CHAPTER THREE

HANDY GENES

While my colleagues and I were digging up the first *Tiktaalik* in the Arctic in July 2004, Randy Dahn, a researcher in my laboratory, was sweating it out on the South Side of Chicago doing genetic experiments on the embryos of sharks and skates, cousins of stingrays. You've probably seen small black egg cases, known as mermaid's purses, on the beach. Inside the purse once lay an egg with yolk, which developed into an embryonic skate or ray. Over the years, Randy has spent hundreds of hours experimenting with the embryos inside these egg cases, often working well past midnight. During the fateful summer of 2004, Randy was taking these cases and injecting a molecular version of vitamin A into the eggs. After that he would let the eggs develop for several months until they hatched.

His experiments may seem to be a bizarre way to spend the better part of a year, let alone for a young scientist to launch a promising scientific career. Why sharks? Why a form of vitamin A?

To make sense of these experiments, we need to step back and look at what we hope they might explain. What we are really getting at in this chapter is the recipe, written in our DNA, that builds our bodies from a single egg. When sperm fertilizes an egg, that fertilized egg does not contain a tiny hand, for instance. The hand is built from the information contained in that single cell. This takes us to a very profound problem. It is one thing to compare the bones of our hands with the bones in fish fins. What happens if you compare the genetic recipe that builds our hands with the recipe that builds a fish's fin? To find answers to this question, just like Randy, we will follow a trail of discovery that takes us from our hands to the fins of sharks and even to the wings of flies.

As we've seen, when we discover creatures that reveal different and often simpler versions of our bodies inside their own, a wonderfully direct window opens into the distant past. But there is a big limitation to working with fossils. We cannot do experiments on long-dead animals. Experiments are great because we can actually manipulate something to see the results. For this reason, my laboratory is split directly in two: half is devoted to fossils, the other half to embryos and DNA. Life in my lab can be schizophrenic. The locked cabinet that holds *Tiktaalik* specimens is adjacent to the freezer containing our precious DNA samples.

Experiments with DNA have enormous potential to reveal inner fish. What if you could do an experiment in

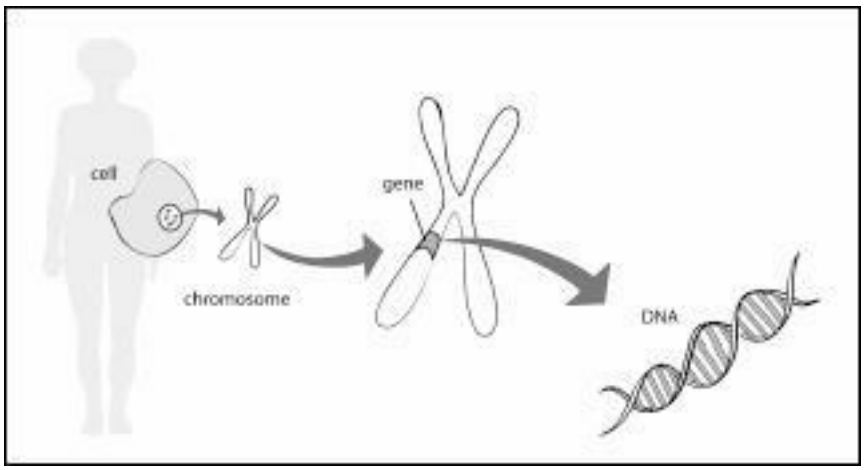
which you treated the embryo of a fish with various chemicals and actually changed its body, making part of its fin look like a hand? What if you could show that the genes that build a fish's fin are virtually the same as those that build our hands?

We begin with an apparent puzzle. Our body is made up of hundreds of different kinds of cells. This cellular diversity gives our tissues and organs their distinct shapes and functions. The cells that make our bones, nerves, guts, and so on look and behave entirely differently. Despite these differences, there is a deep similarity among every cell inside our bodies: all of them contain exactly the same DNA. If DNA contains the information to build our bodies, tissues, and organs, how is it that cells as different as those found in muscle, nerve, and bone contain the same DNA?

