Morphogenesis of the turtle shell: the development of a novel structure in tetrapod evolution

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SUMMARY The turtle shell is an evolutionary novelty that is synapomorphic for cheloniens. The carapace is initiated by the entrapment of the ribs by the carapacial ridge (CR), a lateral bulge of the dorsal ectoderm and dermal mesoderm. The mechanisms by which the CR is initiated, the ribs entrapped and the dorsal dermis ossified, remains unknown. Similarly, the formation of the plastron remains unexplained. Here, we present a series of anatomical investigations into plastron and carapace formation in the red-eared slider, Trachemys scripta, and the snapping turtle, Chelydra serpentina. We document the entrapment of the ribs by the CR and the formation of the plastron and carapacial bones by intramembranous ossification. We note the formation of the ossification centers around each rib, which suggest that the rib is organizing dermal ossification by secreting paracrine factors. The nuchal ossification center is complex and appears to involve multiple bone-forming regions. Individual ossification centers at the periphery of the carapace form the peripheral and pygial bones. The intramembranous ossification of the plastron proceeds from nine distinct ossification centers, and there appear to be interactions between the spicules of apposing centers as they draw near each other.

INTRODUCTION The nature of the turtle shell

The turtle shell is a remarkable evolutionary novelty that is the basal synapomorphy for the order Chelonia. It is composed of two main parts, the dorsal carapace and the ventral plastron, which are connected along the midflanks by lateral bridges. Altogether, the shell contains over 50 dermal bones found in no other vertebrate order, and the presence of this bony casing has necessitated extensive modifications of the tetrapod body plan. Whereas dermal ossification itself is a primitive character for vertebrates (Smith and Hall 1993), the turtle shell represents an extreme development of the dermal skeleton among tetrapods.

The shell clearly has adaptive value for turtles for its physical protection, but it also functions in different species as a pH buffer and as a reservoir for water, fat, or wastes. The embryonic development of the shell involves a dramatic hypertrophy of the dermis in the dorsal body wall and a resultant rearrangement of the typical relationship between the pectoral girdle and the axial skeleton. The neck, skull, and copulatory apparatus are also greatly modified. As we detail below, the key innovation for the cheloniens appears to be the carapacial ridge (CR), a bulge of ectoderm and mesoderm that influences the growth of the ribs. The ribs are enveloped within the dorsal dermis, resulting in their lateral displacement as the dermis rapidly expands. Thus, instead of extending ventrally and enclosing the thorax, the turtle ribs become integrated into the carapacial dermis. The dorsal portions of the vertebrae also fuse with the midline of the carapace (Fig. 1).

The unique relationship of the postcranial axial skeleton and the highly specialized dorsal dermis in turtles poses many questions to the evolutionary developmental biologist. Research has not been extensive on turtle embryos, and some aspects of their development have likely never been formally addressed. We will review the basics of shell anatomy, including what is known about how the carapace and plastron develop. We extend this data with our own studies of the red-eared slider, Trachemys scripta, and the snapping turtle, Chelydra serpentina.

Anatomy of the turtle shell

The character and homology of the bony elements of the turtle shell have a long history of controversy (see discussion). The shell is comprised of the endochondral axial elements of the trunk overlain by a mosaic of dermal bones and an outer epidermal layer made of keratinous scales (also called scutes or shields). All turtles possess 10 thoracic vertebrae associated with the carapace. Each vertebra possesses a single-headed rib that often shares an articulation with the next an-
terior vertebra. The first and tenth ribs are diminutive and normally extend a short distance before making contact with the second and ninth rib, respectively. The tenth rib is often indistinguishable in both embryos and adults, and the presence of a large tenth rib in embryos is a normal variation. The thoracic ribs enter the dermis of the shell a short distance from their articulation with the vertebrae, and they extend laterally within the carapacial dermis, terminating at the periphery (Fig. 1A and 1B; Ruckes 1929). In the dermal layer of the shell, there are generally 59 bones: the carapace has 38 paired and 12 unpaired bones. The plastron contains eight paired and one unpaired bones (Fig. 1C). With the exception of a few key taxa, the only real variations in this general scheme occur as individual variations around the neck and tail where the axial skeleton is not closely joined to the carapace. The shapes and relative sizes of the bones determine the general form of the shell in different genera.

