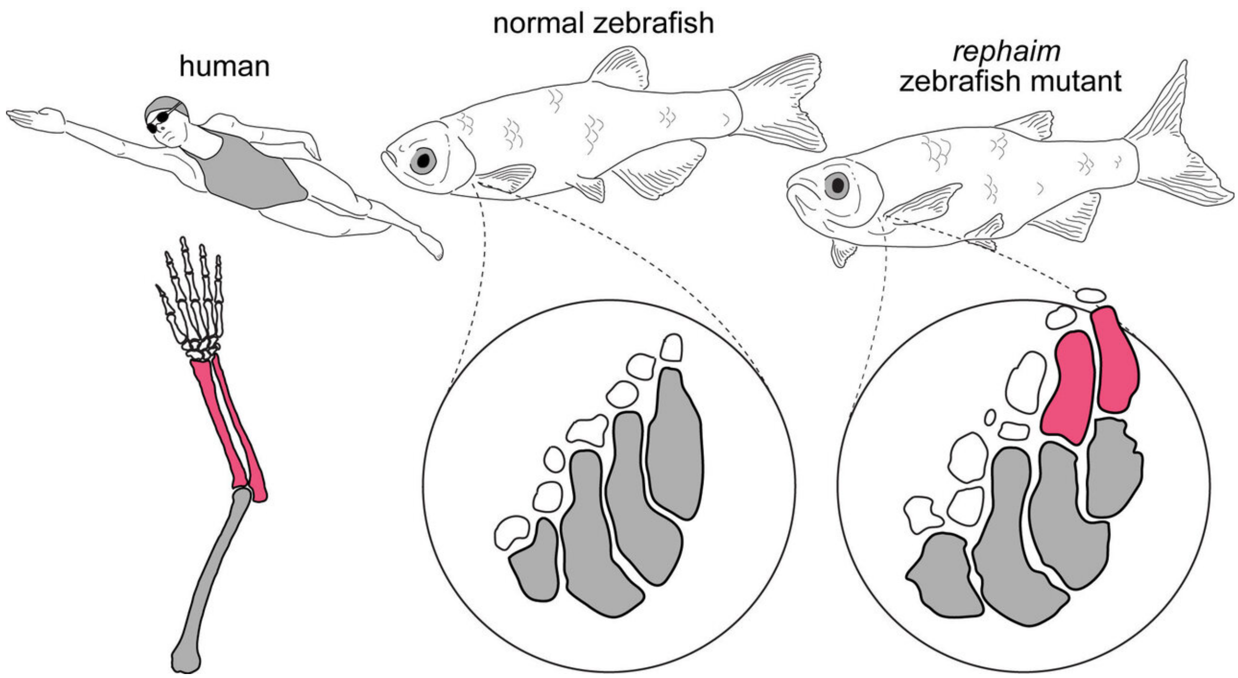
Can a fin become a limb? Single mutations cause zebrafish fins to transform into complex limb-like structures

February 4 2021



Human limbs have multiple long bones that articulate end on end and allow for a wide range of movements (left). Normal zebrafish, on the other hand, have no such articulation in their diminutive fin skeleton (middle). Scientists have now discovered mutant zebrafish that grow new bones away from the body in pattern similar to limbs (right). The new mutant bones develop using the same genes used to make the forearm (pink in left and right skeletons). Credit: M. Brent Hawkins

Fin-to-limb transition is an icon of key evolutionary transformations. Many studies focus on understanding the evolution of the simple fin into a complicated limb skeleton by examining the fossil record. In a paper published February 4 in *Cell*, researchers at Harvard and Boston Children's Hospital examined what's occurring at the genetic level to drive different patterns in the fin skeleton versus the limb skeleton.

Researchers, led by M. Brent Hawkins, a recent doctoral recipient in the Department of Organismic and Evolutionary Biology, performed forward genetic screens in zebrafish looking for mutations that affect the fin skeleton. Unlike tetrapod limbs, which have complex skeletons with many bones that articulate at many joints, zebrafish pectoral fins have a simple endoskeleton that lacks joints. To their surprise, Hawkins and colleagues found mutants that modified their fins into a more limb-like pattern by adding new bones, complete with muscles and joints. These results reveal that the ability to form limb-like structures was present in the common ancestor of tetrapods and teleost fishes and has been retained in a latent state which can be activated by genetic changes.

"We look at some of the developmental aberrations and ask, can they inform us of the processes that were happening underlying some of these large shifts in evolution," said senior author Matthew P. Harris, Associate Professor of Genetics at Harvard Medical School and Orthopedic Research at Boston Children's Hospital. "And when you see something that shouldn't be there, ever, I mean 400 million years type of never, it's a major finding."

