From Developing Limbs, Insights That May Explain Much Else

By CARL ZIMMER

For its first four weeks, a human embryo looks like a crumpled tube. But around its twenty-seventh day of development, four buds bulge from its sides. Over the next few days, the buds grow like tulips, stretching out into flattened stalks and blooming into crowns of fingers and toes. Inside these developing limbs, bones condense. Muscle cells, tendons, blood vessels and nerves all find their respective places. The embryo now has hands with thumbs to suck, legs ready to deliver a kick.

For developmental biologists, the development of limbs captures all that is marvelous about embryos: how a few cells can give rise to complicated anatomy. In fact, biologists understand the development of the limb much better than any other part of the body.

They have been experimenting on developing limbs for almost a century, and today they are figuring out how limb-building genes are organized into a network that almost always manages to build the same structures with the same shape.

In studying limb development, biologists are learning how the diversity of limbs — from bird wings to whale flippers — evolved. They are also getting clues that may someday make it possible to regenerate tendons or even entire limbs. But for many experts on limb development, their most important discoveries are how the rules for limb-building also apply to other parts of the embryo.

“The lessons learned in the limb give you insights into how you build a face, or how you build a heart,” said Clifford Tabin, a developmental biologist at Harvard Medical School.

For centuries doctors and naturalists observed how embryos developed, but it was not until the early 1900s that developmental biologists ran experiments to understand the forces at work.

“This was an era of slice and dice,” wrote Neil Shubin, a University of Chicago biologist, in his 2008 book “Your Inner Fish.” Developmental biologists would snip out pieces of embryos or graft parts together and watch how the development of the embryo was altered.

Limbs proved to be the easiest part of an embryo to study. “The limb is totally external, it’s easy to work with, and it’s totally expendable,” Dr. Tabin said. “No matter what you do to it, the embryo is going to be fine. Having a heart matters a lot to an embryo. But having a limb doesn’t.”

Chickens became a favorite animal for developmental biologists who studied limbs. “You actually break the eggshell and make a window, and you can cover it with tape afterwards,” said Cheryll Tickle of the University of Bath. “The embryo will continue to develop, and you can find out what happens later on.”
In the 1940s, a Johns Hopkins University biologist, John Saunders, discovered through some slicing and dicing that there were two parts of the limb bud that had mysterious powers over the entire limb’s fate.

One of those parts was a translucent ridge that formed along the outer edge, where the fingers eventually form. If Dr. Saunders clipped off the ridge, the entire limb stopped developing. If he grafted a second ridge onto a limb bud, it grew into two arms.

That patch of tissue was called the apical ectoderm ridge.

Dr. Saunders also discovered a zone on the lower edge of the limb bud, around the place where the pinky would later develop. It somehow sent signals across the limb bud, telling the cells where they were along the pinky-to-thumb axis of the hand and thus which digit to become, and it became known as the zone of polarizing activity.

When Dr. Saunders grafted an extra zone to the thumb side of a limb bud, he produced a second set of digits, arranged in a mirror image to the normal ones.

Five decades later, biologists began to pinpoint the signals that these special parts of the limb bud send out.

In 1993, for example, Dr. Tickle and her colleagues discovered that the ridge produces a growth-stimulating molecule called, descriptively, fibroblast growth factor. A limb bud could still grow without its ridge, they found, if they implanted in it a microscopic bead soaked with this growth factor.

In 1993, Dr. Tabin and his colleagues discovered another signaling chemical of major importance in the limb. Geneticists had a little more fun naming the protein, calling it sonic hedgehog, after a video game character.

Over the past 16 years, Dr. Tabin, Dr. Tickle and other researchers have identified more of the crucial limb-building proteins and the genes that carry the instructions to make them.

“We know most of the genes now, so it’s really a system where we can look at more complex things,” said Rolf Zeller of the University of Basel in Switzerland. “We’re trying to understand how these different genes work together.”

Limb development researchers have found that the first steps take place while an embryo is still a crumpled tube. Along the length of an embryo’s flanks, a series of segments forms. Each segment produces chemical signals. And at the places where the shoulders and the hips will be, the signals tell the outer cells to grow rapidly and form little pockets, into which other cells stream.

As the pocket grows, it forms the necessary ridge, which sends out other signals telling the cells just underneath it to multiply. As the limb bud grows, the ridge moves away from the cells at its base, which receive fewer growth factors. Without that stimulation, the cells grow more slowly
and begin to develop into cells that produce cartilage. They form clumps that will eventually turn into limb bones.