The shell’s epidermal layer generally consists of 38 scutes in the carapace and 16 in the plastron. The shield and bone patterns are not in register; each shield covers a particular area of the bony mosaic. The pattern of the sulci that form between neighboring scutes and the sutures that form between neighboring bones form two minimally overlapping patterns. The epidermal shield pattern develops long before the shell bones begin to calcify, and the underlying dermis may play a major role in the formation of the epidermal scutes, similar to the influence of somitic dermis of feather patterns in the chick (Yntema 1970; Cherepanov 1989; Alibardi and Thompson 1999a,b).

**Dermal bones of the carapace**

The unpaired, midline bones of the carapace, called neurals, are attached to the neural spines of the 10 thoracic vertebrae. The first neural is suturally attached to the posterior margin of the nuchal bone, and the last neural to the anterior margin of a pygal. Each neural is laterally attached to a costal bone. The costal bones extend from the neurals towards the periphery. There are eight (or nine) pairs and each is intimately associated with a rib and sutturally in contact with its neighboring costals. The first costal overlies ribs one and two, and the eighth contains rib nine and ten. The distal edge of each costal is attached by sutures to the peripheral bones. This contact often does not occur until later stages of post-hatching growth, leaving open a peripheral ring of fontanelles that surround the distal tips of the ribs (Fig. 1A). The nuchal bone forms the anterior margin of the carapace, which overhangs but is not attached to the posterior cervical vertebra. This bone extends laterally around the margins of the carapace to the level of the second rib (Fig. 1; see also Fig. 3). It is overlain laterally by the first three peripheral bones, and suturally contacts the first costals and neural bone posteriorly.

The pygal and suprapygal bones form the rear of the carapace. These bones have no contact with vertebrae and ribs but project over the sacrum and pelvis. The peripheral bones form the edge of the carapace. Before contacting the costals, they form a socket around the distal tip of ribs two through nine. There are generally 11 pairs of peripheral bones.

**Dermal bones of the plastron**

The plastron is composed of nine bones (Fig. 1C). The paired epiplastron form the anterior border of the plastron and are homologous to the clavicles of other tetrapods. The entoplastron is a single median element, homologous to the interclavicle of other tetrapods.

The remainder of the plastral bones may be homologous to the gastralia (dermal, abdominal “ribs”) of other tetrapods. The hypoplastra form the axillary buttresses and the anterior bridge region. The bridge extension of the bone approaches the carapace at the level of peripheral five and rib four. The bilateral hypoplastra meet each other at the ventral midline and form the anterior rim of the central umbilical fontanel. During embryonic development, this fontanel surrounds the yolk stalk that connects to the gut. The hypoplastra form the inguinal buttress, the posterior bridge region, and the posterior rim of the central fontanel. They approach the carapace
at the level of peripherals six and seven and ribs five and six. The xiphiplastra form the posterior lobe of the plastron.

Development of the turtle shell

Formation of the carapacial ridge

Early stages of turtle embryology have not been extensively studied and remain obscure (reviewed by Ewert 1985). The turtle egg is laid at the midgastula stage, and the embryo forms a notochord and somites in a way similar to those of the chick (Pasteels 1937, 1957). The first sign that the organism is to become a turtle rather than some other tetrapod occurs at Yntema stage 14 (stages for Chelydra, Yntema 1968; stage 14 is approximately equivalent to Hamburger-Hamilton chick stage 24). At this stage, there are the first signs of a ridge on the lateral surfaces of the embryo, dorsal to the limb buds (Ruckes 1929). At first, this ridge is seen between the two limb buds, and only later does it extend anteriorly and posteriorly. This structure has been named the carapacial ridge (CR) (Burke 1989a,b; 1991) and will eventually form the outer edge of the carapace. The CR is formed by a thickening of the ectoderm and is underlain by a condensed mesenchyme (Fig. 2A). This configuration is typical for an epithelial-mesenchymal interaction. Indeed, the accumulations of fibronectin and N-CAM in the carapacial ridge are similar to their distributions in other inductive sites, such as in the early limb bud or feather primordia (Burke 1989a,b; 1991). The histological morphology and the distributions of these adhesion molecules indicate that the induction mechanism that produces the carapace ridge is developmentally analogous to the pathway involved in limb induction.

