through Parkfield and the Cholame tremor zone (*31*) (Fig. 1). The rupture zone of the great 1857 event is composed of multiple fault segments (*32*), including the Cholame segment immediately southeast of the Cholame tremor zone. This segment is now fully locked. Its estimated mean recurrence time is between 85 and 142 years (*32*), and it last ruptured as part of the great 1857 event.

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Evolution of the Turtle Body Plan by the Folding and Creation of New Muscle Connections

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The turtle shell offers a fascinating case study of vertebrate evolution, based on the modification of a common body plan. The carapace is formed from ribs, which encapsulate the scapula; this stands in contrast to the typical amniote body plan and serves as a key to understanding turtle evolution. Comparative analyses of musculoskeletal development between the Chinese soft-shelled turtle and other amniotes revealed that initial turtle development conforms to the amniote pattern; however, during embryogenesis, lateral rib growth results in a shift of elements. In addition, some limb muscles establish new turtle-specific attachments associated with carapace formation. We propose that the evolutionary origin of the turtle body plan results from heterotopy based on folding and novel connectivities.

Turtles are characterized by their shell, and there have been various opinions as to the evolutionary origin of their unique body plan (*1–8*). One possible scenario suggests that acquisition of the osteoderms, the dermal skeletal elements, predated the bony shell (*6*), but other studies have emphasized the importance of more fundamental anatomical changes, especially those associated with the pectoral region, as the decisive factor in turtle evolution (*1–5*). The dorsal part of the turtle shell, or carapace, is derived from the ribs, and the scapula is found beneath the carapace, in contrast to the pattern of other amniotes in which the scapula is outside the rib cage (Fig. 1, A to E). Concomitant with the positional change of skeletal elements, the muscles connecting the trunk and scapula, the serratus anterior (AS) and levator scapulae–rhomboid muscle complex (LSR), also showed aberrant positions and connections in the adult turtle (Fig. 1, D and E, and fig. S1). This unusual topography led to the opinion that the turtle shell represents an evolutionary novelty (*1–5*). At first glance, this evolutionary shift cannot be resolved by gradual changes of shape and size while maintaining the basic architecture of the body, because an intermediate state is impossible. Thus, it was proposed that turtle evolution represents a radical, saltatory evolutionary change (*1–5*). This change has been ascribed to a developmental shift in the ribs, not of the scapula (*7*, *8*). However, the embryonic anatomy of the musculoskeletal system has not been analyzed sufficiently to identify the origin of the turtle-specific body plan.

To understand the developmental changes underlying the origin of the turtle *Bauplan*, we compared the development patterns in embryos of the Chinese soft-shelled turtle (*Pelodiscus sinensis*) and two other amniotes, the chicken (*Gallus gallus*) and the mouse (*Mus musculus*); three to four embryos were examined for each species and stage. As a specialized group of archosaurians, in which turtles are also included (*9*), avians have undergone specialization during evolution (SOM Text 1 and table S1); however, they are expected to exhibit an unaltered developmental pattern reflecting ancestral developmental programs that may have been lost or altered in turtles. Mammals, on the other hand, belong to synapsids, a lineage that diverged before archosaurians (*9*). Thus, the common developmental features shared by chicken and mouse are expected to represent the primitive state of amniotes. We focused on postorganogenetic periods of the embryos when the anatomical variations are thought to be becoming apparent (*10*), and the observations were based on comparative embryological methods.

We used *Sox9* expression as the marker for the skeletal precursor in the late pharyngula stage

