PNAS COMMENTARY

Scales, scutes, and embryonic origins of the vertebrate dermal skeleton

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The story of vertebrate origins is one of novel cell types and shifting germ layer fates—and at the center of this story lies the neural crest. Neural crest cells are a population of stem-like cells that are specified within the dorsal neural tube of vertebrate embryos and that migrate widely throughout the body, giving rise to an array of neuronal and glial, neuroendocrine, pigment, connective tissue, and skeletal cell types (1). While there are hints of neural crest-like cells or "latent" neural crest homologues (2) in nonvertebrate chordates (3), the neural crest exhibits germ layer-scale developmental potential in vertebrate animals and gives rise to many defining features of the vertebrate body plan (4, 5). Importantly, however, in the most widely studied vertebrate lineages, the neural crest does not do everything, everywhere, all at once—rather, there is axial regionalization of the neural crest into cranial, vagal, trunk, and sacral domains, with each exhibiting restricted fate potential and giving rise to distinct derivatives (6). It therefore follows that to understand the origin and early evolution of vertebrates, it is important to consider not only the origin of the neural crest itself but also the evolution of its fate along the embryonic axis. In this issue, Stundl et al. report a paradigm-shifting discovery of trunk neural crest contribution to the postcranial dermal skeleton in fishes that challenges current models of neural crest axial regionalization and evolution (7).

In the mouse and chicken, neural crest cells give rise to most of the dermal skeleton of the head (8, 9)—the osteoblasts that build the flat, plate-like bones of the skull, and the dentine-producing odontoblasts of the teeth—but make no contribution to skeletal tissues in the trunk. This apparently distinct skeletogenic potential of cranial neural crest cells in amniotes is associated with distinct genomic machinery—a cranial neural crest gene regulatory circuit (10)—which underlies spatial restriction of some neural crest derivatives in the embryonic head vs. trunk. But comparative transcriptomic and gene expression studies of developing neural crest in the lamprey, skate, zebrafish, and chick suggest that axial regionalization was acquired in a stepwise manner through vertebrate evolution, with an ancestral neural crest that was perhaps more homogeneous with respect to molecular identity (and, by extension, developmental potential) along the anteroposterior axis than that of amniotes (11). Moreover, many stem-gnathostomes (i.e., fossil taxa more closely related to living jawed vertebrates than to living jawless ones) possessed an extensive postcranial dermal armor (12), and it has been speculated that this armor derived from the neural crest (13–15). This would indicate that both cranial and trunk neural crest were ancestrally skeletogenic but that this fate has been lost from the trunk neural crest of amniotes. Stundl et al. now report evidence that trunk neural crest cells do, indeed, give rise to the postcranial dermal skeleton of a fish, the sterlet sturgeon (*Acipenser ruthenus*), cementing the ancestral skeletogenic fate of this embryonic tissue within jawed vertebrates and highlighting its role in shaping the early evolution of the vertebrate body plan (7).

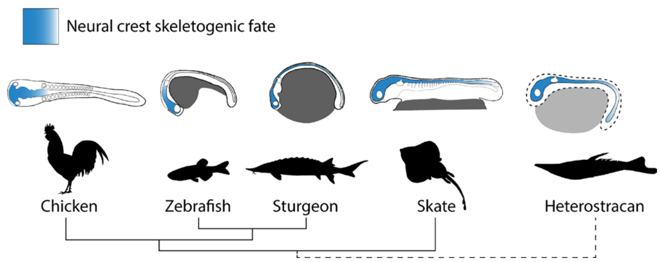

Fig. 1. Evolution of neural crest skeletogenic fate in jawed vertebrates. In amniotes (e.g., chicken) and teleost bony fishes (e.g., zebrafish), only the cranial neural crest contributes to the skeleton, while in sturgeon and skate, skeletal tissues derive from both cranial and trunk neural crest. Histological similarities between trunk neural crest-derived skeletal tissues of sturgeon and skate and the postcranial dermal armor of stem-gnathostomes (e.g., heterostracans) point to a jawed vertebrate neural crest that was ancestrally skeletogenic along its entire axis.

