CHAPTER SIX

THE BEST-LAID (BODY) PLANS

We are a package of about two trillion cells assembled in a very precise way. Our bodies exist in three dimensions, with our cells and organs in their proper places. The head is on top. The spinal cord is toward our back. Our guts are on the belly side. Our arms and legs are to the sides. This basic architecture distinguishes us from primitive creatures organized as clumps or disks of cells.

The same design is also an important part of the bodies of other creatures. Like us, fish, lizards, and cows have bodies that are symmetrical with a front/back, top/bottom, and left/right. Their front ends (corresponding to the top of an upright human) all have heads, with sense organs and brains inside. They have a spinal cord that runs the length of the body along the back. Also like us, they have an anus, which is at the opposite end of their bodies from the mouth. The head is on the forward end, in the direction they typically swim or walk. As you can imagine, "anus-forward" wouldn't work very well in most settings, particularly aquatic ones. Social situations would be a problem, too.

It is more difficult to find our basic design in really primitive animals—jellyfish, for example. Jellyfish have a different kind of body plan: their cells are organized into disks that have a top and bottom. Lacking a front and back, a head and tail, and a left and right, jellyfish body organization appears very different from our own. Don't even bother trying to compare your body plan with a sponge. You could try, but the mere fact that you were trying would reveal something more psychiatric than anatomical.

To properly compare ourselves with these primitive animals, we need some tools. Just as with heads and limbs, our history is written within our development from egg to adult. Embryos hold the clues to some of the profound mysteries of life. They also have the ability to derail my plans.

THE COMMON PLAN: COMPARING EMBRYOS

I entered graduate school to study fossil mammals and ended up three years later studying fish and amphibians for my dissertation. My fall from grace, if you want to call it that, happened when I started to look at embryos. We had a lot of embryos in the lab: salamander larvae, fish embryos, even fertilized chicken eggs. I'd routinely pop them under the microscope to see what was going on. The embryos of all the species looked like little whitish batches of cells, no

more than an eighth of an inch long. It was exciting watching development progress; as the embryo got bigger, the yolk, its food supply, got smaller and smaller. By the time the yolk was gone, the embryo was usually big enough to hatch.

Watching the process of development brought about a huge intellectual transformation in me. From such simple embryonic beginnings—small blobs of cells—came wonderfully complex birds, frogs, and trout comprising trillions of cells arranged in just the right way. But there was more. The fish, amphibian, and chicken embryos were like nothing I had ever seen before in biology. They all looked generally alike. All of them had a head with gill arches. All of them had a little brain that began its development with three swellings. All of them had little limb buds. In fact, the limbs were to become my thesis, the focus of my next three years' work. Here, in comparing how the skeleton developed in birds, salamanders, frogs, and turtles, I was finding that limbs as different as bird wings and frog legs looked very similar during their development. In seeing these embryos, I was seeing a common architecture. The species ended up looking different, but they started from a generally similar place. Looking at embryos, it almost seems that the differences among mammals, birds, amphibians, and fish simply pale in comparison with their fundamental similarities. Then I learned of the work of Karl Ernst von Baer.

In the 1800s, some natural philosophers looked to

embryos to try to find the common plan for life on earth. Paramount among these observers was Karl Ernst von Baer. Born to a noble family, he initially trained to be a physician. His academic mentor suggested that he study chicken development and try to understand how chicken organs developed.

Unfortunately, von Baer could not afford incubators to work on chickens, nor could he afford many eggs. This was not very promising. Lucky for him, he had an affluent friend, Christian Pander, who could afford to do the experiments. As they looked at embryos, they found something fundamental: *all organs in the chicken can be traced to one of three layers of tissue in the developing embryo.* These three layers became known as the germ layers. They achieved almost legendary status, which they retain even to this day.

Pander's three layers gave von Baer the means to ask important questions. Do all animals share this pattern? Are the hearts, lungs, and muscles of all animals derived from these layers? And, importantly, do the same layers develop into the same organs in different species?

Von Baer compared the three layers of Pander's chicken embryos with everything else he could get his hands on: fish, reptiles, and mammals. Yes, every animal organ originated in one of these three layers. Significantly, the three layers formed the same structures in every species. Every heart of every species formed from the same layer. Another layer gave rise to every brain of every animal. And so on. No matter how different the species look as adults, as

tiny embryos they all go through the same stages of development.

To fully appreciate the importance of this, we need to look again at our first three weeks after conception. At the moment of fertilization, major changes happen inside the egg—the genetic material of the sperm and egg fuses and the egg begins to divide. Ultimately, the cells form a ball. In humans, over about five days, the single-cell body divides four times, to produce a ball of sixteen cells. This ball of cells, known as a blastocyst, resembles a fluid-filled balloon. A thin spherical wall of cells surrounds some fluid in the center. At this "blastocyst stage" there still does not appear to be any body plan—there is no front and back, and certainly there are not yet any different organs or tissues. On about the sixth day after conception, the ball of cells attaches to its mother's uterus and begins the process of connecting to it so that mother and embryo can join bloodstreams. There is still no evidence of the body plan. It is a far cry from this ball of cells to anything that you'd recognize as any mammal, reptile, or fish, much less a human.

