An atlas of notochord and somite morphogenesis in several anuran and urodelean amphibians

By B. WOO YOUN, R. E. KELLER and G. M. MALACINSKI

From the Program in Cellular, Molecular and Developmental Biology, Department of Biology, Indiana University

SUMMARY

A scanning electron microscopic, comparative survey of notochord and somite formation including some details of change in cell morphology and arrangement, was made of selected stages of two species of anuran amphibians (Xenopus laevis and Rana pipiens) and two species of urodeles (Ambystoma mexicanum and Pleurodeles waltlii). The ectoderm or neural plate was removed from fixed embryos and the dorsal aspect of the developing notochord and somite mesoderm was photographed. Micrographs of comparable stages of all species were arranged together to form an atlas of notochord and somite formation. Similar morphogenetic events occur in the same sequence in the four species. Notochordal cells become distinguishable from paraxial mesodermal cells by shape, closeness of packing, and arrangement. Notochordal elongation is accompanied by a decrease in cross-sectional area and by cell rearrangement. Somitic mesoderm becomes distinguished from lateral mesoderm by a change in cell shape and orientation, followed by segmentation of somites. The schedule of somite formation was compared and related to the staging series for each species. The urodeles differ from the anurans in that the notochordal region in the early neurula stages is triangular, with the broadest part in the posterior region of the embryo. In anurans it is uniform in width. This difference may reflect differences in gastrulation and in the mechanism of elongation of the posterior part of the embryo in the neurula.

INTRODUCTION

During amphibian morphogenesis, the mesoderm is believed to originate epigenetically from the animal half of the blastula under an inductive influence emanating from the yolky endodermal mass (Nieuwkoop, 1969; Malacinski, Chung & Asashima, 1980). The newly induced mesoderm plays a leading part in further development: it induces the formation of a nervous system in the overlying ectoderm (Spemann, 1938) and develops into various organs and tissues (e.g. notochord and somites). The changes in morphology and the arrangement of the mesodermal cells during gastrulation and subsequent chordamesoderm differentiation have been the subject of extensive studies. Recently, the scanning electron microscope (SEM) has been employed to investigate some of the

1 Author’s address: Program in Cellular, Molecular and Developmental Biology, Department of Biology, Indiana University, Bloomington, Indiana 47401, U.S.A.
details of those changes. Nakatsuji (1975), and Keller & Schoenwolf (1977
described the nature of internal mesodermal cell movements during gastrulation
in terms of changes in cellular morphology and cell–cell contacts. Some pre-
liminary SEM examinations of segmented somites have also been made as
part of a study of the process of early myogenesis (Kordylewski, 1978) and to
elucidate the mechanism of somite segmentation after heat-shock (Pearson &
Elsdale, 1979; Elsdale & Pearson, 1979). However, a thorough, systematic
study of notochord and somite morphogenesis during amphibian development
has not yet been reported, probably due in part to the technical difficulty of
separating ectodermal tissue from the underlying mesoderm. Progress in this
phase of amphibian morphogenesis has awaited the development of improved
techniques for fracturing or dissecting embryos (see Tarin, 1971; Keller &
Schoenwolf, 1977) and examining internal surfaces. In the chick embryo, the
processes of notochord (Bancroft & Bellairs, 1976) and somite (Bellairs, 1979;
Meier, 1979) formation have already been studied with fractured embryos and
the SEM. These studies have provided insight into the temporal sequence of
events associated with primary axis development (see Discussion).

We have succeeded in improving a technique for detaching the overlying
ectoderm from the mesoderm and examining the process of somite and noto-
chord morphogenesis by SEM of these ‘peeled’ and fractured embryos. These
results are summarized here in the form of an ‘atlas’ of notochord and somite
morphogenesis at several developmental stages (early gastrula to tailbud) of
four amphibian species – two anurans (Xenopus laevis and Rana pipiens) and
two urodèles (Ambystoma mexicanum and Pleurodeles waltlii). The results of
this analysis should, therefore, be representative of the most widely used
laboratory amphibia.

The atlas provides novel observations on the earliest developmental stages
at which notochordal and presomitic mesoderm differentiation can be recognized
morphologically. In addition, the comparative analyses indicate that major
differences exist between anurans and urodèles in the pattern of notochord
formation. These observations should be helpful in guiding future studies
directed at the differentiation and function of amphibian mesodermal tissues and
organs.

MATERIALS AND METHODS

Source, handling and staging of embryos: Xenopus laevis embryos were
obtained by gonadotrophic-hormone-induced mating of adults (Gurdon,
1967). The embryos were staged according to the Nieuwkoop & Faber (1967)
series. Embryos were chemically dejellied in a 2% cysteine–HCl solution
(pH 7.4 with Tris buffer) and allowed to develop in dechlorinated tap water
(DTW).

