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Ridge analysis: Function of the carapacial ridge in the development of the turtle shell

June 11, 2007 – Turtles have what no other vertebrate does, a shell. But the means by which this novel structure arose during the evolution of the chelonian (turtle and tortoise) body plan remains imperfectly understood. Studies of turtle embryogenesis have revealed that, contrary to the common misconception that it is some form of thickened and hardened skin, the carapace in fact develops from the turtle's ribs, which grow externally, splay into a fan-like arrangement and fuse to form the final bony structure. How the turtle's ribs came to overlie the scapula (equivalent to our shoulder blades) in such a brief span of evolutionary time, however, is unclear.

Skeleton of juvenile *P. sinensis* stained histochemically with alizarin red, showing the carapace being formed from the ribs.

One candidate for the developmental source of the carapace is an embryonic structure known as the carapacial ridge (CR), which appears on the flank of the pharyngula stage embryo. This structure is interesting in that it is apparently unique to the turtle embryo, and bears some resemblance to another tissue, the apical ectodermal ridge, which serves as an inductive center in the development of vertebrate limbs. Recent embryological and molecular characterization of development in the Chinese soft-shelled turtle, *Pelodiscus sinensis*, performed by Hiroshi Nagashima and colleagues in the Laboratory for Evolutionary Morphology (Shigeru Kuratani; Group Director) have now shown, however, that the carapacial ridge is unique to turtles and cannot be directly likened to any other vertebrate embryonic structure.

Nagashima et al. started by looking closely at the morphology of the region where the carapacial ridge develops in the turtle and comparing it with equivalent regions in other non-chelonian amniote species, such as chicken. Observation of the period during which the ridge forms, revealed that it is an axial structure derived entirely from the somites (transient mesodermal structures that give rise to trunk skeletal muscle, vertebrae and ribs). Unlike in chicken, in which the ribs invade the lateral body wall, the ribs in *P. sinensis* never do so, suggesting that in turtles the ribs (and so the carapace) are confined solely to the axial domain dorsal to the embryonic flank.

The group next looked at the roles of genes that had previously been shown to be expressed specifically in the carapacial ridge. They found that the introduction of a dominant negative version of the CR-specific gene LEF-1 resulted in the abnormal

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development of the carapacial ridge, indicating that this gene plays a role in the formation and maintenance of that structure.

Turning next to classical embryology, Nagashima performed transplantation and ablation experiments to ascertain what role, if any, the carapacial ridge played in determining the turtle's unique pattern of rib growth. Interestingly, the carapacial ridge was found frequently to regenerate and retain its axial position in embryos in which the region was cauterized at stage 14. But examination of the subsequent development of the CR-ablated embryos did show that the fan-shaped pattern of the ribs was partially disturbed. This suggests that while the carapacial ridge is not responsible for the axially restricted growth of turtle ribs, it might rather be involved in the radiation of the ribs in an arc relative to the midline.

"This study showed that the turtle has not added anything new to the ancestral anatomical components—there are no turtle-specific skeletal elements," says Kuratani. "Rather, their ribs are arrested dorsally, in their original position of development and simply grow laterally, which does not happen in other amniotes. The CR might in some way function in changing the direction of growth, but we still do not know how this might be achieved." The lab is continuing to work on anatomical relationships between skeletal and muscle elements in the hopes of identifying the gene or regulatory system that actually altered the direction of rib growth, which will provide a better understanding of 'the making of the turtle'.

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