If we are lucky, our ball of cells has implanted in our mother's uterus. When a blastocyst implants in the wrong place—when there is an "ectopic implantation"—the results can be dangerous. About 96 percent of ectopic implantations happen in the uterine (or fallopian) tubes, near where conception happens. Sometimes mucus blocks the easy passage of the blastocyst to the uterus, causing it

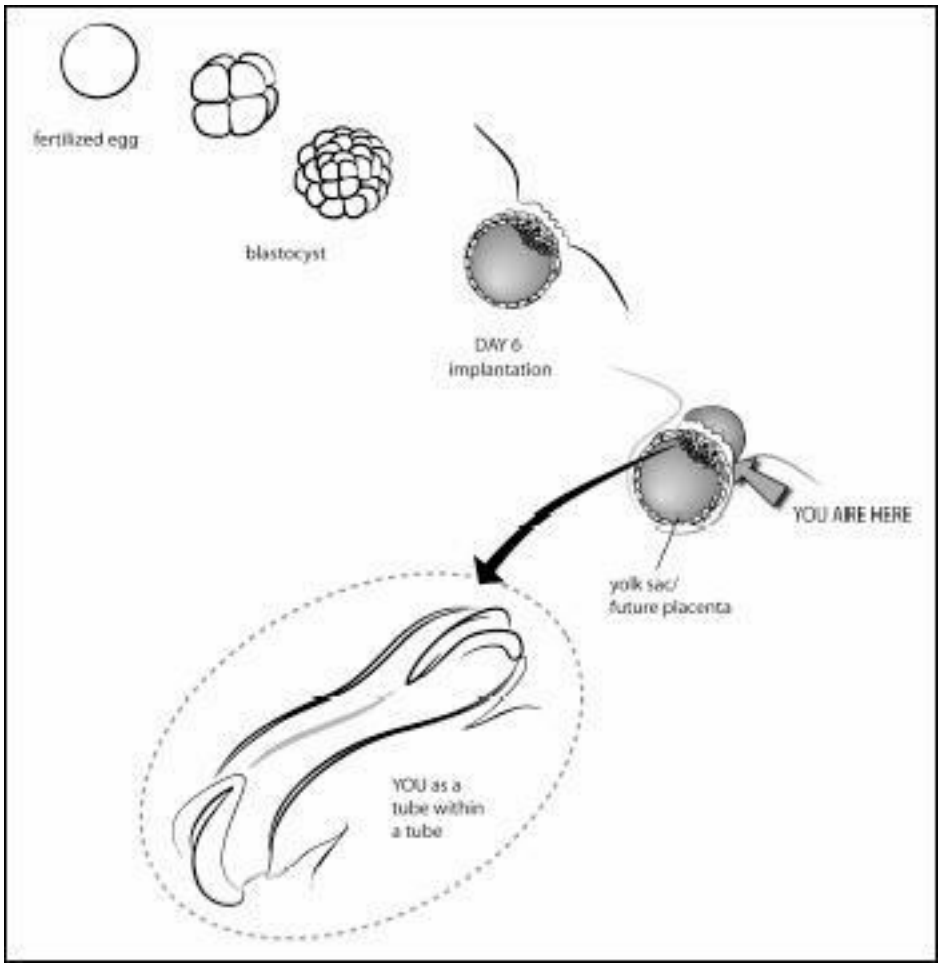
to implant improperly in the tubes. Ectopic pregnancy can cause various tissue ruptures if not caught in time. In really rare cases, the blastocyst is expelled into the mother's body cavity, the space between her guts and body wall. In even rarer cases, these blastocysts will implant on the outside lining of the mother's rectum or uterus and the fetus develops to full term! Although these fetuses can sometimes be delivered by an abdominal incision, such implantation is generally very dangerous because it increases the risk of maternal death by bleeding by a factor of 90, as compared with a normal implantation inside the uterus.

In any event, at this stage of development we are extremely humble-looking creatures. Around the beginning of our second week after conception, the blastocyst has implanted, with one part of the ball embedded in the wall of the uterus, and the other free. Think of a balloon pushed into a wall: this flattened disk becomes the human embryo. Our *entire* body forms from only the top part of this ball, the part that is mushed into the wall. The part of the blastocyst below the disk covers the yolk. At this stage of development, we look like a Frisbee, a simple two-layered disk.

