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Developmental morphology of early vertebrogenesis in Caecilians (Amphibia: Gymnophiona): Resegmentation and phylogenesis

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Summary

The early development of the vertebrae of caecilians from somitogenesis through chondrogenesis sheds light on questions of the nature of resegmentation, vertebral components and their origins, and the relationship of caecilian vertebral structure to that of other amphibians and to amniotes. The relatively greater numbers of cells per somite in caecilians compared to those of frogs and salamanders facilitates resolution of some of these questions. Morphological evidence for resegmentation is clear; transitory sclerofibrils join caudal sclerotomites to cranial sclerotomites of the next posterior segment. The cell-rich posterior sclerotomite contributes the cells that form the neural pedicel rudiments, which are intersegmental, before the perichordal tube is well formed. Centra develop ectochordally. The adult centrum is spool-shaped, and its center retains a small core of notochordal cartilage throughout life. Features of development of ribs and rib-bearers, the atlas, and the cranio-vertebral joint differ more markedly among caecilians, salamanders, and frogs than do those of the neural arch and the centrum, with caecilians and salamanders having more similar patterns. A number of features of caecilian vertebral development resemble those of amniotes at a general level, but differ in many details (with particular comparison to Verbout's [1976, 1985] research on sheep). We interpret our data with reference to Gadow's arcualia theory, paleontological evidence, and current research on the genetics of development of vertebrae in tetrapods.

Introduction

The establishment of head and body segmentation during early development in animals currently is receiving considerable attention. New data and new questions arise from several sources. One such source is the investigation of *Hox*, and other, genes in vertebral specification (e.g., Holland, 1992; Kessel, 1992; Kessel and Gruss, 1991; Lufkin *et al.*, 1992; Monsoro-Burq *et al.*, 1994; Burke *et al.*, 1995; McGrew *et al.*, 1998); a second is the examination of the molecular and cell biology of somitic cells during early development (e.g., Keynes and Stern, 1984; Stern *et al.*, 1988, 1991; Neidhardt *et al.*, 1997; McGrew *et al.*, 1998; see note added in proof). Thirdly, study of the fundamental nature of the somitomere in establishing head and body structures has provided new insights (Jacobson and Meier, 1986; Jacobson, 1988). In addition, new phylogenetic hypotheses of vertebrate relationships have been advanced for which the data are partly or completely those of vertebral structure and development, especially including fossil material (e.g., Parsons and Williams, 1963; Gardiner, 1982, 1983; Løvtrup, 1985; Gauthier *et al.*, 1988; Carroll *et al.*, 1999). Recent advances in these several areas of biology, particularly examination of somitogenesis in various species of vertebrates (e.g., Cooke, 1988; Davidson, 1988; Christ and Wilting, 1992; Wood and Thorogood, 1994; Christ and Ordahl, 1995; Brand-Saberi *et al.*, 1996; Richardson *et al.*, 1998; Tabakhsh and Spörle, 1998), allows reexamination with new insights into a number of issues in morphogenesis investigated for more than a century, but currently not fully resolved.

An idea firmly embedded in the literature of vertebrate morphogenesis is that of the formation of vertebrae by resegmentation of skeletogenous parts of adjacent metameres (caudal sclerotomite [half-somite] associating with the anterior [rostral] sclerotomite of the next-posterior somite). Despite many variations, and several challenges to the con-

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cept, the majority of workers have argued in favor of some kind of resegmentation that involves division and separation of the original metamerically arranged tissue and a joining of separated parts of immediately adjacent somites at approximately intersomitic levels. As a result, muscle extends from one skeletal unit to the next and the vertebral column can be bent (see Jarvik, 1996, and many other authors, but see also Lauder, 1980, who argues that the causal link between resegmentation and the intersegmental position of vertebrae is spurious).

The concept of resegmentation has a long and involved history, extending from the mid-nineteenth century, and has been reviewed by Schauinsland (1905), Gadow (1933), Williams (1959), and Verbout (1976, 1985). Idealistic morphology in an extreme form is seen in the complicated schemes of Gadow (1933) and Piiper (1928), which envisioned division of somites into many parts, each retaining its integrity to a greater or lesser degree into fairly late stages of development. In his excellent critical re-evaluation, Williams (1959) was able to find what he termed "the residue of truth" concerning resegmentation in its simplest form, and he swept away much of the confusion in the literature. Williams focused attention on tetrapods, and concluded that all living tetrapods – the three orders of living Amphibia and all amniotes – have a vertebral centrum derived from perichordal tissue of the caudal half of one sclerotome united with tissue from the cranial half of the next (posterior) sclerotome. Wake (1970) and Wake and Lawson (1973) examined amphibian material, primarily salamander, and found that the evidence for resegmentation was equivocal (see McGowan, 1998, for a summary and evaluation). However, Verbout (1976, 1985) in a thorough examination of an extensive developmental series of mammalian (sheep) material, argued that resegmentation does not take place, and that vertebrae and intervertebral disks arise at their definitive sites.

When we began this work in the 1970's, the notion of resegmentation was generally considered outmoded, in fact erroneous, based largely on Verbout's thoughtful analysis. However, experimental techniques applied to the question in the late 1980's demonstrated resegmentation in chicks and mice, primarily. Bagnall's pioneering work (Bagnall *et al.*, 1988; Bagnall, 1992) established resegmentation unequivocally using chick-quail chimeras; Goldstein and Kalcheim (1992) used the same system to evaluate the contributions of half-somites to vertebral components. Norris *et al.* (1989) identified molecular differences between the sclerotome halves; Ewan and Everett (1992) labelled sclerotome cells early in development with retroviral particles, and unequivocally demonstrated resegmentation in the chick. Monsoro-Burq *et al.* (1994) reviewed the evidence for resegmentation, among other aspects of vertebrogenesis. At the same time, the molecular and developmental geneticists producing these data do not agree on their contribution to establishing the "reality" of a physical resegmentation (see Tajbakhsh and Spörle, 1998). We note that virtually all of this work has taken place on amniotes, and only a very few species have been studied, leaving unresolved the nature of vertebrogenesis in amphibians in particular. At the same time, Borchvardt (1982), Jarvik (1996) and especially Shishkin (1988ab, 1989ab) found evidence for resegmentation in the vertebral structure of Paleozoic and more recent extinct amphibians, and extended this to recent amphibians and to other tetrapods. We therefore thought it timely to examine vertebrogenesis in the caecilian amphibians, to compare the data with those for salamanders and frogs, and with Verbout's (1976, 1982) extensive data for sheep, which had been so influential a few years ago, and finally to compare the data with those for the fossils as well as current molecular and genetic information, in a general assessment of patterns of vertebrogenesis, the issue of resegmentation in amphibians, and the evolution of tetrapod vertebrae.

We have examined vertebral development in members of the amphibian Order Gymnophiona, the limbless, elongate caecilians. Aspects of caecilian vertebral structure and development have been reported by several authors; e.g., Peter (1894), Marcus (1934, 1937), Marcus and Blume (1926), Mookerjee (1932), Ramaswami (1958), Lawson (1963, 1966), Welsch and Storch (1971),Wake (1980), Wake and Wake (1985, 1986), and Renous and Gasc (1989). Most studies of development have focused on only a few stages in single species, especially *Hypogeophis rostratus*, a Seychellean taxon. In this paper, we describe vertebral development based on several species of caecilians, including many stages of several species, and we present new information relevant to the century-old question of resegmentation. We consider in particular the establishment of somites, their differentiation, the fate of the sclerotome, the development of the centrum, and the establishment of the vertebral arches. We also examine the development of the several vertebral processes, the intervertebral disk, the ribs, and the cranio-vertebral joint. We compare these data to those for salamanders and frogs, in order to consider the pattern of evolution in amphibians and tetrapods. These data and analyses form the basis for further study of somitogenesis and vertebrogenesis in caecilians, ongoing presently and using cellular and molecular techniques.

Gymnophiones have proven particularly instructive for studies of vertebral development, for they have rather large and numerous cells, in contrast to the paucity of cells in salamanders and frogs, and their development is highly cephalized. Since they

have 100 or more (to 285) body segments, an individual embryo is also an ontogenetic series, for its somites are in a number of stages of development, advanced anteriorly relative to more posterior segments. Further, several of the species studied, including *Dermophis*, are live-bearers with long gestation periods, so collection of adult pregnant females at different times of the year has provided embryos and fetuses at all stages of development up to birth. We therefore present information and analysis of early vertebrogenesis (through the establishment of the cartilaginous components) with emphasis on the question of resegmentation. A second paper will follow that discusses comparative vertebrogenesis from chondrogenesis through formation of the adult vertebrae among caecilians and other amphibians.

Materials and Methods

Specimens representing embryonic developmental stages of members of two families (Caeciliidae and Typhlonectidae [Duellman and Trueb, 1986; Nussbaum and Wilkinson, 1989]) and six genera of caecilians were available for study. Specimens were prepared as serial sections of whole embryos (4 to 30 mm total length [TL]) or segments of regions of the body (larger embryos and fetuses), and by clearing and staining whole animals with alcian blue for cartilage and alizarin red for bone. Sections were cut at 7–10 µm and stained with hematoxylin-eosin, van Gieson's or picro-ponceau, and/or Mallory's Azan. Our embryonic/fetal material includes: *Dermophis mexicanus* (27 specimens sectioned; total lengths (TL) 5.0 to 80 mm; 11 specimens are 10 mm TL or less; 36 specimens cleared and stained, 29–150 mm TL); *Gymnopis multiplicata* (six specimens sectioned, 10, 12, 15, 30, 54 mm TL; two specimens cleared and stained, 55 and 78 mm TL); *Hypogeophis rostratus* (seven specimens sectioned, 7–38 mm TL); *Geotrypetes seraphini* (one specimen sectioned, 12 mm TL); *Typhlonectes compressicauda* (four specimens sectioned, 5, 10, 15, 30 mm TL; 22 specimens cleared and stained, 17–95 mm TL) and *T. natans* (four specimens sectioned, 15, 18, 38, 76 mm TL; two specimens cleared and stained, 50 and 78 mm TL). Cleared and stained specimens < 50 mm TL were used to guide the histological examination. In addition, large numbers of sectioned embryos of several species of salamanders, particularly plethodontids (see Wake and Lawson, 1973), were available for comparison.

Results

We describe vertebrogenesis in terms of the stages traditionally presented, beginning with the establishment of the somite. We cite the size (total length) of the embryo or embryos in which we see the stages either initially or particularly well. However, development is so cephalized in gymnophiones, and body segments are so numerous, that early stages of development observed in anterior segments in early embryos may be observed posteriorly in advanced embryos; i.e., an individual is an ontogenetic series. We describe the development of structures relevant (often as landmarks) to vertebral morphogenesis, such as dorsal root ganglia, dorsal and ventral roots, the kidney, blood vessels, and aspects of the skin. We emphasize *Dermophis mexicanus* in our descriptions and illustrations because it is our only complete developmental series. However, we indicate when we find the structures in stages/sizes of other species for which we have material, though we lack adequate ontogenetic sequences for full stage-to-stage comparison. Reference in the description and discussion is to *D. mexicanus* unless indicated otherwise. Illustrations are of *D. mexicanus* only in order to present a full and consistent developmental picture. We then compare gymnophione vertebrogenesis with that of frogs and salamanders, and mammals. Finally, we discuss the phylogenetic implications of these data.

