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Rise of the Neurons

In which some embryonic cells become neural stem cells, the founders of the nervous system, and in which we get the first glimpses of the evolution of the brain.

Totipotent Stem Cells

The end of the nineteenth century was a time of tremendous progress in embryology. Questions that had been debated for centuries concerning how an organism with all its parts emerges from a single-cell egg were beginning to be answered by experiments rather than debates. One of the most fundamental of these questions was: When a fertilized egg cell divides to make two cells, does each of the two cells have the capability to make a complete being or do the two cells divide this potential in some way? This was a question that just could never be answered by debate. An experiment on real embryos was clearly necessary to resolve the issue.

In 1888, Wilhelm Roux, working at the Institute for Embryology in Wrocław, took up the challenge of answering this question by using frog embryos at the two-cell stage. He inserted a

heated needle into one of the two cells and then let the embryo develop from the remaining live cell. Most of the experimental embryos ended up looking like halves of animals, for example, a right or left half of an embryo rather than whole one. Based on these results, Roux argued that the capacity to make a whole animal is indeed divided in two at the very first cell division.[1] As Roux's was the first scientific experiment ever to be done on any type of embryo, he is credited with being the father of the entire field of experimental embryology, which has been a cornerstone of developmental biology ever since.

Roux's results were unimpeachable, but his basic interpretation of them drew immediate concern, because it also seemed possible that the dead cell might have affected the development of the single surviving cell next to it. So, a few years later, another embryologist, Hans Driesch, working at a marine biological station in Naples, did a very similar experiment, though he used sea urchin embryos rather than frog embryos. The wonderful thing about the sea urchin embryos is that at the two-cell stage, all it takes is gentle shaking to separate them into single cells. So, in principle, there should be no effects from any neighboring dead cells. The results from Driesch's experiment were the opposite of Roux's. Instead of making half animals, each of the two cells gave rise to an entire sea urchin.[2]

Of course, Driesch's results strengthened suspicions that the presence of the dead cell in Roux's experiments might have affected his results. But it was also plausible that the discrepancy pointed to a fundamental difference in the way that sea urchins and frogs develop. Therefore, it became of major interest to know what would happen if the first two cells of a frog embryo could be fully separated and both cells kept alive. But this experiment was (and still is) extremely challenging, because the cells are not yet fully separated at these stages in amphibian

embryos. Nevertheless, in 1903, Hans Spemann of the University of Würzburg managed to succeed in doing so by fashioning a tiny noose from a fine hair of his newborn baby's head. He positioned the noose between the two cells and began, ever so slowly, tightening it, little by little, minute by minute, with amazing steadiness of hand. When the noose was fully tightened, the two cells fell apart from each other, both alive. In many instances, both these cells formed a whole embryo.[3] It seems that Roux's interpretation of divided potency was indeed wrong and was probably an artefact of the effects of the dead cell, though the biological reason for Roux's results has never really been further investigated.

What about mammals? In 1959, Andrzej Tarakowski at the University of Warsaw separated single cells from a two- or four-cell mouse embryo and then placed each of them into the wombs of foster mothers. These isolated cells often gave rise to healthy baby mice.[4] Similar experiments have now been done with many other mammals. In humans, identical twins result from a single embryo spontaneously splitting into two, and though it is still not known exactly when or how this splitting occurs, the embryonic cells at the time of such splitting are able to make entire humans. Genetic testing of early human embryos that are fertilized in vitro (IVF embryos) is offered to couples who are at risk of carrying severe genetic abnormalities. In such a procedure, one cell of a human embryo at the four- or the eight-cell stage is removed for testing. If no obvious genetic defects are found, the remaining three- or seven-cell embryo can be reimplanted into the womb, as there is little risk that the removal of just one cell has injured the potential of the remaining cells to make an entire human being. So the results are often happy ones. Thus, the embryonic cells at this stage are said to be "totipotent": capable of making it all.