The ensnarement of the ribs by the carapacial ridge

Observations by Ruckes (1929) of turtle development describe two important factors in the development of the shell. First is an accelerated lateral growth of the dorsal dermis of the trunk compared to growth in the dorsoventral plane. Second is the apparent “ensnarement” of the growing ribs by the specialized dorsal dermis (Fig. 2). The involvement of the ribs with the carapacial dermis results in their growth in a predominantly lateral direction. The limb girdles develop in typical tetrapod fashion, but because of the growth trajectory of the ribs, the pectoral girdle ends up ventral to the axial elements.

Yntema (1970) performed a series of somite extirpation experiments on snapping turtles, confirming a somitic origin for the ribs and dermis of the carapace. Post-otic somite pairs 12 through 21 are involved in forming the carapace in Chelydra. A causal role for the CR in rib placement was studied experimentally by Burke (unpublished PhD thesis, 1989c; 1991). Surgical methods were used to (1) remove the CR or (2) prevent CR formation. In the first set of experiments, in those cases where the CR did not regenerate, the rib at the level of the surgery did not grow in its normal trajectory but migrated instead toward a neighboring region that did have a CR. In the second set of experiments, tantalum foil barriers between the somite and the lateral plate mesoderm prevented CR formation. This procedure had extreme results and surviving embryos showed disruption of the body wall as entire regions of the dermal carapace failed to form. The ribs associated with these missing regions interdigitated with the bones of the plastron. Thus, the lateral development of the turtle ribs appears to be directed by the CR, and in the absence of the CR, these ribs grow ventrally and enter the lateral plate, like the ribs of non-chelonian vertebrates.

The investigations described here document the embryonic migration and arrival of the ribs into the carapace ridges of two species of turtle, Trachemys scripta and Chelydra serpentina. We also describe the location and mode of ossification in the plastron and carapace of these turtles during stages of bone formation.

MATERIALS AND METHODS

Specimen fixation

Freshly laid T. scripta eggs were purchased from the Harvey Kliebert Turtle and Alligator Farm (Hammond, LA, USA) and incubated at 24–27°C in a 1:1 mix of water and vermiculite in plastic containers. C. serpentina eggs were obtained from nests in the wild. Hatchlings were housed in aquaria with a source of UV light. Embryos were removed from the egg and washed in Ringer’s solution. Those to be used for bone/cartilage staining were fixed in 4% paraformaldehyde (PFA) in PBS while those destined for Mallory’s Trichrome stain were fixed in 10% neutral buffered formalin. Early embryos were dehydrated in an ethanol series and embedded in paraffin. Coronal sections of 8 μm were mounted on glass slides and stored at 4°C. Hatchlings were anaesthetized with CO₂, and killed by cervical dislocation before being fixed in 4% PFA/PBS. Specimens used for bone/cartilage staining were stored in absolute ethanol at −20°C until use.