How does this oval Frisbee end up with von Baer's three germ layers and go on to look anything like a human? First, cells divide and move, causing tissues to fold in on themselves. Eventually, as tissues move and fold, we become a tube with a folded swelling at the head end and

another at the tail. If we were to cut ourselves in half right about now, we would find a tube within a tube. The outer tube would be our body wall, the inner tube our eventual digestive tract. A space, the future body cavity, separates the two tubes. This tube-within-a-tube structure stays with us our entire lives. The gut tube gets more complicated, with a big sac for a stomach and long intestinal twists and turns. The outer tube is complicated by hair, skin, ribs, and limbs that push out. But the basic plan persists. We may be more complicated than we were at twenty-one days after conception, but we are still a tube within a tube, and all of our organs derive from one of the three layers of tissue that appeared in our second week after conception.

The names of these three all-important layers are derived from their position: the outer layer is called ectoderm, the inner layer endoderm, and the middle layer mesoderm. Ectodermformsmuch of the outer part of the body (the skin) and the nervous system. Endoderm, the inside layer, forms many of the inner structures of the body, including our digestive tract and numerous glands associated with it. The middle layer, the mesoderm, forms tissue in between the guts and skin, including much of our skeleton and our muscles. Whether the body belongs to a salmon, a chicken, a frog, or a mouse, all of its organs are formed by endoderm, ectoderm, and mesoderm.

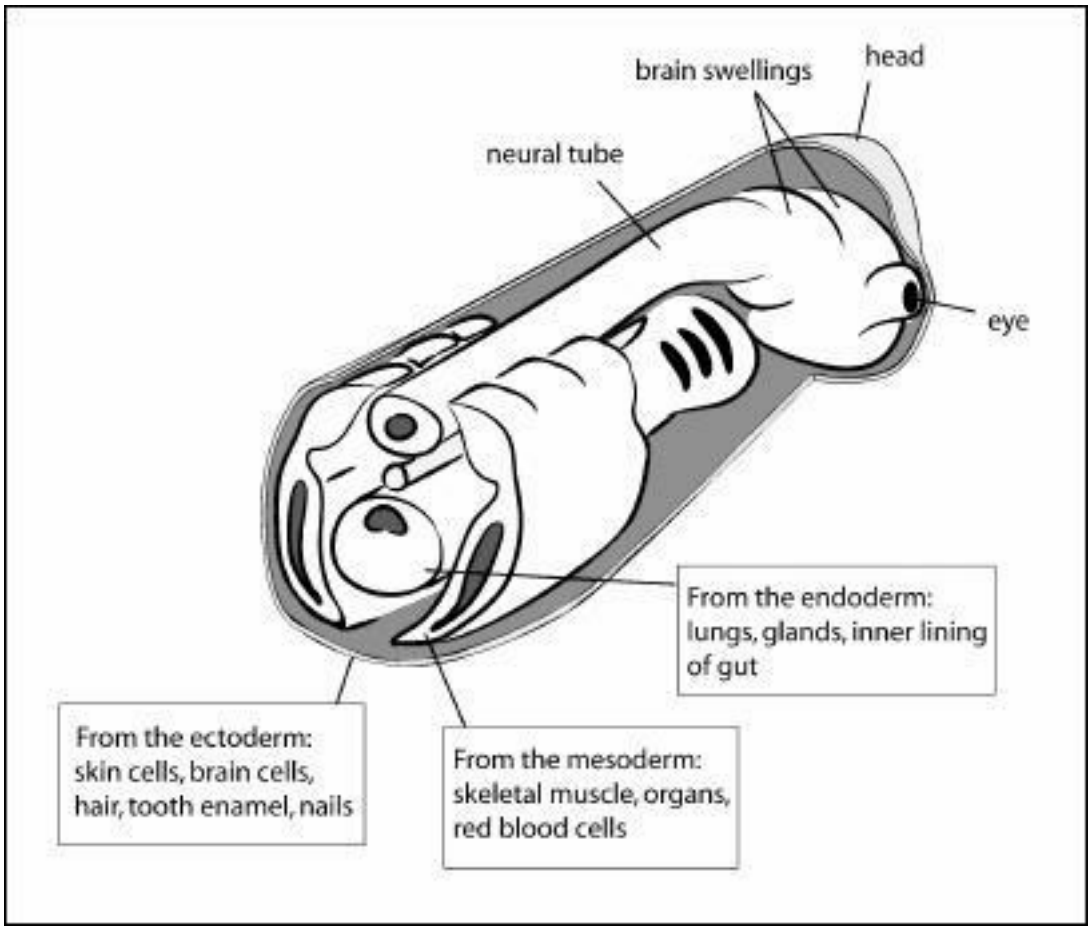


Our early days, the first three weeks after conception. We go from being a single cell to a ball of cells and end up as a tube.

Von Baer saw how embryos reveal fundamental patterns of life. He contrasted two kinds of features in development: features shared by every species, and features that vary from species to species. Features such as the tube-within-a-tube arrangement are shared by all animals with a backbone: fish, amphibians, reptiles, birds, and mammals. These common features appear relatively early in development. The features that distinguish us—bigger brains in humans, shells on turtles, feathers on birds—arise

relatively later.