Establishment and differentiation of somites

The smallest embryos available for examination are 5.0 mm total length (TL) *Dermophis mexicanus*. Approximately 45 somites of a great range of developmental stages are present. In embryos 5–7 mm TL, the dorsal hollow nerve cord is well established, and the notochord is cellular, with vacuolated cells, already beyond the "pile of coins" stage. Posterior somites are small and solid, containing approximately 30 cells in mid-sagittal section (Fig. 1AB). The tail end contains unsegmented mesoderm, and terminates in a knob of tissue. Mid-body somites are elongate vertically, cavitated, and in medial section have a profile of approximately 60 cells, one layer deep (Fig. 1AB). More anterior somites are not only elongate and hollow, but in approximately the first 10 segments the ventral end of the somite has differentiated into a round nephrotome of approximately 12 cells in mid-sagittal profile, the mid-region into parallel-sided sclerotome of approximately 24 cells in profile, and the dorsal part of the somite has differentiated into myotome of approximately 27–28 cells in profile. In several segments, the somitic cavity is continuous, forming a confluent myocoele-sclerocoele-nephrocoele (Fig. 1B) (confirming the observations of Marcus and Blume [1926] on *Hypogeophis*). The myo-sclero-nephrocoele contains fibrous material. In approximately five segments, the nephrotome has split from the sclerotome and is a round, cavitated structure. In these same segments, the myotome has flattened and broadened, and is demarked from the sclerotome by a constriction (Fig. 1B). Both halves of the sclerotomes of these segments (as divided by the sclerocoele) in-

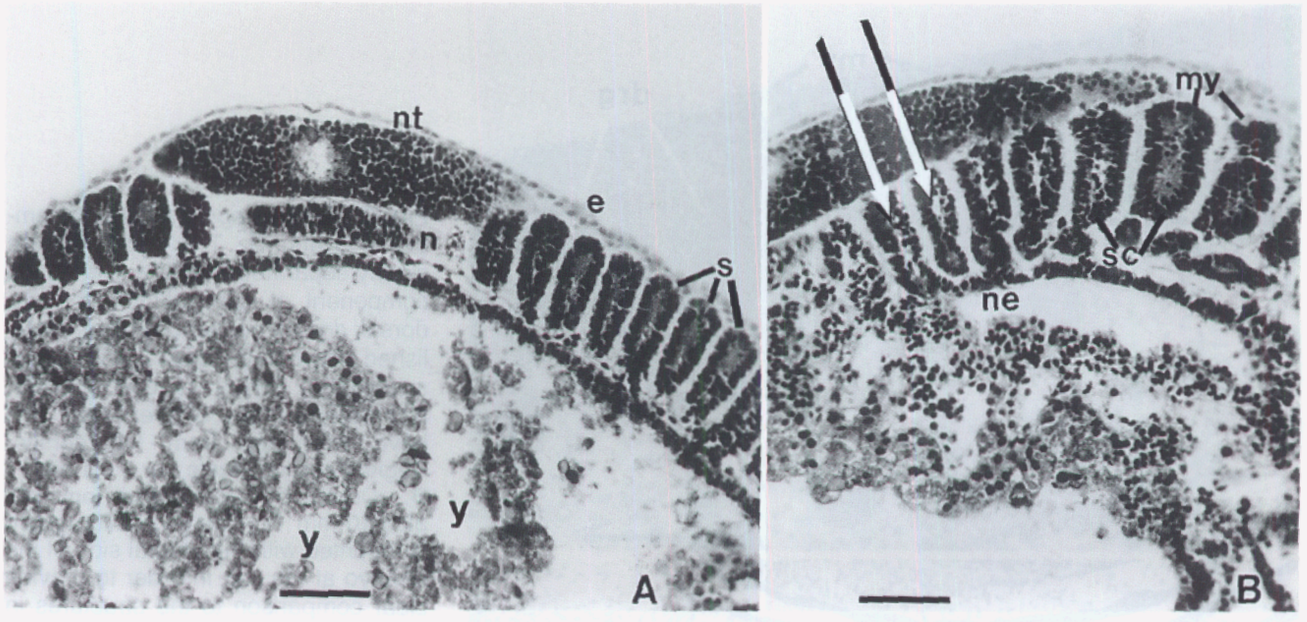

Fig. 1. A. *Dermophis mexicanus* embryo, 5 mm total length (TL). Body segments (somites) are elongate, with a central fluid-filled space; each segment is a three-dimensional structure 1–2 cell layers deep. Oblique transverse section, near anterior end of body. Scale bar = 0.1 mm.
B. Same embryo, illustrating ten anterior segments that have differentiated to have discrete myotome-sclerotome-nephrotome components; at this early stage of development, the segments have a patent and continuous myocoele-sclerocoele-nephrocoele. Scale bar = 0.1 mm. Abbreviations: e – epidermis; my – myotome; n – notochord; ne – nephrotome; nt – neural tube; s – somite; sc – sclerotome; y – yolk. Arrows indicate segments with the continuous cavity.

clude approximately the same number of cells. Anteriormost in the embryo, the otic vesicle is followed by a small segment; the second post-otic segment is large and contains a slight myocoele; neither of these have a nephrocoele. The third post-otic segment has a ventral knot of tissue; the fourth through the eleventh have nephrocoeles that open laterally. More posterior somites have progressively less differentiation of nephrotome, and they lack nephrocoeles. The third through approximately the eighth nephrotomes contribute to the developing pronephric duct.

The notochord is well developed throughout most of the body at the time somitogenesis is proceeding, but it is difficult to distinguish in the terminal part of the body. Anteriorly the notochord has slight segmental constrictions. From about the twelfth through the 20th segments, there are slightly increased numbers of cells ventral to the notochord, which we interpret as those initiating the perichordal sheath.

Differentiation of the sclerotome

Anterior somites in a 10 mm TL *Dermophis mexicanus* (and a 12 mm TL *Geotrypetes* and a 15 mm TL *Gymnopis*) include developing dorsal root ganglia in the anterior (cranial) sclerotomites (Fig. 2A). Cranial and caudal sclerotomites are distinct toward the notochord; laterally the distinction blurs. The cranial sclerotomites contain fewer cells than do the caudal (Fig. 2A). A sclerocoele is no longer present in these sclerotomes. The intersegmental fissures are clear. The myotome curves ventro-laterally. In three anterior segments, scleroblast fibrils cross the borders between segmental halves, and in two segments, run from caudal to cranial sclerotomites (Fig. 3AB). The number of cells around the notochord is increased; there are more ventrally than dorsally, with many laterally and medially, constituting early perichordal tube development (Fig. 3C). Segmental borders in the perichordal tube are obscure. Segmental blood vessels are developed. The kidney is large, and in anterior segments the tubules have proliferated (Fig. 3A).

Initiation of cartilage

In a 10 mm TL *Dermophis mexicanus* that is more advanced developmentally than that described above, discrete masses of procartilage (characterized by concentric rings of many nuclei) have formed in anterior segments. These masses do *not* arise from the perichordal tube, which is not yet fully developed. Each segment has two pair of procartilage masses; one lies laterally on either side

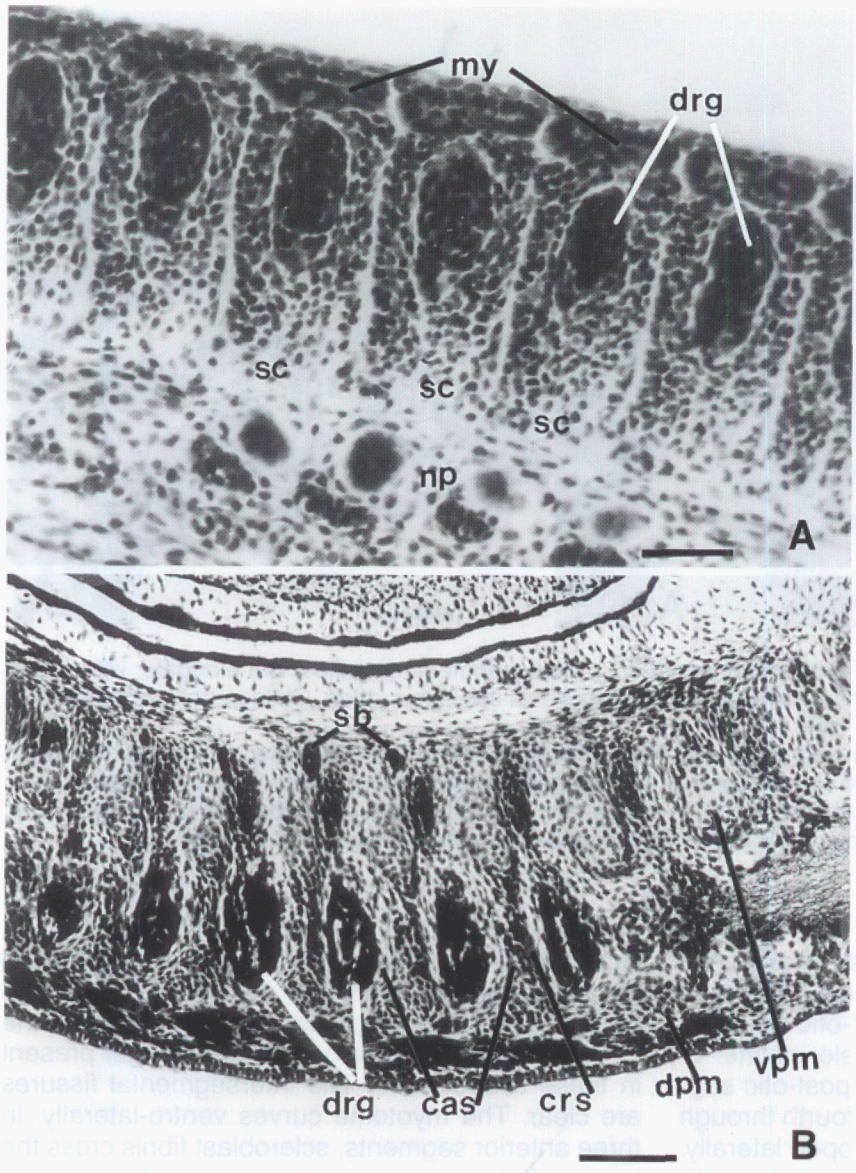

Fig. 2. A. *Dermophis mexicanus* embryo, 10 mm TL. The myotomes are clearly separated from the sclerotome component of each somite and are dorsal; dorsal root ganglia have established between the cranial and caudal sclerotome halves of each somite. Nephrotomes are differentiated and have separated from the somite. Sagittal section; scale bar = 0.1 mm. **B.** *Dermophis mexicanus* embryo, also 10 mm TL. The photomicrograph is mounted with the ventral side of the embryo at the top, in order to provide clear comparison to the structures in A. Myotomes are ventral. Resegmentation is apparent. The cranial sclerotomite (the longer, slenderer, more densely staining half) of the posterior somite is associating with the caudal sclerotomite (the thicker, less densely staining half) of the more anterior somite. Segmental blood vessels lie below each somite. Precartilaginous masses (with dorsal and ventral halves) are differentiating in anterior segments. Sagittal section; scale bar = 0.1 mm. Abbreviations: cas – caudal sclerotomite; crs – cranial sclerotomite; dpm – dorsal precartilaginous mass; drg – dorsal root ganglion; my – myotome; np – nephron; sb – segmental blood vessel; sc – sclerotome; vpm – ventral precartilaginous mass.

of the notochord, the other lies ventrally. Both dorsal and ventral procartilage masses arise in the caudal sclerotome halves (Fig. 2B). The dorsal and ventral masses are not connected except by a few tenuous cells. The cranial sclerotomite appears to contribute cells to the caudal half of the preceding segment (Fig. 2B); the distinction between sclerotomites per sclerotome is still clear. The cell numbers differ, and the long axes of the cells of the two halves have slightly different orientations (Figs. 2B, 3AB).