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Fig. 1. Comparison of turtle, chicken, and mouse. (**A** to **C**) Dorsal views of computerized axial tomography scanned animals. (**D** and **E**) Comparison of deep (as, serratus anterior; lsr, levator scapulae and rhomboid complex) and superficial (ld, latissimus dorsi; p, pectoralis) muscles connecting the trunk and shoulder girdle/forelimb. (**F** to **K**) *Sox9* expression in stages 13 and 15 *P. sinensis*, stages 24 and 28 chicken, and embryonic day 10.5 and 11.5 mouse. (**L** and **M**) Three-dimensional reconstruction of the stage 16 *P. sinensis* and stage 30 chicken. Cartilages are blue, muscle plate (mp) yellow, AS muscle pink, and LSR muscle orange. Scale bar, 500 μm. cr, carapacial ridge; sc, scapula; r, ribs.
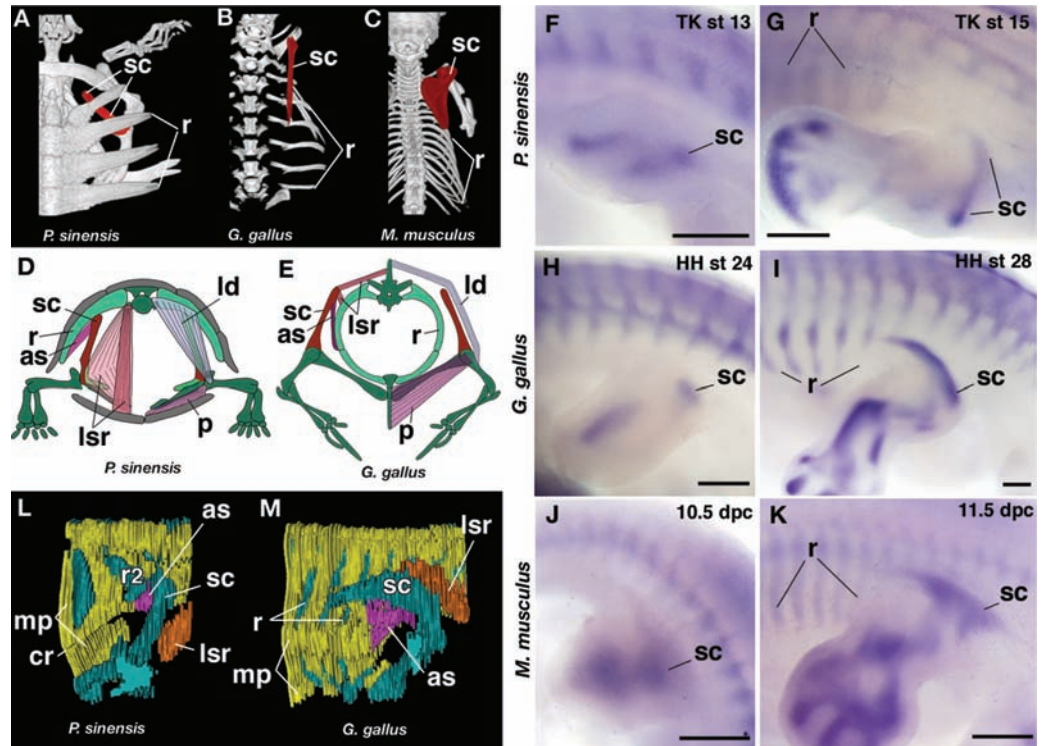
(Fig. 1, F to K) (*11*). In embryos of all three animals, the scapula anlage initially arose lateral to the body wall (somatopleure) into which the muscle plates had grown (Fig. 1, F to M). There were, however, subtle differences; the scapula blade in the stage 28 chicken and 11.5-day mouse began to grow posteriorly, lateral to the developing ribs (Fig. 1, I and K). The *P. sinensis* scapula anlage was located slightly anterior as compared to the chicken and mouse (Fig. 1G). Also, the ribs of *P. sinensis* embryo were comparatively shorter than those in chicken and mouse, in that they never invaded the body wall (*1*, *12*). Slightly later in development, the precursors of AS and LSR muscles also occupied mutually comparable positions in chicken and *P. sinensis*; the AS anlage was found on the ventroposterior aspect of the scapula, and the LSR on the dorsoanterior (Fig. 1, L and M, and movies S1 and S2).

