Preface

During fertilization, the genetic contents of the egg and sperm, each carrying half of the genetic material of the mother and father, respectively, are united in a single cell. This unicellular embryo will give rise to a complete multicellular organism, with its enormous complexity of tissues and multiple types of cells that will appear different from one another and carry out diverse and distinct roles. All the information for this elaborate process is carried within the DNA of that initial single cell that was formed on fertilization, and all cells of the embryo will contain exactly the same genetic information (fig. 1).

The long-standing human desire to understand the instructions and rules of forming an embryo and to unravel "life's blueprint" stems from many sources. First and foremost, we want to understand how we as humans, and all other organisms, build our complex bodies. How do genes shape animals? What are the instructions? How are they carried out in such a reliable and reproducible fashion? Can we use this knowledge to better understand pathological situations in which the genetic program goes awry? And, thinking to the future, can we use the information gained in order to instruct cells to generate tissues and organs that could be used as "spare parts"?

As we set out to explore these fundamental questions, more specific and operational issues come to mind. Are cells preprogrammed by the genome to generate distinct cell types, or do cells have a choice of assuming different fates? How do they decide which fate to choose from the arsenal of tis-

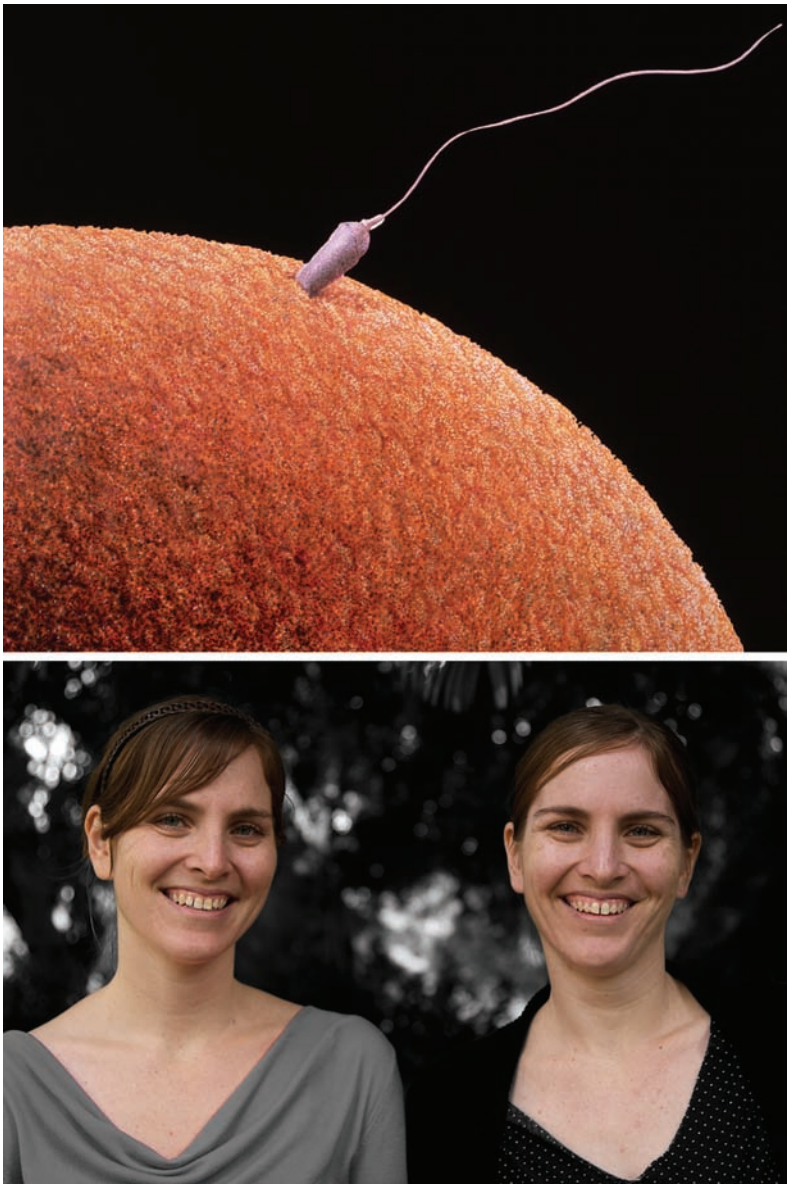


Figure 1. All cells in our body carry identical genetic information
Upon fertilization, the nuclei of the sperm and egg join, and the first cell of the embryo is formed. Throughout the numerous divisions that will follow, all descendants will contain the same genetic legacy. This information dictates the shape of the embryo, as indicated by the fact that identical twins carrying the same genetic material are so similar.

