ZOOLOGICAL RESEARCH

Ancestral developmental potentials in early bony fish contributed to vertebrate water-to-land transition

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The water-to-land transition was a major step in vertebrate evolution and eventually gave rise to the tetrapods, including amphibians, reptiles, birds, and mammals. The first land invasion of our fish ancestors is considered to have occurred during the late Devonian period ~370 million years ago (Mya) (Daeschler et al., 2006). Many fossils from important transitional species, such as Tiktaalik, Acanthostega, and Ichthyostega, have helped to identify key morphological and anatomical structures crucial to vertebrate terrestrial adaptation (Coates, 1996; Johanson & Ahlberg, 2001; Shubin et al., 2006). However, homologous analyses of these body forms and structures in more ancient species have suggested that some of the morphologies related to vertebrate land dispersal were already present in early bony fish species. For instance, the presence of shoulder girdles on the articular surface of the endoskeleton in Late Lochkovian *Psarolepis* indicates that stem sarcopterygians already possessed an endoskeletal fin pattern similar to that of tetrapod stylopods (Zhu & Yu, 2009). In addition, primitive lungs, which originated from the respiratory pharynx and were located on the ventral side of the alimentary tracts, can be observed in several extant basal actinopterygians (bichirs, reedfish) and all extant sarcopterygians, as well as some fossils of coelacanths and salamanders (Cupello et al., 2017; Tissier et al., 2017) (Figure 1). This evidence suggests that, instead of relying on genetic innovations evolving after the first fish left their water habitat, this transition may have been accomplished by adopting physical traits and genetic components that already existed far earlier than when the transition occurred. Whether such an ancestral developmental regulatory network was present or not and how far this ancestral network can be traced in history are challenging questions for paleontologists. Three recent papers published in *Cell* provide new insights into this hypothesis. Wang et al. (2021) sequenced the giant genome of lungfish, the closest fish species to tetrapods, and Bi et al. (2021) sequenced the genomes of multiple early divergent ray-finned fish. Comparative genomic analyses from these two studies confirmed the presence of ancestral genetic regulatory networks that likely played essential roles in the development and evolution of various biological functions related to vertebrate land invasion. Although certain ancestral features have been lost in teleosts, the most derived fish lineage to evolve after whole-genome duplication (Sato & Nishida, 2010), they have been recreated in zebrafish by modifying their genetic makeup to reactivate the ancestral genetic network (Hawkins et al., 2021).

The evolution of walking limbs from swimming fins was one of the most profound morphological changes to occur during water-to-land transition. A key feature of limb morphology is the presence of endoskeletal elements along the proximal-

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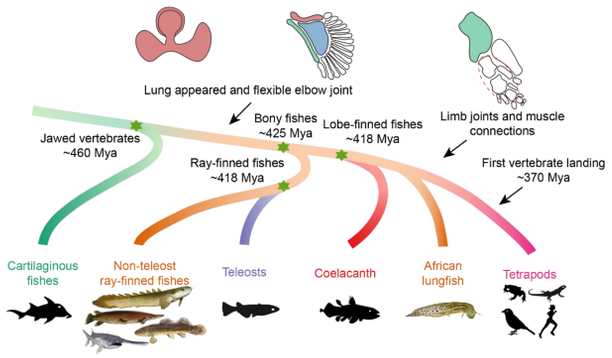

Figure 1 Phylogeny of living jawed vertebrates

Ancestral developmental potential of lungs and limbs was established 400 million years ago.

distal axis. The appendicular skeleton of tetrapods retains the same metapterygium as found in basal ray-finned fish, which formed present-day limbs through the progressive acquisition of bone elements (Zhu & Yu, 2009). However, this structure has been lost in teleosts, whose pectoral fins are only supported by four diminutive bones, called proximal radials (Arratia, 1999). Through comparative genomic study of non-teleost ray-finned fish, we identified a conserved regulatory sequence (mm703) present in all jawed vertebrates, except for teleosts (Bi et al., 2021). This conserved enhancer is involved in the regulation of the *Osr2* gene expressed in the synovial joint connecting the metapterygium and radials (Gao et al., 2011). The presence of this ancestral regulatory element and the synovial joint may have allowed flexible movement of pectoral fins in ancient bony fish. However, this ability has been lost in teleosts. Hawkins et al. (2021) also confirmed the presence of ancestral regulatory pathways in shaping limb-like forms in early fish. They investigated the genetic basis of fin patterning using a forward genetic approach in zebrafish (Hawkins et al., 2021) and discovered two mutation lines, *Reph* and *Wan*, that exhibit the proximodistal segmented endoskeletal radial pattern in the pectoral fin, a character that resembles the metapterygium that has been lost in teleosts. They further identified two genes, *Waslb* and *Vav2*, which corresponded to the mutant phenotypes. Conditional knockout of *Wasl* in mice suggested that this gene regulatory pathway is required for normal limb pattern formation, and its absence results in a range of skeletal phenotypes that match those seen in *Hoxa11*-knockout mutants (Hawkins et al., 2021). This work shows that zebrafish still have the potential to develop limb-like skeletal structures already present in bony fish ancestors, and this inherent capacity could be reactivated by simple genetic changes.