Genesis of the Brain

Written in our genes is an eons-long history of the human brain's evolution. The information there is used to reconstruct an entirely new brain in every single baby. Each of us begins life as a tiny egg, a single cell smaller than a grain of table salt. The cell, like that of its evolutionary ancestors all the way back to the dawn of cellular life 4 billion years ago, is surrounded by a membrane and contains a nucleus. Inside the nucleus of the egg cell are the instructions for making an entire human being. A sperm cell, carrying its own set of complementary instructions, finds the egg and pushes itself inside. With a copy of the genome from each parent, the fertilized egg starts to divide. First, it makes two cells. Two cells become four, then eight, and so on. Soon there is an embryo composed of thousands of cells. Each of these cells contains a nucleus, and each nucleus has access to the full set of instructions.

Some of the instructions for making the brain came from single-cell organisms of the Proterozoic eon.[5] These protozoans sensed their local environment and responded accordingly. They did not have brains themselves—but they had the makings of brains. Many modern protozoans are excitable and motile; they search for food and mates, they adapt to new situations, they store memories of events, and they make decisions. Modern single-cell creatures, such as paramecia, are relics of this ancient eon that preceded the origin of multicellular animals by at least a billion years. When a paramecium swims into a wall, it reorients and heads off in a new direction. It is the synchronized beating of the thousands of tiny cilia all over its body that propels the paramecium forward. The mechanical stimulus caused by the bump opens calcium channels in the paramecium's cell membrane. An electrical current carried by calcium ions begins

to flow through these channels, and this current changes the voltage across the membrane. Other calcium ion channels in the cell's membrane are sensitive to this voltage change, and they open in response. The opening of these voltage-sensitive channels allows even more calcium to flow across the membrane, which changes the membrane voltage further and opens yet more channels. This explosive electric feedback is the essence of a neural impulse of the kind used by the neurons in our brains, except that neurons tend to use sodium ions rather than calcium ions to generate an impulse. What this electrical impulse does for the paramecium is to let calcium ions enter instantly all over the membrane, which leads to the simultaneous disruption of the beating of the cilia of the paramecium, causing it to tumble. When the cell recovers, it is heading in a new direction. The paramecium's channels that are activated by mechanical deformation and those that are activated by voltage are evolutionarily related to the channels found in the neurons of all animals. It seems that many properties that are characteristic of the brain were already encoded in the DNA of our single-cell ancestors. How they got these neural-like properties lies buried even deeper in the early evolution of life on earth.

Protozoans like paramecia have many specialized functions located in distinct compartments of the cell, such as a digestive system, a respiratory system, cilia for motility, a nucleus to carry key information accumulated since the origin of life itself, and an excitable membranous skin capable of making rapid alterations in behavior. Protozoans must do all this, and much more, in a single cell. With the rise of multicellular animals, cells could specialize and divide the labor. A brain is a collection of neurons that communicate with one another using synapses. Nervous systems with real neurons and synapses did not arise, and could not have arisen, until multicellular life began. Jellyfish are

members of a phylum of animals called the cnidarians that arose around 600 million years ago. Cnidarians have networks of interconnected neurons that share many characteristics with the neurons of the bilaterally symmetric animals (aka bilaterians) like us. Bilaterians also arose at one of the earliest of branch points on the tree of multicellular animal life. Cnidarians and bilaterians may have evolved neurons and synapses independently, but it is equally likely that these attributes evolved once in a common ancestor to both groups. The first vertebrate animals arose more than 450 million years ago. These early vertebrates are most related to today's lamprey eels. Lampreys not only have neurons like ours, but they also have a similar layout of the nervous system including a brain with the anatomical and functional beginnings of the cerebral cortex, the region of the brain that is so greatly expanded in humans.[6]

Finding the Neural Stem Cells

When, where, and how do neurons first arise in an animal? About 3.5 billion years ago, single-cell organisms were sometimes joining together to become simple multicellular life forms, which could then afford to divide tasks among themselves. In the multicellular life form known as a human, cells also begin to take on specific tasks. Some will build muscle and bones, some will make skin, some will make the digestive system, and so on. Those that will make the brain and the rest of the nervous system are the neural stem cells.