There is a large mass of cartilage at the occipital condyle, followed by a mass of elevated procartilage, then the first dorsal root ganglion occurs. Its nerve runs anteriorly, presaging the adult condition. In anterior segments, the dorsal root emerges nearly directly below the dorsal root ganglion, which lies against the cranial edge of the caudal sclerotomite. The ventral root arises almost exactly between the ventral cartilaginous masses, and innervates the myotomes. Promuscle cells cross the ganglion, extending from caudal to caudal sclerotomite. Segmental blood vessels lie at segment borders where the myotomes attach to the posterior edge of the caudal sclerotomite. Masses from the extreme postero-ventral margin of the caudal sclerotomite contribute tissue to the kidney. Immediately posterior and dorsal to the kidney is the segmental blood vessel, marking the posterior margin of the segment (Fig. 2AB).

The notochord has segmental constrictions; the procartilage masses lie adjacent to the *widest* parts of the notochord. The dorsal elements are precursors of the neural arch rudiments (neural pedicels, basidorsals). The notochord expands at the segmental borders. Many cells are concentrated in the crevices at the constrictions. The notochord is

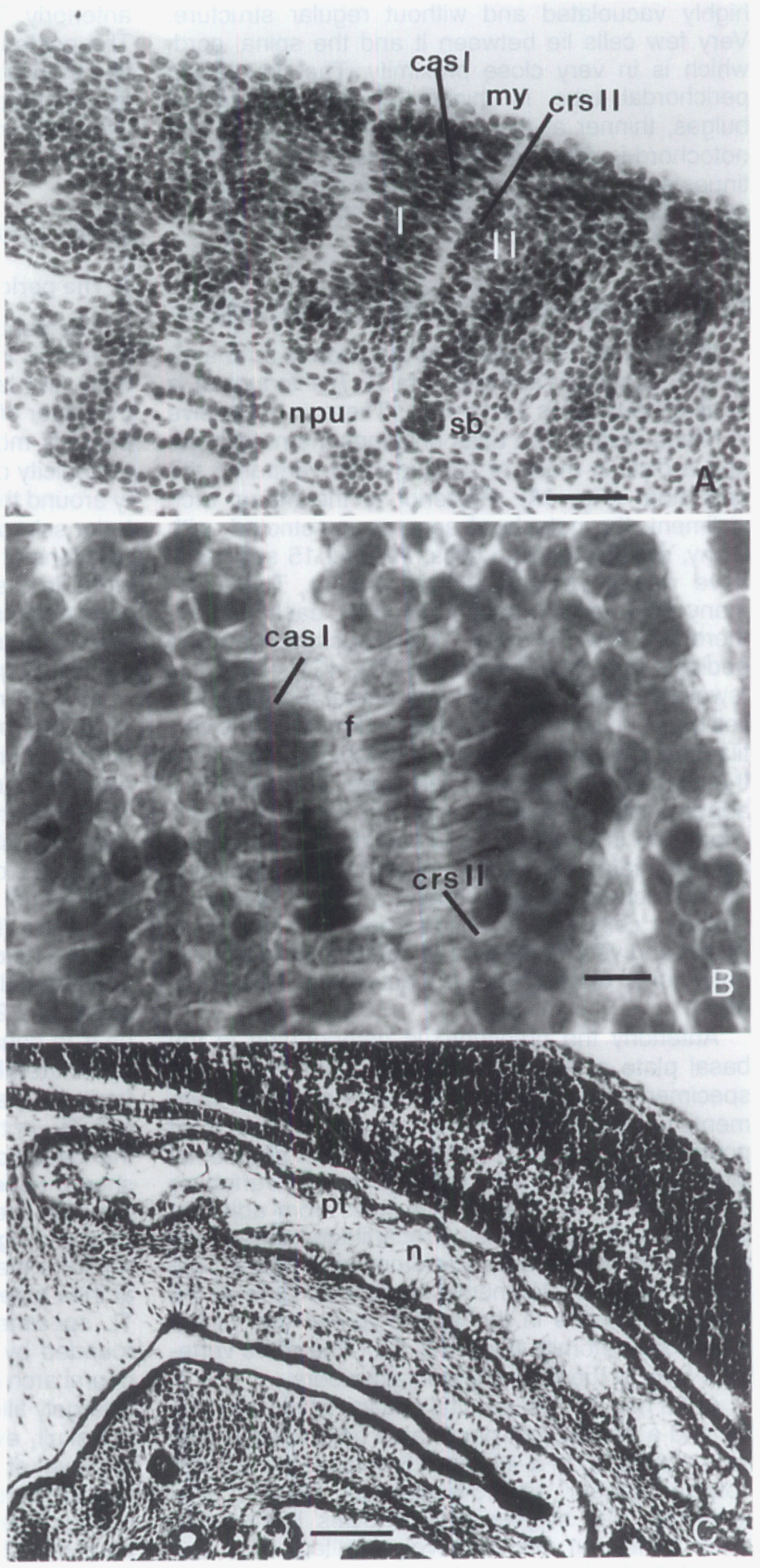

Fig. 3. A. *Dermophis mexicanus* embryo, 10 mm TL. In the anterior part of the body, differentiation of segments is proceeding. For illustration, an anterior segment is labelled I; the one posterior to it is labelled II. Resegmentation is proceeding, and is apparent in these segments. The cranial sclerotomite of the more posterior somite (II) is associating with the caudal sclerotomite of the more anterior somite (I). Oblique sagittal section; scale bar = 0.1 mm.

B. Same embryo, same somites, higher magnification to elucidate details of resegmentation. Cellular fibrils extend from the adjacent sclerotomites, thus effecting the intersegmental association of caudal sclerotomites of anterior somites with the cranial sclerotomites of adjacent, posterior somites. Scale bar = 0.01 mm.

C. *Dermophis mexicanus* embryo, 10 mm TL. Segmental constrictions of the notochord are apparent; the perichordal tube is well developed. Sagittal section; scale bar = 0.1 mm. Abbreviations: cas I – caudal sclerotomite I; crs II – cranial sclerotomite II; f – fibrils; my – myotome; npu – nephric units; pt – perichordal tube.

highly vacuolated and without regular structure. Very few cells lie between it and the spinal cord, which is in very close proximity. The developing perichordal tube is thicker at the notochordal bulges, thinner at the constrictions (Fig. 3C). The notochord is constricted at the skull base, but continues anteriorly into the base of the skull. This overall configuration also is present in a 10 mm TL *Gymnopis* embryo.

Formation of the atlas, and of neural pedicels

In a 14 mm TL *Dermophis mexicanus* (and a 12 mm TL *Gymnopis* and a 15 mm TL *Typhlonectes*), a cartilaginous atlas is formed. There is extensive cartilage matrix. The atlas envelops the notochord, and a distinct mass of cartilage lies ventral to the notochord (Fig. 4A). Posterior to the neural arch rudiment, the notochord appears constricted, with many, densely packed cells. At least 15 segments have neural pedicels/arches forming. There are many cells in the notochord under each pedicel; there are few between. The notochord between pedicels is highly vacuolated (Fig. 4C), giving it a "swollen" appearance. A large dorsal root ganglion lies between each set of pedicels (Fig. 4C). The cartilage is associated with the notochordal sheath in the atlas and subsequent vertebrae. The notochord appears slightly dilated under the dorsal root ganglion, and is expanded and rounded under neural pedicel rudiments by the invading new cartilage. The notochordal sheath appears to be invaded by cartilage cells. In the second and third vertebrae dorsal and ventral masses of cartilage lie within the notochord, displacing the vacuolated cells (Fig. 4BC).

Anteriorly the notochord is deteriorating in the basal plate of the skull. The posterior end of the specimen is beyond the segmental stage; all segments have sclerotomites, promuscle running from posterior to anterior sclerotomite, and segmental blood vessels lie directly under primordial vertebrae almost at the point where muscles from adjacent segments come together. Procartilage masses are associated with caudal sclerotomites in these posterior segments. Segmental blood vessels lie at the segment borders in the antero-ventral end of the cranial sclerotomite (Figs. 4B, 5C). Therefore vertebrae form at the inter-segmental borders.

At 23 mm TL, the atlantal cotyles are in continuity, and a ventral root penetrates before the neural arch connects. In a 44 mm TL animal, the atlantal cotyles appear ventromedial to the occipital condyles. The cotyles and condyles have some outer bone, but are massively cartilaginous. The neural pedicels of the atlas invest the cartilage-filled notochord ventrally. The pedicels surround the atlas, and are tenuously connected at the top anteriorly. The outer covering of the arch is bony. The posterior part of the atlas is much more ossified than anteriorly, and zygapophyses are present. the posterior part of the centrum is a ring of bone with a thin ring of cartilage between it and the notochord.

Centrum development

1. The perichordal tube

The perichordal tube is initiated at approximately 10 mm TL in *Dermophis* by proliferation of cells ventrally and laterally to the notochord. The tube is a thin layer of cells that surrounds the notochord, apparently more limited than in urodeles. There is a periodicity of areas of greater and lesser cell density around the notochord. Denser regions are in posterior sclerotomites and where there are slight constrictions of the notochord. The notochordal expansions lie at segmental borders. The perichordal tube constrictions concomitantly lie at approximately the same points as the intersegmental blood vessels, i.e., the segmental borders.

In a 15 mm TL *Dermophis* embryo (and an 18 mm TL *Typhlonectes*), rings (areas with many cell bodies alternating with areas with few cell bodies) are more fully formed. They alternate with masses forming within the notochordal sheath. The masses within the sheath lie exactly where the neural arch rudiments outside the sheath occur (see below). Perichordal rings alternate with intranotochordal cartilages (Fig. 5B). Some cells are incorporated into the notochordal sheath at the level of the intranotochordal cartilages on both ventral and dorsal sides. In a 20 mm TL embryo, perichordal rings appear to constrict the notochord (Fig. 6A). They expand laterally in sequence so that material derived from the perichordal sheath is continuous. By 22 mm TL, in the extreme anterior end of the column, the developing outer portions of the notochordal sheath have become partially cartilaginous, and a few cells are incorporated into bone of the definitive centrum Fig. 6 BC). The masses of fibroblasts that will form the intervertebral joint have cartilage cells at their anterior and posterior peripheries. A 23 mm TL specimen has the notochord completely surrounded by cartilage at the end of the developing neural arch of the first few vertebrae. The notochord is largely filled with cartilage from the basal plate of the skull, even across the craniovertebral joint, to the level of the neural pedicel of the atlas. The intranotochordal cartilage is in contained masses in each pre-centrum, interrupting the otherwise continuous notochordal cells. These cartilages are rings, which constrict the notochordal cells to membrane remnants. More posteriorly, the notochord is strictly notochordal tissue without incorporated cartilage.

