of the paper[1]). Hydrolysis of ATP molecules by the ATPases could subsequently destabilize the associated splicing factors, allowing the RNA structures in the catalytic core to be remodelled.

The two substrates for the first catalytic step are the 5′SS and an adenosine nucleotide, known as the branch site, within the intron. Although it has long been thought that these two substrates almost certainly interact with each other, to help bring them together as needed for the catalytic step, neither evidence nor models for such an interaction existed. Galej and co-workers' structure reveals intimate interactions between the 5′SS (specifically, its GU sequence, which consists of a guanosine nucleotide next to a uridine nucleotide) and the sequence flanking the branch site; these interactions help to explain the evolutionary conservation of the two sequences. For example, an RNA base triple (a structure analogous to a base pair, but involving three bases) was identified between the uridine of the 5′SS-GU and the helix created by base pairing between the intron sequence flanking the branch site and U2, one of the small nuclear RNAs that forms the spliceosome's active site. This base triple helps to position the 5′SS near the branch-site adenosine, as required for the first catalytic step.

By contrast, in Shi and colleagues' structure[8], the 5′SS and branch site are separated by a large distance (approximately 49 Å). The guanosine of the 5′SS-GU is protected by a pocket formed by a protein subunit of the spliceosome and a first-step splicing factor. Analogously, the branch-site adenosine is positioned in a positively charged pocket of another protein subunit (SF3B1, which is highly mutated in human cancers[10]). These two pockets protect the reactive groups involved in the first catalytic step until the spliceosome has transitioned to a catalytically active conformation.

Galej and colleagues' structure also helps to explain the evolutionary sequence conservation of the branch site–U2 duplex by revealing another base triple interaction between the branch-site adenosine and the intron–U2 RNA helix two nucleotides away. This was presaged in part by interactions observed between the branch-site adenosine and the intron–U2 RNA helix in an RNA-only structure[11] previously determined by nuclear magnetic resonance spectroscopy. This base triple positions the reactive hydroxyl group of the branch-site adenosine outward towards the 5′SS.

The structural insights obtained through the identification of hundreds of RNA–protein and protein–protein interactions in the new structures[1,8,9] suggest innumerable biochemical and genetic experiments to ascertain which splicing step these interactions contribute most to, and for what intron features they are most important. The stage is now set for the exploration and discovery of many other spliceosome structures. Like the explosion of successes that followed the determination of the ribosome structure[12] (the protein-synthesis apparatus), we eagerly await structures not just for normal spliceosome complexes, but also for complexes that include mutations in pre-mRNA substrates or in spliceosomal components, such as those found in many cancers[10]. The future will allow a more comprehensive picture of the basic mechanisms of splicing catalysis, and of how splice sites are recognized and catalysis is regulated. Other achievements may also include the determination of features vital to the alternative splicing regulation found in complex organisms. ■

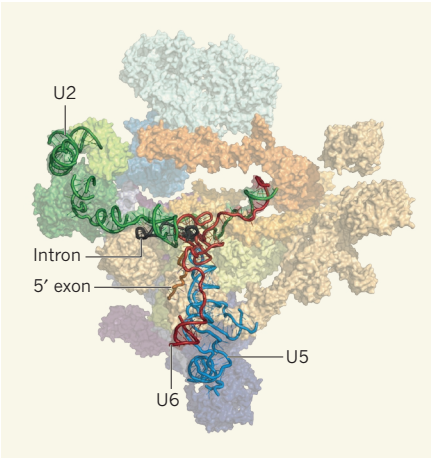

Figure 2 | Model of the catalytically active spliceosome structure. Galej *et al.*[1] report the structure of the spliceosome in complex with an RNA substrate immediately after the first catalytic step of splicing. Most of the spliceosome complex is shown as a fainter surface representation (different colours represent different components). The three small nuclear RNAs (U2, U5 and U6) that form the active site are shown in bold, as are the intron and 5′ exon of the RNA substrate.

Brian Kosmyna *and* **Charles C. Query** *are in the Department of Cell Biology, Albert Einstein College of Medicine, New York, New York 10461-1975, USA.*
e-mails: brian.kosmyna@phd.einstein.yu.edu; charles.query@einstein.yu.edu

1. Galej, W. P. *et al. Nature* **537,** 197–201 (2016).
2. Fica, S. M. *et al. Nature* **503,** 229–234 (2013).
3. Nogales, E. *Nature Methods* **13,** 24-27 (2016).
4. Agafonov, D. E. *et al. Science* **351,** 1416–1420 (2016).
5. Nguyen, T. H. *et al. Nature* **530,** 298–302 (2016).
6. Wan, R. *et al. Science* **351,** 466–475 (2016).
7. Yan, C. *et al. Science* **349,** 1182–1191 (2015).
8. Yan, C., Wan, R., Bai, R., Huang, G. & Shi, Y. *Science* http://dx.doi/org/10.1126/science.aag0291 (2016).
9. Wan, R., Yan, C., Bai, R., Huang, G. & Shi, Y. *Science* http://dx.doi.org/10.1126/science.aag2235 (2016).
10. Yoshida, K. *et al. Nature* **478,** 64–69 (2011).
11. Newby, M. I. & Greenbaum, N. L. *Nature Struct. Mol. Biol.* **9,** 958–965 (2002).
12. Schmeing, T. M. & Ramakrishnan, V. *Nature* **461,** 1234–1242 (2009).