The above data strongly suggested that the common musculoskeletal pattern observed in postpharyngula stage would already have been established in amniotes that gave rise to the turtles. In the chicken and mouse, this pattern is preserved with very little modification in the adult (Fig. 1M and fig. S1C). However, after this stage, the mechanism by which the turtle-specific body plan is generated became apparent. We observed that the second and more posterior ribs grew laterally and anteriorly over the scapula and the AS muscle by folding the dorsal part of the lateral body wall inward (Fig. 2, A to H, and movies S1 and S5). During this process, the same musculoskeletal connectivities were maintained, and as the second rib grew laterally and anteriorly, the AS anlage rotated in a ventral medial direction to
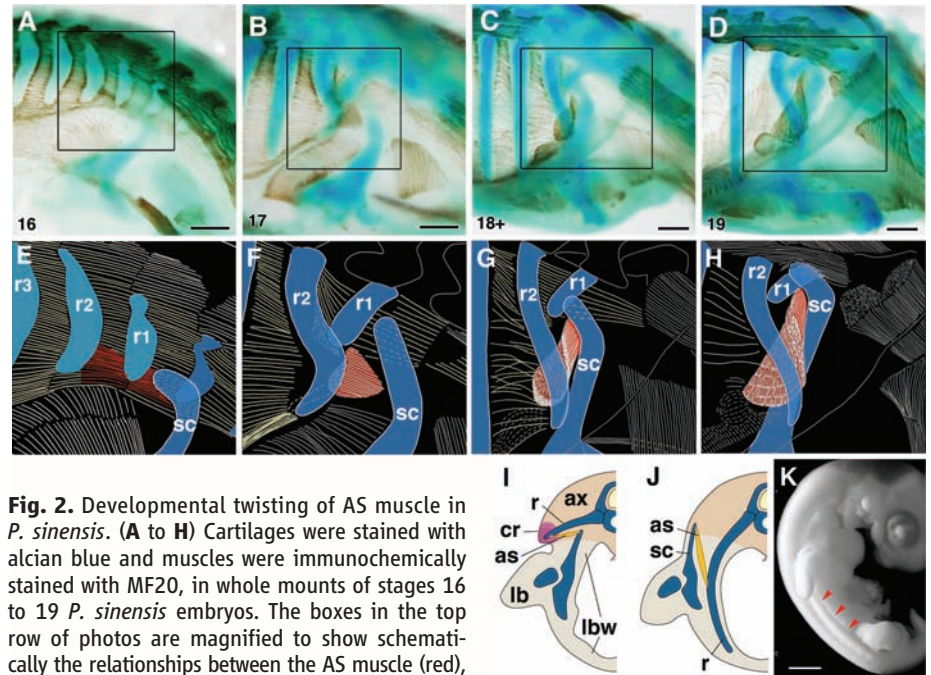

Fig. 2. Developmental twisting of AS muscle in *P. sinensis*. (**A** to **H**) Cartilages were stained with alcian blue and muscles were immunochemically stained with MF20, in whole mounts of stages 16 to 19 *P. sinensis* embryos. The boxes in the top row of photos are magnified to show schematically the relationships between the AS muscle (red), the scapula, and the ribs (r1 to r3) in the middle row. (**I** and **J**) Schematic transverse views to compare the topography of ribs, body wall (lbw), and forelimb bud (lb) with shoulder girdle between the embryos of turtles (I) and generalized amniotes (J). Generally, the amniote ribs grow ventrally into the lateral body wall, whereas in the turtle, they are arrested in the axial part (ax), growing toward the CR. (**K**) The CR (arrowheads) in stage 14 *P. sinensis* embryo. Scale bars, 500 μm for (A) to (D), 1 mm for (K).

assume its final position underneath the carapacial anlage (Fig. 2, A to J). The morphological change of the LSR muscles was less conspicuous (Fig. 3 and SOM Text 2). An analogous rib growth was observed in the pelvic region, and a

similar folding was seen in the posterior part of the obliquus abdominis that spans between the posterior ribs and the pelvic girdle (SOM Text 3).