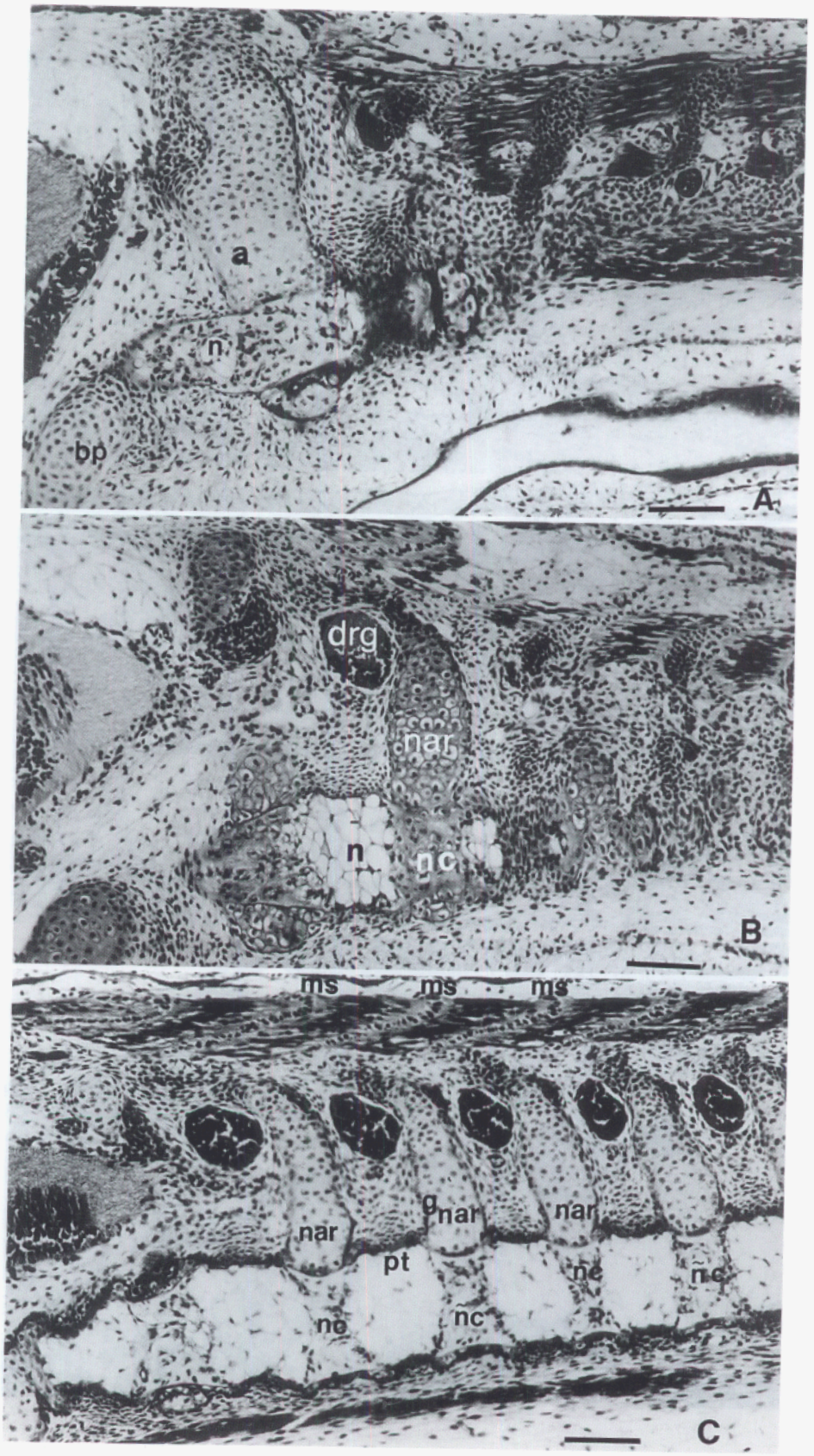

Fig. 4. A. *Dermophis mexicanus* embryo, 14 mm TL. Development of the atlas. Note the vertical structure of the neural arch rudiment that forms the atlas, and the anterior extension of the notochord into the skull. Sagittal section; scale bar = 0.1 mm.
B. Same embryo, illustrating the notochordal cartilage that invades the notochord, and the neural arch rudiments that are forming from the previously precartilaginous masses. Sagittal section; scale bar = 0.1 mm.
C. Same embryo, slightly more posteriorly. A series of vertebrae is forming; segmental dorsal root ganglia are large and apparent. Intersegmental vertebrae are demarked by neural arch rudiments and notochordal cartilages. Neural arch rudiments clearly form from caudal sclerotome halves of adjacent somites. Note the cells of the incipient myotomal septa and their orientation. Scale bar = 0.1 mm. Abbreviations: a – atlas: bp – basal plate of the skull: drg – dorsal root ganglion: ms – myotomal septum: n – notochord: nar – neural arch rudiment: nc – notochordal cartilage: pt – perichordal tube.

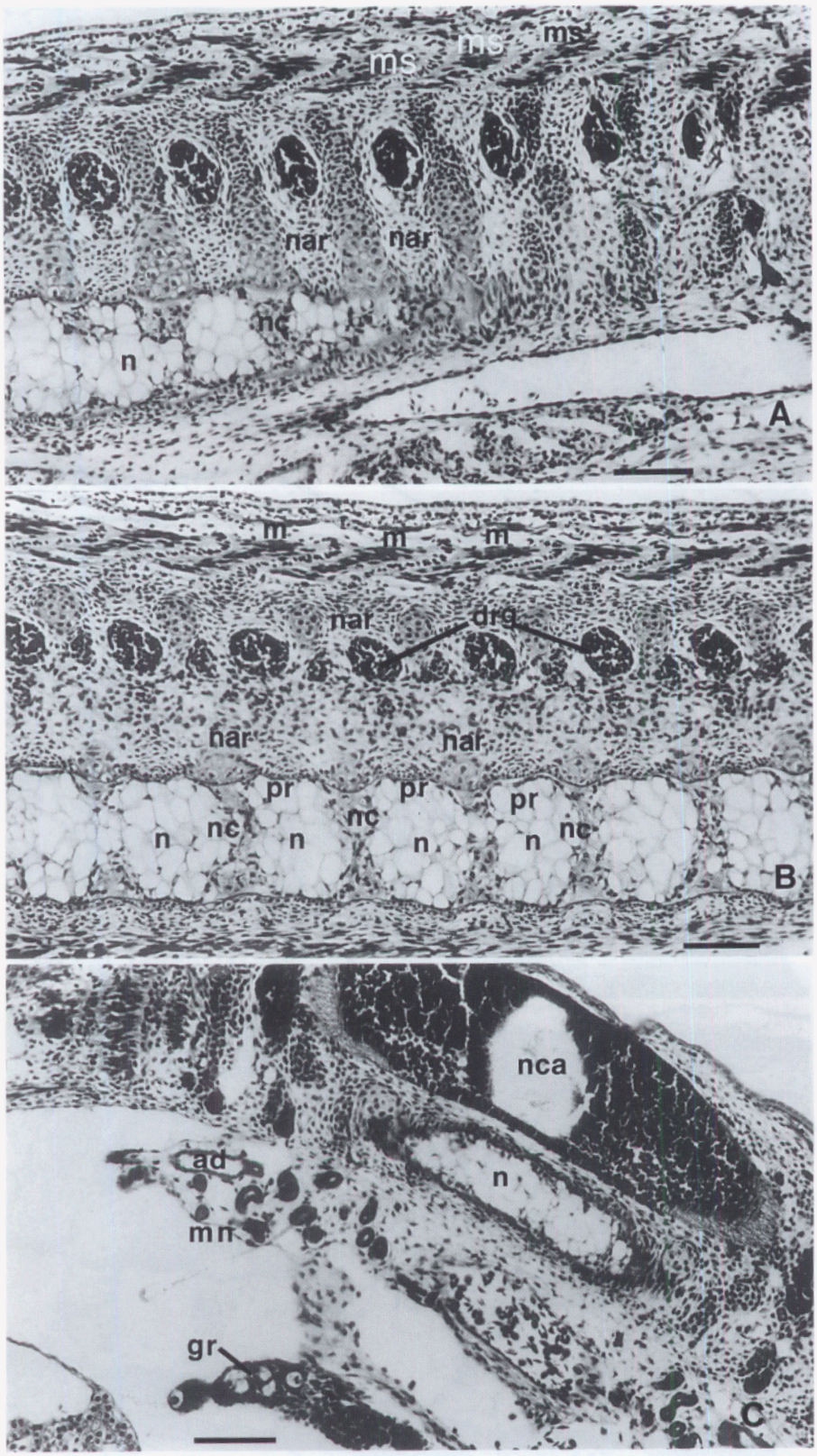

Fig. 5. A. *Dermophis mexicanus* embryo, 14 mm TL. Myotomal septa are demarked by oblique lines of single cells. The myomeres between the septa lie parallel to the borders of the original sclerotomites, but now appear to span the junctures of the developing vertebrae. Neural arch rudiments (cartilages to the sides of the labels [nar]) are curved out of the plane of the section, so that their bases are apparent atop the notochord, and their apices lie between the dorsal root ganglia at intersegmental junctions. Sagittal section; scale bar = 0.1 mm.

B. Same region of the embryo illustrated in A; more lateral section, showing complete neural arch rudiments, their associations with the notochord, the dorsal root ganglia, and the myomeres. Note that the oblique myotomal septa align with the caudal sclerotome half components of the original sclerotomites. Perichordal rings alternate with intranotochordal cartilages. Sagittal section; scale bar = 0.1 mm.

C. Oblique transverse section of the same embryo at approximately body segment 25. The neural tube with a large neural canal and the notochord lying below it are apparent. Mesonephric tubules are forming, the archinephric duct is well developed, and the genital ridge is discrete. Scale bar = 0.1 mm. Abbreviations: ad – archinephric duct; drg – dorsal root ganglion; gr – genital ridge; m – myomere; mn – mesonephric tubules; n – notochord; nar – neural arch rudiment; nc – notochordal cartilage; nca – neural canal; pr – perichordal ring.

