talbot et al., 1995). Much of the zebrafish tailbud fate map is remarkably consistent with cell lineage and gene expression data on the tailbud of other vertebrates (Schoenwolf, 1977; Gont et al., 1993; Catala et al., 1995). However, the optical clarity of the zebrafish embryo makes it an ideal system for the direct observation of labeled cell movements as well as fate mapping analysis. This is crucial for understanding how the cells within lineage-restricted domains arrive at their final tissue locations (Fig. 7C).

### The notochord extends into the tail

The axial mesoderm of the posterior zebrafish arises from cells in the ventromedial region of the anterior tailbud. Cells in more dorsal positions of the epiblast do not give rise to notochord suggesting that ingression/involution does not contribute to axial tissues in the tail and thus the formation of the posterior notochord may be due to the posterior extension of notochord precursors formed during gastrulation. The lack of other tailbud cells contributing to posterior axial mesoderm has also been reported in chick and *Xenopus* (SeEVERS, 1932; Schoenwolf, 1977; Gont et al., 1993). The mechanism of posterior notochord extension may be due to the differentiation of notochord cells, which become large and cuboidal due to vacuolation, and processes involving cell rearrangement and intercalation, as described in the chick and the mouse (Sausedo and Schoenwolf, 1993, 1994). In addition, the cells within the ventromedial region of the tailbud only give rise to notochord, indicating that these cells do not generally intermix with other tailbud cells or exit this region to contribute to other tissues, such as paraxial mesoderm. The lack of ingression/involution movements in the formation of the posterior axial mesoderm distinguishes this process from anterior axial mesoderm formation during gastrulation.

### The formation of paraxial mesoderm and the posterior tailbud domain

Paraxial mesodermal tissue arises from both the anterior and posterior tailbud; however, the cell movements associated with its formation are different in these two regions. Some cells within dorsal regions of the anterior tailbud, colocalizing with spinal cord precursors, continue to move into the hypoblast throughout tail development. The ventral movements of these cells may be a continuation of the ingression/involution cell movements seen during gastrulation (Warga and Kimmel, 1990; Shih and Fraser, 1995). However, unlike gastrulation, these ingressing cells only move to ventrolateral positions, circumventing the ventromedial region, which will become notochord. Mixed populations of neural and muscle precursors

are also observed in the chick tailbud suggesting the continuation of gastrulation movements (Catala et al., 1995). However, involution movements are reported to stop prior to tail elongation in *Xenopus* (Gont et al., 1993).

The cells in the posterior domain of the tailbud are restricted to paraxial mesodermal fates and may correspond to the cells in comparable posterior tailbud regions of other vertebrates, which also give rise to posterior somites (Spofford, 1945; Smithberg, 1954; Catala et al., 1995). In amphibians, the question of how somitic muscle is derived from the posterior neural plate has puzzled embryologists for many years (Spofford, 1945; Smithberg, 1954; Woodland and Jones, 1988). In *Rana* and *Xenopus*, this region is lateral to the blastopore (Fig. 9A), forming the lateral lips of the circumblastoporal collar which eventually becomes the posterior wall of the embryo (Smithberg, 1954; Gont et al., 1993). This region correlates with the posterior end of the tailbud, anterior to the proctodeum anlage, and gives rise to posterior somites as in chick (Catala et al., 1995) and zebrafish.

The ventral-derived cells of the posterior tailbud undergo cell movements that are unique to the tailbud. Subduction is responsible for these cells moving collectively into positions beneath the epiblast layer of the dorsal-derived cells. This novel cell movement results in all of the posterior tailbud cells, even those originally in the superficial regions, assuming positions in the hypoblast layers of the extending tail and developing into paraxial mesoderm. Whether movements similar to subduction occur in the posterior tailbud of other vertebrates is unknown, although this type of process could explain how the comparable region of the posterior chick tailbud, and the posterior neural plate in amphibians, gives rise to posterior somites (Spofford, 1945; Smithberg, 1954; Catala et al., 1995).

Preliminary experiments addressing the commitment of the posterior tailbud cells to their paraxial mesodermal fates have been initiated (data not shown). Although specific domains of the tailbud are restricted to certain cell lineages, their commitment to these fates cannot be assumed a priori. Posterior tailbud cells were transplanted isochronically into dorsomedial regions in the anterior tailbud of host embryos immediately following tailbud formation. In a number of cases, these transplanted cells developed into neurons indicating that, at this stage of development, at least some of the posterior tailbud cells are not yet committed to paraxial mesodermal fates.