If you take a trip to a pond in the woods in early spring and collect some freshly laid frog eggs, one of the first things you might notice about these eggs is that they have a darker half and lighter half (figure 1.1). The darker half is known as the "animal" side and lighter half is known is the "vegetal" side. The imaginary

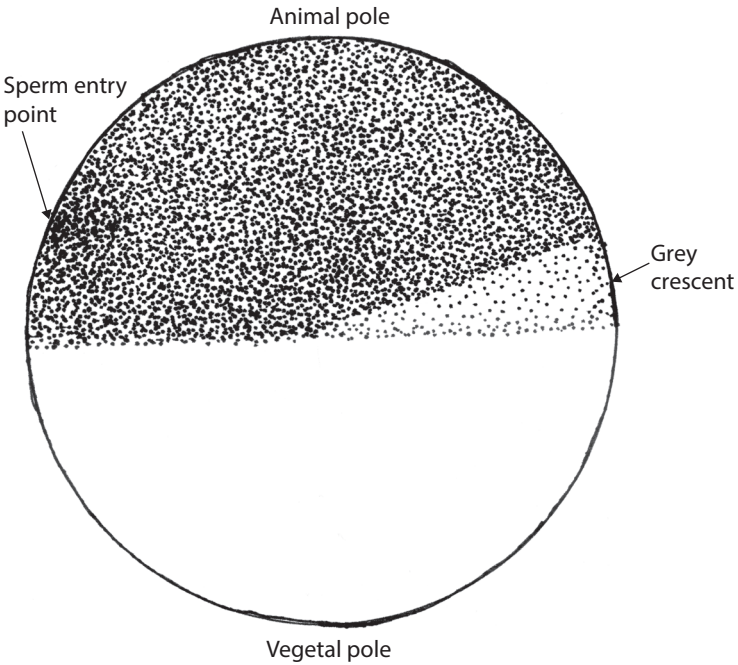

FIGURE 1.1. A frog egg shortly after fertilization. A remnant of the sperm entry point is seen in the aggregation of pigment granules there (near top of figure). The animal pole is at the top and the vegetal pole is at the bottom. The gray crescent forms opposite the sperm entry point in the animal hemisphere near the equator. The gray crescent marks the dorsal or back side of the developing frog embryo.

line from the animal pole to the vegetal pole forms the animal-to-vegetal axis of the embryo. When a sperm fertilizes a frog egg, it initiates a movement of the dark pigment granules toward the point of sperm entry. This movement leads to a lightening on the opposite side of the egg, where one can see what is known as the "gray crescent" rising like a new moon. The gray crescent is on the side of the frog embryo that will become the dorsal or back side of the future tadpole. We can now draw another imaginary line from dorsal to ventral (back

to belly). These dark, light, and gray landmarks remain until the frog embryo reaches a stage of development known as the blastula. The blastula is basically a ball of several hundred cells with a fluid-filled hollow in the middle. Human embryos reach this blastula stage about one week after fertilization.

Embryologists of the late 1800s wanted to understand how this ball of cells transformed itself into a little tadpole, so they began to follow cells that were consistently positioned at certain coordinates along animal-to-vegetal and dorsal-to-ventral axes. They stained the cells with permanent dyes and noted where the dye ended up. Such experiments are now done in embryology courses at universities throughout the world, and students in these courses discover for themselves the origins of the three great germ layers of the vertebrate embryo: the ectoderm, the mesoderm, and the endoderm (from the Greek words for outer, middle, and inner layers). The light-colored vegetal third of the blastula becomes the endoderm and gives rise to the digestive tract and its organ systems. The equatorial third between animal and vegetal poles, which contains the gray crescent on its dorsal side, becomes the mesoderm, which gives rise to muscles and bones. The dark animal portion of the embryo, known as the animal cap, becomes the ectoderm, giving rise to the epidermis and the nervous system. Students in such embryology courses often go further and find that the primordial nervous system comes from just the dorsal half of the ectoderm, the region that lies directly above the gray crescent.

