Evolutionary Morphology

Shigeru KURATANI Ph. D.

Shigeru Kuratani received his masters and Ph. D. from the Kyoto University Department of Zoology. He spent the period from 1988 to 1991 working in experimental embryology in the Department of Anatomy at the Medical College of Georgia before moving to the Biochemistry Department at the Baylor College of Medicine, where he was engaged in molecular embryological research. He returned to Japan in 1994 to take a position as associate professor at the Institute of Medical Embryology and Genetics in the Kumamoto University School of Medicine. He moved to Okayama University to assume a professorship in the Department of Biology in 1997, where he remained until he was appointed team leader at the CDB. He was appointed group director in 2005.

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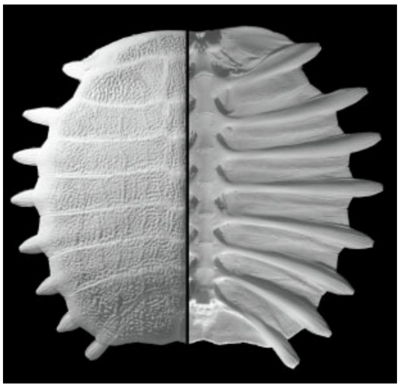
By studying the evolutionary designs of diverse species, I hope to gain a deeper insight into the secrets behind the fabrication of the vertebrate body. Integrating the fields of evolutionary morphology and molecular genetics, our lab seeks to expand the understanding of the relationship between genome and morphology (or body plan) through investigating the evolutionary changes in developmental processes. Our recent studies have focused on novel traits found in the vertebrates, such as the jaw, the turtle shell, and the mammalian middle ear. By analyzing the history of developmental patterns, I hope to open new avenues toward answering as-yet unresolved questions about phenotypic evolution in vertebrates at the level of body plans.

Through the study of evolutionarily novel structures, our lab has identified changes in developmental mechanisms that have obliterated the structural homologies between organisms as evidenced in such novel structures as the jaw in gnathostomes (jawed vertebrates) and the turtle shell. Developmental studies of the cranial region of the lamprey are intended to shed light on the true origins of the vertebrate head and neck, as lampreys lack a number of important features, including jaws, true tongues and trapezius muscles, that are possessed by gnathostomes. We aim to resolve the question of what primary factors that have changed during evolution by comparing the developmental patterns that yield such innovations, and by the experimental construction of phenocopies in one animal that mimic structures in another.

The turtle's shelled body pattern appears suddenly in the fossil record. Our lab's research into turtle carapace development addresses the developmental changes that resulted in this abrupt and dramatic morphological change, and is aimed at identifying genes that function differently in chicken and turtle, which it is hoped will provide a key to discovering the true targets of natural selection in the acquisition of a shell.



Dorsal (left) and ventral (right) views of the carapace (dorsal half of the turtle shell) of Chinese soft-shelled turtle. The carapace forms from laterally expanded ribs.

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Publications

Nagashima H, et al., On the carapacial ridge in turtle embryos: its developmental origin, function and the chelonian body plan, *Development* 134. 2219-26 (2007)

Ota K G, et al., Hagfish embryology with reference to the evolution of the neural crest, *Nature* 446. 672-5 (2007)

Kuraku S, et al., Comprehensive survey of carapacial ridge-specific genes in turtle implies co-option of some regulatory genes in carapace evolution, *Evol Dev* 7. 3-17 (2005)

Takio Y, et al., Evolutionary biology: lamprey Hox genes and the evolution of jaws, *Nature* 429. 1 p following 262 (2004)

Shigetani Y, et al., Heterotopic shift of epithelial-mesenchymal interactions in vertebrate jaw evolution, *Science* 296. 1316-9 (2002)

Hagfish first: Elusive organism sheds light on the emergence of the neural crest


LETTERS

Hagfish embryology with reference to the evolution of the neural crest

Ota K G, et al. Hagfish embryology with reference to the evolution of the neural crest. *Nature* 446. 672-5 (2007)

The possession of a hinged jaw is a trait shared by many, but not all vertebrates. The cyclostomes, such as lampreys and hagfish, are vertebrate taxa but feed by means of circular mouthparts, which can be used for either predation or scavenging. Even within the Cyclostomata, considerable diversity can be found; indeed the hagfish, in which many primitive traits are preserved, are sometimes considered to be markedly distinct from both the jawed vertebrates (gnathostomes) and the lampreys. It has long been suspected that an analysis of hagfish embryonic development would shed light into the question of its true phylogenetic position, which conceivably is near the branch point of the vertebrate divergence, but their seafloor habitat is little explored and hagfish eggs have proven extremely difficult to obtain. So difficult, in fact, that the last major research into hagfish development dates back to an 1899 treatise by Bashford Dean.