Rana pipiens females were induced to ovulate by injection of minced pituitary
glands and progesterone. Eggs were artificially inseminated according to the
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methods outlined by DiBerardino (1967). Manually dejellied embryos were reared in DTW, and staged according to Shumway (1940, 1942).

Ambystoma mexicanum and Pleurodeles waltlii eggs were collected from natural spawnings, and kept in DTW until the embryos to be examined were selected at appropriate stages of development. Jelly layers were removed manually, and the staging of embryos followed Harrison (1969) for Ambystoma mexicanum, and Gallien & Durocher (1957) for Pleurodeles waltlii.

Preparation for SEM: Embryos were fixed and processed for SEM with the methods described by Keller & Schoenwolf (1977). Briefly, embryos were fixed overnight in 2% glutaraldehyde (0.1 M cacodylate buffer, pH 7.6) and washed in the same buffer for 24 h. The ectoderm, neural ectoderm, or epidermis (depending on the area and developmental stage) was separated from the mesoderm and endoderm using a fine steel knife and forceps. In some cases, in order to expose the cells of the interior, the embryos were broken into halves near the mid-sagittal plane or the mid-transverse plane. The dissected embryos were dehydrated in increasing concentrations of ethanol and infiltrated with amyl acetate. Embryos obtained from three to five spawnings were employed and 10–20 embryos at various developmental stages were dissected and processed for SEM.

All specimens were then critical-point dried using liquid CO₂. They were mounted on aluminium stubs with conducting silver paint, and coated with gold–palladium (60:40) in a Denton 503 vacuum evaporator. Specimens were observed with an Etec Autoscan U-1 scanning electron microscope and photographed on Polaroid Type 55 positive–negative film.

RESULTS

At each developmental stage, the morphology of the notochord and somite will be described and illustrated with scanning electron micrographs. In order to make the comparative analysis easily understood, the photographs are arranged so that the sequential differentiation pattern of the notochord and the somite segmentation pattern can be examined at comparable stages in four species (see Fig. 1). Due to the use of different staging series for each species, it was difficult to assign a meaningful stage designation to each group of embryos. Three main phases of development were, therefore, analysed: early to late gastrula; neural plate to neural fold; and neural groove to neural tube. Each main phase was further divided into four subphases in which distinctive morphological changes in the notochord and the somite could be recognized. A comparative table is shown which relates the stage numbering of various normal tables to the subphases assigned here to each main phase in the four different species (Table 1). As the results will demonstrate, similar morphogenetic events appeared to take place in all four species of embryo in approximately the same sequence.
Table 1. Comparative table of anuran and urodele normal tables

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<td>11^+–12</td>
<td>12–12^+</td>
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<td>Ambystoma</td>
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<td>12–12^+</td>
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<tr>
<td>Pleurodeles</td>
<td>10–11</td>
<td>11–11^+</td>
<td>12–12^+</td>
</tr>
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</table>

(a) The same morphogenetic events seem to occur in phases I and III of Xenopus and Ambystoma, in terms of the blastoporal lip formation and the neural-tube closure. Therefore, the stage numberings are similar in both species in these phases. However, different stage numberings occur in phase II, since the timing of neural-fold rising appears to be different between the two species. In Xenopus, the neural folds begin to rise earlier than in Ambystoma, and the morphology of the neural folds is not as clearly defined as in Ambystoma.

(b) Stage designations were given according to the comparative tables of Nieuwkoop & Faber (1967) for Rana and of Gallien & Durocher (1957) for Pleurodeles.
The notochord forms in a progressive fashion in a postero-anterior direction along the midline of the embryo. Photographs of early- to late-gastrula stage embryos were therefore taken from a postero-dorsal view in order to best show the structure of the posterior part of the involuting mesoderm. Once the anterior tip of the notochord is laid down in the head mesenchyme area at the neural plate stage, the somites begin to undergo a sequential segmentation process in an antero-posterior direction on each side of the notochord. Accordingly, photographs of neural plate and latei stage embryos were taken from a dorsal view to best reveal this process.

It was discovered that, of the four amphibian species studied, *Xenopus laevis* provides the best material for examining cellular morphology and arrangement during early notochord and somite morphogenesis. Anuran mesoderm in general, especially *Xenopus laevis* mesoderm, provides the best material for examining cellular morphology and arrangement during early notochord and somite morphogenesis. Anuran ectoderm was more easily peeled off and the cellular morphology, contacts and arrangement in the mesoderm were more clearly seen with the SEM than in the urodele species. Difficulty was encountered in detaching the ectoderm from the underlying mesoderm of the early urodele embryos. Even the best specimens displayed considerable amounts of broken cells and debris on the surface of the involuting mesoderm, especially in the notochord area (see Fig. 1). In urodele embryos these two layers are probably more tightly attached to one another. Therefore, most of the observations on details of changes in cell shape and arrangement were made on *Xenopus* embryos.