The answer lies in understanding what pieces of DNA (the genes) are actually turned on in every cell. A skin cell is different from a neuron because different genes are active in each cell. When a gene is turned on, it makes a protein that can affect what the cell looks like and how it behaves. Therefore, to understand what makes a cell in the eye different from a cell in the bones of the hand, we need to know about the genetic switches that control the activity of genes in each cell and tissue.

Here's the important fact: these genetic switches help to assemble us. At conception, we start as a single cell that contains all the DNA needed to build our body. The plan for that entire body unfolds via the instructions contained in

this single microscopic cell. To go from this generalized egg cell to a complete human, with trillions of specialized cells organized in just the right way, whole batteries of genes need to be turned on and off at just the right stages of development. Like a concerto composed of individual notes played by many instruments, our bodies are a composition of individual genes turning on and off inside each cell during our development.



Genes are stretches of DNA contained in every cell of our bodies.

This information is a boon to those who work to understand bodies, because we can now compare the activity of different genes to assess what kinds of changes are involved in the origin of new organs. Take limbs, for example. When we compare the ensemble of genes active in the development of a fish fin to those active in the development of a human hand, we can catalogue the genetic differences between fins and limbs. This kind of comparison gives us some likely culprits—the genetic

switches that may have changed during the origin of limbs. We can then study what these genes are doing in the embryo and how they might have changed. We can even do experiments in which we manipulate the genes to see how bodies actually change in response to different conditions or stimuli.

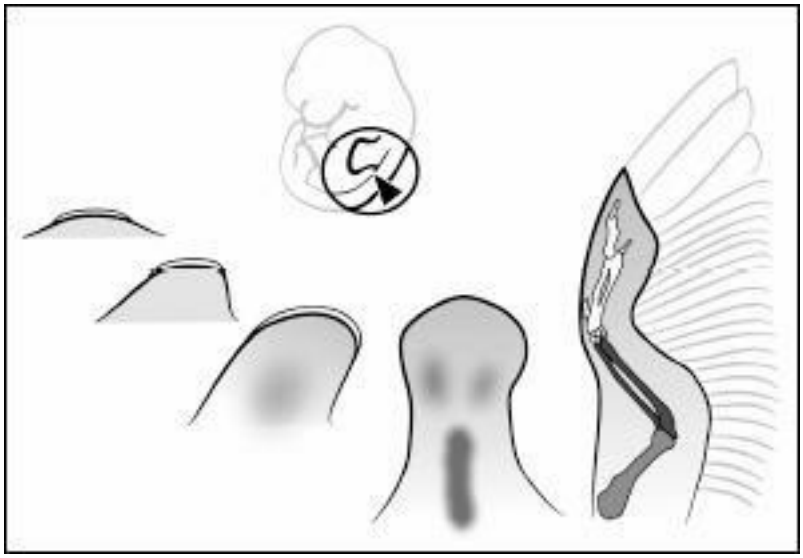
To see the genes that build our hands and feet, we need to take a page from a script for the TV show *CSI: Crime Scene Investigation*—start at the body and work our way in. We will begin by looking at the structure of our limbs, and zoom all the way down to the tissues, cells, and genes that make it.

MAKING HANDS

Our limbs exist in three dimensions: they have a top and a bottom, a pinky side and a thumb side, a base and a tip. The bones at the tips, in our fingers, are different from the bones at the shoulder. Likewise, our hands are different from one side to the other. Our pinkies are shaped differently from our thumbs. The Holy Grail of our developmental research is to understand what genes differentiate the various bones of our limb, and what controls development in these three dimensions. What DNA actually makes a pinky different from a thumb? What makes our fingers distinct from our arm bones? If we can understand the genes that control such patterns, we will be

privy to the recipe that builds us.

All the genetic switches that make fingers, arm bones, and toes do their thing during the third to eighth week after conception. Limbs begin their development as tiny buds that extend from our embryonic bodies. The buds grow over two weeks, until the tip forms a little paddle. Inside this paddle are millions of cells which will ultimately give rise to the skeleton, nerves, and muscles that we'll have for the rest of our lives.



The development of a limb, in this case a chicken wing. All of the key stages in the development of a wing skeleton happen inside the egg.