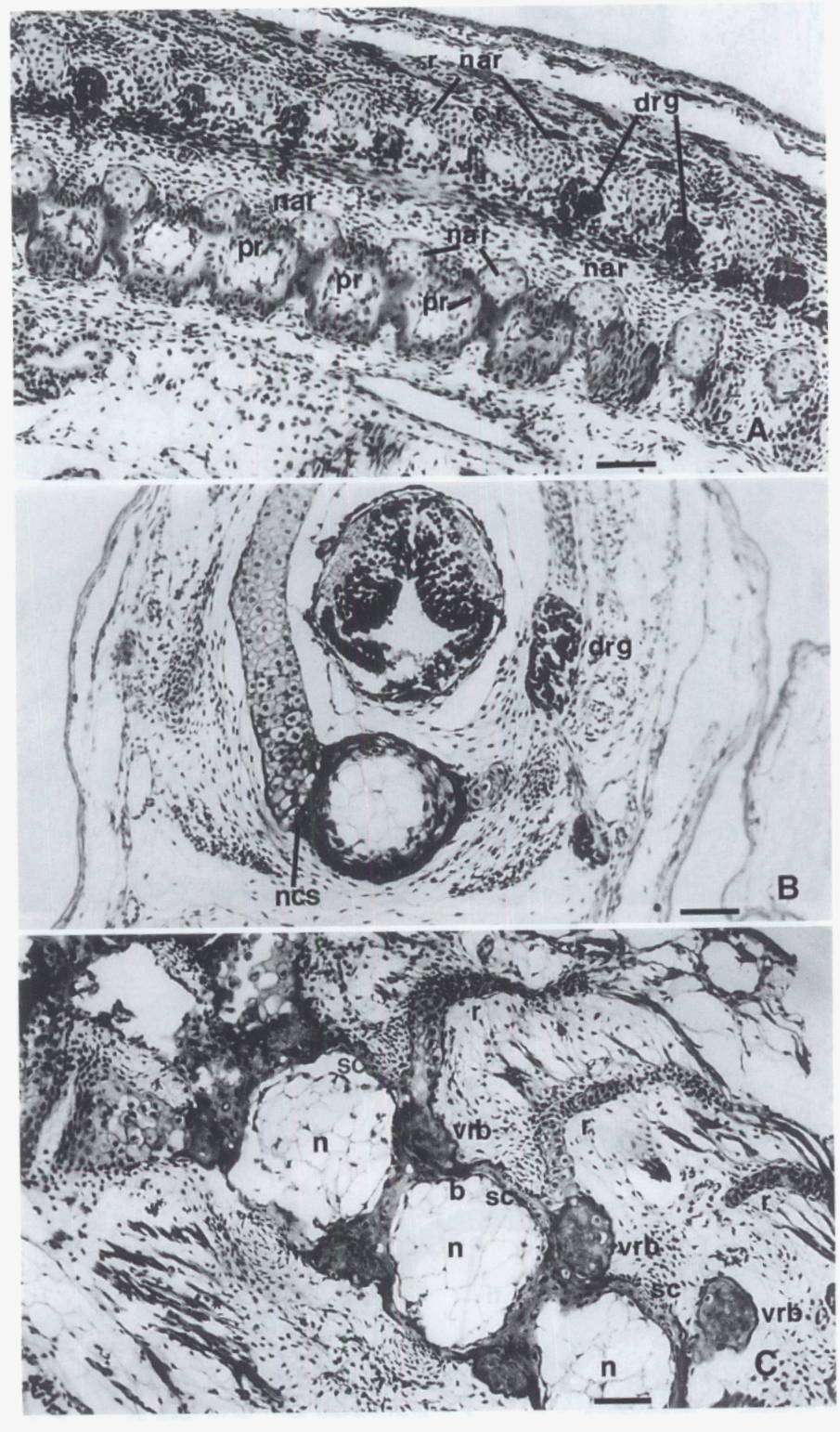

Fig. 6. A. *Dermophis mexicanus* embryo, 20 mm TL. The perichordal rings (pr) now appear to constrict the notochord dorsally and ventrally. Compare neural arch structure with that in figure 5 A. Sagittal section; scale bar = 0.1 mm.
B. *Dermophis mexicanus* embryo, 23 mm TL. The neural arch is well developed. In this slightly oblique transverse section, the dorsal root ganglion (drg) lies posterior to the arch; the cells of the edge of the arch are apparent on the left arch component. A distinct neurocentral suture is apparent. Mineralization is beginning in the outer edge of the centrum. Scale bar = 0.1 mm.
C. *Dermophis mexicanus* embryo, 25 mm TL. Cartilage cells are apparent in the notochordal sheath. A few cells are incorporated into the bone of the definitive centrum. Ventral rib bearers attach to vertebrae in the clefts between the lower part of the basidorsal (neural arch rudiment) and the anterior part of the centrum. Cartilaginous ribs attach to the bearers. Frontal section; scale bar = 0.1 mm. Abbreviations: b – bone; drg – dorsal root ganglion; n – notochord; nar – neural arch rudiment; ncs – neurocentral suture; pr – perichordal ring; r – rib; sc – cartilage in sheath; vrb – ventral rib bearer.

2. The definitive centrum

In a 25 mm TL specimen, the centra are very short, and the anterior ones are partly ossified. There is little definitive centrum tissue before ossification. The bone of the centrum does not form in direct contact with the notochord, but is separated from it by a thin (two cells maximum, usually one) layer of cartilage.

The centra of a 35 mm TL fetus remain very short (also in a 30 mm TL *Gymnopis* and a 38 mm TL *Typhlonectes*). The ends of the centra have begun to

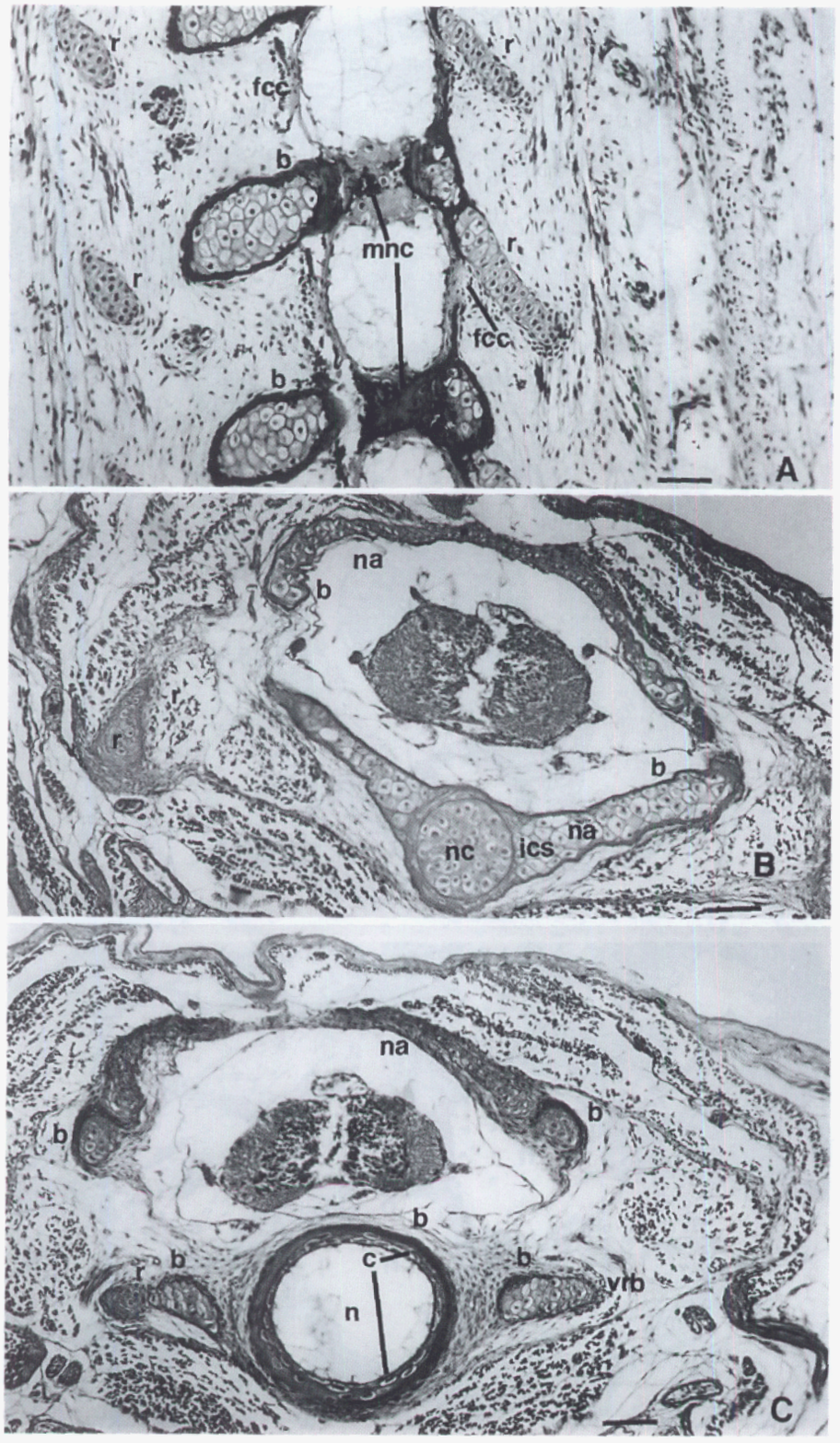

Fig. 7. A. *Dermophis mexicanus* embryo, 35 mm TL. The notochordal cartilage is partly mineralized. Bone surrounds the rib-bearers; the ribs remain cartilaginous. Fibrocartilage cells are concentrated at the inter-vertebral joint region. The section is frontal, and tilted ventrally to the left. Scale bar = 0.1 mm.
B. *Dermophis mexicanus* embryo, 44 mm TL. The neural arch is complete dorsally. Dense notochordal cartilage lies at the neural arch/centrum juncture; there is a pronounced intercentral suture at this stage. Transverse section; scale bar = 0.1 mm.
C. *Dermophis mexicanus* embryo, 44 mm TL. Thin cartilage lies between the outer bone of the centrum and the notochord, which is rapidly eroding. Bone is developing peripherally on the outer margins of the neural arch , the centrum and the rib-bearers. Transverse section; scale bar = 0.1 mm. Abbreviations: b – bone; c – cartilage; fcc – fibrocartilage cells; ics – intercentral suture; mnc – mineralized notochordal cartilage; na – neural arch; nc – notochordal cartilage; r – rib; vrb – ventral rib bearer.

attain a cone-shape, and the notochordal tissue seems to pull apart as the cones diverge (Fig. 7A). The notochordal cartilage is partly mineralized (Fig. 7A). In a 44 mm TL specimen, the centrum of the atlas is ossified, especially posteriorly where the centrum is a ring of bone with a thin ring of cartilage between it and the notochord. The centrum of the first trunk vertebra has a thicker bony ring surrounding the layer of cartilage that lies against the notochord. The second trunk vertebra resembles the first. The notochord lacks cartilage until the neural arch (see below) contacts the centrum, then it fills

with dense cartilage (Fig. 7B). The centrum has a thin layer of cartilage between the outer bone and the inner notochord (Fig. 7BC), and in the rear of the centrum the notochord again loses cartilage as the neural arch is passed. At the mid-vertebral level there is a continuous ring of cartilage all around the nerve cord. There is a distinct intercentral suture (Fig. 7B) and intervertebral cartilage (Fig. 7A). In mid-body of the same specimen, the vertebrae are less well developed, but with one important difference. There is less cartilage between the bony centrum and the notochord at the mid-vertebral level than in more anterior centra, both dorsally and ventrally, and there are places where the bone is in direct contact with the notochordal sheath, with no intervening cartilage; still there is a complete ring of cartilage around the nerve cord.