Histological staining

Cross-sections of early turtle embryos were stained with Mallory’s Trichrome stain (Everett and Miller 1973). Differential bone/cartilage staining of whole embryos and hatchlings was carried out with a modified version of Wassersug’s (1976) and Dingerkus and Uhler’s (1977) Alizarin-red/Alcian-blue protocols, where bone matrix stains red and cartilage matrix stains blue. Modifications were necessary to overcome the pigmentation and keratinization of the scales covering the shell. Thus, the turtles were initially washed in distilled water for at least two days and incubated in the Alcian-blue staining solution for 36 h. Subsequent washes in a descending ethanol series and trypsin digestion were each carried out for at least 24 h. Alizarin-red staining took place for 48 h, and destaining in KOH-glycine was performed from three to four weeks.
RESULTS

The ossification of the carapace in \textit{Trachemys}

In the red-eared slider, the CR can be seen on day 23 of development as a bulge on the dorsal flanks of the embryo (Fig. 2). The ribs will enter these bulges between days 25 and 29. In \textit{Trachemys}, the initial rib is cartilaginous throughout (Figs. 3A and 3B) but by 45 days (CL 1.5 cm), the central region of the rib loses its Alcian-blue staining. Moreover, it does not become calcified bone matrix until later. This leaves a colorless region in the Alcian-blue/Alizarin-red-stained ribs (Figs. 3C and 3D). Thus, the cartilaginous matrix of the mid-shaft of the ribs is resorbed before calcium deposition begins, and this resorption precedes the onset of ossification of the periosteum. The distal portion of the ribs remain cartilaginous beyond the border between pleural and marginal scutes, and they do not make contact with the peripheral bones until later in life (Figs. 3G and 3H). There is an anterior-posterior polarity in that the anterior ribs begin remodeling earlier. As the turtles grow, the region of cartilage is seen at the tips of the ribs, separated from the proximal bony region by a thin colorless band (Figs. 3E and 3F).

Although the ribs begin to ossify in ovo, the dermal bones of the carapace develop after hatching. The rate of osteogenesis, and perhaps to some degree the pattern, is influenced by environmental conditions (Ewert 1985). Size and age are both important parameters for bone pattern. Turtles of the same age can be at developmentally different stages, and there is significant variation even among turtles of the same size. Hatching time is also variable, and specimens are described by their carapace length (CL) as well as by their age since the egg was laid.

The formation of dermal carapacial bone in \textit{Trachemys scripta} is shown in Figures 3E through 3H. A 70-day (CL \textasciitilde 3.0 cm) hatchling has well-defined and pigmented ectodermal scutes. However, these hatchlings show only the beginnings of dermal bone formation—a small nub on the margin of the anterior ribs and an extension of bone from the nuchal region. In the carapace, the nuchal bone begins to extend from the most anterior vertebra that is embedded in the dermis.
Meanwhile, the anterior ribs show expanded regions of bone around them. The ribs are visible beneath these bony plates, and they appear to be inducing new bone from the dermis around and above them. The ossification appears to be extending from the ribs into the dermis, but it is not uniform. Rather, a spur of ossification is first seen emanating from the ribs at a region slightly removed from the vertebrae. By 118 days (CL 3.1 cm), the ossifying dermis between the ribs has coalesced. It appears that each costal bone of Trachemys is derived from the rib (or ribs) beneath it. The regions continue to grow and fuse until the full bony armor has formed.

In addition to the nuchal (anterior) and costal (rib-associated) ossification centers, the ossifications that produce the peripheral bones are also seen to begin in the largest of the new hatchlings. The peripheral ossification centers are first seen in the anterior of the carapace (starting with peripheral pair one) on large day-78 turtles (CL 3.1 cm) and as the turtle grows, more peripheral ossification centers can be seen caudally on the shell (Figs. 3G and 3H). These ossification centers form on the outer edge of the carapace and expand both laterally and internally as they grow. The pygal bone forms in sequence as the last peripheral, therefore the last bone to ossify.

The ossification of the dorsal dermis in Chelydra

As in Trachemys, the ribs of Chelydra enter the CR, and dermal bone begins to extend outward from the perioseal collar of the rib at the point where the rib enters the dermis, slightly proximal to the pleural/vertebral sulci. The earliest embryo to show costal formation is at stage 21 (CL 1.5 cm). The costal bones expand anterioposteriorly toward one another and distally along the shaft. The proximal part of the ribs are free from the dermis and remain cartilaginous longer (Fig. 3K). Contact between adjacent costals does not occur until the carapace has reached a length of approximately 4 cm. Ossification spreads distally and laterally, with the site of initial ossification, under the pleural vertebral sulci, achieving intercostal contacts earliest. As in Trachemys, the distal extremes of the ribs beyond the marginal/pleural sulci also remain cartilaginous, and growth proceeds by apposition.