Zebrafish belong to the teleost lineage of ray-finned fishes. Teleosts are a diverse lineage of about 30,000 species that includes goldfish, salmon, eels, flounder, clownfish, pufferfish, catfish and zebrafish. There are more teleost species than all birds, mammals, reptiles, and amphibian species combined. Yet, despite the large number of species and wide ranges of shapes, sizes, and habitats, the pectoral fin of teleosts is surprisingly simple and unchanged.

The fin-to-limb transition in the tetrapod lineage modified and elaborated the ancestral fin to include many bones that articulate end on end. From the same ancestral starting state, teleost fins were reduced and simplified, such that there is no end-on-end articulation, only the side-by-side arrangement of the proximal radials. This structure allowed researchers to determine which aspects of development are uniquely limb and which features are common across teleost and tetrapod fins and limbs.

Study co-author Katrin Henke, Boston Children's Hospital, performed forward genetic screens to mutate DNA at random and identify genes that control the formation of the fin skeleton. When a mutation caused interesting changes to the zebrafish skeleton, researchers then worked backwards to genetically map the mutation and determine which genes were affected. In this case, they discovered that mutations in the waslb and vav2 genes cause the fin phenotype. This was a surprising finding as these genes have not previously been known to play roles in patterning the body.

"It was a big question as to how waslb and vav2 were changing fin patterning," said Hawkins. "These genes were not known to interact with any of the very well characterized pathways that guide limb development. However, we found that these mutations cause an increase in the expression of the gene hoxa11b. This gene is very exciting because the Hox genes are in part responsible for patterning the vertebral column as well as the regions of the limb. And the Hox11 genes in particular are required to make the forearm."

The researchers used an advanced CRISPR knock-in approach and inserted a marker into the genome that shows where a particular gene is active. They replaced the hoxa11b gene with a red fluorescent protein, and cells that express hoxa11b glow red. This tool allowed them to determine that the mutants increase the expression of hoxa11b to form the new bones.

Histological analysis revealed that the new bones had muscle attachment, which occurs extensively in limb bones, but not in the fin. In teleost fishes there are no muscles attached to the bones. Instead, the bones provide an intermediate support in the fin and the muscles extend directly from the shoulder out to the bony fin rays, bypassing the bones all together. The new bones are fully integrated into the fin, complete with joints for articulation and attachment to the fin muscles.

Much work has been done in the field of limb development and it provides a good understanding of what genes are present and required to make the limb form. In this study however, researchers flipped traditional approaches by focusing on the small simple zebrafish fin and asking what genetic changes could elaborate the appendage and increase its complexity. "Prior to this there aren't any examples where we have genes or mutations that actually elaborate the structure and make it even more complicated," said Hawkins. "Even in the case of limbs we only know how to make a limb smaller or less complex, but we didn't have any information on how add elements to a fin or a limb."

"That was very surprising as well," confirmed Harris. "We had no hard experimental examples where you take a gene, turn it up, make it work more and get a more complex mature structure at the end. In our findings we actually found some of the dials that can turn up the genetic pathways and get a more complicated structure in the end."

Previously, other researchers removed the Hox11 genes in mice and found this prevented the radius and ulna from fully forming. "Given that both the new bones in our mutant and the forearm of the limb are located in the middle part of the appendage, our result suggests that fins and limbs both use Hox11 cues to specify this region," stated Hawkins.

This finding also fits well with another recent discovery that Hox13 genes are required to form the distal regions of both fins and limbs. Altogether these discoveries reveal that the appendage Hox code was likely present in the common ancestor of tetrapods and teleost fishes and is not specific to the tetrapod lineage.

This study shows that both fins and limbs use the same genetic mechanisms to specify the middle portion of the appendage. Going forward, Hawkins hopes to explore the question, do fins and limbs make other appendage regions in the same way?

"There's still the humerus in the limb and we don't know what the corresponding part in the fin is for that, in terms of the genetic requirements," said Hawkins. "We know there are proximal cues that determine where the humerus should go, but we don't know if the fish use these cues or not. We want to know in a developmental sense how those cues arose in limbs and if those cues are present in some form in the fish fin already. And hopefully we can fill out the correspondence between fins and limbs."

More information: *Cell*, Hawkins et al.: "Latent developmental potential to form limb-like skeletal structures in zebrafish" www.cell.com/cell/fulltext/S0092-8674(21)00003-9 , DOI: 10.1016/j.cell.2021.01.003

Provided by Harvard University