During the turtle-specific folding, ancestral connectivities were maintained between the ribs,
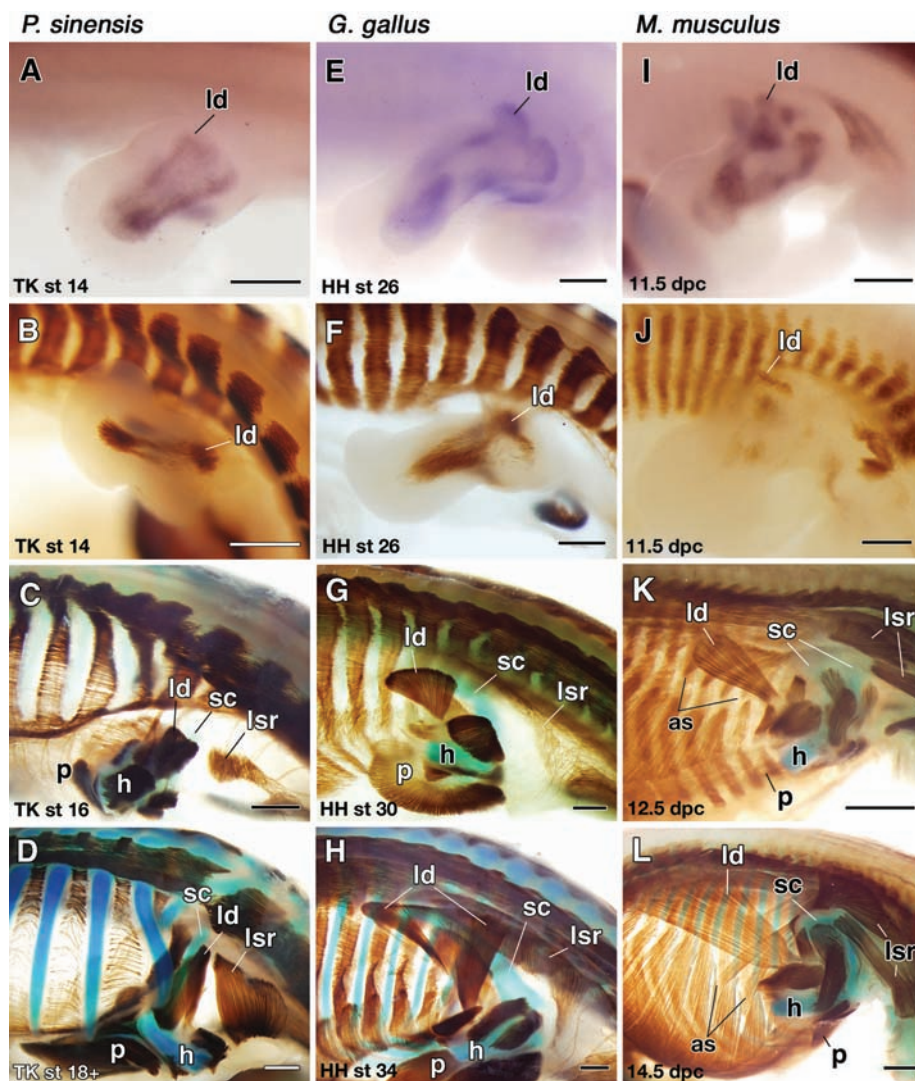
Fig. 3. Comparison of muscle development. (**A** to **D**) *P. sinensis* embryos, (**E** to **H**) chicken embryos, and (**I** to **L**) mouse embryos. (A, E, and I) Expression of *Lbx-1* in the right forelimb buds before muscle differentiation. *Lbx-1*–positive proximal cells represent latissimus dorsi muscle primordia. (B to D, F to H, and J to L) Immunostained embryos to show muscle and peripheral nerve development. Distal parts of the forelimb buds have been removed at the level of the humerus (h). Scale bar, 500 μm.

the anlage grew dorsally and anteriorly to circumvent the carapace and to connect to the nuchal plate, which is the dermal skeleton that covers the cervicodorsal transition (Fig. 3, A to D, fig. S1B, and movie S3). The homologous muscles in the chicken and mouse grew more posteriorly to expand over the back, as in other amniotes (Fig. 3, E to L, fig. S1D, and movie S4). Similarly, pectoralis in *P. sinensis* attached to the dorsal aspect of the plastron, not the ventral aspect of the sternum as in other amniotes (fig. S2). These morphologically divergent attachments were specific to these in-and-out muscles in turtles. Thus, turtles appear to have used these muscles' flexibility in establishing new connections, particularly to invent novel morphological patterns specific to this taxon. Establishment of the turtle-specific new attachments was more conspicuous in the pectoral than in the pelvic region (SOM Text 3).

In summary, the turtle body plan can be explained by a combination of folding with conserved connectivities and the establishment of new connectivities. Therefore, morphological connectivities (i.e., morphological homologies) are partly conserved and partly disturbed. The latter may be regarded as a case of heterotopic shift that yielded novelty in the turtle, at the cost of some homologies (*17, 18*), similar to the evolution of the gnathostome jaw (*19*).