I. Early- to late-gastrula stages

Figure 1 displays a developmental sequence depicting the comparative features of the morphological differentiation pattern of the involuting mesoderm during late gastrulation. The notochord is the first mesodermal organ to be morphologically defined in all four species of amphibian embryos. The first sign of its differentiation appears at stage 11+–12 in all species. In the anuran embryos, the notochord is characterized by closer packing and fewer intercellular spaces, particularly in *Xenopus* (see arrow at stage 11+–12 of *Xenopus*, Fig. 1). These differences are subtle, and the boundary between the prospective notochord and the paraxial mesoderm is vague at this stage. The notochord region in the urodele embryos, on the other hand, is defined somewhat earlier and more definitely. It appears primarily as a region in which debris resulting from cellular breakage is prominent, possibly because of its tighter attachment to the removed ectoderm (see pointer at stage 12–12+ of *Ambystoma*, Fig. 1). The urodele notochordal area appears to be depressed below the level of the adjacent paraxial mesoderm. This trough is not caused by fixation or processing, since it is also observed in the living embryo.

At stage 12–12+, the anuran notochord becomes separated from the paraxial mesoderm in the posterior region, while in the urodele it remains in its previous
Fig. 1. An atlas of notochord development from early- to late-gastrula stages as seen in postero-dorsal view. Diagrammatic sketch in each subphase shows the progress made by the blastopore of *Ambystoma* gastrulae viewed ventrally and externally. Approximate staging numbers are also indicated above the sketches. Arrow at stage 11*-12* of *Xenopus* shows tight packing of presumptive notochordal cells. Pointer at stage 12-12+ of *Ambystoma* indicates broken cell debris in the notochord region. Bars represent 0.2 mm for *Xenopus*, and 0.4 mm for *Rana, Ambystoma* and *Pleurodeles*. 
configuration. With further development (stage 12+−13), the notochord becomes elongated in the postero-anterior direction and is morphologically clearly separated from the adjacent mesoderm. The anuran notochord is more or less uniform along its length, while the urodele notochord tapers gently from its widest extent posteriorly to its narrowest extent anteriorly (stage 12+−13, Fig. 1 and stage 13+−14, Fig. 3). The importance of this difference will be discussed later (see Discussion).

Higher-magnification micrographs in *Xenopus* show that notochordal cells are flatter and more tightly packed than those of the paraxial mesoderm (Fig. 2a−c). Notochordal and paraxial mesodermal cells both have numerous protrusions and points of apparent intercellular contact. Notochordal cells, however, are not separated by large intercellular spaces, and they have numerous lamellar protrusions which are closely applied over large areas of neighbouring cells (see arrows, Fig. 2b). In contrast, paraxial cells are separated by large intercellular spaces and are connected by filiform protrusions (see pointer, Fig. 2c). The internal structure of the notochord of *Xenopus* late gastrulae (stage 12+−13) is shown in embryos fractured transversely about midway along the notochord (Fig. 2d), or fractured sagittally along the side of the notochord (Fig. 2e). It appears to consist of approximately four layers of cells which are rather irregular in shape but perhaps are elongated and oriented dorso-ventrally (Fig. 2e). In contrast, the paraxial mesoderm is double-layered and composed of polyhedral cells, which are perhaps slightly greater in height than width (Fig. 2d). It should be noted that a continuous layer of endoderm underlies the notochord of *Xenopus* (see pointers, Fig. 2e).

II. Neural-plate to neural-fold stage

As development proceeds beyond gastrulation, the borders of the neural plate begin to elevate to form the neural folds, which eventually bend medially to form a trough – the neural groove. Figure 3 displays the further morphological differentiation of the notochord and the somites during neural-fold elevation. In all species, the notochord continues to elongate, and at about stage 13+−14 (13−13+ in *Rana pipiens*) its anterior tip lies in the head mesenchyme region (see pointer at stage 13+−14 of *Xenopus*, Fig. 3). The anuran notochord is rod-like, except at its anterior region where it broadens slightly, and the lateral edges become distinct from the adjacent head mesenchyme. In contrast, the paraxial mesoderm is double-layered and composed of polyhedral cells, which are perhaps slightly greater in height than width (Fig. 2d). It should be noted that a continuous layer of endoderm underlies the notochord of *Xenopus* (see pointers, Fig. 2e).
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The micrographs in Fig. 3 also demonstrate that somite segmentation begins to occur as early as at stage 13+–15 (13+–14 in Rana). The appearance of deep clefts along the paraxial mesoderm indicates that somite segmentation is in progress. The first somite always seems to form in the mid-trunk region, which corresponds to the narrowest part of the elevating neural folds. Additional somites are sequentially added in the posterior direction. At stage 16, the segmentation of the first two somites of *Xenopus* is visible by the formation of mediolateral grooves (see pointers in Fig. 4). The third somite segmentation process is being undertaken as indicated by the faint groove formation (see arrow in Fig. 4).