To study how this pattern emerges, we need to look at embryos and sometimes interfere with their development to assess what happens when things go wrong. Moreover, we need to look at mutants and at their internal structures and genes, often by making whole mutant populations

through careful breeding. Obviously, we cannot study humans in these ways. The challenge for the pioneers in this field was to find the animals that could be useful windows into our own development. The first experimental embryologists interested in limbs in the 1930s and 1940s faced several problems. They needed an organism in which the limbs were accessible for observation and experiment. The embryo had to be relatively large, so that they could perform surgical procedures on it. Importantly, the embryo had to grow in a protected place, in a container that sheltered it from jostling and other environmental disturbances. Also, and critically, the embryos had to be abundant and available year-round. The obvious solution to this scientific need is at your local grocery store: chicken eggs.

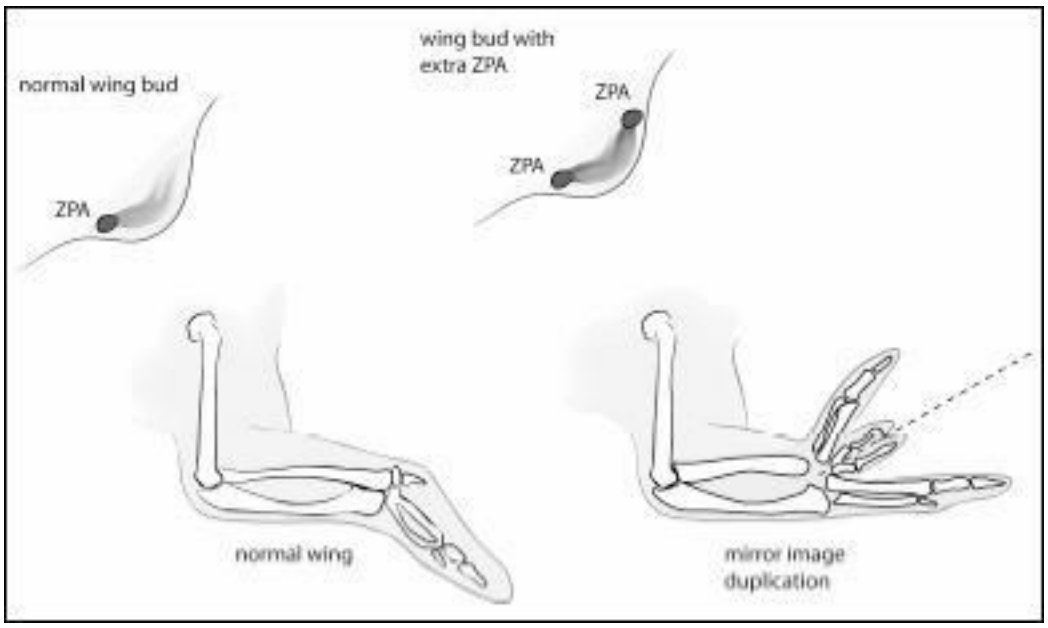
In the 1950s and 1960s a number of biologists, including Edgar Zwilling and John Saunders, did extraordinarily creative experiments on chicken eggs to understand how the pattern of the skeleton forms. This was an era of slice and dice. Embryos were cut up and various tissues moved about to see what effect this had on development. The approach involved very careful microsurgery, manipulating patches of tissue no more than a millimeter thick. In that way, by moving tissues about in the developing limb, Saunders and Zwilling uncovered some of the key mechanisms that build limbs as different as bird wings, whale flippers, and human hands.

They discovered that two little patches of tissue

essentially control the development of the pattern of bones inside limbs. A strip of tissue at the extreme end of the limb bud is essential for *all* limb development. Remove it, and development stops. Remove it early, and we are left with only an upper arm, or a piece of an arm. Remove it slightly later, and we end up with an upper arm and a forearm. Remove it even later, and the arm is almost complete, except that the digits are short and deformed.