Additional ossification and growth produces the spool-shaped adult centrum. Its constricted center contains a small core of notochordal cartilage throughout life. That cartilage arose from clear lines of cells that lay within the notochord at the segmental borders, as observed in a 20 mm TL specimen (Fig. 6A).

Neural arch development

Procartilaginous masses noted above are formed from the caudal sclerotomite, distinctly larger than and separated from the perichordal tube. A 14 mm TL *Dermophis* and 15 mm TL *Gymnopis* and *Typhlonectes* have well formed neural arch rudiments (Fig. 4BC). The notochord is more constricted where the neural arch pedicels (basidorsals) lie against it (Fig. 4C). Large dorsal root ganglia lie between pedicels and over notochordal constrictions. A 20 mm TL embryo has a long series of basidorsals (Fig. 5A). These cartilages rest laterally against cartilage cells inside the notochord, then rise dorsally and bend posteriorly. This configuration is as though there is a basiventral (but they are never truly ventral) element formed of the new intra-notochordal cells with a basidorsal attached laterally. There appears to be a joint between the ventral and lateral cartilaginous masses, because of differences in cell axis orientation. The dorsal mass is clearly the precursor of the basidorsal/neural pedicel, and appears continuous with a series of cartilage cells, the interdorsal element of Gadow (see Discussion). The basidorsals arise exactly at the point of the notochordal cartilage in more posterior elements, rather than slightly posterior as in anterior vertebrae. There is a neurocentral suture where the basidorsal attaches at the lateral midpoint of the notochord. A 23 mm TL embryo includes better developed neural arches. The anterior arches are nearly complete at the top; i.e., the paired basidorsals have extended almost completely dorsally and medially. The dorsal root does not appear to penetrate the neural arch, but to pass posterior to it (Fig. 6B). The neural arch of the atlas has large posterior-extending flanges. The neural arch of the first trunk vertebra commences with the basidorsal attachment at approximately 67% of the dorso-ventral diameter of the notochord. There is a distinct neurocentral suture (Fig. 6B). At the level of the middle of the nerve cord, there is a distinct lateral expansion in the paired neural arch components; i.e., they are thicker at their midpoints. Neither the suture nor the expansion occur in urodeles. In the first two trunk vertebrae, bony material from the developing centrum extends into the bases of the neural arch elements internally. In the third trunk vertebra, there is no neurocentral suture because the basidorsal sits directly on the notochordal sheath. The basidorsals appear to form in the intersegmental cleft regions (Figs. 4C, 5 AB, 6A), from posterior sclerotomite tissue plus some contribution from the anterior sclerotomite behind it. As development procedes, the myotomal border is pulled forward as the anteriorly directed rib bearer and rib form.

There is cartilaginous continuity of the neural arch halves over the nerve cord for the first time in a 35 mm TL specimen. Both dorsal and ventral rib bearers are present.

A 44 mm TL specimen has neural pedicels that surround the atlas, and are tenuously connected dorsally over the nerve cord on many more posterior vertebrae. The outer covering of the arch is bony, but the core remains cartilaginous (Fig. 7BC). The posterior end of the arch is much more ossified than anteriorly. The neural arch of the first trunk vertebra is mainly cartilage, which continues to the cartilage-filled notochord, but cartilage does not surround the cord. The arch has outer and inner husks of bone. The upper rib bearer arises from the swollen lateral side of the neural arch and extends ventrolaterally. The neural arch dorsal contact is thin, one capsule thick, on the first vertebra, but is thicker over more posterior vertebrae.

Intervertebral cartilage

At 22 mm TL, the masses of fibroblasts that will later form the intervertebral joints have cartilaginous cells at their anterior and posterior boundaries. These masses lie at the level of the notochordal bulges. A 25 mm TL specimen has formed intervertebral discs anteriorly, but this is not yet a distinct intervertebral cartilage. At their ends, centra assume a cone-shape and the notochordal tissue pulls apart and joins the margins of the spindle-shaped intervertebral cartilage. At 35mm TL, there is still no definitive intervertebral cartilage, but there are fibrocartilage cells concentrated at the joint region that have a capsular organization (Fig. 7A). At 44 mm TL, the ring-like "intervertebral cartilage" is really a

fibrocartilage joint. It is never more than five fibrocartilage capsules thick, and the capsules are very flattened with little extracellular matrix. There are several plies to the fiber layer that lies external to the main cartilage.

Ribs and rib bearers

Rib bearers are first apparent at 20 mm TL. They lie ventral to the dorsal root ganglia and anterior and ventral to the lower parts of the basidorsals. The dorsal rib bearers develop as bulges of cartilage laterally on the neural arch halves, and are apparent at 22 mm TL. Basiventral pieces lie laterally just before the intervertebral joints, and connect to the lower part of the more posterior neural pedicel. Rib rudiments lie dorso-laterally and anteriorly to the bearer rudiments, and form at the level of the intersegmental blood vessel, i.e., the segmental border, just anterior to the point where the basidorsal arises. The rib then lies just anterior to the segmental blood vessel, the basidorsal just posterior to it. The segmental blood vessel changes position as well, so there are no absolute fixed points. In a 25 mm TL specimen, the ventral rib bearer is sharply angled anteriorly, so that myotomic muscle effectively runs from one intercentral joint to the other at this level. The ventral rib bearer attaches to the vertebra in the cleft between the lower part of the basidorsal and the anterior part of the centrum (Fig. 6C). By 35 mm TL, both dorsal and ventral rib bearers are definitive in *Dermophis*, as well as in *Gymnopis* and *Typhlonectes*. The dorsals are attached to the midpoint of the neural arch and are essentially continuous with the cartilage of the arch. The ventral bearers are far separated from the dorsals, and are attached to the centrum immediately at its anterior end (Fig. 7A). There is a thin ossified cylinder around each largely cartilaginous bearer. The rib is still entirely cartilaginous, but elongate. It has its strongest attachment to the dorsal rib bearer, and a tenuous, fibrous attachment to the ventral bearer. Yet, it appears that the lower rib bearer is the dominant one and it is attached both to the anterior part of the centrum and to the neural arch. At 44 mm TL, the lower rib bearer of the first trunk vertebra remains largely cartilage, but gains a stout bony cylinder as it approximates the centrum, to which it is attached by a bony strut. The upper rib bearer is cartilaginous. The medial component of the rib is an elbow-like structure, mainly cartilaginous but with a surrounding bony tube, presaging the bicipital structure of the rib head. The second trunk vertebra has the ventral rib bearer ringed with thick bone, and the dorsal rib bearer is poorly organized cartilage. The ventral bearer still has only a tenuous strut connecting it to the centrum. The rib is progressively bony distally, and extends to a point. At mid-body, the ventral rib bearer is noticeably less well developed than on more anterior vertebrae.

Zygapophyses

Rudiments of the prezygapophyses are present at 22 mm TL as masses of cartilage just dorsal and slightly anterior to the dorsal root ganglia. In a 23 mm TL specimen, series of procartilaginous masses are precursors of pre- and postzygapophyses. At 44 mm TL, pre- and postzygapophyses are present on anterior vertebrae.

The cranio-vertebral joint

Even at 44 mm TL, the notochord extends well into the skull. It is filled with cartilage and appears somewhat swollen. It appears to lack lateral attachments in the anterior skull region. More posteriorly in the skull, the notochord lies atop a triangular mass of cartilage that joins more lateral cartilage to form the basal plate. The basal plate is broad, flat, and cartilaginous, with the round, cartilage-filled notochord in a mid-dorsal portion. The notochord becomes free posteriorly and the occipital condyles form independently. There is no sign of a tuberculum interglenoideum. The atlantal cotyles lie ventromedial to the occipital condyles. The cotyles and condyles have some outer bone, but are massively cartilaginous. The outer covering of the neural arch of the atlas is bony by this stage.

Discussion

Evidence for resegmentation

We have demonstrated the presence of a sclerocoele during somitogenesis in gymnophiones. We also presented evidence for the establishment of cell-rich caudal sclerotomites and cell-scant cranial sclerotomites, and showed that transitory sclerofibrils join caudal sclerotomites to cranial sclerotomites of the next posterior segment. Our sections show that the cell-rich posterior sclerotomite contributes the cells that differentiate into the neural arch rudiment (basidorsal), and that the pro-cartilage masses that give rise to these rudiments are established before the perichordal tube is well formed. The neural arch rudiments are intersegmental, in origin and final position, appropriate to inferences of resegmentation. We demonstrated that promuscle and muscle cells extend from posterior sclerotomite to posterior sclerotomite, also suggesting a positional reorganization of the somite. Without studies following labelled cells, we cannot identify the contributions of sclerotomites more specifically. We find no particular evidence for the compo-

nents of the centrum as defined by Gadow (1906) and Gardiner (1983). Some elements (e.g., basidorsals, interdorsals, perhaps basiventrals: we adopt the Gadovian terminology for this discussion) clearly are present, but others are not.

Wake (1970) pointed out that no clear evidence of segmentation, then resegmentation, of the sclerotome had been presented for salamanders or frogs, despite much work by many people on vertebrogenesis. A sclerocoele has not been observed in members of these groups, though a myocoele has been reported (cf. Wake and Lawson, 1973). Sclerotome tissue is very scanty in frogs and salamanders. Differences in the density of cells in anterior and posterior segmental halves have been observed (Mookerjee, 1930, 1931; Wake and Lawson, 1973), and this is much of the basis for inferences about sclerotome halves and their reassociation.

Gymnophiones have many more cells per segment than do frogs or salamanders. For example, at comparable stages of development with apparent sclerotome halves, a *Pseudoeurycea* embryo has approximately 55 cells in sagittal section profile, while a *Dermophis* has approximately 150 cells. The *Pseudoeurycea* has five segments in 1.12 mm body length, and the *Dermophis* 12 segments in the same distance. Nuclei in cells of both genera are the same size (varying from 13u x 5u to 10u dia in each). Their genomes are also approximately the same size (Sessions and Larson, 1987; M. Wake and Sessions, unpubl. data). Therefore the density of sclerotomites in gymnophiones relative to those of salamanders is a phenomenon of increased cell number. In salamanders near obliteration of the anterior sclerotome half occurs early in development, with growth of the dorsal root ganglia, but the anterior sclerotome half persists much longer in gymnophiones, so its associations are more easily assessed. Gatherer and del Pino (1992) present data that show that, at a particular stage of development, there is considerable variation among taxa of frogs in cell numbers per somite, the large-egged *Gastrotheca* and *Bombina* having six times more cells than the small-egged *Xenopus*, the former near the range for mice, and three times the number for chicks. They note that the salamander *Ambystoma mexicanum* has few, large cells that have much more nuclear DNA than occurs in frogs, and speculate that this is an attribute of somitogenesis. Their data are not directly comparable to ours, and our comparison of caecilians and salamanders with the same genome size suggests that cell number during development may not be a function of genome size.

