4 All from a Single Cell: How a Fertilized Egg Develops into a Baby

Nine months after a human egg is fertilized, a baby's lungs fill with air and she bawls out her first lusty cry. Just thirty-eight weeks ago she was a single cell, created by the union of one of her father's sperm and one of her mother's eggs. How did one cell give rise, in that short span of time, to an organized mass of human flesh with limbs and lungs in the correct places, with the proper number of fingers and toes, and with eyes and ears and everything else working properly? It seems like a miracle. While it is marvelous, it's not miraculous: biologists have learned the principles of the process that produces a complex organism from a single cell.

This process has intrigued scientists for a long time. An early theory to explain human development, dating back more than two thousands years, is that of preformation. This theory provided a simple answer: we already contain in our bodies very small but fully formed members of the next generation, who merely grow within the mother until they reach the size of a baby able to survive outside the womb. Many scientists thought they saw this tiny person—which they called a homunculus—when they peered at sperm through the first microscopes in the seventeenth century.

This explanation sidestepped the seemingly intractable issue of how complexity unfolds. But there's a big problem with this theory of little people: the homunculus must carry its own sperm that shelter an even smaller version of the person who will be born in the next generation, and that prehomunculus must have in its sperm an even smaller pre-prehomunculus that is to be born two generations hence, and so on ad infinitum for all future generations of humankind. By the nineteenth century embryologists had come to see this fundamental flaw in the preformation theory. The alternative view was that the egg was formless, and,

after fertilization, goes through a series of transformations that result in a fully formed individual.

But how? Enter now the fruit fly, *Drosophila melanogaster*. The humble fruit fly seems to appear like magic whenever we leave an open bottle of wine on the table or neglect to toss out a banana peel, calling to mind another discredited theory—spontaneous generation—the idea that life forms can spring from nonliving material. *Drosophila* species are cosmopolitan, having hitchhiked from place to place along trade routes, and spread west in North America with the migration of people and their fruits and vegetables and garbage.

The fruit fly also populates thousands of research laboratories, serving as an ideal subject for the investigation of all sorts of biological phenomena. With its small size (a mere 0.1 inches head to tail), short generation time (just a couple of weeks), large litters (hundreds of eggs per mom), and low feeding and housing costs (quite happy to spend their lives in milk bottles feeding on yeast), *Drosophila* has been a fond object of biologists' attention for more than a century. And it is this fly that has yielded many of the secrets of embryonic development.

That a fly would be key to unlocking the path from egg to adult seemed unlikely in the early part of the twentieth century. Tiny *Drosophila* made its name not in developmental biology but in genetics, while larger animals like the frog and the sea urchin were the darlings of embryologists. For a period of about thirty years, beginning around 1910, researchers in the laboratory of Thomas Hunt Morgan—first at Columbia University, later at the California Institute of Technology—made groundbreaking genetic discoveries using the fly. These included showing that genes lie on chromosomes, uncovering the process by which chromosomes exchange pieces of themselves, and figuring out that sex-linked traits are specified by the X chromosome, discoveries that we will discuss shortly, and that garnered a Nobel Prize for Morgan in 1933.

In the 1940s, following its heyday in Morgan's laboratory, *Drosophila* was eclipsed by even smaller creatures as the objects of geneticists' attention. Taking its place in the new field of molecular biology were the bread mold *Neurospora crassa* and the intestinal bacterium *Escherichia coli* and its viruses. Experiments on these rapidly dividing organisms revealed the nature of the gene, the genetic code, the process of protein production, and the principles of gene function.

Beginning in the 1970s, *Drosophila* began its comeback, led by a young German biologist, Christiane Nüsslein-Volhard, who dazzled developmental biologists with her work showing how a single cell turns into a fully formed organism with trillions of cells. In partnership with a young American biologist, Eric Wieschaus, Nüsslein-Volhard tackled a project so audacious in its concept that another geneticist wondered, "Does she have the whole German army working for her?" But it was just Nüsslein-Volhard and Wieschaus, sitting across from each other at a small table in their lab in Heidelberg, Germany for an entire year isolating mutant flies—ones with changes in their DNA sequence that produce deformed embryos—in the hope that learning what goes wrong in each mutant would reveal how the normal flies do it right.