Even prior to the initiation of somite segmentation, differences in cell shape arise at about stage 12+ which distinguish the presomitic cells from the lateral mesodermal cells (Fig. 5). The surfaces of presomitic mesodermal cells bounding the ectoderm appear to be smaller and more elongated dorso-ventrally than those in the lateral mesoderm (compare Figs. 5a and 5b). These differences become more accentuated at stage 13+: the elongated shape and mediolateral orientation of presomitic cells in *Xenopus* is particularly evident (Figs. 5a and 5d). In contrast, no major changes in morphology or arrangement were observed in the lateral mesoderm cells (compare Figs. 5b and 5d) during these developmental stages (from stage 12+ to stage 13+).

A micrograph of a stage-13+ *Xenopus* embryo fractured transversely approximately midway along the length of the notochord (Fig. 6) shows significant changes that have taken place within the notochord since stage 12+ (compare Fig. 2d). In cross section it appears smaller in area and consists of fewer cells. It is usually two cells in width and approximately four cells in height. The upper and lower cells exhibit a tendency to be triangular shaped with their apices lying centrally and their bases lying at the periphery of the notochord. The cells of the middle layers are variable in shape in the cross-sectional view. All the cells appearing in the cross sections appear to be flattened. A side view of the mid-body region of the notochord at stage 13+ (Fig. 7a) shows these cells to extend a relatively short distance in the antero-posterior direction and much

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Fig. 2a. Dorsal view of involuted mesoderm of a stage-12 *Xenopus* gastrula showing the beginning of notochord formation (centre). Bar represents 50 μm.
Fig. 2b. Higher magnification view of the notochord region in Fig. 2a. The close packing of notochord cells and the prevalence of broad, flattened protrusions (arrows) are shown. Bar represents 10 μm.
Fig. 2c. Higher magnification view of the paraxial mesoderm in Fig. 2a showing the prevalence of larger intercellular spaces and filiform protrusions (pointer). Bar represents 10 μm.
Fig. 2d. Cross-sectional view of stage 12+ *Xenopus* gastrula, showing the cell morphology and arrangement of the notochord (centre) and paraxial mesoderm. Bar represents 50 μm.
Fig. 2e. Lateral surface of the *Xenopus* notochord at stage 12+. Endodermal roof of the archenteron (E) is also shown below pointers. Bar represents 50 μm.
Fig. 3. An atlas of notochord and somite development from the early neural plate to the neural-fold stage in dorsal view. Diagrammatic sketch in each subphase displays the progress made by the neural folds of Ambystoma early neurulae viewed antero-dorsally. Approximate staging numbers are given above each sketch. Pointer at stage 13+–14 of Xenopus indicates the anterior tip of the notochord lying in the head mesenchyme. Also shown is the typical morphology of the urodele notochord at stage 13+–14 of Ambystoma, which is triangular posteriorly (pointer), long and narrow in the middle (thin arrow), and broadens anteriorly (thick arrow). Staging numbers of Rana are indicated in parentheses. Bars represent 0.4 mm.
farther in the dorso-ventral direction. Thus these cells are indeed flattened in the antero-posterior direction and are arranged in a somewhat radial array in the cross-sectional aspect of the notochord. Similar observations have been reported for the chick embryo by Bancroft & Bellairs (1976). Numerous small cellular protrusions and contacts are found between notochordal cells (see pointers, Fig. 7a). No intercellular spaces are obvious, as was the case in the earlier stages (see Fig. 2d). As previously reported (Hamilton, 1969), the paraxial mesoderm appears to be double-layered, except where the upper and lower layers meet in the middle (Fig. 2d).

At approximately stage 14+ of Xenopus, the number of cell layers (four) along the side of the notochord seems to remain constant. A considerable amount of extracellular material surrounds the notochord, and makes the observation of cell boundaries difficult (Fig. 7b). When the notochord is viewed antero-dorsally at this stage, extracellular fibrils are observed in small numbers on the surface of the notochord and crossing the space between the notochord and the paraxial mesoderm (Fig. 8a). These increase in number and are more conspicuous by stage 15-16 (Fig. 8b). The entire notochord becomes surrounded by a sheath, and a dense meshwork of extracellular fibrils are found in the ‘perinotochordal space’.
Fig. 5. A comparison of the paraxial mesoderm of *Xenopus* at stage 12+ (a) and 13+ (c) with lateral mesoderm at stages 12+ (b) and 13+ (d) shows the tendency for the former to align dorso-ventrally (in the direction of the arrow). Bar in (b) represents 10 μm for each.