The appearance of five digits was important for the emergence of tetrapods. Hox genes, particularly *Hoxa13* and *Hoxd13*, are essential for digit morphogenesis (Zákány & Duboule, 1999). In a comparative genomic study on the African lungfish genome, Wang et al. (2021) identified a tetrapod-specific regulatory element, located upstream of *Hoxa11*, which may be a key element for the morphogenesis of the pentadactyl limb. They also discovered many unique changes related to the fin-to-limb transition, including a 40 amino acid fragment at the beginning of *Hoxb13* lost in tetrapods, the gradual loss of actinotrichia proteins in lungfish and tetrapods, and the gain of two tetrapod-specific regulatory elements in the upper region of *Hoxc10* essential for lumbar motoneuron development.

In addition to limbs, the development of air-breathing lungs to supply oxygen efficiently was an essential innovation to support terrestrial life. However, lungs were not an evolutionary innovation that appeared during the water-to-land transition. Lobe-finned fish, including coelacanths and lungfish, and bichirs have a pair of lungs that allow them to breath out of water. Consistent with this anatomical feature, we found that non-teleost ray-finned fish and lobe-finned fish carry two olfactory receptor genes that can detect water-soluble odorants and airborne smells, respectively. Furthermore, through clustering and phylogenetic analysis of the multi-organ expression profiles, our study suggests that lungs evolved much earlier than the swim bladder. In addition, several lung-specific expressed genes also already appeared in sharks (Bi et al., 2021). This collective evidence suggests that the molecular basis for the formation of “proto-lungs” can be dated back to cartilaginous fish.

The pulmonary alveoli structure can increase surface tension during lung respiration, which is a challenge for organs with air-breathing function. Pulmonary surfactants with a balanced cholesterol/phospholipid ratio are critical for reducing surface tension during respiration (Liem, 1988). Wang et al., (2021) discovered a series of evolutionary steps that led to the evolution of genes associated with pulmonary surfactants. Surfactant protein B (SP-B) is the most ancient member of the SP family, appearing in the ancestors of bony fish, consistent with the finding that bony fish ancestors already had a preliminary ability to breathe air. The SP-C gene emerged from the ancestors of lobe-finned fish, a vertebrate group with enhanced air-breathing ability. Furthermore, the SP-A and SP-D genes emerged from the ancestors of tetrapods, indicating further improvement in the respiratory ability of terrestrial vertebrates. In addition, the *Slc34a2* gene, which evolved in the common ancestor of lungfish and tetrapods, is highly expressed in the lungs (Wang et al., 2021). This gene participates in the transportation of phosphate released from phospholipids during pulmonary surfactant recycling (Izumi et al., 2017), thus also plays an important role in the development of pulmonary surfactants.

Cardiac function co-evolved with the respiratory system during vertebrate evolution to deliver oxygen to the whole body efficiently. In co-adaptation with the lung-circulatory system, tetrapod hearts evolved specialized chambers to separate oxygenated and oxygen-depleted blood and could direct blood flow within the arterial structures of the right ventricle. While most air-breathing fish did not evolve a separate chamber and mix deoxygenated and oxygenated blood in their circulatory system (Ishimatsu, 2012), it has been hypothesized that the conus arteriosus is an ancestral

characteristic of the cardiac outflow tract in early fish to direct blood flow (Icardo et al., 2002; Lorenzale et al., 2018). According to comparative anatomical analyses, this structure was present in early jawed vertebrates but has been lost in most teleost species (Icardo, 2006). Our study identified a conserved regulatory element upstream of the *Hand2* gene in all jawed vertebrates, except the Neoteleostei teleost lineage. Through genome-editing technology, we knocked out this element in mice. The mutation resulted in heart hypoplasia and congenital death. We detected a significant decrease in *Hand2* expression in the right ventricle of mutant mice and mutant mice also developed thinner and smaller right ventricles, suggesting that *Hand2* plays an important role in right ventricle formation with a conus arteriosus. The natural loss of this enhancer in advanced teleost groups may correspond to the complete loss of the conus arteriosus structure in these fish (Bi et al., 2021).

Together, the above studies support the hypothesis that many ancestral characteristics in early jawed fish were lately adopted in the development of biological functions associated with terrestrial adaptation in tetrapods. This has revolutionized our traditional view that genetic innovations appearing during evolutionary transition played a central role in the evolution of functional advances. Instead, the above studies suggest that ancestral developmental potentials provided essential genetic networks and facilitated later adaptation events.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

X.B. and G.Z. conceived and prepared the draft. All authors read and approved the final version of the manuscript.

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