Nüsslein-Volhard and Wieschaus's mutant flies, first described in 1980 in the international scientific journal *Nature*, were crucial to solving the mystery of development, because they led to the identification of the key proteins that decide each cell's fate by turning particular genes on or off. The two biologists analyzed the flies' cells as an investor might analyze a new company to predict whether it is going to be successful: identify the key executives, find out what critical decisions they are making, and observe how the company responds to their strategic mistakes. Nüsslein-Volhard and Wieschaus were shrewd investors: their acumen won them the 1995 Nobel Prize in Physiology or Medicine.

What was striking about Nüsslein-Volhard's approach was its simplicity: it required only a commercially available chemical to cause mutations in the flies, an ordinary microscope for observing the fly embryos, and standard genetic analysis—all of which were available as far back as 1930. Why did no one think to try this approach in the intervening four decades?

Nüsslein-Volhard had been trained as a biochemist; she wrote her doctoral dissertation on her studies of an RNA-synthesizing enzyme from bacteria. She turned to *Drosophila* because she wanted to apply genetics to the problem of development, and found that she "immediately loved working with flies. They fascinated me, and followed me around in my dreams." As a newcomer to the field of developmental biology, Nüsslein-Volhard was unencumbered by the constraints that limited the thinking of other scientists interested in these problems. "I, compared to other people working in this field, came up with ideas. They were blocked in

their minds. Other biologists would say, "'This is not done. We don't do that in our field.' . . . I did things that were completely unconventional."

Nüsslein-Volhard knew that the different types of cells in an organism are different because they deploy (biologists say "express") different sets of genes to produce different kinds of proteins. How did she know that? Isn't it possible that a cell develops into a liver cell rather than a lung cell because it possesses a different set of genes than the lung cell? Might not unspecialized cells of a developing organism lose genetic information as they divide, retaining different sets of genes that determine the type of cell they will eventually become? A cell destined to become a liver cell might retain only those genes that are needed to specify a liver cell; a cell destined to become a lung cell might retain a different set of genes that cause it to become a lung cell. Might it work like that?

This very reasonable idea—that development of one cell type might proceed by loss of genes for all other cell types—was ruled out in the 1960s by the English scientist John Gurdon, who showed that specialized cells possess all the genetic information necessary to specify all the other types of cell in an animal; specialized cells do not lose any genes while assuming their particular identity. Gurdon established this principle with a clever and technically impressive experiment with frogs: he used the genetic information present in a single specialized frog cell to program the development of an entire frog. He was the first person to clone an organism.

Gurdon began by removing the chromosomes from an unfertilized frog egg, literally reaching into the egg with a very thin straw and sucking out its nucleus, the part that contains the DNA wrapped up into chromosomes. He then put into that DNA-denuded egg a nucleus he had similarly extracted from a cell he obtained from a frog's gut. If the specialized gut cell had acquired its identity because it retained only gut cell genes, then its genetic material should not be able to program that egg to develop into a frog, because it would be missing genes necessary for making other types of cells. But Gurdon saw complete, normal frogs develop from some of the eggs he had manipulated. He concluded that specialized cells carry all the genetic information necessary to specify an entire animal.

Because Gurdon did these experiments with frogs, his conclusion was met with some skepticism. Some people said the rules for frog development might be different from the rules for other animals. Frogs, after all, are

cold-blooded—very different from us and our warm-blooded cousins. Thirty years later, Ian Wilmut, a Scottish veterinarian, quieted any remaining doubters when he cloned a sheep. Employing Gurdon's methods, Wilmut replaced the nucleus of a sheep egg with a nucleus taken from a cell of an adult sheep's mammary gland. The reprogrammed egg was placed into a ewe who served as a surrogate mom, and five months later Dolly burst into the world, proving that the mammary gland cell carried all the information necessary to create a fully formed, normal lamb. This experiment has since been successfully repeated with many other kinds of animal using several different kinds of specialized cell as the source of the nucleus that programs the egg, proving beyond a reasonable doubt that virtually every one of our cells carries the same complete set of genes.