Meanwhile, cells near what will become the pinky start making sonic hedgehog. That molecule spreads across about half the limb bud, to where the middle finger will later form.

Cells that produce sonic hedgehog are exposed to the protein the longest. The neighboring cells become separated as the limb bud grows, and so they are only briefly bathed in it.

Some experiments suggest that being exposed for a long time turns limb bud cells into pinkies. No time exposed to sonic hedgehog turns them into thumbs.

Each part of the limb knows what it should develop into thanks to sets of genes, each laying down the coordinates in one of three dimensions, and all working together. It turns out, for example, that the cells in the ridge can function only if the limb bud can make sonic hedgehog. Dr. Zeller and his colleagues have discovered why: sonic hedgehog switches on a gene in nearby cells called gremlin. Gremlin, in turn, inhibits a protein called BMP4 (for bone morphogenetic protein). At high enough levels, BMP4, can shut down the production of the growth factor in the ridge. So by keeping BMP4 levels low, sonic hedgehog lets the ridge continue to function.

Once the limb has reached the right proportions, it must quickly stop growing. Experiments carried out by Dr. Tabin and his colleagues point to the brake on limb development. The limb bud gets so big that the gremlin-producing cells drift farther and farther away from the cells that make sonic hedgehog. As their supply of sonic hedgehog drops, the cells cannot make gremlin proteins. The level of BMP4 rises, and it shuts down the ridge. Without the ridge’s help, the limb bud can no longer make sonic hedgehog. In other words, all the crucial genes for the development of the limb shut one another off.

Today, researchers still have much left to learn about the development of limbs. “Your knuckle and your humerus are the same size when they first form,” Dr. Tabin said. “Why does the humerus grow so much bigger than the knuckle? We don’t know.”

Dr. Tickle and other researchers are screening all the genes that are active in limb bud cells to find those that are essential for the development of limbs. She is optimistic that before long scientists will chart the entire path by which limb buds develop into fully formed limbs. “It’s just a question of having enough people plugging away at it,” she said.

Dr. Tabin shares that optimism. “It’s definitely clear that we’re going to get there,” he said. “We’re going to understand it from beginning to end.”

But Dr. Tabin argues that long before scientists find the complete pattern of limb growth, they will discover many important insights. It is now clear, for example, that genes involved in making limbs (like BMP4) are important for building other parts of the body as well.

“Within an embryo the same molecules are used over and over again,” Dr. Tabin said. “No one would have expected there would have been so few signals used to form an embryo. If you have a
signal that says make a heart, you wouldn’t expect it to make a limb. But that’s exactly what you find.”

Deciphering the development of limbs may also lead to treatments for injuries and birth defects. In the near term, scientists are searching for the signals that cause tendons to develop and attach to bones. The signals might be able to cause cells in a dish to form extra tendon tissue, as well, which could be surgically implanted in arms or legs.

Eventually, it may even be possible to apply the right signals that can turn stem cells into limb buds and, ultimately, full-blown arms and legs.

“I’m optimistic it’s going to happen,” Dr. Tabin said. “If you can get the initial conditions right and the cells know what they’re supposed to do, you can turn them loose. It’s a self-organizing system. You don’t have to come back in and say you’ve got to split the muscle in two here. That will happen by itself.”

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Directions: Respond to the following questions on a separate sheet of paper. Use complete sentences and refer to the text to support your answer.

Questions...

1. Why is limb development the most prevalent area of study by biologists and why are chicken embryos most commonly used?

2. What were scientists able to learn by “slicing and dicing?”

3. Diagram the relationship between the apical ectoderm ridge, the zone of polarizing activity, fibroblast growth factor, sonic hedgehog, gremlin and BMP4 as they relate to limb growth.

4. The article states that “all the crucial genes for the development of the limb shut one another off.” Use your diagram to explain what this means.

5. What insights into the development and formation of other parts can be gleaned from the studies on limb growth?

*Critical Thinking…

6. How might research into embryonic development be applied in the future? How do the potential treatments for injuries and birth defects stack up against the objections by groups that oppose the use of stem cells and human embryos for research? In your opinion, should scientists attempt to grow “full-blown arms and legs” in a laboratory setting? Why or why not? What limits, if any, should be placed on the study and manipulation of human embryos?