As already reported, carapacial formation is based on the ribs attached to the vertebrae that are defined as dorsal (thoraco-lumbar) in terms of the conserved role of Hox (*20, 21*). The growth of these ribs, however, is axially arrested by an unidentified mechanism and never invades the body wall (*12*). The latter situation results not only in ribs that grow over the scapula but also in the curious inward folding of the body wall. Along the folding line, the turtle-specific embryonic structure called the carapacial ridge (CR) (Fig. 1L and Fig. 2, I and K) develops through turtle-specific regulation of genes in the flank (*22–25*) and later grows anteriorly and posteriorly to form a circle that differentiates into the carapacial margin. The CR does not appear to induce the axial arrest of the ribs, as suggested previously (*2, 26*), but rather functions in the flabellate expansion of the turtle ribs in late development (*12*), which characterizes the turtle-specific anatomy (Fig. 4). Although it remains to be determined which mechanism gives rise to the CR, it is likely that the CR is tightly linked to the axial arrest of the ribs in establishing the turtle body plan.

Such a developmental perspective is consistent with the morphology of the recently discovered oldest turtle, *Odontochelys*, which possessed a plastron but no carapace. It cannot be ruled out that the carapace of this animal merely underwent a secondary degeneration (*27*); however, if it really possessed the precarapacial dorsal ribs as reconstructed (*28*) (Fig. 4), the evolution of the turtle body plan would be consistent with the embryonic development of the modern turtle. At least, the dorsal ribs of *Odontochelys* appear to

scapula, and AS and LSR muscles that arise as derivatives of the muscle plate (trunk muscles) (*13*) (Fig. 2, I and J). As shown in Fig. 2, the turtle scapula hung over the first rib, which does not extend or participate in carapace formation. Thus, in the turtle, the scapula arises primarily anterior to the ribs. Because the turtle ribs are confined to the axial domain (axial arrest of ribs), they can only grow laterally and anteriorly to cover the scapula dorsally, while the AS rotates to follow the rib (Fig. 2) (*12*). Still, this growth never altered the connectivities between the anatomical elements, and the positions of the folded muscle plate and rotated AS muscle indicate that the scapula was always morphologically outside the prospective rib cage that is indicated by the position of the muscle plate (movies S3 and S5). In other words, even though the turtle shell can be

regarded as an evolutionary novelty, the changes accompanying its development are not so radical as to disrupt the morphological homologies of the above structures (*14*). Rather, the true novelty in turtles is in the axial arrest of the rib growth as well as in the folding at the hinge between the axis and body wall (Fig. 2, I and J).