If all cells in an organism have the same genes, specialized cells must acquire their particular identity by using only some of those genes. A liver cell is what it is because it uses only a subset of its genes, those that provide the proteins that make a liver cell and carry out its tasks. It does not express genes for making brain or bone cells. Lung cells deploy a different set of genes, which give them their unique characteristics; they do not express genes for making skin or spleen cells. And so on for the hundreds, perhaps thousands, of different types of cells in our bodies. So now the key question becomes: How do cells in the developing embryo come to use some genes but not others and thereby become a specific type of cell, eventually leading to the organized mass of tissues we call an organism?

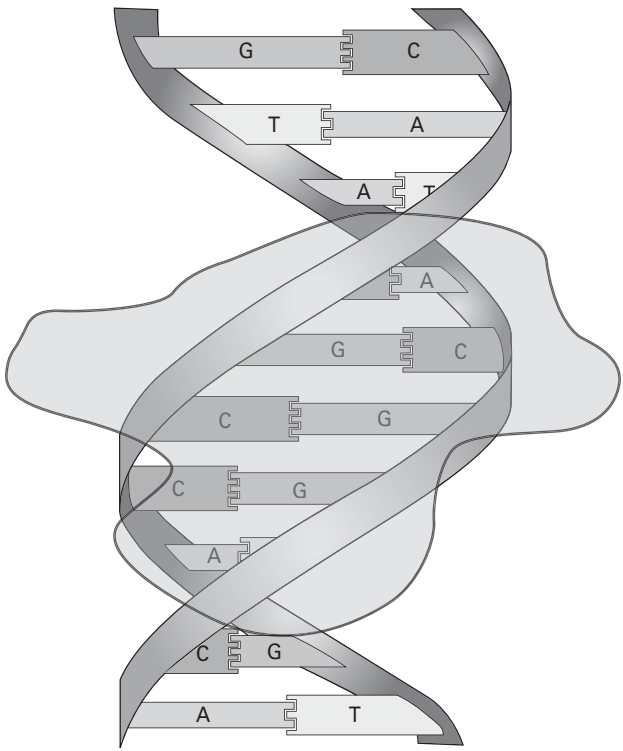
As we discussed in chapters 2 and 3, genes are stretches of DNA that contain the information for making a protein. Genes direct the synthesis of a protein by first being copied into a molecule called RNA, which is very similar to DNA but consists of only one strand of bases rather than the two of the double helix. This first step in the expression of a gene is carried out by a special protein machine in the cell that transcribes the sequence of the DNA into RNA copies that are then translated into proteins, much as medieval monks transcribed sacred texts onto papyrus for translation by their colleagues.

Only some of the genes in each cell are used like this: maybe only ten thousand of the twenty thousand or so genes in each cell get expressed. Each gene has a switch that controls whether it is "on" or "off." If the switch is in the "on" position the gene will spring into action and be transcribed

into RNA; if the switch is in the "off" position the gene will remain at rest. The switches of some genes are in the "on" position only in muscle cells, while the switches of other genes are flipped "on" only in nerve cells.

What determines whether a gene's switch is on or off? The decision is made by a class of proteins that we can think of as the executives: their job is to decide whether certain genes are to be on or off. They do this by recognizing and binding to specific DNA sequences near particular genes and regulating their transcription into RNA. Hence their name: transcription factors.

Each transcription factor recognizes one particular short DNA sequence (usually six to twelve base-pairs in length) that is present near the genes it controls. A remarkable property of transcription factors is that they can find their short recognition sequence among the other three billion base-pairs of DNA in the human genome (see figure). They rapidly search through the genome—much the way Google searches through billions of web pages—until they find their sequence, and then they glom on to it.



Most genes contain recognition sequences for several transcription factors. The sum of the effect of each transcription factor bound to the gene determines the state of the gene's switch. Some transcription factors act to turn transcription on, others strive to turn it off. The transcription factors are like the transistors that constitute the motherboard of a computer, integrating the input they receive and responding with the coordinated output you see on your screen. This integrated circuitry of transcription factors bound near a gene constitutes the switch that turns the gene on or off.