The Organizer

Knowing which cells of the blastula will become the neural stem cells allowed Hans Spemann, now working in Freiburg, to devise an experiment to test whether these cells are also capable

of giving rise to other tissues or whether they have become restricted to making only the nervous system. Spemann thought of testing this by taking groups of cells from a particular position on one embryo and transplanting them to a different position on another embryo. As was his style, Spemann invented a variety of new microtools for these experiments, including incredible fine-glass pipettes with fingertip control that could be used to transfer tiny fragments of embryonic tissue carefully between embryos, and superfine scalpels to cut out such fragments. With such tools and his extreme dexterity, Spemann was able to perform precise cut-and-paste experiments on amphibian embryos. In one series of experiments, he transplanted bits of one blastula to different positions on another. When he transplanted a piece of the dorsal ectoderm from the blastula of a newt embryo (i.e., the piece of the embryo that would have become its nervous system if left in its original position) to a different position in the blastula of another newt embryo, nothing extraordinary happened. The resulting animal developed normally. It did not, for example, have an extra bit of brain tissue. The transplanted cells simply switched or ignored their previous fates and integrated beautifully into their new positions. They still appeared to be totipotent and flexible at this stage.

The breakthrough came at the next stage of development, just two to three hours later. This is called the "gastrula stage." Human embryos reach this stage at about week three of gestation, when there are thousands of cells. The gastrula stage of development begins when the cells of the mesoderm start to move into the hollow in the center of the blastula. Developmental biologists say that they begin to "involute." Imagine holding a soft balloon in your left hand; now push the fingers of your right hand into the balloon. The first mesodermal cells to involute are the most dorsal ones (figure 1.2). These are the cells of

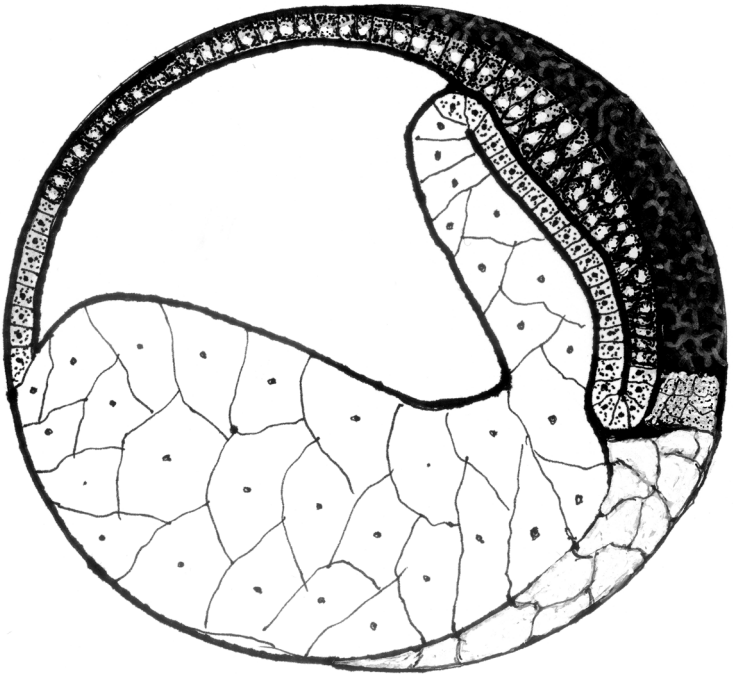

FIGURE 1.2. A cross section of an amphibian embryo during gastrulation and neural induction. The involuting mesoderm (gray stippling) is moving under the dorsal ectoderm (dark) and inducing the latter to become neural ectoderm, which can be seen thickening up as the neuroepithelium.

the gray crescent. When Spemann transplanted just a small piece of this involuting dorsal mesoderm at the very beginning of gastrulation from one donor newt embryo to the ventral side of another host embryo, something remarkable happened. Spemann was stunned! The host animal did not look normal, as happened when he did this experiment at the blastula stage. Nor did it have an extra bit of out-of-place mesoderm, as one might have suspected if the transplanted tissue had become restricted. What Spemann saw was that a whole new secondary

embryo developed in these hosts.[7] This second embryo was often joined belly-to-belly with the host embryo, like face-to-face Siamese twins!

What happens during gastrulation is absolutely critical for the organization of an embryo. Without gastrulation, any frog or even any human embryo would not have much of a body and no brain at all. This is why Lewis Wolpert, the British developmental biologist, whom will be discussed in the next chapter, often told his audiences at lectures: "It is not birth, marriage, or death, but gastrulation which is truly the most important time in your life." How to explain this incredible result in terms of cells, tissues, and biological mechanisms was Spemann's next challenge. There were two main possibilities. One was that the transplanted piece of dorsal mesoderm was still totipotent, and that the trauma of being transplanted somehow stimulated these cells to make an entire new embryo. The other possibility was that the transplanted tissue somehow induced the nearby host tissue to form the new embryo around it.

