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GEOSCIENCES

Special Topic: Paleontology in China

Bone gain and loss: insights from genomes and fossils

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In the human body, bones are dynamic and highly tuned organs that allow efficient movement, protect vital organs and house the bone marrow [1]. Among the four skeletal tissue types (bone, dentine, enamel, cartilage) in vertebrates, bone is the most distinct and diversified mineralized tissue that is patterned to provide maximal strength with minimal mass [2]. Based on the bone formation or ossification types in the embryos and young of vertebrates, bones are classified into the membrane (achondral) and chondral bones, the latter comprising perichondral and endochondral bones (Fig. 1) [3].

In living vertebrates, in contrast to the widespread distribution of cartilage, bone is only present in osteichthyans or bony vertebrates (bony fishes and tetrapods including ourselves). This raises the question whether the absence of bone in living chondrichthyans (cartilaginous fishes) [4,5] is a primitive retention or a derived trait due to the bone loss from a bony ancestor. The past decade has witnessed a dramatic increase in our understanding of the molecular and genomic bases of mineralized tissues [6–8] as well as that of early vertebrate evolution [9–12]. New breakthroughs from these two independent lines of research have provided unparalleled insights into the molecular mechanism and pacing of bone gain and loss in vertebrates (Fig. 2) [5].

One aspect of the mineralized tissue biology that has received extensive attention from molecular geneticists is the correlation between the phenotypic complexity of hard tissues in vertebrates and the SCPP (secretory calcium-binding phosphoprotein) gene family [6–8]. SCPP genes have a common ancestor, *sparcl1* (*sparc*-like 1). Recent research suggests that at around the same time that early jawless fishes first evolved a mineralized skeleton, *sparcl1* arose from *sparc* (gene for secreted protein, acidic and cysteine-rich) as a by-product of a whole-genome duplication (WGD); *sparc* has been found to occur in a wide range of both protostomes and deuterostomes. Two WGDs are thought to have taken place, the first in stem vertebrates (WGD1) and the second in stem gnathostomes (WGD2). The genes more specific to various mineralized tissues (genes for Pro/Gln-rich and acidic SCPPs) arose subsequent to the genome duplications by genomically local tandem duplications [7]. In tetrapods, acidic SCPPs are used in bone and dentine formation, whereas Pro/Gln-rich SCPPs participate in enamel formation [7]. Because SCPP genes have been found in teleosts, coelacanths and tetrapods to date, the initial SCPP gene must have originated before the divergence between actinopterygians (ray-finned fishes) and sarcopterygians (lobe-finned fishes and tetrapods) (Fig. 2).

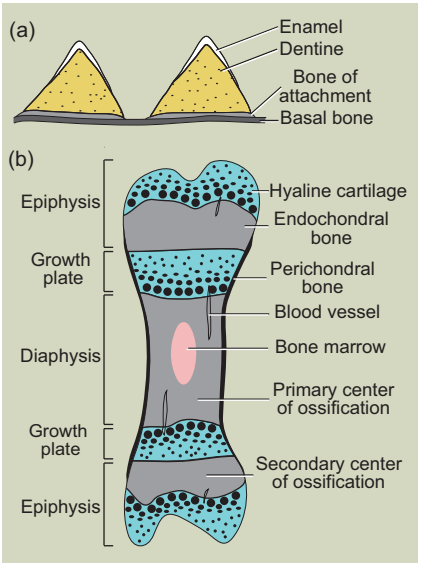

Figure 1. Illustrative drawings of the hard tissues that comprise the dermal skeleton (a) and the endoskeleton (b). Bone, dentine and enamel form a mineralized-tissue continuum based on genomic data. In (a), bone of attachment and basal bone comprise the membrane bone that is formed by intramembranous ossification. In (b), perichondral bone and endochondral bone comprise the chondral bone.

Noteworthy in this line of research is the whole-genome analysis of a cartilaginous fish, the elephant shark (*Callorhinchus milii*), that provides intriguing data regarding the innovations of SCPP genes and the genetic basis of bone formation [8]. The *C. milii* genome contains both *sparc* and *sparcl1* as in humans and other bony vertebrates, but lacks any SCPP genes that have a critical role in the formation of endochondral bone and intramembranous (dermal) bone as tested by knockdown experiments on *spp1* (secreted phosphoprotein 1 gene) in zebrafish. The genomic resources available for other chondrichthyans, as well as the genome assembly of the jawless sea lamprey, do not contain any SCPP genes either. Because osteichthyans have extensive endochondral bone [10] whereas chondrichthyans have only a cartilaginous endoskeleton, Venkatesh and colleagues [8] suggested that the tandem duplication of *sparc1* that gave rise to SCPP genes occurred in the common ancestor of osteichthyans and after the osteichthyan–chondrichthyan split.