Actually, these switches are more like rheostats that can be turned up or down, the brightness or dimness of the rheostat's setting being determined by the particular combination of transcription factors that are bound to the gene. Since the human genome encodes about fifteen hundred different transcription factors, the number of different combinations of them is huge, so the rheostats can be set to an almost limitless number of levels. And since the settings of the rheostats on all 20,000 genes determine the identity of a cell, the great diversity of cell types in the human body should no longer be a surprise.

Wise investors know that too many executives often spell doom for a company, so we may wonder why successful organisms such as humans have so many transcription factors. But a complex organism has to make many more decisions than even the largest of companies, and we need all those transcription factors to do that. The factors ask questions about what's going on inside and outside the cell: Are there enough nutrients? What are the cells next door up to? Is there a big demand in the rest of the body for things this cell makes? And many, many other important questions.

The transcription factors learn the answers to these questions, integrate that information, and take action by turning on the genes that are needed (and turning off those that are not needed) by a cell that finds itself in that specific situation at that particular time. The diversity of transcription factors allows many questions about cellular fitness to be asked simultaneously and continuously. The answers to those questions comprise a huge amount of data that the transcription factors process in deciding which genes should be active, and thus which proteins will be present at that specific time in that particular cell.

The decision to turn a gene on or off is like the choice an editor must make whether to run a story about a big fire with a banner headline on

page 1 or to go with a more modest mention on an interior page. The editor gets input from several reporters: some at the fire watching a rescue in progress, others at the mayor's press conference hearing what the city's emergency teams are doing, and a few at the hospital listening to the stories of victims. The editor integrates this input and decides the story will run on page 3, but with a large headline. Transcription factors are the cells' editorial staff, collecting information from a press corps of proteins that gather a huge amount of news as they survey the situation.

We can see, then, that cells become different by expressing different sets of genes, which results from each kind of cell having a unique collection of transcription factors. Liver cells have a corps of transcription factors that turn on genes necessary to make a liver cell and turn off genes necessary for making other cell types; lung cells have a different corps of transcription factors that are responsible for turning on the genes lung cells need and for turning off the genes used by other types of cells.

The whole developmental program, from the first division of the fertilized egg to the birth of a fully formed organism consisting of trillions of cells, is largely a diversification of the transcription factor collections in cells as they divide. How does a cell that is destined to contribute to the iris of the eye come to possess *just* the right set of transcription factors to ensure that the genes for making an iris (and *not* genes for making a retina, or lens, or cornea) are expressed at *just* the right levels and at *just* the right time as the embryo develops? In other words, how do different cells come to possess different transcription factors?

Cells assemble their complement of transcription factors by expressing the genes that encode those transcription factors. Each transcription factor is a protein encoded by a different gene, and the subset of those transcription factor genes that a cell expresses determines the collection of transcription factors it contains.

What determines which transcription factor genes get expressed in a cell? The same mechanism that determines the expression of every other gene in the cell: the particular collection of transcription factors it contains.

Oh, oh . . . We seem to have boxed ourselves into a corner: different cells express different genes because they possess different combinations

of transcription factors. But they possess different combinations of transcription factors because the genes that encode those transcription factors are acted upon by yet other combinations of transcription factors. A bit circular, isn't it?

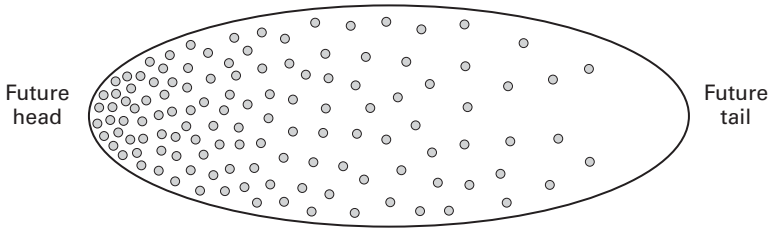
The genes that encode transcription factors, like all genes, have rheostats that govern their output, and, as is the case for all genes, those rheostats are set by transcription factors. So the set of transcription factors that a given cell has at any given moment is the result of which particular transcription factor genes were expressed during the course of development of that cell. This logic makes the developing organism seem like a set of nested Russian dolls: to determine why a set of transcription factors came to be present in a liver cell, you have to look at the transcription factors in the cell that gave rise to the liver cell, and to determine why that particular set of transcription factors came to be present in *that* cell you have to look at its precursor cell, and so on, all the way back to the original fertilized egg.