This article was published online on 10 August 2016.

EVOLUTIONARY BIOLOGY

Fin to limb within our grasp

There was thought to be little in common between fish fin bones and the finger bones of land-dwellers. But zebrafish studies reveal that *hox* genes have a surprisingly similar role in patterning the two structures. SEE LETTER P.225

ADITYA SAXENA & KIMBERLY L. COOPER

The next time you gaze at fish in an aquarium, or order a whole trout at your favourite restaurant, you may wish to ponder how the dozens of thin, delicate bones in the fish pectoral fins that lie just behind the gills compare with your own fingers. Although scientists have long known that the human arm evolved from the pectoral fin of our fish ancestors, the relationship between the bones of the two strikingly different skeletons has remained mysterious. Nakamura *et al.*[1] address this issue on page 225 and provide evidence that fish fin-ray bones and human fingers have more in common than was previously thought.

There are two types of bone, and they form in different ways. Most of the bones in our skeleton, including our limbs, start out in the embryo as rod-shaped pieces of cartilage that build a mineralized scaffold on which the bone grows, in a process known as ossification. Bone that develops using a cartilage template is called endochondral bone and includes the short, broad radial fin bones in fish.

The other type of bone is dermal bone, which is found in human shoulder blades and in the plate-like bones that form the roof of our skulls. Dermal-bone formation does not use a cartilage scaffold, but instead proceeds by depositing bone material directly on the innermost layer of skin, the dermis. Although the fin rays of fish and the bones of our fingers may seem superficially similar because they

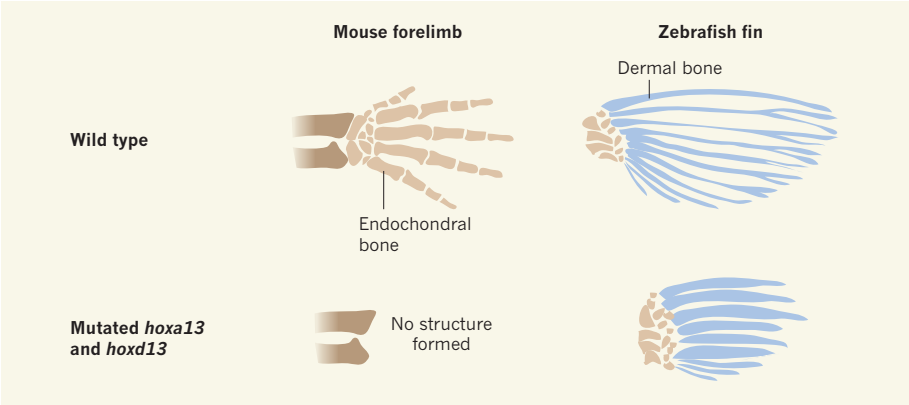

Figure 1 | Fin and limb development. Fin rays and mouse digits are formed from different types of bone — mouse digits are made of endochondral bone and fish fins are made of dermal bone. The genes *hoxa13* and *hoxd13* are expressed in cells that will become the mouse digits, and mice with mutations in these two genes do not form wrist or digit structures in their forelimb[9]. Nakamura *et al.*[1] assessed the effect of loss-of-function mutations of *hoxa13* and *hoxd13* in zebrafish, and found that the mutant fish fins had dermal-bone structures that were reduced in length and had extra endochondral-bone structures, indicating that these *hox13* genes are required for both tetrapod digits and fish fin rays.

are both rod-like structures oriented away from the body, fish fin rays are dermal bones, whereas our fingers are endochondral bones.

It has long been thought[2] that the digits of our earliest four-legged (tetrapod) vertebrate ancestors were a structural innovation when they first appeared in aquatic species, and that fin rays were lost. Digits form at the end of a limb skeleton that has three segments: the upper arm, the lower arm, and the wrist and finger area (also known as the autopod). Formation of these segments in the developing embryo depends on the function of a few key members of the large group of Hox-family transcription-factor proteins[3].

In tetrapods, regions of *hox* gene expression shift in space and time from an early pattern of nested areas across the anterior–posterior axis of the developing limb to a late pattern that is characterized by restriction of *hoxa13* and *hoxd13* expression to the autopod region[4,5]. Zebrafish also express *hox* genes in the cells that will form the endochondral skeleton[2,6]. However, in transgenic mouse embryos, none of the identified regulatory DNA sequences of the zebrafish *hox* genes seem to be active in the region where digits will form[7,8]. This had led researchers to think that the wrist and digits were a tetrapod innovation that arose as a result of a newly acquired region of *hox* expression.

