Lungfish fins reveal how limbs evolved

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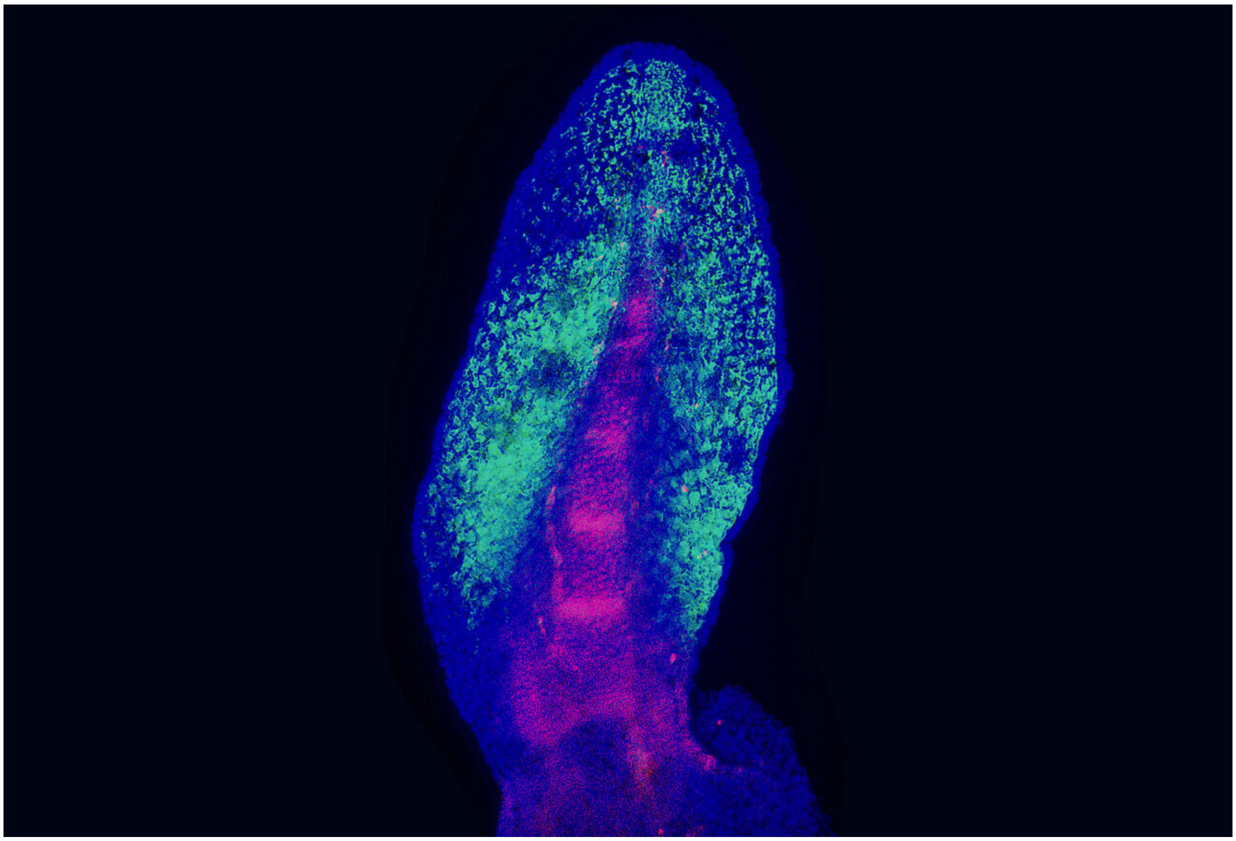

An embryonal fin of the Australian lungfsh as used in the study. The fin has been stained with fluorescent markers to visualise the developing internal skeleton (red), from which the limbs of land animals evolved, and the position of the external skeleton (green) that is specific to fish. Credit: Joost Woltering

The evolution of limbs with functional digits from fish fins happened approximately 400 million years ago in the Devonian. This morphological transition allowed vertebrates to leave the water to conquer land and gave rise to all four-legged animals or tetrapods—the evolutionary lineage that includes all amphibians, reptiles, birds and mammals (including humans). Since the nineteenth century several theories based on both fossils and embryos have been put forward trying to explain how this transformation unfolded. Yet, exactly how hands with digits originated from fish fins remained unknown.