That is precisely what Nüsslein-Volhard set out to do: go all the way back to the first few cells of the embryo and identify the transcription factors they have that make them different, then learn how the cells produced in successive divisions come to possess different combinations of transcription factors that cause them to express unique sets of genes and thus become increasingly specialized.

The genes she discovered that control this process operate by a few general principles. While these principles are simple, the complex process of development is anything but. We'll illustrate the principles that govern development in the fly; human development operates a bit differently, but the fundamental principles are similar.

One principle is that the fly egg, even before it ever sees a sperm, is already subdivided into specialized areas: one end will give rise to the head, the other end to the tail; one part will become the top of the fly, another part the bottom. The basis of this polarity of the egg is chemical gradients in the egg.

Certain proteins in the egg get synthesized by the mother at one end of the egg—say, the end that will become the head—and their levels diminish as they spread outward toward the other end of the egg. If one of these proteins is a transcription factor, it would be most effective

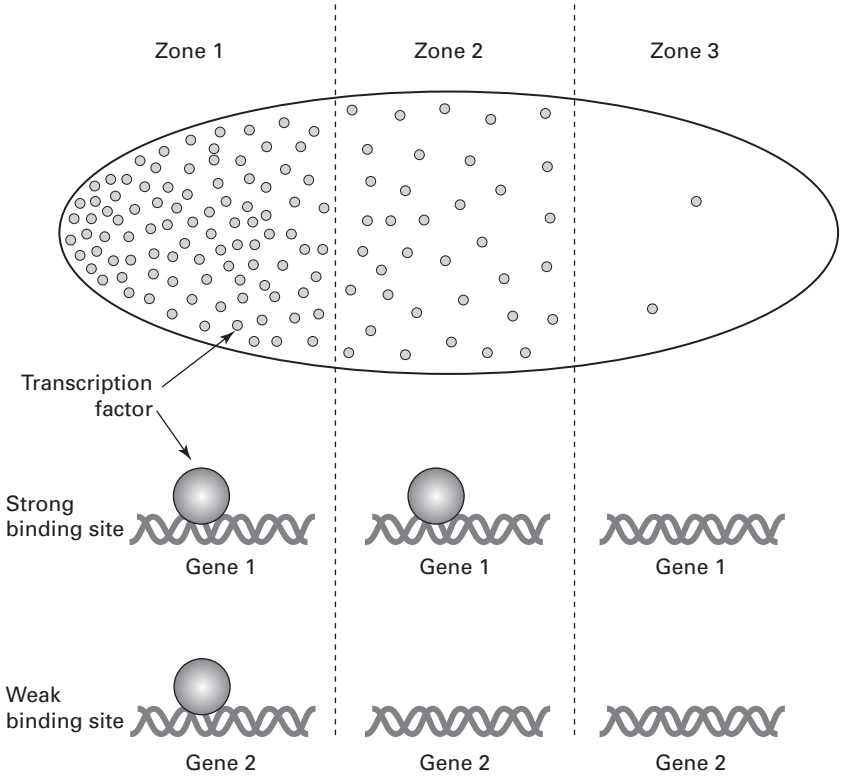


controlling the activity of genes in cells that lie near the end of the embryo destined to become the head, with decreasing effectiveness as its concentration diminishes toward the end of the egg that will form the tail (see figure).

This can be visualized by imagining that you've opened up a can of blue paint in preparation to repaint your kitchen. It sits peacefully in a corner while you gather the brushes and track down the tarp. Just then your teenager zooms in to demonstrate her latest skateboard maneuver, tipping over the can as she glides across the room. The blue paint pours across the floor, a thick puddle in the region nearest the corner where the can stood, thinning out as it spreads across the floor. There is now a gradient of paint that spreads from one end of the kitchen to the other.