Von Baer's approach is very different from the "ontogeny recapitulates phylogeny" idea you might have learned in school. Von Baer simply compared embryos and noted that the embryos of different species looked more similar to each other than do the adults of those species. The "ontogeny recapitulates phylogeny" approach championed decades later by Ernst Haeckel made the claim that each species tracked its evolutionary history as it proceeded through development. Accordingly, the embryo of a human went through a fish, a reptile, and a mammal stage. Haeckel would compare a human embryo to an adult fish or a lizard. The differences between the ideas of von Baer and Haeckel might seem subtle, but they are not. In the past one hundred years, time and new evidence have treated von Baer much more kindly. In comparing embryos of one species to adults of another, Haeckel was comparing apples to oranges. A more meaningful comparison is one where we can ultimately uncover the mechanisms that drive evolution. For that, we compare embryos of one species to embryos of another. The embryos of different species are not completely identical, but their similarities are profound. All have gill arches, notochords, and look like a tube within a tube at some stage of their development. And, importantly, embryos as distinct as fish and people have Pander and von Baer's three germ layers.



At four weeks after conception, we are a tube within a tube and have the three germ layers that give rise to all our organs.

All of these comparisons lead us to the real issue at stake. How does the embryo "know" to develop a head at the front end and an anus at the back? What mechanisms drive development and make cells and tissues able to form bodies?

To answer these questions required a whole new approach. Rather than simply comparing embryos as in von Baer's day, we had to find a new way of analyzing them. The latter part of the nineteenth century ushered in the era,

which we first discussed in Chapter 3, when embryos were chopped, grafted, split, and treated with virtually every kind of chemical imaginable. All in the name of science.

EXPERIMENTING WITH EMBRYOS

Biologists at the turn of the twentieth century were grappling with fundamental questions about bodies. Where in the embryo does the information to build them lie? Is this information contained in every cell or in patches of cells? And what form does this information take—is it a special kind of chemical?

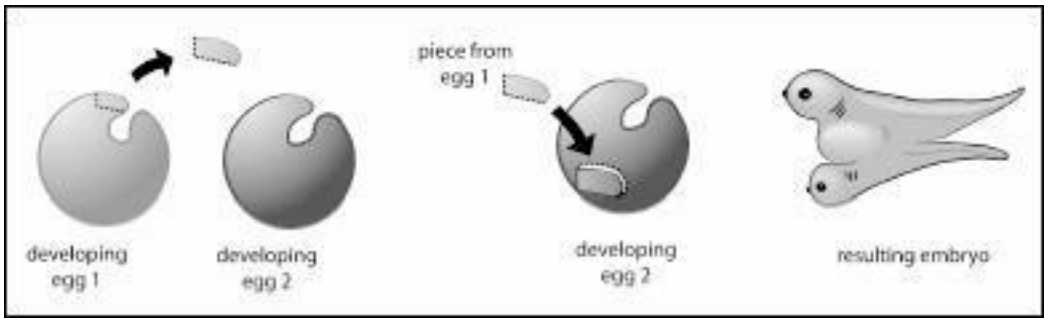
Beginning in 1903, the German embryologist Hans Spemann began to investigate how cells learned to build bodies during development. His goal was to find where the body-building information resides. The big question for Spemann was whether all the cells in the embryo have enough information to build whole bodies, or whether that information is confined to certain parts of the developing embryo.

Working with newt eggs, which were easy to obtain and relatively easy to fiddle with in the lab, Spemann devised a clever experiment. He cut off a strand of his infant daughter's hair and made a miniature lasso out of it. Baby hair is remarkable stuff; soft, thin, and pliant, it made the ideal material for tying up a tiny sphere such as a newt egg. Spemann did exactly that to a developing newt egg,

pinching one side off from the other. Manipulating the nuclei of the cells a bit, he let the resulting contraption develop and watched what happened. The embryo formed twins: two complete salamanders emerged, each with a normal body plan and each entirely viable. The conclusion was obvious: from one egg can come more than one individual. This is what identical twins are. Biologically, Spemann had demonstrated that in the early embryo some cells have the capacity to form a whole new individual on their own.

This experiment was only the beginning of a whole new phase of discovery.

In the 1920s Hilde Mangold, a graduate student in Spemann's laboratory, started to work with small embryos. The fine control she had of her fingers made her able to do some incredibly demanding experiments. At the stage of development with which Mangold worked, the salamander embryo is a sphere about a sixteenth of an inch in diameter. She lopped off a tiny piece of tissue, smaller than a pinhead, from one part of the embryo and grafted it onto the embryo of another species. What Mangold transplanted wasn't just any patch, but an area where cells that were to form much of the three germ layers were moving and folding. Mangold was so skilled that the grafted embryos actually continued to develop, giving her a pleasant surprise. The grafted patch led to the formation of a whole new body, including a spinal cord, back, belly, even a head.