The zebrafish is the most commonly used model fish, for which well-established genetic approaches and laboratory techniques are available. However, among the fishes, zebrafish are said to be highly derived because they have evolved many traits that are not thought to have been present in ancestral species. The spotted gar fish and zebrafish share a common ancestor with tetrapods, but, in some ways, the spotted gar has changed less than the zebrafish in comparison with their ancestor. An enhancer DNA sequence for *hoxA* from the spotted-gar genome can promote a late phase of gene expression both in the digit-forming region of developing mouse limbs and, surprisingly, in the distal fin of zebrafish[8]. Fish don't have fingers, so what do these cells become in the zebrafish that can respond to the same regulatory sequence that is active in developing mouse digits?

To answer this question, Nakamura and colleagues used the spotted-gar *hoxA* enhancer DNA sequences to develop a genetic marker system with which to trace the development of the population of cells near the tip of the zebrafish fin that respond to the enhancer. The authors found that these cells go on to contribute exclusively to the dermal skeleton of the fin rays. Although this is not evidence that fin rays and mouse digits are the same, or even that tetrapod digits evolved from the rays of fish, it does show that there is much more similarity between the structures than was previously thought. This further supports the hypothesis that autopod evolution may have occurred by the hijacking of some of the developmental processes that were already shaping the fins of our ancestors.

The *hoxa13* and *hoxd13* genes are more than mere identifiers of the developing tetrapod digits; they are also essential for autopod development, and mice that lack the two proteins encoded by these genes do not form autopods[9]. However, testing the role of these genes in zebrafish has been difficult because the species has undergone full-genome duplication, and so there are multiple copies of many genes. This can hinder loss-of-function studies using conventional mutation and breeding approaches, and the effect of loss of function of *hoxa13* and *hoxd13* on the zebrafish fin was not known.

To study loss of function of *hoxa13* and *hoxd13* in zebrafish, Nakamura and colleagues used CRISPR–Cas9 genome-editing technology, which offers a fast and specific way to create mutations both in the *hoxa13* duplicate genes (*hoxa13a* and *hoxa13b*) and in the single copy of *hoxd13*. The resulting mutant fish have a dermal-fin-ray skeleton that is dramatically reduced in length, together with an increased number of distal endochondral radial bones (Fig. 1).

This result is interesting because it is a transformation of the fish fin that is in some ways similar to what is expected to have occurred in the earliest tetrapods that lost their dermal-fin-ray skeleton and elaborated an endochondral skeleton to include true digits. Tetrapod endochondral digits were previously thought[10] to be homologous with the distal row of fish endochondral radial bones that are adjacent to the dermal-fin rays. However, the loss of rays and gain of true digits are thought[2] to be the result of further elaboration, not loss, of the late phase of *hox13* expression in tetrapods.

Some caution should be taken in the interpretation of these data. Because zebrafish are highly derived compared with more-basal fishes, it is possible that the role of *hox13* transcription factors in the development of fin rays is a recent zebrafish acquisition. It will be important, where possible, to perform some of the same fate-mapping and gene loss-of-function experiments in fish species, such as the paddlefish and gar, that diverged closer to the shared ancestor with tetrapods and that have fin skeletons with more similarities to ancestral tetrapods. Fortunately, these exciting questions are emerging just as CRISPR–Cas9 genome-editing technologies are becoming options for a variety of unusual model species. The answers may soon be within our grasp. ■

Aditya Saxena *and* **Kimberly L. Cooper** *are in the Division of Biological Sciences, University of California, San Diego, La Jolla, California 92093, USA.*
e-mails: adsaxena@ucsd.edu; kcooper@ucsd.edu

1. Nakamura, T., Gehrke, A. R., Lemberg, J., Szymaszek, J. & Shubin, N. H. *Nature* **537,** 225–228 (2016).
2. Sordino, P., van der Hoeven, F. & Duboule, D. *Nature* **375,** 678–681 (1995).
3. Zakany, J. & Duboule D. *Curr. Opin. Genet. Dev.* **17,** 359–366 (2007).
4. Dollé, P., Izpisúa-Belmonte, J. C., Falkenstein, H., Renucci, A. & Duboule, D. *Nature* **342,** 767–772 (1989).
5. Nelson, C. E. *et al. Development* **122,** 1449–1466 (1996).
6. Ahn, D. & Ho, R. K. *Dev. Biol.* **322,** 220–233 (2008).
7. Woltering, J. M., Noordermeer, D., Leleu, M. & Duboule, D. *PLoS Biol.* **12,** e1001773 (2014).
8. Gehrke, A. R. *et al. Proc. Natl Acad. Sci. USA* **112,** 803–808 (2015).
9. Fromental-Ramain, C. *et al. Development* **122,** 2997–3011 (1996).
10. Boisvert, C. A., Mark-Kurik & Ahlberg P. E. *Nature* **456,** 636–638 (2008).

This article was published online on 17 August 2016.