A second principle is that different genes respond to different amounts of a transcription factor. One gene might need a high level of a transcription factor to be turned on, a level present only at the region of the egg that will give rise to the head. This may occur because the DNA sequences in the gene that that transcription factor binds to are not very good matches to the sequence it recognizes, so that many copies of the transcription factor are necessary to ensure that some of them recognize and latch on to the partial recognition sequence. Another gene might contain a DNA sequence that is a close match to the sequence recognized by that transcription factor and may therefore require less of the transcription factor to be switched on. As a consequence, that gene will be turned on in cells farther away from the head-forming end of the embryo.

A concentration gradient of a single transcription factor will already define three zones of the fertilized egg: a zone of high concentration at one end of the egg (say, where the head of the fly will form), where the factor turns on genes containing strong and weak recognition sequences



for the transcription factor; a zone of medium concentration near the middle of the egg, where it turns on only genes that have close matches to the recognition sequence (strong binding sites for the transcription factor); and a zone of low concentration near the opposite end of the egg (where the tail of the fly will form), where there is not enough of the transcription factor to turn on either kind of gene (see figure).

A third principle is that cells talk to one another, and these conversations influence which genes get expressed, much as conversations in the hall of a high school influence who is going to the prom with whom. Neighboring cells communicate with each other through proteins they display on their cell surfaces, which act like molecular feelers, or antennae. When these antennae make contact with a neighboring cell, or detect molecules given off by neighboring cells, they send signals into the cell that affect the function of certain transcription factors that result in changes in gene expression.

Among the genes whose expression is affected by these signals are those that encode transcription factors. Since cells in different parts of the developing embryo get different cues from their neighbors, their antennae generate different signals, and thus different cells come to express different sets of transcription factor genes, which eventually cause them to express different genes, which determine the fate of the cell.

These kinds of intercellular conversations are constantly going on in the developing embryo. It's a veritable cacophony. Let's listen in: "Hi neighbor! I've decided to become a cell of the iris, but I can't form the iris all by myself so I'd like you to join me. Hey, you over there! Listen up and get with the program! I'm sending you a signal, so pay attention. And after you receive it make sure you pass it on to your neighbors. We're going to need some of them to become cells for a cornea." By means of these intercellular conversations cells continually refine the set of transcription factor genes they express, ultimately causing them to express the specific set of genes that results in their taking on very specific functions.

Why was it that Christiane Nüsslein-Volhard, rather than some other biologist, had the idea to seek the *Drosophila* mutants whose analysis would reveal these principles? Evelyn Fox Keller points out that as a German scientist, Nüsslein-Volhard was less affected by the gene-centric view of biology typified by the Americans, and was more willing to consider how the other components of the cell participated in the process. Furthermore, as a molecular biologist she was impatient, unlike many developmental biologists; she was accustomed to getting quick results from her experiments. Most critically, she had the imagination to come up with novel ideas.

If everything goes right with the gradients of transcription factors, with the combinatorial interplay of proteins sitting on the DNA, with the intercellular conversations and negotiations, and with the many other things that go into the developmental process, then a complete organism is eventually born, with limbs and lungs in the right places and with the proper number of fingers and toes and with irises and eyelids that work.

Most of the time it does go right—remarkably so, given the complexity of the process. But things can go wrong. A very large percentage of human pregnancies—perhaps 30 to 50 percent—spontaneously abort before the

pregnancy is detected because something goes very wrong soon after fertilization of the egg. Fifteen to 20 percent of known pregnancies also result in miscarriages, most of them probably due to mistakes in the developmental program. And the parents of one out of every twenty-eight babies get the distressing news that their child has a birth defect. But when one considers everything that must go right with the process for a healthy child to be born, it's remarkable that we're here at all, let alone with all of our organs and limbs in their proper places and working correctly. And all from a single cell!

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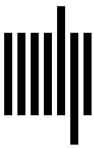

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