### The formation of the posterior spinal cord

The zebrafish spinal cord was derived exclusively from dorsal cells in the epiblast of the anterior tailbud. Since posterior tailbud cells do not contribute to neural tissues, the spinal cord precursor cells in the anterior tailbud, like the notochord precursors, must become distributed along the entire length of the developed tail. Their posterior movements may in part be explained by the proliferation of cells in dorsomedial regions as well as the intercalation of cells associated with convergence. Convergence movements begin during gastrulation and continue into posterior development. Presumptive spinal cord cells in dorsolateral positions move medially forming the neural keel (Schmitz et al., 1993; Papan and Campos-Ortega, 1994), and intercalation of these cells contributes to the A-P extension of the embryo (see below).

Although the posterior spinal cord of the zebrafish was

derived from the anterior tailbud, cell transplantation experiments in the chick show that spinal cord tissue can be derived from the posterior tailbud (Schoenwolf, 1977; Catala et al., 1995). One possible explanation for this apparent difference between chick and zebrafish may involve the differential posterior movement of the dorsomedial cells. Cell transplantation experiments in the chick were performed as the tailbud was beginning to evert (HH stage 15). During the stage of zebrafish tailbud protrusion and eversion, the cells in the dorsomedial region of the anterior tailbud (spinal cord precursors) are seen to move caudally over the posterior tailbud cells. This rearrangement of cells places the spinal cord precursors into more caudal regions of the tailbud, which would be consistent with the transplantation results from the chick tailbud at the comparable stage of tail development. Evidence for the preferential caudal movement of spinal cord precursors relative to paraxial mesoderm has been reported in the chick (Gaertner, 1949; Schoenwolf, 1977; Catala et al., 1995). It is possible that this type of cell rearrangement during the eversion of the chick tailbud could account for the distribution of neural precursors into the posterior tailbud of the chick at this stage.

### Mechanisms of tail extension

The caudal movement of cells in the dorsomedial region of the tailbud plays a role in several aspects of zebrafish tail development. The stronger posterior movement of dorsomedial cells is responsible for their caudal distribution relative to cells in more lateral and ventral positions and may implicate this region as a driving force in the process of tail elongation. The caudal displacement of dorsomedial spinal cord cells relative to ventrolateral muscle cells may be a conserved feature of posterior development (Gaertner, 1949; Schoenwolf, 1977; Catala et al., 1995). Preferential dorsomedial extension may also contribute to the initial ventrad curvature of the everting tail, perhaps in conjunction with the arrest of posteriorly moving ventral cells, the proctodeal precursors, at the caudal tip of the tailbud. After tail eversion, cells in ventral regions continue to move caudally; however, the cells that are close to the proctodeum anlage remain in this location at the end of the future hind yolk tube.

Cell rearrangements, such as the convergence of cells from relatively lateral positions towards the midline, and their subsequent intercalation, contributes to the posterior extension of the tailbud (Schoenwolf and Alvarez, 1989). The convergence and intercalation of cells within the tailbud occurs until it reaches the point of tail eversion. Tail eversion brings about the physical separation of the distal tail from the yolk cell, which prevents converging cells along the yolk ball from continuing to contribute directly to posterior tail tissues. Convergence within the everted tail probably does not contribute significantly to tail extension since the overall diameter of the everting tail does not appear to decrease as it extends (data not shown). However, rostral to the point of tail eversion, the convergence and intercalation of cells along the yolk ball may continue to contribute to posterior tail extension. These observations suggest that convergent-extension is either reduced or not occurring within the tail once it has everted off of the yolk cell.

The reduced convergence of cells within the everted tail may suggest a possible reason for the different forms of neurulation observed in the anterior and posterior region of the chick and

the mouse (Criley, 1969; Schoenwolf, 1984). In the chick and the mouse, the anterior neural tube develops by the formation of neural folds and their subsequent dorsal closure over the neural groove, while the posterior neural tube forms by cavitation. It is possible that the mechanism of anterior neural tube formation relies upon convergent cell movements while cavitation does not. In the zebrafish, the entire neurocoel forms by cavitation in an anteroposterior direction, at much later stages (17-18 h.p.f.; Schmitz et al., 1993), when convergent cell movements are significantly reduced in the spinal cord. Cavitation may therefore provide a mechanism for the generation of the neural tube in zebrafish, and the posterior neural tube in cases of secondary neurulation, in the absence of convergence.

Different rates of cell proliferation within subregions of the tailbud may also contribute to tail elongation. Cell proliferation at the posterior tip of the zebrafish tailbud is relatively low suggesting that, like other vertebrate species, the tail does not extend by the addition of cells at its posterior end (Pasteels, 1943; Gaertner, 1949; Schoenwolf, 1977; Mills and Bellairs, 1989). However, the relatively higher rate of mitoses in the dorsomedial region of the anterior tailbud, corresponding to presumptive spinal cord, may contribute to the stronger posterior movement of cells in this domain and their placement into relatively caudal tail positions during tail migration and eversion (Fig. 8A,B).