The nuchal bone shows two distinct modes of development in two distinct phases of ossification that fuse into the final structure. We will refer to these phases as primary and secondary, referring to both the modes of ossification and the elements themselves. This pattern of primary and secondary ossification is also seen in the plastron and may have phylogenetic significance (see discussion).

The primary portion of the nuchal forms early (CL 1.4 cm, Yntema stage 20–21), appearing as a thin band of condensed cells within the dermis, and is continuous across the midline and extends laterally around the margin to the level of the third marginal (Figs. 3I and 3J). The band is visible deep in the dermis before actual deposition of calcium and alizarin staining. It underlies the marginal/vertebral sulci, which is clearly visible at this stage. Calcium deposition, as evidenced by positive staining with alizarin, starts bilaterally at the level of the first marginal scute and spreads along the bars medially and laterally.

The second phase of nuchal ossification involves the nuchal plate. This begins to form in embryos of CL 1.8 cm. The nuchal plate forms as a loose lattice-work of bone, much like the pattern seen in the initial stages of ossification in the skull roofing bones. It begins in contact with the anterior-medial nuchal bar and extends laterally along the bar and posteriorly into the dermis above neural spines of the last two cervical vertebrae. This posterior extension of secondary dermal bone forms the main body of the nuchal and lies under the first vertebral scute. It will eventually form a suture with the first neural bone, which develops around the neural spine of the first thoracic vertebra.

In specimens of CL 2.6 cm, the nuchal is fully developed and ossified. The lateral bars of the primary ossification extend to the mid-point of M4, to the level of contact with the cartilaginous distal tip of the second rib. It underlies the sulci, separating the marginals from the first vertebral and costal scutes. The lateral extensions of the primary nuchal bone are never in association with the secondary nuchal bone, but rather come to be overlain by the first and second peripheral bones.

The smallest specimen to show signs of peripheral bone formation is when CL = 2.6 cm. Small crescents of bone, concave outward, appear in the dermis on the extreme edge of the carapace immediately subjacent to the intermarginal sulci. The first peripheral appears under the sulci of the first two marginal scutes.

Contrary to Suzuki (1963), who reported that the neural bones are the first dermal bones to show signs of ossification in the carapace of Trachemys, we find in Trachemys and Chelydra that the costal ossification is underway without any sign of neural bone formation. The neural bones form in association with the neural spines of the thoracic vertebrae in a manner identical to the ribs and costal bones. Neurals are not seen in Chelydra embryos as large as CL 2.6 cm, but are visible in ones of CL 3.4 cm. There is much variation in the timing of bone development in these species.