Spemann had a brilliant young graduate student, Hilde Proescholdt, who took up the challenge of disentangling these possibilities as her thesis project. It was clear that if the transplanted dorsal mesoderm grew into one of the twins by itself, then this twin would be composed of donor-derived cells. However, if the transplant somehow induced the surrounding tissue to make an embryo, then this second embryo would be composed mostly of host-derived cells. So, Proescholdt addressed this issue by using embryos of two species of newts, one that was lightly pigmented (which she used as the donors) and ones that were darkly pigmented (which she used as the hosts). The cells of the light embryos could be identified in a microscope from their lack of pigment granules. Then, just as Spemann had

done, she transplanted this special piece of the dorsal mesoderm from one early gastrula to the ventral side of another—the only difference being that this time, the donor cells were light, and the host cells were dark.

Her experiments immediately settled the issue. She found that the transplanted cells made only a minor contribution to the second embryo (figure 1.3). Most of the second embryo, including the brain and spinal cord, was made of host rather than donor cells.[8] With this one experiment, she proved that this small piece of dorsal mesoderm, taken at the beginning of gastrulation, can induce the tissue around it to make an entire embryo. Spemann said it in this way: "This experiment shows, therefore, that there is an area in the embryo whose parts, when transplanted into an indifferent part of another embryo, there organize the primordia for a secondary embryo."[9] Spemann called this tissue "the organizer." The discovery of the organizer is one of the most fundamental findings in all developmental biology.

After writing up her PhD thesis on this work, Proescholdt married Otto Mangold and moved with her husband and their new baby to Berlin. Tragically, soon after the move, a gas heater exploded in their new home. She suffered horrific burns and did not survive to see the publication of her famous thesis in 1924 nor the award of the Nobel Prize to Hans Spemann in 1935 for their joint discovery of the organizer.

The organizer region of a frog embryo is similar to what is known in a mammal embryo as the "node." The mammalian node, like Spemann's organizer, is a region of dorsal mesoderm that involutes and induces the overlying ectoderm to make neural stem cells. The node or organizer region must work in a similar way in all vertebrate animals, as the node from a chick

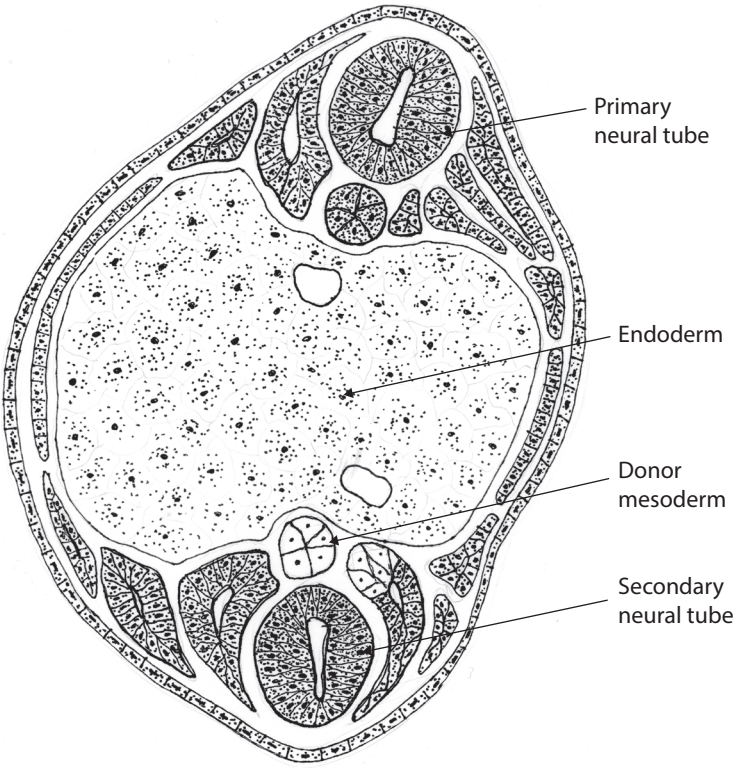

FIGURE 1.3. A result from Spemann and Mangold's 1924 experiment. Hilde Mangold (née Proescholdt) made cross sections of the pigmented newt embryos that had organizer transplants from unpigmented donor embryos. What she often saw, as shown here, was the unpigmented donor mesoderm underneath the host-derived secondary neural tube.