During the same developmental period, some other muscles formed phylogenetically new connectivities. These muscles belong to the 'in-and-out muscles' primarily classified as limb muscles (*15*). In amniotes, they arise as *Lbx1*-positive, migrating muscle precursors (Fig. 3) (*16*), that is, these muscles invade the limb bud and grow out again to establish attachments onto the trunk. In all the amniote embryos observed, latissimus dorsi primordia came out of the forelimb buds to establish proximal connections (Fig. 3). In the turtle,
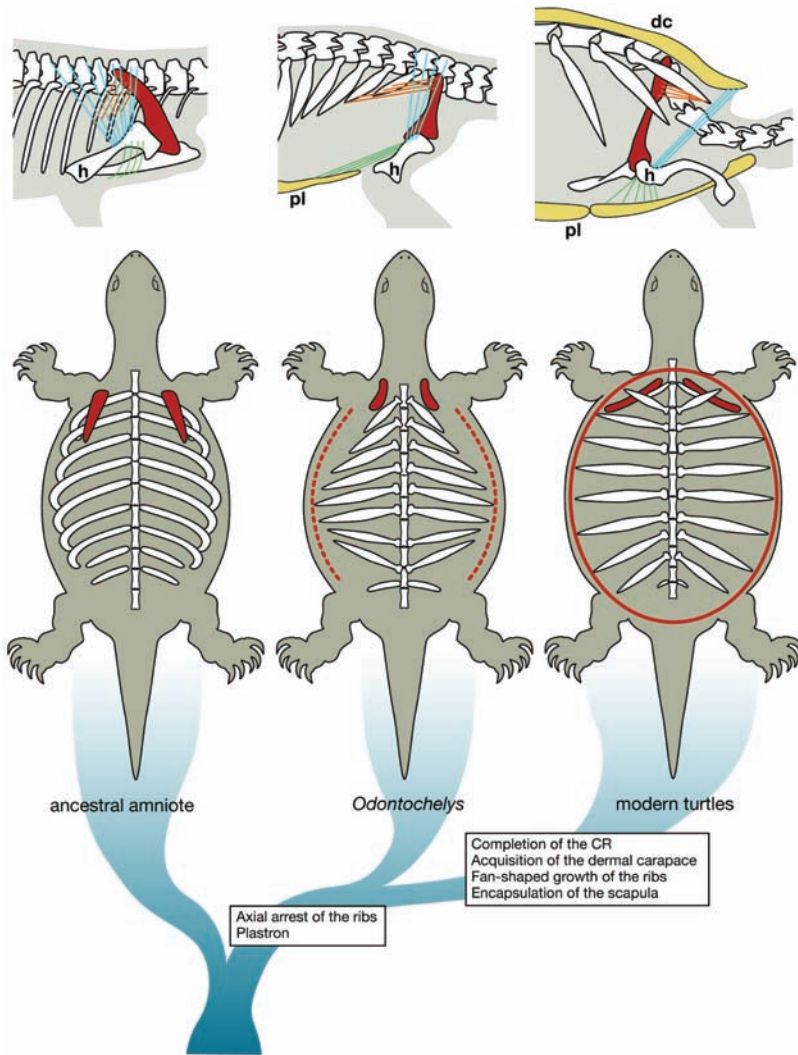
Fig. 4. Evolution of the turtle body plan. (**Top**) A hypothetical sequence of changes in musculoskeletal connectivities. The scapula is red, AS muscle orange, latissimus dorsi blue, and pectoralis green. In *Odontochelys*, the AS would have connected the scapula and distal tips of anterior ribs antero-posteriorly. The pectoralis would have connected the humerus and the plastron (pl). (**Bottom**) A phylogenetic consideration. In *Odontochelys*, the CR (red broken line) may have developed only temporarily and incompletely in the embryo. In the modern turtle, the CR (red solid line) forms a complete circle, inducing the fan-shaped growth of the ribs. dc, dermal carapace.

have already been arrested axially because they never bend strongly ventrally. As previously mentioned, this leads to the presumption that the CR-like ridge would have been acquired in the flank in their embryos (Fig. 4). However, it does not seem to have persisted and encircled the carapacial margin in later development as in the modern turtles, because the flabellate arrangement of the second to eighth ribs is not apparent in *Odontochelys*, that is, the tips of the ribs approximated to each other distally. Consistently, the anterior ribs grew posteriorly in the *Odontochelys*, the scapula remained anterior to the ribs, and the AS muscle would have been stretched to the anterior and posterior (Fig. 4, top), a pattern that is reminiscent of early *P. sinensis* embryos (Fig. 2, A and E).

Odontochelys reconstructed by Li *et al.* (*28*) resembles the embryonic modern turtles in some respects (Fig. 2, A and E, and Fig. 4), and this animal may represent an ancestral state. The *Odontochelys*-like, ancestral pattern is still retained in the first rib in modern turtles (Fig. 4, right). Although it remains to be seen whether latissimus dorsi of *Odontochelys* was shifted rostrally (Fig. 4, middle), its pectoralis would have established a new attachment to the dorsal aspect of the plastron (Fig. 4, middle). Thus, the developmental sequence of *P. sinensis* may not wholly recapitulate the suggested evolutionary sequence of turtles. Nevertheless, the above suggests that the dorsal arrest of ribs can now be assumed to have taken place by the common ancestor of *Odontochelys* and modern turtles

(*28*), and in the latter, the completed CR would have allowed for emergence of the carapace (Fig. 4, bottom). The modern turtles have acquired their unique body plan by passing through an *Odontochelys*-like ancestral state during embryonic development. Our embryological study may help to explain the developmental changes involved in both the pre- and post-*Odontochelys* steps of turtle evolution, from an evolutionary developmental perspective.

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