A major hurdle on the path to developing a more certain understanding of hagfish ontogeny and phylogeny was cleared with a report by Kinya Ota et al. of the Laboratory for Evolutionary Morphology (Shigeru Kuratani; Group Director) of the first significant new study of hagfish development in more than 100 years. The study, published in *Nature*, described the obtainment and analysis of multiple pharyngula-stage embryos of the hagfish, *Eptatretus burgeri*, yielding new insights into their early development, particularly that of the neural crest.

To collect viable specimens, the group placed eel traps in the waters off the coast of Shimane Prefecture, collecting around 50 live hagfish. “We were lucky to work with some local fisherman who knew the best spots to look for egg-carrying females,” says Ota. “It turns out that it may be easier to catch them in Western Japan, rather than the Pacific Coast, as the costal shelf slopes more gradually there.”

On returning to the CDB, they kept the animals in special aquarium tanks designed to replicate the deep-sea environment of the hagfish as closely as possible, and were rewarded for their efforts with a few dozen eggs, seven of which were fertilized. This in itself was something of an achievement, as hagfish have never successfully been bred in captivity. “We’re not sure what the critical factor in keeping

Figure 1

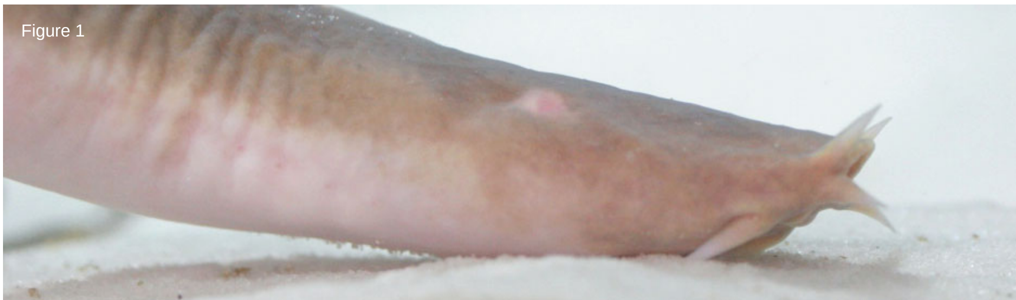
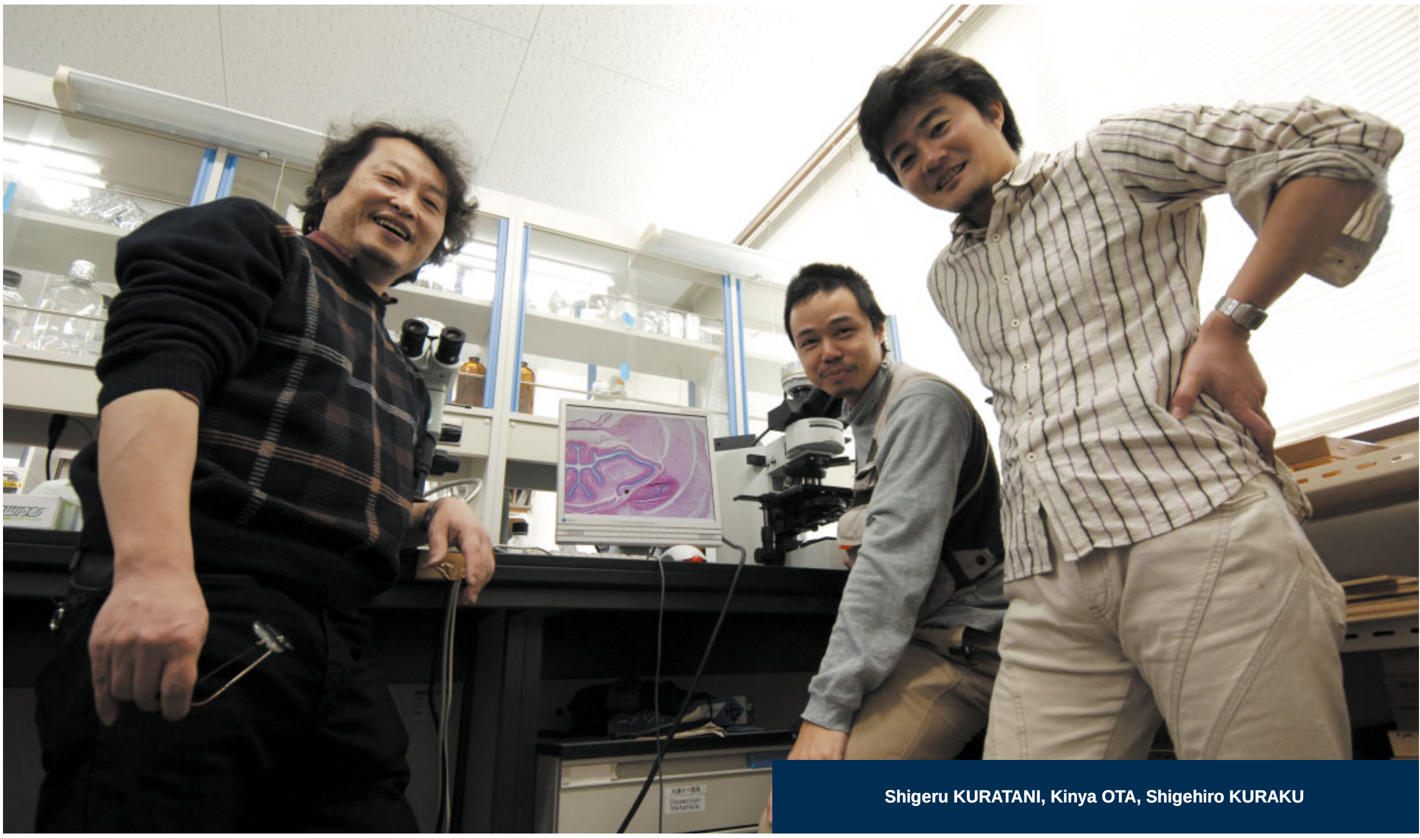