Another experiment, initially done by Mary Gasseling in John Saunders's laboratory, led to a powerful new line of research. Take a little patch of tissue from what will become the pinky side of a limb bud, early in development, and transplant it on the opposite side, just under where the first finger will form. Let the chick develop and form a wing. The result surprised nearly everybody. The wing developed normally except that it also had a *full duplicate set of digits.* Even more remarkable was the pattern of the digits: the new fingers were mirror images of the normal set. Obviously, something inside that patch of tissue, some molecule or gene, was able to direct the development of the pattern of the fingers. This result spawned a blizzard of new experiments, and we learned that this effect can be mimicked by a variety of other means. For example, take a chicken embryo and dab a little vitamin A on its limb bud, or simply inject vitamin A into the egg, and let the embryo develop. If you supply the vitamin A at the right concentration and at the right stage, you'll get the same mirror-image duplication that Gasseling, Saunders, and

Zwilling got from the grafting experiments. This patch of tissue was named the zone of polarizing activity (ZPA). Essentially, the ZPA is a patch of tissue that causes the pinky side to be different from the thumb side. Obviously chicks do not have a pinky and a thumb. The terminology we use is to number the digits, with our pinky corresponding to digit five of other animals and our thumb corresponding to digit one.



Moving a little patch of tissue called the ZPA causes the fingers to be duplicated.

The ZPA drew interest because it appeared, in some way, to control the formation of fingers and toes. But how? Some people believed that the cells in the ZPA made a molecule that then spread across the limb to instruct cells to make different fingers. The key proposal was that it was the concentration of this unnamed molecule that was the

important factor. In areas close to the ZPA, where there is a high concentration of this molecule, cells would respond by making a pinky. In the opposite side of the developing hand, farther from the ZPA so that the molecule was more diffused, the cells would respond by making a thumb. Cells in the middle would each respond according to the concentration of this molecule to make the second, third, and fourth fingers.

This concentration-dependent idea could be tested. In 1979, Denis Summerbell placed an extremely small piece of foil between the ZPA patch and the rest of the limb. The idea was to use this barrier to prevent any kind of molecule from diffusing from the ZPA to the other side. Summerbell studied what happened to the cells on each side of the barrier. Cells on the ZPA side formed digits. Cells on the opposite side often did not form digits; if they did, the digits were badly malformed. The conclusion was obvious. Something was emanating from the ZPA that controlled how the digits formed and what they looked like. To identify that something, researchers needed to look at DNA.

THE DNA RECIPE

That project was left to a new generation of scientists. Not until the 1990s, when new molecular techniques became available, was the genetic control for the ZPA's operation unraveled.

A major breakthrough happened in 1993, when Cliff Tabin's laboratory at Harvard started hunting for the genes that control the ZPA. Their prey was the molecular mechanisms that gave the ZPA its ability to make our pinky different from our thumb. By the time his group started to work in the early 1990s, a number of experiments like the ones I've described had led us to believe that some sort of molecule caused the whole thing. This was a grand theory, but nobody knew what this molecule was. People would propose one molecule after another, only to find that none was up to the job. Finally, the Tabin lab came up with a novel notion, and one very relevant to the theme of this book. Look to flies for the answer.

Genetic experiments in the 1980s had revealed the wonderful pattern of gene activity that sculpts the body of a fly from a single-celled egg. The body of a fruit fly is organized from front to back, with the head at the front and the wings at the back. Whole batteries of genes are turned on and off during fly development, and this pattern of gene activity serves to demarcate the different regions of the fly.

Tabin didn't know it at the time, but two other laboratories—those of Andy MacMahon and Phil Ingham—had already come up with the same general idea independently. What emerged was a remarkably successful collaboration among three different lab groups. One of the fly genes caught the attention of Tabin, McMahon, and Ingham. They noted that this gene made one end of a body segment look different from the other. Fly geneticists

named it *hedgehog*. Doesn't the function of *hedgehog* in the fly body—to make one region different from another—sound like what the ZPA does in making the pinky different from the thumb? That parallel was not lost on the three labs. So off they went, looking for a *hedgehog* gene in creatures like chickens, mice, and fish.

Because the lab groups knew the structure of the fly's *hedgehog* gene, they had a search image to help them single out the gene in chickens. Each gene has a distinctive sequence; using a number of molecular tools, the researchers could scan the chicken's DNA for the *hedgehog* sequence. After a lot of trial and error, they found a chicken *hedgehog* gene.