Ossification of the plastron

The plastron begins to ossify before the time of hatching. In the embryonic turtle (CL = 10 mm in Trachemys, CL = 20 mm in Chelydra), the future plastron can be identified by nine ossification centers in the ventral dermis of Trachemys and Chelydra (Figs. 4A, 4B, and 4G). No Alcian-blue staining is seen presaging these sites. In Trachemys, the three ossification centers corresponding to the three anterior plastron bones appear to fuse around day 78 (CL 22 mm; Fig. 4C). The two epiplastral bones form a suture with one another,
Fig. 3. Development of the carapace. All embryos and hatchlings are stained with Alcian-blue and Alizarin-red: (A–D) Endochondral ossification of the ribs and vertebrae in *Trachemys*. (A) 25-day (CL = 6 mm) embryo. The forelimbs had come loose. (B) 36-day (CL = 1.2 cm) embryo. The initiation of the replacement of cartilage by bone in the vertebrae and proximal ribs is seen. (C, D) 45-day (CL = 1.5 cm) embryo. The transition between cartilage and bone in the ribs is presaged by a region where neither matrix is seen (asterisk). Arrow in (C) shows bone matrix in a long bone of the limb. (E–H) Dermal bone formation in *Trachemys* hatchlings. (E) Ventral view of 90-day (CL = 3.1 cm) carapace, showing intramembranous ossification of the nuchal bone and around the anterior ribs. (F) Lateral view of the same carapace, showing region of rib chondrogenic growth (blue) and transition zone (white) between cartilage and bone (red). (G) Dorsal view of 118-day (CL=3.1 cm) carapace showing expanded nuchal bone region, the fusion of the costal ossification centers, and the peripheral bone ossification centers that start anteriorly. The pigmentation of the epidermal scutes can be seen. (H) dorsal view of 185-day (CL = 4.5 cm) carapace showing fusion of marginal ossification regions anteriorly and the pygal ossification center posteriorly. The costal ossification centers have created bony armor dorsally. (The blue staining is beneath the carapace). (I–K) Ossification of
whereas the entoplastron bone forms more internally and projects caudally. As the hatchling turtle gets larger, the six peripheral ossification centers of the plastron grow toward one another and fuse (Figs. 4D through 4F). Condensed mesenchyme is seen in advance of the calcified tissue. These sites contain both Alizarin-red-stained bony spicules and a region of condensed mesenchyme that has already migrated out in the stellate arrays seen later to be stained for bone matrix.

One of the interesting things we observed about plastron ossification is that the bony spicules cross the midline. This was not expected, since the midline forms a division between the paired bones on opposite sides of the plastron. However, the midline was not respected by the developing spicules (Fig. 4E). Moreover, the spicules, as they crossed the midline, did not immediately fuse. Rather, it appears as if the ossifying spicules on either side avoided one another, altering their course of ossification such that they interdigitated rather than run into each other. This is very likely a prerequisite for continued growth.

A similar situation is seen in *Chelydra* (Figs. 4G and 4H). The plastral bones appear with a slight anterior-posterior bias, the epiplastra and entoplastron first and the xiphiplastron last. They are all present in specimens of CL 1.5 cm, preceded only by the appearance of the primary nuchal bar. Like the nuchal bone, the plastral bones show two phases of development. They first appear as slender bars of condensed cells that then calcify from their centers outward (Fig. 4I).

**DISCUSSION**

**Classical arguments of shell homology**

The character and homology of the bony elements of the turtle shell have a long history of controversy joined by some of the great nineteenth-century morphologists. Cuvier’s (1800) functionalist approach led him to interpret the carapace as no more than expansions of the ribs and vertebral spines. E. Geoffroy Saint-Hilaire (1818) agreed with Cuvier’s assessment of the carapace, and claimed the plastron as the endoskeletal sternum in its greatest state of development. K.G. Carus (1834) was perhaps the first to suggest that the carapace and plastron involved both the endo- and the exoskeletal (dermal) tissue. He proposed that the endoskeletal vertebrae, ribs, and sternum were overlain by dermal ossifications, Rathke (1848), in an extensive monograph on turtle development, confirmed the dual nature of the carapace but argued that the plastron belonged exclusively to the exoskeleton and was in no way homologous to the sternum. Richard Owen (1849) found his own satisfying explanations through the vertebral archetype. He also correctly recognized the presence of both dermal and endochondral bone in the carapace, but adherence to his ideal caused him to homologize the plastral bones with thoracic haemapophyses and, as such, part of the endoskeleton. Gegenbaur (1859) considered the ribs to be greatly expanded transverse processes overlain with dermal ossifications. More recent histological studies confirmed Rathke’s assessment that the bones of the plastron all ossify intramembranously without any cartilaginous precursors and, therefore, by definition belong to the dermal, integumental, or exoskeleton (Rathke 1848; Zangerl 1969). Goette (1899) made more detailed histological studies of the carapace and believed the dermal carapace bones to form as outgrowths of the periosteal collar around the ribs and vertebrae.