embryo can act like an organizer when it transplanted into a frog embryo, and the node from a mouse embryo can induce a secondary chick embryo. Similar results have now been found with mouse-to-frog, chick-to-fish, fish-to-frog, chick-to-mouse, and mouse-to-chick transplants.

The Neural Inducer

As soon as Mangold (née Proescholdt) and Spemann published their findings, biologists immediately wanted to know how the organizer worked. How can a small piece of tissue orchestrate the building of an entire embryo around it? How does the organizer communicate with neighboring cells, and what does it tell them? Does it, for instance, tell some of them to make the brain? Such questions became a major preoccupation of developmental biology laboratories around the world. It was quickly discovered that the organizer tissue did not have to heal into place and involute to induce a second embryo; one could just stuff it into the hollow center of a blastula, and it was still able to induce a second embryo from the surrounding host tissue. It even worked if the organizer tissue was separated from host tissue by a piece of filter paper, so direct cell-to-cell contact was not essential. These experiments made it seem likely that the organizer was releasing some diffusible signaling molecules. The interspecies node-transplant experiments suggested that these signaling molecules were a fundamental and ancient aspect of how balls of cells become organized embryos, so there was great interest in discovering the nature of these magic molecules.

The host cells that were closest to the transplanted organizer generally became the central nervous system of the secondary embryo, so the search for the organizer substance became, in some laboratories, the search for the "neural inducer," a hypothetical substance released by the organizer that was responsible for turning totipotent cells of the blastula into the neural stem cells of the gastrula.

Some laboratories tried to find organizer substances or neural inducers through biochemical analysis of organizer tissue, but the miniscule amount of starting material stifled progress

using this approach. Other laboratories searched for other tissues that might have organizer properties; they found that bits of liver and kidney were capable of acting like the organizer if they were stuffed inside a blastula. But after a while, it became clear that just too many different tissues had neural-inducing capabilities. In 1955, a disheartened Johannes Holtfreter, one of those on the hunt for the neural inducer, said in despair that "fragments from practically every organ and tissue from various amphibians, reptiles, birds and mammals, including man, were inductive."[10] Even random chemicals off the laboratory shelf were sometimes inductive. It seemed the problem was that the animal cap cells of newt embryos were somehow poised to become neural, so finding the thing that normally induced them was going to be a huge challenge. As a result, the hunt for the real neural inducer went cold for decades.

A small digression is now warranted. In 1927, a British endocrinologist, Lancelot Hogben, relocated to rural South Africa and found himself surrounded by multitudes of claw-toed frogs, known as *Xenopus*. Hogben immediately took advantage of their abundance for his research on hormones. He injected adult female *Xenopus* with an extract from the pituitary gland of an ox, and to his astonishment saw that the injected frogs soon started laying an abundance of eggs. Hogben knew that the urine of pregnant women also carried some pituitary hormones, so he and his colleagues tested the effects of injecting concentrated urine from potentially expectant mothers into adult female *Xenopus* and found that egg-laying predicted pregnancy very accurately. As a result, *Xenopus* became used for pregnancy tests throughout the world until the 1960s.

More important to the field of developmental biology was the fact that one could get *Xenopus* eggs on demand throughout the year, just by injecting females with hormones, rather than

seasonally, as was the case for newts and salamanders. Early in my own career, I worked with salamander embryos, so my embryological experiments were restricted to springtime. I must say, I liked the seasonal pace of the work. Later, I switched to *Xenopus* embryos, because they were so much more readily available, and work could proceed faster. The luckiest thing about *Xenopus*, however, for those who were still searching for the neural inducer, was that the animal cap cells of *Xenopus* offered a clean experimental system for a new molecular approach to searching for the organizer. If one cuts out the animal cap of a *Xenopus* embryo and puts it in a petri dish, it does not make any neural tissue, unlike the case for tissue from newts and salamanders, where even this small insult is enough to do so. The *Xenopus* animal cap, when isolated in a petri dish, becomes pure epidermis. If, however, one waits a couple hours until gastrulation is in progress and then puts these same animal caps in a petri dish, they make neural tissue. This clear change in the commitment from epidermal to neural tissue that can be seen in isolated *Xenopus* animal caps offered a new way to search for the elusive neural inducer.