Figure 1
Hagfish maintained in laboratory aquarium

Figure 2
EFT: Hagfish pharyngula-stage embryo (anterior to left, gills visible); RIGHT: Sox9 gene expression in embryonic trunk. The stained Sox9-expressing region corresponded to neural crest.

Figure 2



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them alive in captivity is," Ota admits, "but we know that hagfish like cold water and are extremely sensitive to changes in salinity – even a small dilution of salt content can kill them. Their breeding habits are also still a complete mystery."

Histological staining and in situ hybridization of the embryos enabled the Kuratani group to address the question of neural crest development in these phylogenetically important animals. The vertebrate neural crest is a population of delaminated migratory cells, which serves as the source for such a variety of cell types that it is sometimes referred to as the fourth germ layer. Non-vertebrate chordates (such as tunicates and lancelets), however, develop only pockets of relatively immobile epithelial cells. The crucial question for the hagfish was whether this aspect of its development would more closely resemble that of the gnathostomes and lampreys, or that of the non-vertebrates (one previous study had speculated that it would be the latter). Looking at the expression of *Sox9* and several other neural crest maker genes, Ota found that the distribution patterns were closely matched to those seen in neural crest development, indicating that the genetic programs at work in the hagfish crest are similar to those in other vertebrates.

This new work, made possible by the first-ever artificial breeding of this elusive species, strongly supports the phylogenetic grouping of hagfish with the lampreys and other vertebrates. "Although they appear to be quite primitive, the hagfish actually use the same basic developmental program common to the vertebrates, including humans," notes Kuratani. "This genetic architecture has been preserved over at least 500 million years of evolutionary history, since the time of the divergence of the jawed gnathostomes from the more basal cyclostomes." The new availability of breeding techniques and viable embryos for this key organism opens up new inroads for molecular embryology toward a better understanding of vertebrate evolution.

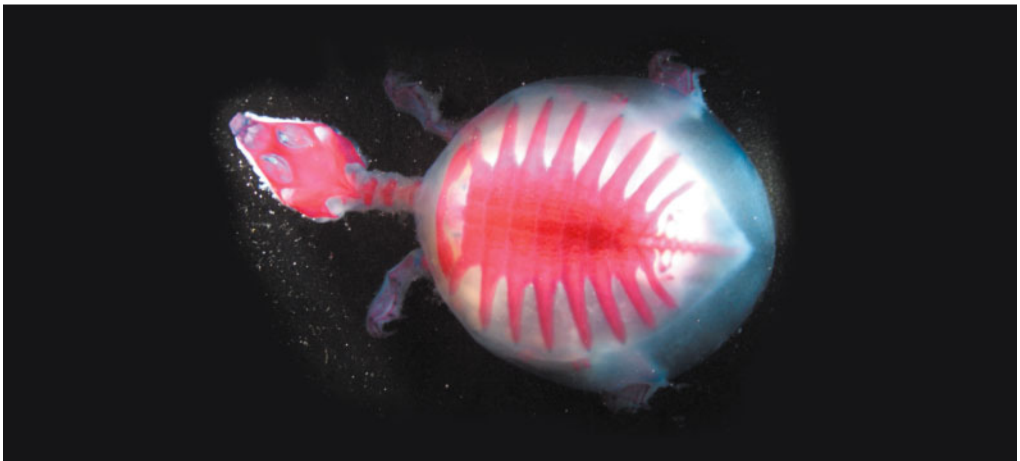
Ridge analysis: Function of the carapacial ridge in the development of the turtle shell


On the carapacial ridge in turtle embryos: its developmental origin, function and the chelonian body plan

Nagashima H, et al. On the carapacial ridge in turtle embryos: its developmental origin, function and the chelonian body plan. *Development* 134. 2219-26 (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.

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. 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 shown, however, that the carapacial ridge is unique to turtles and cannot be directly likened to any other vertebrate embryonic structure.



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

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 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 et al. 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 re-

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tain 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.'