Just by moving a small patch of tissue in the embryo, Mangold produced twins.

Why is all this important? Mangold had discovered a small patch of tissue that was able to direct other cells to form an entire body plan. The tiny, incredibly important patch of tissue containing all this information was to be known as the Organizer.

Mangold's dissertation work was ultimately to win the Nobel Prize, but not for her. Hilde Mangold died tragically (the gasoline stove in her kitchen caught fire) before her thesis could even be published. Spemann won the Nobel Prize in Medicine in 1935, and the award cites "his discovery of the Organizer and its effect in embryonic development."

Today, many scientists consider Mangold's work to be the single most important experiment in the history of embryology.

At roughly the same time that Mangold was doing experiments in Spemann's lab, W. Vogt (also in Germany) was designing clever techniques to label cells, or batches of them, and thus allow the experimenter to watch what happens as the egg develops. Vogt was able to produce a

map of the embryo that shows where every organ originates in the egg. We see the antecedents of the body plan in the cell fates of the early embryo.

From the early embryologists, people like von Baer, Pander, Mangold, and Spemann, we have learned that all the parts of our adult bodies can be mapped to individual batches of cells in the simple three-layered Frisbee, and the general structure of the body is initiated by the Organizer region discovered by Mangold and Spemann.

Cut, slice, and dice, and you'll find that all mammals, birds, amphibians, and fish have Organizers. You can even sometimes swap one species' Organizer for another. Take the Organizer region from a chicken and graft it to a salamander embryo: you get a twinned salamander.

But just what is an Organizer? What inside it tells cells how to build bodies? DNA, of course. And it is in this DNA that we will find the inner recipe that we share with the rest of animal life.

OF FLIES AND MEN

Von Baer watched embryos develop, compared one species to another, and saw fundamental patterns in bodies. Mangold and Spemann physically distorted embryos to learn how their tissues build bodies. In the DNA age, we can ask questions about our own genetic makeup. How do our genes control the development of our tissues and our

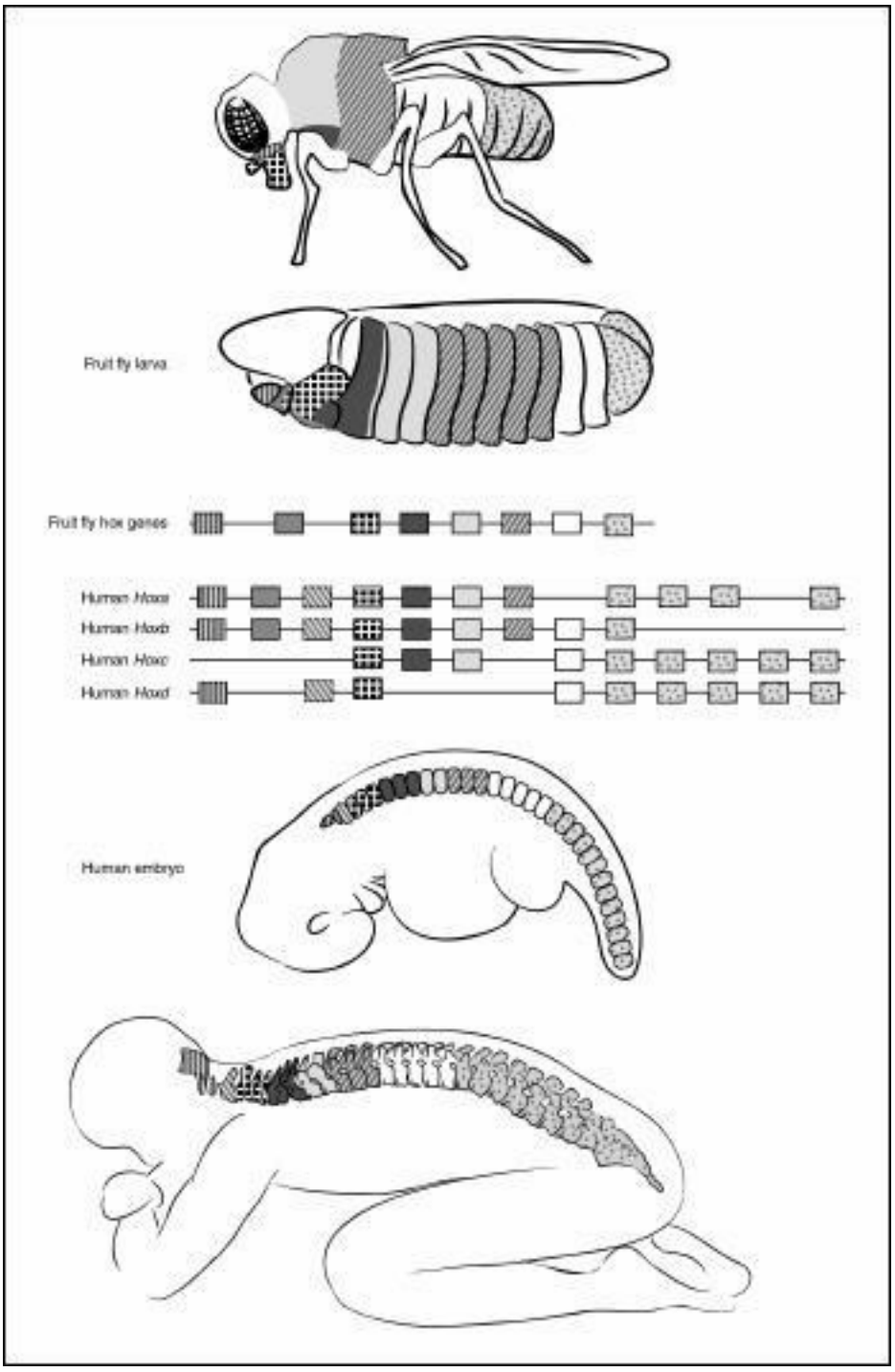
bodies? If you ever thought that flies are unimportant, consider this: mutations in flies gave us important clues to the major body plan genes active in *human* embryos. We put this kind of thinking to use in the discovery of genes that build fingers and toes. Now we'll see how it tells us about the ways entire bodies are built.