Sixty-eight years passed between the first report of the organizer by Mangold and Spemann and the moment in 1992, when Richard Harland and his group at the University of California, Berkeley, taking advantage of *Xenopus* embryos and modern molecular biological strategies, announced the discovery of the first active component of Spemann's organizer.[11] It was a neural inducer. Harland and colleagues called the protein they had discovered "Noggin," which is slang for "head." Noggin is made and secreted by cells of the organizer and it is able to directly induce totipotent embryonic stem cells to become neural stem cells.

The Secret of Neural Induction and Growing Human Mini-brains

Most developmental neurobiologists, including me, assumed that when they were eventually discovered, neural inducers would turn out to be molecules that instruct cells to become neural stem cells. So, we figured, this was probably what Noggin was doing. But this assumption was wrong. This kind of thing often happens in biology. You are biased to suspect that something works one way, but it turns out that it works in almost exactly the opposite way. So it was for neural induction. The first part of this reversal of general expectations came from Doug Melton's laboratory in the Department of Biochemistry and Molecular Biology at Harvard University. Melton was searching for a signaling protein that, when applied to animal caps of *Xenopus* embryos, turned them into mesodermal tissue: muscle and bone. They had narrowed down their search to a class of signaling proteins. A postdoc in Melton's lab, Ali Hemmati-Brivanlou, found a way block the reception of this potential mesoderm-inducing signal. As he and Melton hoped, the animal caps of embryos that were treated in this way did not become mesoderm even when exposed to the mesoderm-inducing signal. But the thing that came as a surprise to everyone was that these animal caps became neural just like they did when they were exposed to neural inducers like Noggin.[12]

This new result raised what seemed like a shocking possibility: Noggin might *not* be instructive; it might *not* induce cells to become neural. Instead, it might simply stop them from becoming something else. Indeed, this turned out to be the case. There is a signal that percolates through the animal cap telling cells to become epidermal. Noggin works by blocking this signal.

Noggin is not instructive; it does not tell cells to become neural stem cells; it simply prevents them from becoming epidermal. So the simple secret of "neural induction" is that the term "induction" is completely inappropriate, because inducing cells to become neural is exactly what the neural inducer does not do. The cells will become neural stem cells by default as long as the "neural inducer" prevents them from being induced to become epidermal.

Neural inducers like Noggin (several others were subsequently found) are now known to work by blocking a set of signaling molecules known as bone morphogenetic proteins (BMPs).[13] BMPs are secreted proteins that induce ectodermal cells to become epidermal. BMPs were named for their ability to induce the formation of bone, but they have since been found to have effects throughout the body, especially in early development. The mechanism by which Noggin and other neural inducers block BMP signaling is simple. They disguise themselves as receptor molecules for BMPs, and they sponge up all the BMPs that are floating around nearby, thereby preventing BMPs from finding their true receptors. Cells that are not in the vicinity of the organizer, however, are not protected by these BMP sponges, and so they receive a dose of BMP signal that results in their turning on genes that commit them to an epidermal fate. Epidermal cells make even more BMPs and release them onto their neighbors, creating a wave of epidermal induction that spreads across the whole of the animal cap, turning cells into epidermal stem cells. Were it not for the molecules of Noggin and other anti-BMPs protecting some of these cells from being influenced by the spreading wave of BMP, there would be no nervous system, no brain. Anti-BMPs like Noggin are released from the nodes of bird and mammalian embryos, which is why nodes are able to induce neural tissue across species boundaries.