Just as paleontologists get to name new species, geneticists get to name new genes. The fly geneticists who discovered *hedgehog* had named it that because the flies with a mutation in the gene had bristles that reminded them of a little hedgehog. Tabin, McMahon, and Ingham named the chicken version of the gene *Sonic hedgehog,* after the Sega Genesis video game.

Now came the fun question: What does *Sonic hedgehog* actually do in the limb? The Tabin group attached a dye to a molecule that would stick to the gene, enabling them to visualize where the gene is active in the limb. To their great surprise, they found that only cells in a tiny patch of the limb had gene activity: the ZPA.

So the next steps became obvious. The patterns of activity in the *Sonic hedgehog* gene should mimic those of

the ZPA tissue itself. Recall that when you treat the limb with retinoic acid, a form of vitamin A, you get a ZPA active on the opposite side. Guess what happens when you treat a limb with retinoic acid, then map where *Sonic hedgehog* is active? *Sonic hedgehog* becomes active on both sides—pinky and thumb—just as the ZPA does when it is treated with retinoic acid.

Knowing the structure of the chicken *Sonic hedgehog* gave other researchers the tools to look for it in everything else that has fingers, from frogs to humans. Every limbed animal has the *Sonic hedgehog* gene. And in every single animal that we have studied, *Sonic hedgehog* is active in the ZPA tissue. If *Sonic hedgehog* hadn't turned on properly during the eighth week of your own development, then you either would have extra fingers or your pinky and thumb would look alike. Occasionally, when things go wrong with *Sonic hedgehog,* the hand ends up looking like a broad paddle with as many as twelve fingers that all look alike.

We now know that *Sonic hedgehog* is one of dozens of genes that act to sculpt our limbs from shoulder to fingertip by turning on and off at the right time. Remarkably, work in chickens, frogs, and mice was telling us the same thing. The DNA recipe to build upper arms, forearms, wrists, and digits is virtually identical in every creature that has limbs.

How far back can we trace *Sonic hedgehog* and the other bits of DNA that build limbs? Is this stuff active in building the skeleton of fish fins? Or are hands genetically completely different from fish fins? We saw an inner fish in

the anatomy of our arms and hands. What about the DNA that builds it?

Enter Randy Dahn with his mermaid's purses.

GIVING SHARKS A HAND

Randy Dahn entered my laboratory with a simple but very elegant idea: treat skate embryos just the way Cliff Tabin treated chicken eggs. Randy's goal was to perform all the experiments on skates that chicken biologists had performed on chicken eggs, from Saunders and Zwilling's tissue surgeries all the way to Cliff Tabin's gene experiments. Skates develop in an egg with a kind of shell and a yolk. Skates even have big embryos, just as chickens do. Because of these convenient facts, we could apply to skates many of the genetic and experimental tools people had developed to understand chickens.

What could we learn by comparing the development of a shark fin to that of a chicken leg? Even more relevant, what could we learn about ourselves from all this?

Chickens, as Saunders, Zwilling, and Tabin showed, are a surprisingly good proxy for our own limbs. Everything that was discovered by Saunders and Zwilling's cutting and grafting experiments and by Tabin's DNA work applies to our own limbs as well: we have a ZPA, we have *Sonic hedgehog,* and both have a great bearing on our well-being. As we saw, a malfunctioning ZPA or a mutation in *Sonic*

hedgehog can cause major malformations in human hands.

Randy wanted to determine how different the apparatus is that builds our hands. How deep is our connection to the rest of life? Is the recipe that builds our hands new, or does it, too, have deep roots in other creatures? If so, how deep?

Sharks and their relatives are the earliest creatures that have fins with a skeleton inside. Ideally, to answer Randy's question, you would want to bring a 400-million-year-old shark fossil into the laboratory, grind it up, and look at its genetic structure. Then you'd try to manipulate its fossil embryos to learn whether *Sonic hedgehog* is active in the same general place as in our limbs today. This would be a wonderful experiment, but it is impossible. We cannot extract DNA from fossils so old, and, even if we could, we could never find embryos of those fossil animals on which to do experiments.