The differentiation and growth of cells within the developing tail contribute to the elongation of the tail. Cellular differentiation occurs in an anterior-to-posterior direction, such that the proximal cells in the tail develop before the cells in more distal regions. The vacuolation and rearrangement of presumptive notochord cells are in part responsible for the posterior extension of this axial structure (Keller and Tibbetts, 1989; Sausedo and Schoenwolf, 1993, 1994). The development and extension of the notochord make it a very good candidate for participating in the mechanism of tail elongation. This is consistent with the *no tail* zebrafish mutant, which lacks a notochord and also exhibits a severely reduced tail (Halpern et al., 1993). However, another zebrafish mutant called *floating head* has recently been isolated that also lacks a notochord, yet its tail at 24 hours exhibits relatively normal extension (Talbot et al., 1995; Halpern et al., 1995). Therefore it is unlikely that the developing notochord is solely responsible for the elongation of the tail.

### Medial-lateral cell movements in the tailbud

An interesting change in the directionality of cell movements takes place during posterior body development. Once the tailbud is formed, cell movements containing laterad components that diverge away from the midline begin to be observed, in addition to the continued mediad movements of convergence seen during gastrulation. The laterad movement of posterior tailbud cells circumventing the posterior-advancing cells of the anterior tailbud demonstrates divergent cell movements away from the midline of the tailbud. Divergent cell movements are also observed as cells move laterally out of the flow of posterior-moving dorsomedial regions (Fig. 7C). The bilateral distribution of clones originating in medial positions of the tailbud are the result of these divergent cell movements. The bilateral distribution of clones was not observed in gastrula fate map studies of anterior body development (Kimmel and Warga, 1987) and may therefore reflect the initiation of divergent cell

movements during posterior development. Although most bilateral clones were initially located in medial positions, cells were observed to converge from further lateral positions into the midline before diverging from one another and giving rise to bilateral progeny (data not shown).

There is no strict correlation between a cell's A-P level within the tailbud and its eventual A-P level within the developed tail. This is in part due to the unique movements of the ventral-derived cells in the posterior tailbud (Fig. 7C), but also to the continuation of convergence during tailbud extension. Although some caudally moving cells in relatively dorsomedial regions move laterally and exit from this posterior flow of cells, other cells continue to move medially due to convergence. Convergence can cause cells to enter into dorsomedial regions and potentially travel further caudal than cells that are initially situated relatively posterior to them. Thus, although a correlation of A-P relationships is generally maintained over the entire tailbud, local reversals of cell positions with respect to A-P levels can occur due to convergence and the relative position of cells with respect to the dorsomedial region of posterior cell movement (see Fig. 5).

### Implications of distinct domains within the developing tailbud

An intriguing observation is that the original boundary between the dorsal- and ventral-derived tissue over the blastopore appears to be maintained through tail development. No morphological distinction between these two domains can be detected yet they are clearly distinct with respect to cell movements, cell fates and gene expression. The subduction of ventral-derived cells and the movement of dorsal-derived cells into the hypoblast appear to occur at this cryptic boundary suggesting that these cells continue to recognize the borders of their respective gastrula-stage marginal zones throughout tail development. Several zebrafish homologs of genes such as brachyury (T) (Schulte-Merker et al., 1994), snail (Thisse et al., 1993) and *eve1* (Joly et al., 1993; Barro et al., 1995) are expressed exclusively in the posterior domain of the tailbud, consistent with their association with presumptive mesodermal fates. The expression of the T gene has been shown to result in changes at the cell surface affecting cell adhesion and migration (Chesley, 1935; Wilson et al., 1995). Thus the lack of cell mixing between the posterior tailbud cells and the epiblast of the anterior tailbud cells may reflect differences in cell adhesion/repulsion properties at the surface of these cells and correlate to domain-specific gene expression. Interestingly, some of the dorsal-derived cells from the anterior tailbud must be capable of altering these cell surface properties when they ingress and mix with the ventral-derived cells in paraxial mesodermal tissues. Furthermore, preliminary experiments transplanting posterior tailbud cells into anterior tailbud regions indicate that these cells are not committed to mesodermal fates despite their initial expression of these genes.

The correlation of gene expression domains within the tailbud to cell movements and eventual tissue fates is currently in progress. This analysis is crucial since gene expression patterns can be transient during development and do not necessarily indicate cell movement or commitment to specific cell lineages. The correlation between the movements of cells and the expression of developmentally relevant genes is necessary

before addressing potential inductive processes in the tail and their comparison with gastrulation. Furthermore, the examination of cell movements, lineage and gene expression in mutant zebrafish lines exhibiting posterior defects will certainly contribute to our understanding of how the posterior vertebrate body develops.

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