It is also interesting to examine the lateral outline of the rising neural folds (see Fig. 3). Their contours seem to correspond exactly to those of the underlying mesoderm. The mesodermal contours are the most conspicuous in *Ambystoma*, less in *Pleurodeles* and *Rana*, and the least obvious in *Xenopus*. At about stage 13+–14 of *Xenopus*, the contours are difficult to observe. However, two elevated ridges of the paraxial mesoderm are clearly visible on each side of the notochord. At about the same stage of other species there seems to exist two elevated ridges of the paraxial mesoderm between the contours and the notochord. Later the mesodermal contours move mediad, and become indis-
Fig. 6. Cross-sectional view of stage 13+ of a *Xenopus* embryo showing neural ectoderm (NE), notochord (N) and paraxial mesoderm (PM). Bar represents 25 μm.

...tistinguishable from the paraxial mesodermal ridges. These observations may suggest that the mesoderm plays an important role in the initial folding of the neural plate. However, the paraxial mesoderm does not seem to directly participate in that event (see Discussion).

III. Neural-groove to neural-tube stage

During this phase the edges of the neural folds meet and fuse, forming a hollow neural tube. The embryo continues to elongate antero-posteriorly and the number of somites increases. The notochord continues to stretch anteroventrally. By about stage 18 (14+-15 of *Rana pipiens*), the anterior end of the notochord shows marked differences from the earlier stages (Fig. 9). The anterior part of the notochord is now rod-like and has completely separated from the adjacent head mesenchyme and has a regular, smooth appearance due to the extracellular sheath material now found in this region. As the somite number increases the notochord loses its characteristic lengthwise uniformity and shows variation in width. It is widest at the intersomitic cleft and narrowest at the antero-posterior midpoint of each somite, similar to the alternation of wide and narrow regions seen in chick embryos by Bancroft & Bellairs (1975, 1976). The posterior part of the urodele notochord, which until now was triangular in shape, has become rod-like during this phase (see pointer at stage...
Fig. 7a. Ventro-lateral view of the notochord (N) of *Xenopus* at stage 13+. Below the notochord is the endodermal roof of the archenteron (E). Bar represents 25 μm.

Fig. 7b. Dorso-lateral view of the notochord (N), paraxial mesoderm (PM) and endoderm (E) of *Xenopus* at stage 14+. Bar represents 25 μm.

Fig. 8a. Postero-dorsal view of the anterior region of the notochord (N) of *Xenopus* at stage 14+. Bar represents 10 μm.

Fig. 8b. Mid-dorsal view of the notochord (N) of *Xenopus* at stage 15–16. Bar represents 20 μm.

18 of *Ambystoma*, Fig. 9). At stage 18 of *Xenopus* much more extensive distribution of the extracellular fibrils can be observed between the notochord and the paraxial mesoderm (Fig. 10a).

A transverse view of the *Xenopus* notochord and the unsegmented paraxial mesoderm at the mid-body region is shown in Fig. 10b. The notochord cells
Fig. 9. An atlas of notochord development from neural-groove to neural-tube stages in antero-dorsal view. Diagrammatic sketch in each subphase displays the progress made by the neural folds of *Ambystoma* late neurulae viewed dorsally. Approximate staging numbers are shown above each sketch. Staging numbers of *Rana* are in parentheses. Pointer at stage 18 of *Ambystoma* indicates the rod-like notochord posteriorly. Bars represent 0.4 mm.
Fig. 10a. Antero-dorsal view of the notochord (N) and somite mesoderm (SM) of *Xenopus*. Bar represents 20 μm.

Fig. 10b. Postero-dorsal view of the notochord of *Xenopus* at stage 18 showing the development of the fibrous matrix between the notochord (N) and somite mesoderm (SM). Bar represents 50 μm.
appear to retain their tendency to be tapered towards the centre of the notochord where their apices meet. The somitic cells assume a double-layered arrangement. As the neural folds rise, the cells of the prospective sclerotome and myotome region elongate and are arranged more or less radially around the myocoel, whereas the prospective dermatomal cells form a sheet over the lateral surface of the prospective somite. At about stage 22 of *Xenopus* the somitic cells have rotated and are oriented in an antero-posterior direction (Fig. 11). This process of somite formation in *Xenopus* and other amphibian species has been previously described by Hamilton (1969).