Living sharks and their relatives are the next best thing. Nobody would ever confuse a shark fin with a human hand: you couldn't ask for two more different kinds of appendages. Not only are sharks and humans very distantly related, but also the skeletal structures of their appendages look nothing alike. Nothing even remotely similar to Owen's one bone–two bones–lotsa blobs–digits pattern is inside a shark's fin. Instead, the bones inside are shaped like rods, long and short, thin and wide. We call them bones even though they are made of cartilage (sharks and skates are known as cartilaginous fish, because their skeletons never turn into hard bone). If you want to assess whether *Sonic*

hedgehog's role in limbs is unique to limbed animals, why not choose a species utterly different in almost every way? In addition, why not choose the species that is the most primitive living fish with any kind of paired appendage, whether fin or limb? Sharks fit both bills perfectly.

Our first problem was a simple one. We needed a reliable source for the embryos of sharks and skates. Sharks proved difficult to obtain with any degree of regularity, but skates, their close relatives, were another matter. So we started with sharks and used skates as our supply of sharks dwindled. We found a supplier who would ship us every month or two a batch of twenty or thirty egg cases with embryos inside. We became a virtual cargo cult as we waited each month for our shipment of precious egg cases.

Work by Tabin's group and others gave Randy important clues to begin his search. Since Tabin's work in 1993, people had found *Sonic hedgehog* in a number of different species, everything from fish to humans. With the knowledge of the structure of the gene, Randy was able to search all the DNA of the skate and shark for *Sonic hedgehog*. In a very short time he found it: a shark *Sonic hedgehog* gene.

The key questions to answer were Where is *Sonic hedgehog* active?, and, even more important, What is it doing?

The egg cases were put to use as Randy visualized where and when *Sonic hedgehog* is active in the development of skates. He first studied whether *Sonic hedgehog* turns on at

the same time in skate fin development as it does in chicken limbs. Yes, it does. Then he studied whether it is turned on in the patch of tissue at the back end of the fin, the equivalent of our pinky. Yes again. Now he did his vitamin A experiment. This was the million-dollar moment. If you treat the limb of a chicken or mammal with this compound, you get a patch of tissue that has *Sonic hedgehog* activity on the opposite side, and this result is coupled with a duplication of the bones. Randy injected the egg, waited a day or so, and then checked whether, as in chickens, the vitamin A caused *Sonic hedgehog* to turn on in the opposite side of the limb. It did. Now came the long wait. We knew that *Sonic hedgehog* was behaving the same way in our hands and in skates' and sharks' fins. But what would the effect of all this be on the skeleton? We would have to wait two months for the answer.

The embryos were developing inside an opaque egg case. All we could tell was whether the creature was alive; the inside of the fin was invisible to us.

The end result was a stunning example of similarity among us, sharks, and skates: a mirror-image fin. The dorsal fins duplicated their structures in a wonderful front-to-back pattern, the same kind we saw with experiments in limbs. Limbs duplicate a limb structure. Shark fins duplicate a shark fin structure as do skates. *Sonic hedgehog* has a similar effect in even the most different kinds of appendage skeletons found on earth today.