Comparisons to frogs and salamanders

The vertebrae of basal frogs, *Ascaphus* and *Leiopelma*, resemble those of caecilians and salamanders more closely than do those found in other more derived families of frogs (frog phylogenetic relationships from Ford and Cannatella, 1993). Moffat (1973) argued that the presence of an uninterrupted notochord and the absence of ball and socket joints in these genera were retentions of ancestral conditions, not the result of derived heterochonic evolution from a holochordal ancestor, as Inger (1967) believed. With respect to these two features, caecilians, salamanders and frogs share the same conditions, which we believe to be ancestral for each group. Accordingly, they constitute two potential synapomorphies for the Lissamphibia or possibly symplesiomorphies (see below, phylogenetic implications).

Another potential synapomorphy of the three amphibian taxa is the presence of notochordal cartilage in the basal plate of the skull during ontogeny (Wake, 1970). Notochordal cartilage in vertebrae is found in some amniotes as well, and it seems likely to be a synapomorphy at the level of tetrapods. There is no vertebral notochordal cartilage in frogs, but cartilage does appear in the portion of the notochord in the basal plate of *Ascaphus* during development. Whether this is the sole remnant of notochordal cartilage in frogs, or if instead it represents a unique synapomorphy with caecilians and salamanders cannot be determined at this time. Notochordal cartilage has not been reported in the basal plate of amniotes.

The intervertebral joint has unique developmental patterns in each of the three amphibian taxa. In particular, the distinctive tuberculum interglenoideum of salamanders is absent in frogs and caecilians (see Wake, 1970; McGowan, 1998).

Available information suggests that frogs as a group have a pattern of vertebral development that differs from that of caecilians and salamanders with respect to the proportion of the vertebral centrum that is preformed in cartilage. Most studies of vertebral formation in frogs have been conducted on relatively derived taxa (e.g., Mookerjee, 1931; Mookerjee and Das, 1935; Brustus *et al.*, 1976; Dey *et al.*, 1989; Smit, 1953). However, work on leiopelmatids and ascaphids demonstrates that they have holochordal centra that have been called "ectochordal" (Griffiths, 1963), in that they ossify around a persistent notochord (Ritland, 1955; Stephenson, 1952, 1960; Moffat 1973, 1974), whereas the holochordal vertebrae of some other anurans (e.g., ranids; Mookerjee, 1930; Brustus *et al.*, 1976) incorporate a resorbed notochord into a relatively solid centrum (this is called "perichordal" development; Gadow, 1896). In other taxa (e.g., pipids [Smit, 1953], pelobatids) vertebral development is "epichordal", with the notochord and portions of the perichordal tube lateral and ventral to it largely disappearing by resorption during development. In the

great majority of frogs the resulting centrum is a solid and relatively dense structure. The centra of leiopelmatids and ascaphids, and rhinophrynids, in contrast, appear hollow when prepared as skeletons, as do those of caecilians and most salamanders (Wake, 1970). Ectochordal vertebral formation has been proposed for the fossil taxa *Prosalirus* and *Vieraella*, and the vertebrae of the former clearly preserve evidence of a persistent notochord because they are hollow (Jenkins and Shubin, 1998). A well defined neurocentral suture is evident in amniotes (Williams, 1959), but no such suture is found in salamanders or caecilians. Williams (1959) also reported a neurocentral suture in *Leiopelma*, and the presence of separate centers of ossification for the neural arch and centrum in that genus (Stephenson, 1960) supports his contention.

In order to make an appropriate comparison among the three major amphibian taxa it may be best presently to set aside the highly derived patterns of development found in all anurans except the leiopelmatids and ascaphids. The latter two groups resemble caecilians and salamanders in that the fundamental developmental plan involves relatively little cartilage formation in the prospective centrum region. The neural pedicel rudiments form directly opposite the middle of the vertebra and rest on the notochordal sheath. However, there is an important difference between these frogs and both caecilians and salamanders. The neural arch rudiments of caecilians and salamanders are tightly circumscribed in a persistent perichordal tube of connective tissue. This tube forms a cylinder, which arises on the dorsolateral surface of the notochordal sheath. The tube at its origin is at about its smallest diameter; as it ascends and grows around the nerve cord it remains narrow at first, gradually broadening until it gives rise to the rudiments of the zygapophyses. In *Leiopelma* the rudiment of the neural pedicel is far less spatially restricted. Where it arises from the perichordal tube it is broad and it becomes narrower dorsolaterally (Moffat, 1974, fig. 4); in the earliest stages available the bases of the rudiment are more restricted, but apparently they are never bounded as in caecilians and salamanders. Furthermore, there is far more involvement of cartilage during centrum development in *Leiopelma* than in caecilians and salamanders (e.g., Moffat, 1974, fig. 9). In *Leiopelma* there is sufficient cartilage in the centrum that centers of central ossification are separated from ossification associated with the neural arch (Stephenson, 1960, fig. 3D), a situation never encountered in caecilians or salamanders. In the latter groups the neural pedicels and centra, and often the rib-bearers as well, ossify as a continuous unit. Ossification begins in the vicinity of the point of contact of the neural pedicel rudiment with the notochordal sheath and radiates outward in all directions, but ossification of the centrum completely encircles the notochordal sheath from the beginning. We find no evidence of any discrete centers of ossification in caecilians, nor is there for salamanders (Wake, 1970; Wake and Lawson, 1973). In both caecilians (this paper) and salamanders there are contributions from very small strips of cartilage to the developing vertebrae (Schmalhausen, 1957, 1958; Wake and Lawson, 1973).

Perhaps the only feature in which frogs and salamanders uniquely resemble one another is in the relatively scanty sclerotomal tissue that is present, which makes it nearly impossible to detect any developmental resegmentation that might take place (Wake, 1970). Caecilians are more similar to amniotes in having a relatively cell-rich sclerotomal region and in manifesting resegmentation during development (see above, and Wake and Wake, 1986).

There are many similarities in vertebral formation among salamanders and caecilians, but there are also important differences. The dorsal rib bearer of caecilians arises in close proximity to the base of the prezygapophyses, essentially as an outgrowth of the neural pedicel rudiment, while the ventral rib bearer is far removed from it and formed from a distinct rudiment that arises cranial to the neural pedicel rudiment. The two rib bearers are oriented anteriorly, and the rib heads are oriented posteroventrally, an acute angle is formed by the rib bearer just before the articulation point. Bone is added to this angle so that it becomes a spinous, cranially directed process that forms the most anterior end of the vertebra. The articulation is near the anterior end of the vertebra. The vertebra, as a consequence, lies largely in the cranial part of each myotomal segment. In salamanders the two rib bearers are more closely associated with each other and arise separately from but exactly lateral to the neural pedicel rudiment. The rib lies in a more central to slightly caudal position on the vertebra, so that the vertebra as a consequence lies mainly in the caudal part of each myotomal segment. While ribs are present in basal frogs, including fossil taxa, they are not clearly bicipital and the rib bearer arises from the neural pedicel, as do the transverse processes (Moffat, 1974; Jenkins and Shubin, 1998).

While caecilians and salamanders both have centra that are ectochordal and form with very little cartilage preformation, those of salamanders often are more complicated internally. The notochord remains more fully intact in most salamander families, and there are diverse cartilage contributions to the complete centrum. While both caecilians and salamanders have a mid-vertebral intranotochordal cartilage, it is small and relatively insignificant (very narrow) in caecilians, but typically larger in salamanders. In addition, salamanders may have large and well developed intervertebral cartilages that

may become mineralized to form a modified ball and socket opisthocoelous vertebral articulation. The condyle part of this cartilage may contain a second intranotochordal cartilage (Wake and Lawson, 1973). In some salamanders (e.g., salamandrids) the intervertebral cartilage ossifies. Intervertebral cartilages are relatively insignificant in caecilians, and ball and socket type joints never form.

While there is now widespread acceptance that living amphibians form a monophyletic group (the Lissamphibia: Parsons and Williams, 1963), analysis of the pattern of morphological evolution within the group is made problematic as a result of two facts. First, assuming the monophyly of the group, there is no clear living sister-taxon, and any candidate taxon would be phylogenetically remote. While there is no consensus concerning the likely sister-taxon within the Paleozoic amphibians (Carroll, 1997), attention has focused on the dissorophoid temnospondyls (Bolt, 1991) and on microsaurs (Carroll and Currie, 1975; Carroll *et al.*, 1999). Second, the three members of the Lissamphibia, the Caudata, the Salientia, and the Gymnophiona, are strongly differentiated from one another both with respect to morphology and DNA sequences, and there is no robust phylogenetic hypothesis. However, support for a Caudata-Salientia sister-taxon relationship has been found in morphological data (Milner, 1988; Trueb and Cloutier, 1991). In contrast, molecular data suggest a Gymnophiona-Caudata sister-taxon relationship (Larson, 1991; Hedges and Maxson, 1993). With respect to details of vertebral morphogenesis (detailed above) as well as many aspects of neurobiology (Roth *et al.*, 1993), caecilians and salamanders are more similar to each other than either is to frogs. These similarities may be the result of common ancestry, but it is unclear if they are symplesiomorphies or synapomorphies. At least some of the similarities may be secondary, resulting from simplification of morphogenesis arising from paedomorphosis (Roth *et al.*, 1993). What is needed is a total evidence analysis of all available data for those taxa for which relatively complete data sets exist. Such an analysis would help unravel the complexities of morphological evolution in these divergent taxa and identify those features that are homoplastic from those that are truly synapomorphic.

Comparison with mammals

Our observations of early vertebrogenesis in gymnophiones differ markedly from those of Verbout (1985) on sheep (the only data available for appropriate comparison regarding these issues), in contrast to the more subtle differences noted between gymnophiones and other amphibians. We attribute most of these differences to vastly different developmental rates. For example, Verbout found that in sheep the "somite cavity" appears lost before myotome and especially sclerotome differentiate in his Stage I (ca 3 mm crown-rump length [CR]). He does not comment on the nephrotome. We find a continuous and patent myo-sclero-nephrocoel in somites of 5 mm TL gymnophiones that have the three regions of the somite clearly differentiated (fig. 3).

We summarize Verbout's data in order to make comparisons of his material with ours. In Verbout's Stage II (ca 3.5 mm CR), segments have "looser" cranial and denser caudal parts. In Stage III (ca 4 mm CR), he notes a differential reorganization in the cranial part of the segment, because of development of the peripheral nervous system ventral root anlage. This aspect gives rise to Verbout's notion of two segmentation processes, one in the cranial half and associated with neural development, and one in the caudal half (see below for further discussion). Verbout follows development in both halves, and notes that in Stage IV (ca 4.5 mm CR) caudal half tissue "embraces the neural tube", and that axial tissue is still differentiating. By Stage V (ca 5 mm CR), cells of the caudal halves of segments give rise to a dorsal process and a costal process. These expanding mesodermal condensations do not pass beyond segment boundaries, and there is still unsegmented loose axial mesoderm around the notochord. In the cranial half, "structuralization" of mesoderm procedes due to peripheral nervous development. In Stages VI and VII (ca 5.5 and 6 mm CR), Verbout reports a medio-lateral differentiation gradient in the cranial parts of segments, and a latero-medial gradient in the caudal half. In the cranial part, differentiation is restricted to the lateral area adjacent to the caudal half. Segmentation progresses medially in the axial mesoderm toward the notocord. There is now a zone with cells concentric to the notochord that is the "perichordal zone". Stage VIII (ca 6.5–7 mm CR) features rapid development of a continuous cellular perichordal tube. There is no segmentation of the tube. The cell density in the caudal halves of segments increases, but is reduced near the chord. Verbout rejects earlier ideas (summarized by Baur, 1967, 1969) that there is an anteriorward shift of cells from the caudal level. Also at this stage, the anlage of the intervertebral disc appears in the caudal portion of the cranial part of the segment, so that the highest cell density is at mid-segmental level. At Stage IX (ca 7.5–8.5 mm CR), zones of higher density develop at regular intervals in the perichordal tube. They extend laterally far outside the tube. The chordal process connects to the perichordal tube caudal to the high-density region.