That all vertebrate animals use the same basic molecular mechanisms to generate neural tissue raises the possibility that these mechanisms predate even the origin of vertebrates. At the beginning of the eighteenth century, the French naturalist Étienne Geoffroy Saint-Hilaire emphasized a fundamental similarity among all animals. He noted, as many others had before him, that all animals are composed of essentially the same organs and parts. All animals have digestive systems, circulatory systems, secretory systems, musculoskeletal systems, outer coverings (skin or cuticle), nervous systems, and so forth. The systems may look different in a worm, a fly, a squid, and a human, but they each have all these parts.

A possibly apocryphal story is that, at a dinner party where lobster was served, Saint-Hilaire entertained his dinner guests by observing that the cooked invertebrate animals lying on their backs on the dinner plates looked remarkably like vertebrates in some ways. In a right-side-up lobster, the nervous system is ventral, and the organs of the digestive system are dorsal, opposite to the case in vertebrates. So the upside-down lobsters had the same arrangement of parts as a right-side-up vertebrate. This speculation became known as Saint-Hilaire's inversion hypothesis. The inversion hypothesis was ridiculed and then ignored over the course of the next 150 years. Then, in 1996, a reexamination of the inversion hypothesis was triggered by a study by Ethan Bier, working at the University of California, San Diego. Bier discovered that the fruit fly embryo expresses a BMP dorsally and anti-BMPs ventrally.[14] He showed that blocking BMP signaling ventrally is necessary for the nervous system to form there. It is the same molecular logic as in vertebrates but just inverted, flipped belly-to-back. The resurrection of Saint-Hilaire's inversion hypothesis has led evolutionary biologists to seriously entertain the possibility of a "flip" that led to the

origin of vertebrate animals in the Cambrian Period, about a half billion years ago.

In 2012, John Gurdon shared the Nobel prize with Shinya Yamanaka for their work showing how almost any cell in the body could be reprogrammed to become more like a totipotent embryonic stem cell. The ability to reprogram cells to this embryonic state means that we can now clone animals. Gurdon was the first to clone a new animal from the nuclei of an adult.[15] It was a claw-toed frog, a *Xenopus*. Since then, sheep (Dolly), horses, cats, dogs, and monkeys have been cloned. In the futuristic comedy "Sleeper," a botched attempt was made to clone the great leader from some surviving cells from in his nose. A few years later, workers at Columbia University were able to clone a whole mouse using a reprogrammed olfactory neuron.[16]

There has been huge excitement over the past several decades as developmental biologists have learned more about how to grow totipotent stem cells in tissue culture and how to control the differentiation of these cells, especially into different brain regions. It is now possible to remove a few cells from any human, expose them to a regime of molecular reprogramming so that they become like embryonic stem cells, and then expand these cells in tissue culture, and when there are enough of them, "induce" them to become neural stem cells by exposing them to neural inducers that block BMP signaling. In 2011, Yoshiki Sasai of the Riken Center for Developmental Biology, in Kobe, Japan, found that by using techniques learned from developmental biology, he could induce embryonic stem cells to form layered neural structures, such as the retina and cerebral cortex.[17] Sasai was a hero of mine both for his extraordinary work on the early development of the nervous system and for his breakthroughs in making neural tissues in culture. Thanks in large part to Sasai's work, scientists recognized the huge potential of using such

strategies to study human development and disease. Sadly, we lost Sasai, because a postdoc in his laboratory sought instant fame by publishing a simple way to reprogram adult cells by dipping them briefly in an acidic solution. As the postdoc expected, his papers made headlines, but other labs could not reproduce the results, and an internal investigation by the Riken Center found out why they could not: The postdoc had made them up! Though Sasai himself was cleared of having any involvement with the phony data, he was held responsible for a failure of oversight. Sasai was deeply ashamed, he became depressed and committed suicide just six months after the publication of the papers. What a loss! A few years later, the now tried-and-tested biochemical methods that Sasai helped develop are regularly used to reprogram cells in many labs and hospitals. Cells from patients with genetically caused neurological disorders are being used to make microscopic mini-brains that float around in a petri dish. These miniature bits of brain often display similar problems to the patient, speeding medical progress.[18]

Though it is so exciting to be able to make and study mini-brains in a dish, only the neural stem cells inside a human embryo can make an entire human brain. It is the next step in multi-generational stories of these primordial neural stem cells and their descendants that we follow in chapter 2.

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