The somite numbers in all four species were counted and compared between the neural-plate and neural-tube stages. It was found that each of the amphibian species examined here has the same number of somites at comparable stages. The somite counts obtained from the scanning electron micrographs are compared with those appearing in the various staging series (Table 2). Some discrepancies are found in the urodele somite numbers. For example, the number of somites at stage 22 of *Ambystoma* has been listed as six in the staging series. Our SEM studies reveal eight to ten, indicating that the urodele somite number may have been underestimated by other authors (e.g. Bordzilovskaya & Dettlaff, 1979).
Table 2. Comparison of the somite numbers as seen by the scanning electron micrographs with those from various staging series

<table>
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<td>Bordzilovskaya &amp; Dettlafl (1979)</td>
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<td><em>Pleurodeles</em></td>
<td>Gallien &amp; Durocher (1957)</td>
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<td>All four species as seen by SEM in this study</td>
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<td>1-2</td>
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**DISCUSSION**

**I. Notochord morphogenesis**

A summary of the pattern of morphogenesis of the notochord is included in Fig. 12. When the notochord begins to develop (stage 11+–12), it is not sharply delineated from the paraxial mesoderm at either side (Fig. 12a). Even before the delineation takes place, the prospective notochord cells are different from the prospective paraxial mesoderm cells (Fig. 2). Other studies (e.g. Nieuwkoop & Faber, 1967; Hama, 1978; Elsdale, Pearson & Whitehead, 1976) have also reported early formation of the notochord during gastrulation. However, this report provides the earliest evidence for notochord formation as seen by the SEM. With further development (stage 12+–13), the notochord becomes elongated and separated from the adjacent mesoderm in a postero-anterior direction, and acquires a clear outline (Fig. 12b). At this stage, the principal difference in the pattern of notochord formation between anurans and urodeles can be clearly seen. The anuran notochord is more or less uniform along its length, while the urodele notochord is widest posteriorly and narrowest anteriorly. This difference may reflect the fact that *Xenopus laevis* gastrulation movements are different from urodele movements and perhaps also different from other anuran movements. In *Xenopus*, the prospective mesoderm is located internally in a ring-shaped collar lying in the deep marginal zone of the early gastrula (Nieuwkoop & Florschütz, 1950; Nakatsuji, 1975; Keller, 1975, 1976). By the time the blastopore appears, some of the mesoderm has already moved over itself and upward on the blastocoel wall—the so-called ‘internal or cryptic gastrulation’ (see Nieuwkoop & Florschütz, 1950; Keller & Schoenwolf, 1977).

In contrast, the prospective mesoderm of urodeles and other anurans is
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Fig. 12. Comparison of notochord development between anurans and urodeles. Broken lines indicate immature delineation between the notochord and the adjacent mesoderm. Solid lines indicate that the notochord becomes separated from the adjacent mesoderm, and acquires a clear outline. Also shown are outlines of the neural folds (mesodermal contours) indicated by thinner solid lines. Staging numbers of *Rana* are in parentheses.

considered to occupy the superficial cell layer of the marginal zone of the early gastrula (Vogt, 1929; Pasteels, 1942). During gastrulation, this prospective mesoderm and the adjacent 'suprablastoporal' endoderm are involuted to form the roof of the gastrocoel. At some point, the lateral endodermal crests migrate dorsally and medially, covering this superficial mesoderm and fusing at the midline to form an endodermal archenteron roof (see Vogt, 1929). Lovtrup (1966, 1975) has questioned whether the early fate maps of these species are accurate, and has argued that the prospective mesoderm of urodeles is also located in the deep marginal zone, Over and above this question of the
origin of the mesoderm, it is likely that there is real variation among the amphibians in the mode of the mesodermal movement of the mesoderm (Keller, 1976). These differences may account for the different shapes of the notochord observed here. Differences in the shape of the notochordal anlage before gastrulation, differences in the rate of movement of the prospective mesoderm before and after turning over the dorsal lip, and differences in the amount of notochordal elongation (extension) completed at each stage may be related to differences between anurans and urodeles in notochordal shape.