**General patterns of ossification**

The studies of Kälin (1945) and Vallen (1942) confirmed those of Goette and showed the cartilaginous ribs lying between two layers of the stratum compactum in the thick dermis of the carapace. These layers unite between the ribs, and costal ossification is initiated within the double layer of stratum compactum. Suzuki (1963) noted that in *Trachemys*, the cartilaginous matrix of the ribs degenerates within the periosteum, consistent with our observations on *Chelydra* and *Trachemys* (Figs. 3A, 3D, and 3H). Although Kälin (1945) thought that discrete dermal ossifications later associated with the ribs and spinous processes, our observations also support Suzuki’s (1963) description of ossification of the costal bones. Ossification of these elements is initiated as spicules of bone extending from the intramembranous, periosteal collar surrounding the rib cartilages. The spicules develop into the typical reticulated pattern of trabeculae surrounding hematopoietic elements. The ribs themselves grow by apical apposition, invested in a dense “periostoeochondrogenic” membrane. These observations indicate that the ribs act as initiation centers for the dermal ossification of costal bones. The ossifying regions of the dermis extend toward one another to eventually fuse. The ossification center for the neural bones appears to reside in the vertebrae.

Of particular interest are the two strikingly different modes of ossification seen in the development of the nine plastral bones and the nuchal bone in the carapace. These bones develop in two very different stages. The first signs of morpho-
The order Chelonia emerges abruptly in the late Triassic, around 200 Mya, with the fossil species Proganochelys (Jaekel 1914). This animal had a full carapace and plastron, the synapomorphy that defines the order. Interestingly, both the carapace and plastron of Proganochelys have more dermal bones than most subsequent forms. For full description, see Gaffney (1990). The Chelonian Bauplan appears in the fossil record without intermediates, and the relationship of turtles to other amniote groups is not certain. In fact, turtles represent one of the most extreme examples of Watson’s rule of mosaic evolution: the observation that new taxa are composed of combinations of primitive and derived characters (de Beer 1954). Proganochelys has primitive anapsid skull characters that have been used to link it to various groups of primitive amniotes or “stem reptiles.” However, because of their highly derived trunk morphology, Carroll (1988) comments that turtles might well be placed in their own subclass of the Amniota.

The phylogenetic origin of turtles remains extremely controversial. Different sets of characters generally produce different hypotheses about relationships of turtles to other “stem reptile” groups. Osteological characters have generally placed turtles as very basal members of the amniote tree, or as “parareptiles” along with various extinct groups. Most of these other parareptiles and other basal reptile groups have been suggested as including the ancestor of turtles. Cranial characters have been used to link turtles to the captorhinomorphs (Gaffney 1980, 1984; Gaffney and Mckenna 1979), or procolophonids (Laurin and Reisz 1995). Dermal armor, flattened ribs, and shape of the scapular blade are claimed to link the turtles to the pareiasaurs (Lee 1996, 1997a,b).

However, using different character sets (e.g., polypeptide sequences, nuclear and mitochondrial DNA sequences), it has been argued that turtles are modified diapsids within the
reptilian clade (Platz and Conlon 1997; Hedges and Poling 1999; Zardoya and Meyer 1998; Mannen and Li 1999). Rieppel and Reisz (1999) also argue for the diapsid nature of turtles, strictly on the basis of morphological evidence (e.g., placement of scapula, hooked fifth metatarsal bone, modified tarsus formation). Phylogenetic hypotheses rely on accurate discrimination between true homology and convergence. In turtle phylogeny, it has been argued that both morphological and molecular characters can be homoplasic (see Rieppel and Reisz 1999).