Flies have a body plan. They have a front and a back, a top and a bottom, and so on. Their antennae, wings, and other appendages pop out of the body in the right place. Except when they don't. Some mutant flies have limbs growing out of their heads. Others have duplicate wings and extra body segments. These are among the fly mutants that tell us why our vertebrae change shape from the head end to the anal end of the body.

People have been studying abnormal flies for over a hundred years. Mutants with one particular kind of abnormality got special attention. These flies had organs in the wrong places—a leg where an antenna should have been; an extra set of wings—or were missing body segments. Something was messing with their fundamental body plan. Ultimately, these mutants arise from some sort of error in the DNA. Remember that genes are stretches of DNA that lie on the chromosome. Using a variety of techniques that allow us to visualize the chromosome, we can identify the patch of the chromosome responsible for the mutant effect. Essentially, we breed mutants to make a whole population where every individual has the genetic error. Then, using a variety of molecular markers, we

compare the genes of individuals with the mutation to those without. This allows us to pinpoint the region and the likely stretch of chromosome responsible for the mutant effect. It turns out that a fly has eight genes that make such mutants. These genes lie next to one another on one of the long DNA strands of the fly. The genes that affect the head segments lie next to those that affect the segments in the middle of the fly, the part of the body that contains the wings. These bits of DNA, in turn, lie adjacent to the ones that control the development of the rear part of the fly. There is a wonderful order to the way the genes are organized: their position along the DNA strand parallels the structure of the body from front to back.

Now the challenge was to identify the structure of the DNA actually responsible for the mutation. Mike Levine and Bill McGinnis, in Walter Gehring's lab in Switzerland, and Matt Scott, in Tom Kauffman's lab in Indiana, noticed that in the middle of each gene was a short DNA sequence that was virtually identical in each species they looked at. This little sequence is called a homeobox. The eight genes that contain the homeobox are called *Hox* genes. When the scientists fished around for this gene sequence in other species, they found something so uniform that it came as a true surprise: *versions of the* Hox *genes appear in every animal with a body.*



Hox genes in flies and people. The head-to-tail organization of the body is under the control of different *Hox* genes. Flies have one set of eight hox genes, each represented as a little box in the diagram. Humans have four sets of these genes. In flies and

people, the activity of a gene matches its position on the DNA: genes active in the head lie at one end, those in the tail at another, with genes affecting the middle of the body lying in between.

Versions of the same genes sculpt the front-to-back organization of the bodies of creatures as different as flies and mice. Mess with the *Hox* genes and you mess with the body plan in predictable ways. If you make a fly that lacks a gene active in a middle segment, the midsection of the fly is missing or altered. Make a mouse that lacks one of the genes that specifies thoracic segments, and you transform parts of the back.

Hox genes also establish the proportions of our bodies—the sizes of the different regions of our head, chest, and lower back. They are involved in the development of individual organs, limbs, genitalia, and guts. Changes in them bring about changes in the ways our bodies are put together.

Different kinds of creatures have different numbers of *Hox* genes. Flies and other insects have eight, mice and other mammals thirty-nine. The thirty-nine *Hox* genes in mice are all versions of the ones that are found in flies. This similarity has led to the idea that the large number of mammalian *Hox* genes arose from a duplication of the smaller complement of genes in the fly. Despite these differences in number, the mouse genes are active from front to back in a very precise order just as the fly genes are.