As development proceeds beyond gastrulation, the anuran notochord maintains its rod-like shape, except in its anterior region, which becomes broadened (Fig. 12c). In contrast, the urodele notochord is triangular in the posterior region, long and narrow in the middle, and broadens anteriorly. The anterior broadened region later becomes rod-like and the triangular posterior region is stretched antero-posteriorly, as the whole embryo elongates during neurulation (Figs 12d and 12e). These observations confirm the results obtained from the vital dye mapping experiments. In *Xenopus*, the mesoderm continues to involute even after 'gastrulation' ends (Keller, 1976; Cooke, 1979). During neurulation, the source of the mesoderm added to the posterior portion of the mesodermal mantle is the thick 'circumblastoporal collar' which has not yet involuted by stage 12+–13. As cells are added posteriorly from the circumblastoporal mesoderm, the increased length probably occurs in the posterior end of the notochord without any posterior stretching. Conversely, in the case of urodeles, posterior stretching during neurulation has been reported by the vital dye mapping experiments of Jacobson & Löfberg (1969) and Hama (1978). The posterior triangular region of the urodele notochord is believed to contribute to this stretching, presumably by increase in its length at the expense of width. Compared to anurans, less involution of mesoderm occurs during neurula stages of urodeles (Vogt, 1929). In general, the antero-posterior elongation of the notochord is easily recognizable in both anurans and urodeles by vital dye mapping (Jacobson & Löfberg, 1969; Keller, 1976; Hama, 1978). The elongation occurs at the expense of mediolateral extent and is coincident with the movement of the mesodermal mantle dorsally ('dorsal convergence') during neurulation (Vogt, 1929; Jacobson & Löfberg, 1969; Keller, 1976). Dorsal convergence of the mesoderm results in the thickening of the dorsal mesoderm as well as its lengthening.

Examinations of sagittally and transversely fractured *Xenopus* embryos have indicated that notochord elongation is accompanied by extensive cell rearrangements, once the prospective notochord is separated from the adjacent prospective paraxial mesoderm. During late gastrulation (stage 12+), the notochord is many cells wide, and approximately four cells deep along its side (Fig. 2). During the period of early neurulation (stage 13+–14), the notochord becomes one or two cells wide, but maintains its four-cell-layered configuration (Fig. 6). It is possible that the reduction in the number of cells in the width of the noto-
chord (by medial migration) and the maintenance of a constant number of cell layers in its depth are the fundamental mechanisms for increasing notochordal length. Jacobson & Gordon (1976) have proposed that the notochord elongates by cell rearrangements which occur without any change in the number of cells of the notochord area. Conversely, however, it has been suggested that mitotic division plays a role in increasing the length of the notochord (Mookerjee, Deuchar & Waddington, 1953; Jurand, 1962; Bancroft & Bellairs, 1976). We have not, however, made any special studies of the mitotic cells or their distribution in the notochord. In addition to SEM studies, a conventional histological analysis using light microscopy and transmission electron microscopy should be made to determine whether and when mitoses occur in the notochord cells during development. That is, a further analysis of the changes in the number of notochord cells should be carried out to gain insight into the issue of how the notochord elongates.

Notochord elongation is also accompanied by changes in cell shape and cell-cell contacts. At about stage 13+-14 of *Xenopus*, cells within the notochord are flattened in the antero-posterior direction and begin to exhibit a radial arrangement. They also become closely packed, with few intercellular spaces. Perhaps close packing is an important factor in causing the notochord to become rod-shaped (Mookerjee et al. 1953; Jurand, 1962).

Since the notochord forms during neural induction and becomes a part of the primary embryonic axis, there have been many studies attempting to explain its functional role. According to these studies, the notochord promotes the 'keyhole shape' movements of neural plate cells (Jacobson & Gordon, 1976), induces the formation of neural tube and somites (Hay, 1973), maintains the integrity of the somites (Lipton & Jacobson, 1974), induces various mesodermal differentiations underneath (Yamada, 1940), specifies the type of overlying neural tissue (Takaya, 1977), and stimulates chondrogenesis in somatic mesoderm (Kosher & Lash, 1975). We have recently begun to re-examine the functional roles of the notochord, using ultraviolet (u.v.) irradiation and the SEM. U.v. irradiation of the vegetal hemisphere of fertilized uncleaved amphibian eggs causes deficiencies in axial structure development (Baldwin, 1915; Grant, 1969; Malacinski, Benford & Chung, 1975; Malacinski, Brothers & Chung, 1977). Our as yet unpublished observations indicate that the notochord is the most u.v.-sensitive target. The primary effect of increasing doses of irradiation is a reduction in the size of the notochord. The neural tube and somites are usually, however, intact; further analyses of the axial structure development in 'notochordless' embryos will be reported in another paper (Youn & Malacinski, 1980).