Verbout emphasizes that the perichordal tube is the blastema from which the axial vertebral column

arises. The high-density zones become the intervertebral discs; the lighter zones the vertebral bodies. He states that the perichordal tissue mass is surrounded by loose-meshed vascular mesenchyme *because* laterally, perineural (cranial half) tissue is looser and richer in vessels. Arcual and costal processes develop into well differentiated blastemic condensations. Dorsally, below the future intervertebral disc, neural arch blastemas arising from both sides unite with the perichordal blastema. The somite margin bends ventro-laterally caudally, and the rib anlage lies obliquely "in front of" the perichordal tube. The rib anlage remains within the caudal part of the segment. Disc condensation takes place midway between intersegmental vessels. In the cranial region of the vertebral column, the rib blastema connects to the perichordal tube blastema in the same transverse plane as the neural arch, "ventrally and below" the intervertebral disc. Notochordal undulation is determined by axial segmentation, according to Verbout. In Stages X–XII (ca 9–16 mm CR), Verbout describes incorporation of neural arch anlagen and rib anlagen by "inflowing" of tissue from the several blastemas. Chondrification is first observed in Stage XII (ca 13–16 mm CR). Examination of the mouse specimens used in work on vertebral identities (Kessel, 1992; Kessel and Gruss, 1991, and their more recent work), though not generated with regard to these issues, might provide an illuminating comparison.

Clearly there are major differences in developmental pattern and rate between gymnophiones and sheep. In addition to the maintenance of the myo-sclero-nephrocoel in gymnophiones mentioned above, the transitory crossing by sclerofibrils of caudal to cranial halves of adjacent segments apparently does not occur in sheep. The perichordal tube is a dominant contributor to the vertebra in sheep; it is a minor factor and far less a contributor in gymnophiones. Neural arch blastemas apparently develop much earlier in gymnophiones than in sheep, and differential chondrification occurs earlier as well. The incorporation of cartilage within and without the perichordal tube in gymnophiones has a very different pattern than in sheep.

We interpret the position of development of the intervertebral discs, the intravertebral suture, the position of the dorsal root ganglion and the dorsal and ventral roots, and the attachment of the neural arch rudiments (basidorsals) as evidence for resegmentation, based on our data that indicate that neural arch rudiments arise largely from caudal sclerotomite tissue, that the perichordal tube is scanty, that there is a clear association of posterior halves of anterior somites with anterior halves of adjacently posterior somites, and the relative position of neural development. Both we and Verbout use the position of the intersegmental blood vessels as indicators of segmental borders, but we urge that this be done with caution, for we note some relative positional changes during the course of development. We consider that there are no truly "fixed" points. Because Verbout did not observe several of these phenomena in his material, he stated that resegmentation did not occur, and that vertebrae arise segmentally. In fact, we consider that it is possible that resegmentation may have been lost in amniotes, as a consequence of the altered rate and pattern of early vertebrogenesis, and the emphasis on perichordal tube contribution to the vertebral body. The latter may explain as well why Wake (1970) reported no clear evidence for resegmentation in salamanders. We assume that gymnophiones retain aspects of the ancestral condition, and that salamanders, frogs, and amniotes reflect derived states. The basis for this assumption has to do with the cell-richness of both halves of somites that allows us to trace aspects of early vertebrogenesis. In fact, resegmentation may be a property of cell density (see below).

Phylogenetic implications

The vertebrae of Paleozoic amphibians have diverse vertebral structure, and fossils often preserve vertebrae that contain separate neural arch and centrum ossifications. The centrum may contain two or more ossifications. The homologies of these components have been the subject of debate for many years (e.g., Panchen, 1967, 1977; Parrington, 1967; Rage and Janvier, 1982; Borchvardt, 1982, 1983; summarized by Shiskin, 1989ab, and by Carroll, 1989). Caecilian vertebrae most closely resemble those of some taxa that are grouped as lepospondyls, in particular the nectrideans and äistopods. These latter taxa are the only lepospondyls that have a single centrum per segment throughout the body and also have the neural arch continuous with the centrum. Nectrideans also have haemal arches fused to the centrum as do basal-most caecilians (Wake, 1987; Wake, in press). Haemal arches are absent in äistopods. However, the spinal nerves of these lepospondyls exit through the neural arches, rather than between them as in modern caecilians.

A central issue in debates over homologies of the vertebrate centrum relates to the issue of resegmentation. There are two dimensions to the debate. The first has to do with the trans-segmental nature of the vertebrae and the degree to which different parts of composite vertebrae have reoriented and shown patterns of differential growth during phylogeny. The second has to do with the degree to which the phylogenetic shifts are reflected in ontogeny. The most useful summary of these arguments is that of Shishkin (1989b), whose view is

that resegmentation has occurred during phylogenesis, and that lepospondylous vertebrae and those of all of the living amphibians are resegmented. For caecilians this means that a vertebra has homology with the neural arch and two sets of hemicentra of Paleozoic amphibians and rhipidistians. This vertebra is trans-segmental. A key element of Shishkin's argument is that caecilians (and salamanders) have bicipital ribs which in caecilians arise from well separated rib bearers. He postulates two heterochronic events during development that have affected different taxa, causing them to diverge from a pure recapitulation in which resegmentation is evident as a result of sclerotomite formation, separation by sclerocoel formation, and subsequent merger of caudal and cranial sclerotomites of adjacent vertebrae. The first is acceleration of the process to early stages of vertebral development so that it is condensed and less evident, if present at all. The second heterochronic event is the reduced role of the intermyotomal septum in retarding anterior growth of the pleurocentrum; acceleration of development means that the septum is an ineffective constraint because of its weak development when centrum formation is taking place. Thus in salamanders resegmentation is thought to occur prior to sclerotomal differentiation, which does not occur anyway because of the scanty amount of sclerotomal material present (the same argument would hold for frogs). However, in caecilians, as we have shown, caenogenetic evolution has not occurred to the extent that it has in other living amphibians, and development continues to recapitulate the main features of resegmentation which otherwise are evident only in the trans-segmental position of the unitary vertebra and the widely separated rib heads.

Sclerotome halves in vertebrogenesis – resegmentation

Evidence from cell and molecular biology clarifies the real differences between anterior and posterior halves of somites (e.g., Bagnall *et al.*, 1988; Bagnall, 1992; Goldstein and Kalcheim (1992; Norris *et al.*, 1989; Ewan and Everett, 1992), and suggests that these differences likely are widespread among vertebrates. Though virtually all of this work has been done on chicks, it provides a cellular, biochemical, and mechanistic explanation for many of the phenomena reported, particularly by Verbout (1985), and in many ways for development in tetrapods generally. Keynes and Stern (1984) demonstrated that motor axon growth is from the neural tube through the anterior half of each somite in chicks. Rotation of the somitic mesoderm 180° caused the axons to grow through the posterior half (the original anterior half) of the somite. Loring and Ericson (1987) and Tosney (1988) showed that the assymmetry of laminin in somites allows growth of axons through the anterior halves of the somites. Stern *et al.* (1988), and Stern and Keynes (1986, 1987) have used cell lineage and chimera studies to determine cellular contributions to vertebrogenesis, e.g., that a single somite can contribute to two vertebrae. The several demonstrations of biochemical differences between the sclerotome halves in chicks (e.g., Norris *et al.*, 1989), provides a basis for determining the significance of those differences.

Bagnall, *et al.* (1988) presented especially important evidence that resegmentation indeed occurs in amniotes. Using chick-quail chimaeras, they found that quail donor cells were generally located in one half of each of two adjacent vertebrae, and in the intervertebral disc, of the chick host. The horizontal plane of division of each vertebra fell through the center of the vertebral body, and divided the neural arch into rostral and caudal halves through the rostral border of the caudal notch. They interpreted this as evidence for a realignment of segmentation between the somite and vertebral stages of development. They cited evidence in *Drosophila*, in which the segmental embryonic pattern is shifted by half a segment relative to the metameric pattern. Bagnall, *et al.* suggested that attention should shift to focus on the intersomitic clefts and the clusters of cells that lie rostral and caudal to the clefts as "representative of the metameric pattern in the mature animal". We concur with this suggestion, and Bagnall's (1992) DiI labelling study and Ewan and Everett's (1992) retroviral labelling experiment in particular make contributions, but specific information regarding contributions to adult structure is not available for most, nearly all, species. Goldstein and Kalcheim (1992) found that each vertebra includes a pedicel-containing area apparently derived from the caudal sclerotomite, and a pedicel-free zone, the intervertebral foramen, derived from the anterior sclerotomite. The contributions differ, but this in general is a similar conclusion to our data for amphibians that show that most of the structure of the adult vertebra is derived from the posterior sclerotome. Monsoro-Burq *et al.* (1994) elucidate the complex interactions of sclerotome, notochord, superficial ectoderm and roof plate to establish vertebrae. Tajbakhsh and Spörle (1998) extensively review the cellular, molecular, and genetic information available, including much of the work that we have cited. They conclude, contrary to our interpretations, that these studies continue to produce conflicting data because resegmentation was not observed in some of the chick/quail chimeras and experiments that labelled adjacent somites with fluorescent stains. They state that "The issue (resegmentation) therefore remains unresolved." We interpret the evidence differently, when placed in an evolutionary context.

Conclusions

On the basis of descriptive developmental data for a number of amphibian species, compared to data for sheep, as well as the paleontological, the molecular, and the genetic information, we conclude that resegmentation does occur, but it is a subtle phenomenon, partly dependent on cell size and cell number. In amphibians the contribution to vertebral development of the anterior sclerotome half is limited, and the anterior sclerotomite apparently is extinguished early in development. That extinction may contribute to the segmental 're-positioning' that we observe. Therefore resegmentation may be transitory, and the contribution of the two sclerotome halves to the definitive vertebra may vary extensively among major groups of vertebrates. The evidence favors resegmentation, whether apparent in blocks or streams of cells moving about or not. Gymnophiones provide evidence that is more diagrammatic than that seen in other groups, and illustrates the plesiomorphic state of early development, not present in later stages.

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Note added in proof: Too late for inclusion in our analysis, two important volumes on somitogenesis were published that include papers relevant to our conclusions. Especially noteworthy are those by Brand-Saberi and Christ, Monsoro-Burk and Le Douarin, and Keller in C. Ordahl (ed) "Somitogenesis", Current Topics in Developmental Biology, vol 47–48, Academic Press, New York and San Diego.

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