The ancestral postcranial dermal skeleton of jawed vertebrates consisted of a *basal bony layer* and a *superficial dental layer*, and these tissue layers have been variously retained (albeit, often in a reduced form) as the spectacularly diverse array of scales, scutes, plates, and denticles of fishes (16). To date, limited lineage tracing data from fishes have pointed to distinct embryonic origins of the bony and dental layers of the ancestral postcranial dermal skeleton: The scales of teleost fishes (e.g., zebrafish and medaka) are composed entirely of bone and derive from paraxial mesoderm (17, 18), while the tooth-like dermal denticles of cartilaginous fishes (sharks, skates and rays) are composed almost entirely of dentine and derive from trunk neural crest (19). Stundl et al. use histology and microCT imaging to survey the tissue composition of the scales of the Senegal bichir and the spotted gar, the denticles of an armored catfish, and the scutes of the sterlet sturgeon. They find that while the dental layer of these elements appears to be variable and highly evolutionarily labile, a basal bony layer is

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ever present. Interestingly, however, comparative transcriptomic and hierarchical clustering analysis reveals that bichir scales and sturgeon scutes (the latter composed entirely of bone) have a gene expression signature that is quite different from that of the mesodermally derived scales of zebrafish—one that contains features of the neural crest gene regulatory network. Since gene expression of differentiated structures alone is not a proxy for cell lineage, the authors take their study further and directly test for a neural crest contribution to sturgeon scutes. By labeling trunk neural crest cells of sturgeon embryos with a lipophilic dye, the authors were able to track the progeny of these cells as they migrate and differentiate into osteoblasts of the developing scutes. The authors also performed comparable experiments to label the paraxial mesoderm of developing sturgeon embryos and found no contribution from this tissue to scute osteoblasts.

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This study clearly demonstrates that trunk neural crest cells in a nonteleost bony fish give rise to osteoblasts—and this, alongside data from cartilaginous fishes, points to a jawed vertebrate neural crest that ancestrally gave rise to bone and dentine along its entire axis, with restriction of this skeletogenic fate to the cranial region in amniotes (Fig. 1). From these findings follow fascinating questions about whether restriction of trunk neural crest developmental potential is *causal* or *consequential* with respect to the amniote postcranial skeleton: Did changes in the developmental potential of trunk neural crest cells alter the organization of the amniote dermal skeleton, or did a reduction of the postcranial dermal skeleton relax constraint on trunk neural crest skeletogenic fate, ultimately leading to its loss? And in amniotes that have secondarily acquired an extensive postcranial dermal skeleton (e.g., turtles and crocodilians), has this skeleton evolved by "reawakening" a lost or dormant trunk neural crest skeletogenic programs, or do these elements derive from mesoderm? A neural crest origin has been speculated for these structures, but cell lineage tracing data are still lacking.

And finally, how do we reconcile the distinct embryonic origins of bony scutes in sturgeon and the scales of teleost fishes? Perhaps the mesodermal origin of teleost scales reflects the ancestral embryonic origin of the basal bony layer of the jawed vertebrate postcranial dermal skeleton, with origin shifting to trunk neural crest in some bony fish lineages—after all, the neural crest has a track record of acquiring cell fates that ancestrally fell within the mesodermal wheelhouse. Or perhaps the scales of teleost fishes are not homologous with the bony scutes of sturgeon. Although both scales and scutes are often regarded as derivatives of the basal bony layer of a once more extensive dermal armor, perhaps the distinct embryonic origins of these elements reflect their independent evolutionary histories. Or perhaps trunk neural crest- and mesoderm-derived mesenchyme are equally competent to give rise to postcranial dermal bone, with topology, timing, and spatial availability of mesenchyme ultimately dictating which germ layer gives rise to postcranial skeletal elements. This seems to be the case, for example, at the head-trunk interface of jawed vertebrates, where both cranial neural crest- and lateral mesoderm-derived mesenchyme intermingle and contribute to cartilages of the posterior pharynx (20). The latter explanation would imply that some as-yet-undetermined property of "competence" may supersede germ layer origin as an indicator of anatomical homology in the vertebrate skeleton. Whatever the explanation, the discovery of Stundl et al. illustrates the primacy of experimental embryology in strategically selected model systems in resolving the evolution of neural crest cell fate. Further neural crest lineage tracing experiments in a plethora of other vertebrate taxa combined with rapidly emerging single cell "-omic" approaches promise to reveal the diverse anatomical fates and transcriptional complexity of an evolving neural crest.

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