Can we go even deeper in our family tree, finding similar

stretches of DNA involved in making even more fundamental parts of our bodies? The answer, surprisingly, is yes. And it links us to animals even simpler than flies.

DNA AND THE ORGANIZER

At the time when Spemann won the Nobel Prize, the Organizer was all the rage. Scientists sought the mysterious chemical that could induce the entire body plan. But just as popular culture has yo-yos and Tickle Me Elmo dolls, so science has fads that wax and wane. By the 1970s, the Organizer was viewed as little more than a curiosity, a clever anecdote in the history of embryology. The reason for this fall from grace was that no one could decipher the mechanisms that made it work.

The discovery of *Hox* genes in the 1980s changed everything. In the early 1990s, when the Organizer concept was still decidedly unfashionable, Eddie De Robertis's laboratory at UCLA was looking for *Hox* genes in frogs, using techniques like Levine and McGinnis's. The search was broad and it netted many different kinds of genes. One of these had a very special pattern of activity. It was active at the exact site in the embryo that contains the Organizer, and it was active at exactly the right time of development. I can only imagine what De Robertis felt when he found that gene. He was looking at the Organizer, and there in the Organizer was a gene that seemed specifically to control it

or be linked to its activity in the embryo. The Organizer was back.

Organizer genes started popping up in laboratories everywhere. While doing a different kind of experiment, Richard Harland at Berkeley found another gene, which he called *Noggin*. *Noggin* does exactly what an Organizer gene should. When Harland took some *Noggin* and injected it into the right place in an embryo, it functioned exactly like the Organizer. The embryo developed two body axes, including two heads.

Are De Robertis's gene and *Noggin* the actual bits of DNA that make up the Organizer? The answer is yes and no. Many genes, including these two, interact to organize the body plan. Such systems are complex, because genes can play many different roles during development. *Noggin,* for example, plays a role in the development of the body axis but is also involved with a host of other organs. Furthermore, genes do not act alone to specify complicated cell behaviors like those we see in head development. Genes interact with other genes at all stages of development. One gene may inhibit the activity of another or promote it. Sometimes many genes interact to turn another gene on or off. Fortunately, new tools allow us to study the activity of thousands of genes in a cell at once. Couple this technology with new computer-based ways of interpreting gene function and we have enormous potential to understand how genes build cells, tissues, and bodies.

Understanding these complex interactions between

batteries of genes sheds light on the actual mechanisms that build bodies. *Noggin* serves as a great example. *Noggin* alone does not instruct any cell in the embryo about its position on the top–bottom axis; rather, it acts in concert with several other genes to do this. Another gene, *BMP-4,* is a bottom gene; it is turned on in cells that will make the bottom, or belly side, of an embryo. There is an important interaction between *BMP-4* and *Noggin*. Wherever *Noggin* is active, *BMP-4* cannot do its job. The upshot is that *Noggin* does not tell cells to develop as "cells on the top of the body" instead, it turns off the signal that would make them *bottom* cells. These off-on interactions underlie virtually all developmental processes.

AN INNER SEA ANEMONE

It is one thing to compare our bodies with those of frogs and fish. In a real sense we and they are much alike: we all have a backbone, two legs, two arms, a head, and so on. What if we compare ourselves with something utterly different, for example jellyfish and their relatives?

Most animals have body axes defined by their direction of movement or by where their mouth and anus lie relative to each other. Think about it: our mouth is on the opposite end of the body from our anus and, as in fish and insects, it is usually in the direction "forward."