An international team of biologists based at the University of Konstanz (Germany), Macquarie University in Sydney (Australia) and the Stazione Zoologica Anton Dohrn in Naples (Italy) has determined how limbs have evolved from fins using embryos of the Australian lungfish (Neoceratodus forsteri) for their study. The Australian lungfish is the closest living fish relative of tetrapods and is often considered a "living fossil" as it still resembles the fishes that were around at the time when the first four-limbed vertebrates began to walk on land. For these reasons the fins of lungfish provide a better reference to study the evolutionary transition of fins into limbs than any other extant fish species.

The team's research, which is reported in the latest issue of *Science Advances*, shows that a primitive hand is present in lungfish fins but at the same time suggests that the unique anatomy of limbs with digits only evolved during the rise of tetrapods through changes in embryonic development.

Insights from embryonic development: limb architect genes

To solve the puzzle of how limbs emerged from fins during evolution researchers have focused on embryonic development. "During embryogenesis, a suite of 'architect' genes shapes an amorphous group of precursor cells into fully grown limbs," explains Dr. Joost Woltering, first author on the study and an assistant professor in the Evolutionary Biology group at the University of Konstanz led by Professor Axel Meyer. The very same "architect" genes also drive fin development. However, because evolutionary changes have occurred in the activity of these genes, the developmental process produces fins in fish and limbs in tetrapods.

To compare this process in fins and limbs, the team studied such "architect" genes in the embryos of the Australian lungfish. "Amazingly, what we discovered is that the gene specifying the hand in limbs (hoxa13) is activated in a similar skeletal region in lungfish fins," explains Woltering. Importantly, this domain has never been observed in the fins of other fish that are more distantly related to tetrapods. "This finding clearly indicates that a primitive hand was already present in the ancestors of land animals."

Developmental patterns: differences and similarities

The lungfish "hand," in spite of this modern genetic signature, only partially resembles the anatomy of tetrapod hands because it lacks fingers or toes. To understand the genetic basis for this difference the team went on to analyze additional genes known to be associated with the formation of digits, finding that one gene important for the formation of fingers and toes (hoxd13—a "sister gene" to the above-mentioned hoxa13) appeared to be switched on differently in fins.

During tetrapod limb development, the hoxd13 gene is switched on in a dynamic manner. It first becomes activated in the developing pinky finger and then expands all the way throughout the future hand towards the thumb. This process coordinates the correct formation of all five fingers. While Joost Woltering's team observed a similar activation pattern of this gene in lungfish fins, it did not show this expansion but only remained activated in exactly one half of the fin. Additional differences were found for genes that are normally switched off in digits. In lungfish fins these genes remain active, but on the opposite side of the domain where hoxd13 is activated.

Old hypotheses—future directions

"All of this goes to show that while lungfish fins unexpectedly have a primitive hand in common with tetrapods, the fins of our ancestors also needed an evolutionary 'finishing touch' to produce limbs. In this sense it looks as if the hand was there first, only to be complemented with digits later during evolution," says Woltering. One influential hypothesis regarding the evolution of limbs first put forward by early 20th-century paleontologists Thomas Westoll and William Gregory, and in the 1980s famously developed further by Neil Shubin, postulates that fingers and toes arose through an expansion of the skeletal elements on one side of the fins of the tetrapod ancestor. This inferred expansion of fin elements corresponds exactly to the differences the team found in the expansion of the digit genes between lungfish fins and tetrapod limbs. The team's observations on the activation and deactivation of limb "architect" genes in lungfish fins thus provides evidence in support of this classical transformational model.

In the future, to fully understand what causes this domain to expand, making our limbs so different from fish fins, the researchers plan to conduct further analyses on the development of fins and limbs, using lungfish but also more modern fish species such as cichlids as their embryos are easier to investigate using techniques like CRISPR. "To complete the picture of what happened in our fish ancestors that crawled onto land hundreds of millions of years ago, we really rely on currently living species to see how their embryos grow fins and limbs so differently," concludes Woltering.

More information: J.M. Woltering el al., "Sarcopterygian fin ontogeny elucidates the origin of hands with digits," *Science Advances* (2020). DOI: 10.1126/sciadv.abc3510 , advances.sciencemag.org/lookup … .1126/sciadv.abc3510

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