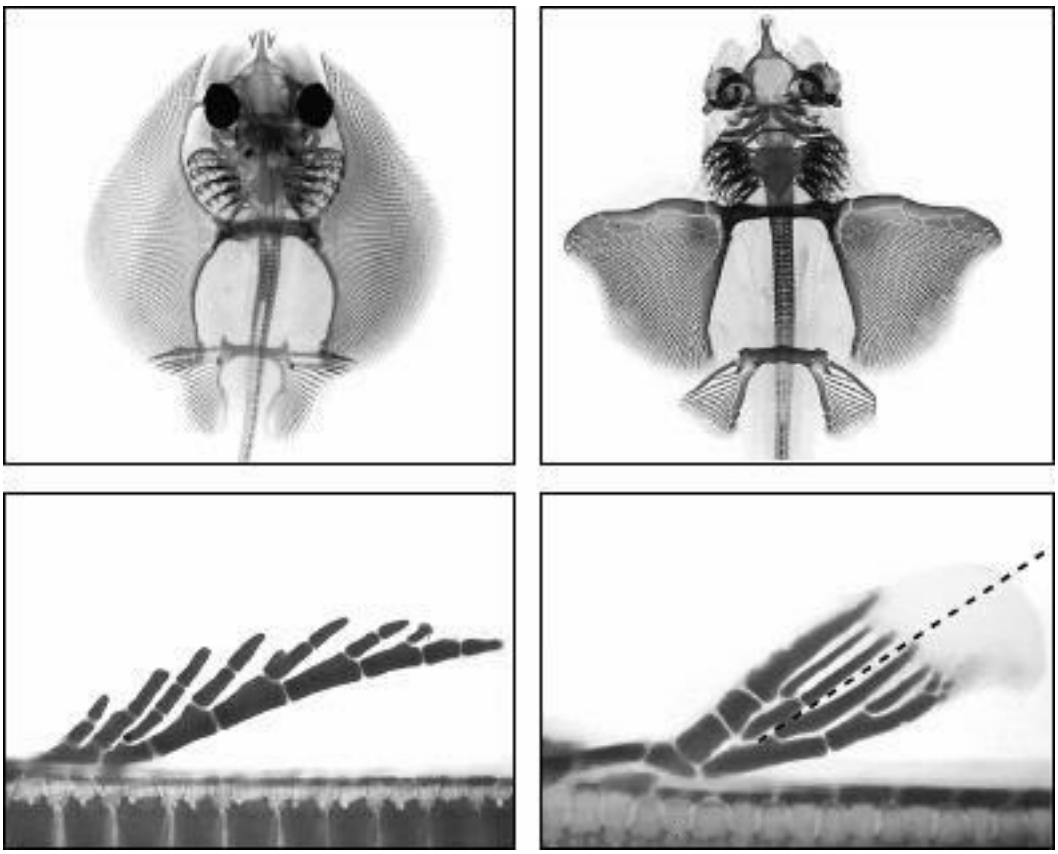
One effect of *Sonic hedgehog,* you may recall, is to make

the fingers distinct from one another. As we saw with respect to the ZPA, what kind of digit develops depends on how close the digit is to the source of *Sonic hedgehog.* A normal adult skate fin contains many skeletal rods, which all look alike. Could we make these rods different from one another, like our digits? Randy took a small bead impregnated with the protein made by *Sonic hedgehog* and put it in between these identical skeletal rods. The key to his experiment is that he used mouse *Sonic hedgehog.* So now we have a real contraption: a skate embryo with a bead inside that is gradually leaking mouse *Sonic hedgehog* protein. Would that mouse protein have any effect on a shark or a skate?

There are two extreme outcomes to an experiment like this. One is that nothing happens. This would mean that skates are so different from mice that *Sonic hedgehog* protein has no effect. The other extreme outcome would present a stunning example of our inner fish. This outcome would be that the rods develop differently from one another, demonstrating that *Sonic hedgehog* does something similar in skates and in us. And let's not forget that since Randy is using the protein from a mammal, it means that the genetic recipe would be really, really similar.

Not only did the rods end up looking different from one another, they responded to *Sonic hedgehog,* much as fingers do, on the basis of how close they were to the *Sonic hedgehog* bead: the closer rods developed a different shape from the ones farther away. To top matters off, it was the

mouse protein that did the job so effectively in the skates.



Normal fins (left) and Randy's treated fins. The treated fins showed a mirror-image duplication just as chicken wings did. Photographs courtesy of Randall Dahn, University of Chicago.

The "inner fish" that Randy found was not a single bone, or even a section of the skeleton. Randy's inner fish lay in the biological tools that actually build fins. Experiment after experiment on creatures as different as mice, sharks, and flies shows us that the lessons of *Sonic hedgehog* are very general. All appendages, whether they are fins or limbs, are built by similar kinds of genes. What does this mean for the problem we looked at in the first two chapters—the

transition of fish fins into limbs? It means that this great evolutionary transformation did not involve the origin of new DNA: much of the shift likely involved using ancient genes, such as those involved in shark fin development, in new ways to make limbs with fingers and toes.

But there is a deeper beauty to these experiments on limbs and fins. Tabin's lab used work in *flies* to find a gene in *chickens* that tells us about *human* birth defects. Randy used the Tabin lab discovery to tell us something about our connections to *skates.* An "inner fly" helped find an "inner chicken," which ultimately helped Randy find an "inner skate." The connections among living creatures run deep.