How can we try to see ourselves in animals that have no

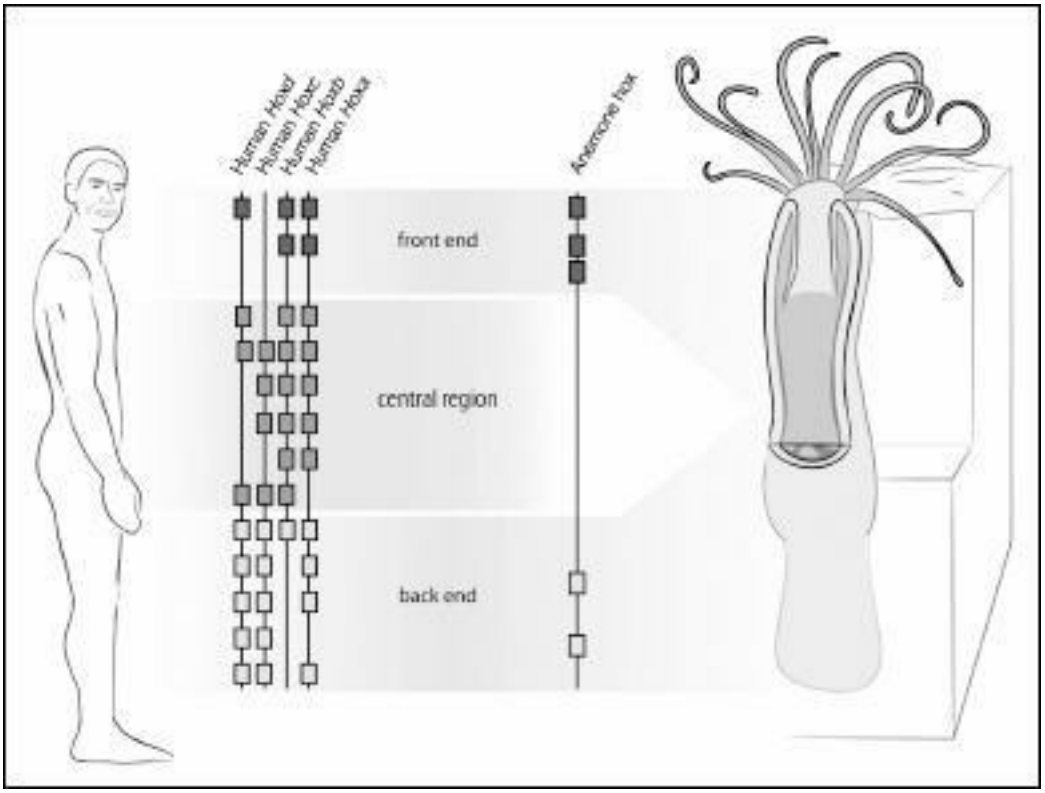
nerve cord at all? How about no anus and no mouth? Creatures like jellyfish, corals, and sea anemones have a mouth, but no anus. The opening that serves as a mouth also serves to expel waste. While that odd arrangement may be convenient for jellyfish and their relatives, it gives biologists vertigo when they try to compare these creatures to anything else.

A number of colleagues, Mark Martindale and John Finnerty among them, have dived into this problem by studying the development of this group of animals. Sea anemones have been remarkably informative, because they are close relatives of jellyfish and they have a very primitive body pattern. Also, sea anemones have a very unusual shape, one that at first glance would seem to make them worthless as a form to compare to us. A sea anemone is shaped like a tree trunk with a long central stump and a bunch of tentacles at the end. This odd shape makes it particularly appealing, since it might have a front and a back, a top and a bottom. Draw a line from the mouth to the base of the animal. Biologists have given that line a name: the oral–aboral axis. But naming it doesn't make it more than an arbitrary line. If it *is* real, then its development should resemble the development of one of our own body dimensions.

Martindale and his colleagues discovered that primitive versions of some of our major body plan genes—those that determine our head-to-anus axis—are indeed present in the sea anemone. And, more important, these genes are

active along the oral–aboral axis. This in turn means that the oral–aboral axis of these primitive creatures is genetically equivalent to our head-to-anus axis.

One axis down, another to go. Do sea anemones have anything analogous to our belly-to-back axis? Sea anemones don't seem to have anything comparable. Despite this, Martindale and his colleagues took the bold step of searching in the sea anemone for the genes that specify our belly-to-back axis. They knew what our genes looked like, and this gave them a search image. They uncovered not one, but many different belly-to-back genes in the sea anemone. But although these genes were active along an axis in the sea anemone, that axis didn't seem to correlate with any pattern in how the adult animal's organs are put together.



Jellyfish relatives, such as sea anemones, have a front and a back as we do, a body plan set up by versions of the same genes.

Just what this hidden axis could be is not apparent from the outside of the animal. If we cut one in half, however, we find an important clue, another axis of symmetry. Called the directive axis, it seems to define two distinct sides of the creature, almost a left and a right. This obscure axis was known to anatomists back in the 1920s but remained a curiosity in the scientific literature. Martindale, Finnerty, and their team changed that.

All animals are the same but different. Like a cake recipe passed down from generation to generation—with enhancements to the cake in each—the recipe that builds our bodies has been passed down, and modified, for eons. We may not look much like sea anemones and jellyfish, but the recipe that builds us is a more intricate version of the one that builds them.

Powerful evidence for a common genetic recipe for animal bodies is found when we swap genes between species. What happens when you swap a body-building gene from an animal that has a complex body plan like ours with one from a sea anemone? Recall the gene *Noggin,* which in frogs, mice, and humans is turned on in places that will develop into back structures. Inject extra amounts of frog Noggin into a frog egg, and the frog will grow extra back structures, sometimes even a second head. In sea anemone embryos, a version of *Noggin* is also turned on at

one end of the directive axis. Now, the million-dollar experiment: take the product of Noggin from a sea anemone and inject it into a frog embryo. The result: a frog with extra back structures, almost the same result as if the frog were injected with its own Noggin.

Now, though, as we go back in time, we are left with what looks like a huge gap. Everything in this chapter had a body. How do we compare ourselves with things that have no bodies at all—with single-celled microbes?