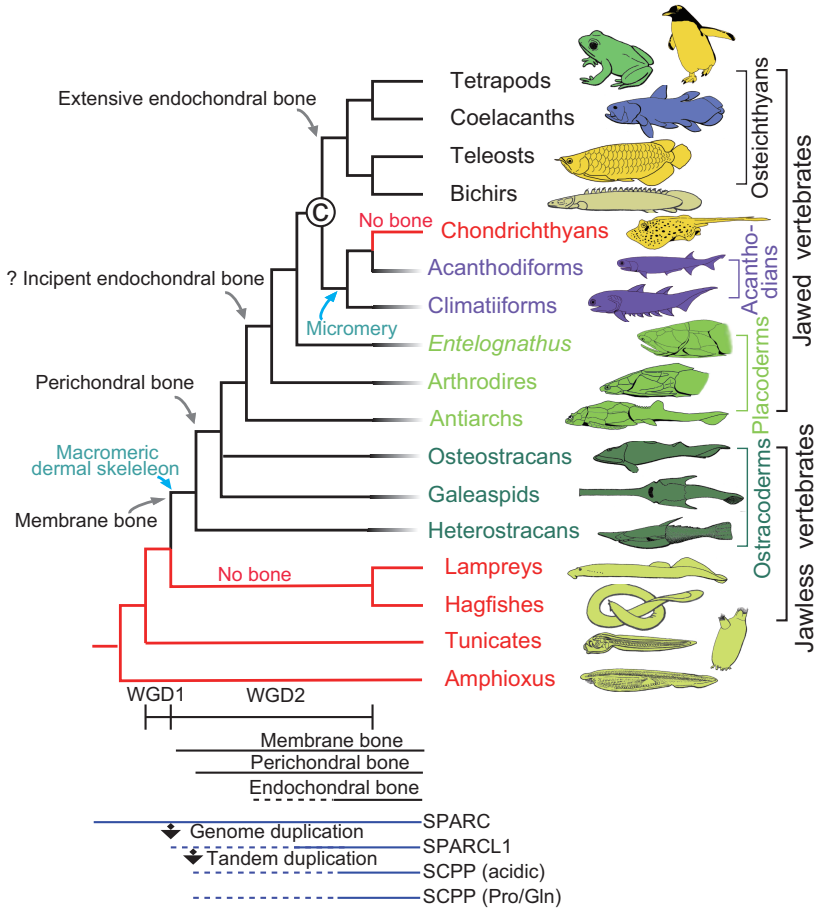

Figure 2. Vertebrate phylogeny, macromery versus micromery of dermal skeleton, and genetic events underlying the evolution of skeletal mineralization (modified from [6] and [8]). The phylogeny of jawed vertebrates is based on [11], with C denoting the last common ancestor of osteichthyans and chondrichthyans. The new scenario shows that the micromery (i.e. dermal skeleton only with tiny scales) and lack of bone in chondrichthyans are derived, rather than primitive, corresponding to the absence of any SCPP genes in chondrichthyans [8]. Extinct groups are shown as faded lines, and groups that lack bone as red lines.

Beyond doubt, the absence of SCPP genes from chondrichthyans is related to the unossified nature of their endoskeleton. Considering the zebrafish *spp1* knockdown impacting on the formation of both membrane and endochondral bones [8], whether this lack of bone in chondrichthyans is primitive or secondary cannot be ascertained in the family tree of vertebrates because bone has a broader distribution in extinct fish groups such as placoderms and jawless ostracoderms [9].

Recent research on early gnathostomes has identified a unique role of new fossils [11] and the re-examination of old ones [10] in the understanding of innovations or losses of key morphological traits as well as our family tree. Conventionally, gnathostomes comprise chondrichthyans, osteichthyans and their extinct relatives (placoderms and acanthodians). Osteichthyans and placoderms differ from chondrichthyans and acanthodians in having macromeric dermal skeletons with a stable pattern of bone sutures. It was assumed [10] that the macromeric dermal skeleton of placoderms was replaced by an acanthodian-like tessellate condition in the common ancestor of chondrichthyans and osteichthyans, with subsequent *de novo* acquisition of a non-homologous macromeric dermal skeleton in osteichthyans. The discovery of an astounding placoderm from the Silurian of China, *Entelognathus* [11], has challenged this shark-like-ancestor hypothesis and provided a new framework for studying the crown gnathostome divergence. *Entelognathus* is a placoderm [11], but bears dermal marginal jaw bones (premaxilla, maxilla and dentary) that were previously thought to be restricted to osteichthyans [10], thus lending strong support to the homology of macromeric skeletons between placoderms and osteichthyans. The new phylogenetic scenario shows that the chondrichthyans arose from a placoderm-like ancestor, and the condition displayed by chondrichthyans and their kin (e.g. reduction of large dermal plates, absence of bone) is evolutionary novel, rather than ancestral [11, 12].