II. Somite morphogenesis

The scanning electron micrographs in Fig. 5 clearly show that changes in cell morphology and cell arrangements take place in the presomitic cells, even prior to the initiation of somite segmentation. From the late gastrula to
the early neurula stage of *Xenopus*, the presomitic cells begin to elongate and orient mediolaterally. At the same time the paraxial mesoderm starts to form a longitudinal ridge on either side of the notochord, apparently due to the dorsal convergence of the mesoderm (see above). These results support a hypothesis that there is a programming of the paraxial mesoderm at earlier stages. Detailed studies on such programming have been made in the chick embryo by Meier (1979). In chick embryos the early programming was demonstrated to consist of repeating circular domains (157 μm in diameter) of paraxial mesoblast (‘somitomere’) progressively aligned in tandem on either side of the axial mesoblast. Each paraxial somitomere appeared as a slightly hollowed, squat cylinder, composed of tapering mesenchyme cells whose long axes are directed towards the core centre. During neurulation, somitomeres undergo morphogenesis to become somites. In the chick embryo, therefore, somites appear to emerge from the pre-existing somitomeres. However, in the amphibian embryo, we have not yet found any evidence for somitomere-like structures. The somite segmentation process is characterized by the progressive formation of deep clefts extending medio-laterally among the presomitic cells (see Fig. 4).

Examination of the deep cleft in the paraxial mesoderm indicates that the first somite becomes segmented as early as stage 13+–15 of *Xenopus*, *Ambystoma* and *Pleurodeles*, and stage 13–13+ of *Rana*. To our knowledge, these observations represent the earliest stage that somite formation has been seen by SEM. The results of this study also show that the first somite always forms in the mid-body region, an observation also made by Jacobson & Löfberg (1969) from their vital dye experiments on the urodele mesoderm. Later this somite is found in the cephalic region of the embryo, which suggests that a strong anterior stretching movement can be found not only in the notochord but also in the paraxial mesoderm.

Recently, somite segmentation has attracted substantial attention. In the chick embryo, extensive SEM studies of somite segmentation have been done by Bellairs (1979) and Meier (1979). In the amphibian embryo, however, no detailed SEM description of somite segmentation exists. An SEM study of somite segmentation was beyond the scope of our present study. But we believe that the techniques employed in this report will provide a basis for further studies on the changes of cellular morphology, contacts and arrangements which occur during the process of somite segmentation.

The last point for discussion is the role of the mesoderm in neural fold formation and elevation. Previous studies indicated that forces which bring about the initial folding of the neural plate originate in the neural plate itself or the non-neural ectoderm lateral to the neural tissue. Yet the results of others have suggested that these forces originate in the underlying mesoderm (reviewed by Karfunkel, 1974). The scanning electron micrographs in Fig. 3 show that there exists an elevated ridge at each side of the lateral mesoderm, which appears to resemble the contours of the neural folds. The elevated ridges
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are most sharply defined in Ambystoma, less so in Rana and Pleurodeles, and not easily detectable in Xenopus. With further development, those mesodermal contours migrate mediad as the neural folds do, which confirms the results of vital dye mapping experiments of Jacobson & Lofberg (1969), and Keller (1976) on the mediad migration of the mesoderm (i.e. dorsal convergence, see above). This observations may indicate that the mesoderm plays an important role in the initial folding of the neural plate, and that this mediad migration of the mesoderm on which the neural folds sit contributes to the formation of the neural folds. However, it is not clear at this point whether such elevation and migration of the mesoderm are ‘results’ or ‘causes’ of the neural-fold formation.

The scanning electron micrographs in Fig. 3 also show that there exists an elevated ridge of the paraxial mesoderm between the lateral mesoderm contours and the notochord at about stage 13+-14. of Xenopus, Pleurodeles and Ambystoma, and stage 13-13+ of Rana. The fact that two structures, the lateral mesodermal contours and the paraxial mesodermal ridges, are separable may indicate that the ‘paraxial mesoderm does not directly participate in the initial folding of the neural plate. The elevation of the paraxial mesodermal regions on either side of the notochord seems to be caused by the dorso-ventral elongation of the double-layered presomitic cells (compare Fig. 2d and 6). As the neural folds rise, the mesodermal contours migrate mediad and the paraxial mesodermal ridges are no longer visible. At the same time, the cells of the prospective sclerotome and myotome region elongate further dorso-ventrally, which causes the thickening and elevation of the segmented somites and unsegmented mesoderm (see Figs. 6 and 9). It is possible that further mediad migration of the neural folds is enhanced by that process, at least in the case of Xenopus (Schroeder, 1970). The changes of the mesodermal contours, as neurulation advances and dorsal convergence continues, lead us to conclude that the paraxial mesoderm may play a part in neural-tube closure.

We wish to thank Dr Sally Frost, Fran Bacher (I.U. Axolotl Colony) and Dot Barone for providing Pleurodeles, Ambystoma and Rana eggs, respectively. This work was supported by NSF PCM 77-04457.

REFERENCES


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(Received 3 January 1980, revised 21 April 1980)