Regardless of the group chosen to include the ancestor of turtles, there is dramatic evolutionary transition between the chelonian and the non-chelonian body plans. Mayr (1960) identified the origin of evolutionary novelties as a distinct and neglected problem, but the prevalence of gradualism and adaptationalistic explanations may have prevented its analysis until recently. Müller and Wagner (1991) define evolutionary novelties as structures that are neither homologous to any other structure in the ancestral species nor a duplication of any other structure. The turtle shell is comprised of primitive structures (axial skeleton, dermis), but as a complete morphological entity it clearly fits the definition of a novelty. The developmental processes that form the turtle shell are likely also homologous to processes in the ancestral organisms. The evolutionary innovation is likely to be found in the timing and positioning of common mechanisms, resulting in altered contexts for developmental events and ultimately the altered body plan.

The absence of intermediates or transitional forms in the fossil record could indicate that turtles arose saltationally. The recognition of a simple developmental mechanism, namely an epithelial-mesenchymal interaction, at the initiation of carapace development provides a basis for hypotheses about the rapid evolution of this body plan (Burke 1989b).

**Turtle embryos in the age of molecular developmental biology**

Knowledge of the developmental mechanisms by which the shell is formed may help discriminate between those hypotheses predicting a gradualist origin of turtles through a common ancestor with dermal-armored pareiasaurs (Lee 1996, 1997a) and those that predict a more saltatory origin of the shell. The main questions to be addressed are: (1) How does the carapacial ridge form?; (2) How does the carapacial ridge trap the developing ribs?; (3) Do the entrapped ribs induce shell formation, and if so, how?; (4) How do the primary dermal bones differ from the secondary?; and (5) Is the neural crest involved in shell morphogenesis?

The recruitment (cooption) and reassortment of existing developmental pathways has been proposed as a major mechanism for generating novel structures (Jacob 1977; Raff 1996; Duboule and Wilkins 1998), and the turtle provides an example of both heterochrony and heterotopy in development (Hall 1999). The carapacial ridge is considered to be the key innovation for turtle evolution, and its similarity to the limb bud has been noted earlier (Burke 1989a,b,c). Since the tetrapod limb induction pathway is initiated through the interaction of fibroblast growth factors FGF10 and FGF8 (Crossley et al. 1996; Vogel et al. 1996; Ohuchi et al. 1997; Szebenyi and Fallon 1999; Yonei-Tamura et al. 1999), the expression of these genes during early turtle development will be of interest (Loredo et al., in preparation). Similarly, FGFs function as chemotactic factors in the limb bud and elsewhere in the vertebrate embryo (Park et al. 1998; Li and Muneoka 1999; Kubota and Ito 2000) and thus may provide a mechanism for the entrapment of ribs. Our results suggest that the ribs are inducing the ossification of the carapacial dermis. The ability of a cartilagenous rod to induce bone around itself is thought to happen in the formation of the mandible from Meckel’s cartilage (Chun et al. 1995), and the ossification program of the turtle ribs appear to recapitulate the Meckel’s cartilage mode of ossification (Suzuki 1963). The main candidates for these substances released by the rib are bone morphogenetic proteins (BMPs) that are known to be able to induce bone formation in dermal tissue (Takaoka et al. 1994; Shafritz et al. 1996; Nifui and Noda 1999; Kim et al. 1998).

Smith and Hall (1993) have outlined a developmental model for the evolution of the various elements of the exoskeleton. One of their postulates is that all exoskeletal structures, from teeth to scales to fin rays, are derived from the neural crest. They also outline many of the molecular players currently known to participate in the differentiation of dermal bone. The role of the neural crest in turtle postcranial skeleton has not been studied and may reveal specific developmental characters of the primary and secondary dermal ossifications in the shell. Comparative, molecular developmental studies of dermal ossification in various taxa including turtle will also generate new characters for the evolutionary analysis of shell structure.

The turtle shell represents a classic evolutionary problem: the appearance of a major structural adaptation. The place of turtles amongst the other amniote groups is a classic question in systematics, due to the “instantaneous” appearance of this evolutionary novelty. The fossil record may yet provide a morphological missing link between Proganochelys and the generalized tetrapod body plan. In the meantime, the application of developmental studies to this evolutionary puzzle may help resolve these questions as to the ontogeny and phylogeny of the ancient order of Chelonia.

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