This new scenario allows refined perspectives on bone evolution. Membrane bone, albeit absent in chondrichthyans, has a very early origin at the root of jawless ostracoderms [9], possibly mediated by *sparc1* resulting from the WGD2. Perichondral bone is absent in some basal groups of ostracoderms (e.g. heterostracans); however, its wide presence in jawless galeaspids and osteostracans, as well as jawed acanthodians, placoderms and osteichthyans, definitely indicates its secondary loss in chondrichthyans. The distribution of endochondral bone still awaits further clarification from the fossil record. Although extensive endochondral bone is exclusively found in osteichthyans, the paleontological data show that incipient endochondral bone is probably present in some placoderms such as petalichthyids and arthodires. Considering the evolutionary losses of membrane and perichondral bone in chondrichthyans, endochondral bone might have undergone similar loss resulting from the same genetic basis (i.e. absence of any SCPP genes). Further investigation on hard tissues in placoderms might tell whether the bone-specific SCPP gene *spp1* has a role in the formation of endochondral bone [8] or just a general role in bone development [5]. Based on available genomic data, the Pro/Gln-rich SCPP genes that are responsible for the formation of enamel originated after

the split between actinopterygians and sarcopterygians [7, 8]. However, the tandem duplications of *sparc1* genes that gave rise to acidic SCPP genes might have happened before the split between chondrichthyans and osteichthyans (unlike [8]), possibly at the node where perichondral bone arose [5].

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The end-Permian mass extinction: a still unexplained catastrophe

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The end-Permian mass extinction is widely regarded as the largest mass extinction in the past 542 million years with loss of about 95% of marine species and 75% of terrestrial species. There has been much focus and speculation on what could have caused such a catastrophe. Despite decades of study, the cause or causes remain mysterious. Numerous scenarios have been proposed, including asteroid impact, Siberian flood basalt volcanism, marine anoxia and euxinia, sea-level change, thermogenic methane release and biogenic methane release due to explosive growth of a methanogenic microbe.

It is now clear that a number of major environmental perturbations are approximately coincident with the end-Permian mass extinction. These include global negative excursions of both $\delta^{13}C_{carb}$ and $\delta^{13}C_{org}$ near the extinction interval (see a review by Korte and Kozur [1] and a recent study by Shen *et al.* [2]); distinctive calcium isotope excursions [3]; a sudden expansion of microbialites [4]; a rapid temperature rise of $\sim 8^{\circ}C$ in the extinction interval [5] followed by a long 'hothouse' period in the Early Triassic [6], large regression followed by rapid transgression [7], evidence for wildfires and cyanobacteria blooms [8], etc. There remains disagreement over the nature, timing and duration of the environmental perturbations and how they relate to detailed patterns of extinction, resolution of which is critical for understanding the causative mechanism(s).

TIMING AND DURATION

The timing and duration of the end-Permian extinction at the Meishan sections in South China has been studied for over two decades. In many ways, successive publications track the evolution of high-precision U-Pb geochronological techniques that have led to increasingly precise and accurate constraints on the extinction.

Burgess *et al.* [9] review the evolution of increasing precise and accurate geochronological constraints on the age and duration of the extinction. For example, the published ages of Bed 25 at Meishan have varied from 251.4 ± 0.3 to >254 Ma and the duration of the extinction from 500 to 61 ± 48 kyr. The latter reflects the latest work using EARTHTIME protocols [9]. In addition to U-Pb zircon geochronology of ash beds, the floating astronomical time scale (ATS) has been applied to the extinction interval at Meishan and Shangsi. The ATS is based on recognizing astronomically forced stratigraphy and the latest effort has yielded an extinction duration of 112 kyr at Meishan and 83 kyr at Shangsi in South China [10]. Thus, both the most recent high-precision CA-TIMS U-Pb dates and the ATS are consistent with a catastrophic event that occurred in less than 100 kyr. Higher temporal resolution estimates from Shangsi and Meishan will likely be limited by the condensed nature of the sections.

EXTINCTION PATTERN

In addition to timing of the extinction, another fundamental issue is how to reconstruct and understand patterns of biological diversity. A single catastrophic event between Bed 25 and 28 was proposed based on detailed paleontological