Top: Scanning electron micrograph of fertilized human egg (F. Leroy/Science Source); *bottom:* Identical twins (B. Shilo, Rehovot, Israel)

sues they are capable of forming? Does every species have its own rules and logic of embryonic development, or, alternatively, can we identify common themes among disparate species? The scientific discoveries of the past three decades have revolutionized our knowledge and understanding of embryonic development in all multicellular organisms. This book conveys these striking findings and the emerging rules of executing development.

The analysis of embryonic development relies strongly on microscopic images that we obtain and analyze. These images include fluorescent multicolor depictions of different embryonic tissues, scanning electron microscope images, and movies of live tissues and embryos showing dynamic processes in real time. The images are not only highly informative but often also aesthetically striking. Their visual beauty is often central in attracting many researchers to the field.

For me, these visual scientific stimulations are combined with another personal passion. Since my high school years, I have been interested in photography and how we view and depict our surroundings. In my early work, I felt more comfortable photographing still objects and how they interact with light. In the past fifteen years, as a result of frequent trips to foreign destinations, including the countries of the Far East and India, and possibly also as a consequence of a more mature view of the world, I have concentrated on photographing people. I try to portray universal aspects that unite people of all races and ages, as well as the unique features of individuals in their surroundings.

To visually present the emerging concepts of embryonic development in this book, I have assembled scientific images of diverse organisms that display the central concepts of embryogenesis from the laboratories of colleagues and from my own lab. Each of these images is paired with an image from our "macro" world taken by me, highlighting a similar theme and reflecting my personal metaphors. In seeing these juxtaposed images, I hope that readers will be able to understand each paradigm in an intuitive way, letting our everyday experiences and recollections resonate with and inform our comprehension of the scientific image.

It is interesting to note that the inception of the word *cell* as a biological term was formulated by Robert Hooke in 1665 and was based on a visual analogy to the macro world. Hooke looked at the bark of a cork oak using


B
A

a microscope he developed. He saw the regular shapes of the nonliving cell walls and noted that they resemble the cells in which monks live. I envisage that through the pictorial analogies, the reader will "feel" like a cell within the developing embryo (fig. 2).

The goal of this book—to present a coherent and simplified description of the complicated process of embryonic development—has forced me to define to myself the true essence of each aspect of life's blueprint. The juxtaposition of metaphors from the human world with these biological processes has also required a continuous examination of each concept in order to crystallize its essence and employ the most appropriate analogy. Finally, my efforts to present a global view of embryonic development have mandated a critical evaluation of the relative importance of each concept from a broader perspective. As a scientist, my work involves delving deeper and deeper into defined biological problems. Stepping back and viewing the entire scope of embryogenesis in a global manner has been both refreshing and illuminating.

◀ *Figure 2. Analogies between cells and the human world*
From the inception of the word by Robert Hooke in 1665, the cell has been defined by analogy to the human world. Hooke coined the word *cell* because he felt that the structures he saw in his microscope resembled monks' cells. In this book I will be making analogies between the environment of cells and the human world. I would like the viewer to "feel" like a cell as it participates in forming the complex structure of the embryo by relating this process to visual and conceptual similarities in the human world.

Top: Drawing of cork oak bark by Robert Hooke in *Micrographia; bottom:* Child and cell (B. Shilo, Exploratorium, San Francisco)

CHAPTER 4

How Cells Talk and Listen to Each Other

In the previous chapter, I discussed the implications of Spemann and Mangold's experiment of 1924, which revealed that cell fates are not predetermined during development. In the course of deciding what they will become when they "grow up," cells actually communicate constantly with one another. In other words, a cell is not oblivious to its environment as it carries out its destiny during development; it relies on the environment to receive continuous instructions. This feature resembles the contrast between the old class system, where a person's fate and occupation were determined from birth, and today's modern society in which we trust that a person's qualifications and interactions with his or her environment will shape the individual's ultimate destiny.

The implications of this notion for our perception of embryonic development cannot be overstated. To understand embryogenesis, we have to discover those instructions that cells are continuously transmitting to and receiving from one another. We must be able to comprehend the language of the cells. It means that the information in the DNA defines the final outcome only by setting the rules of interactions among cells, but there is no micromanagement.

By leaving the decisions open to the cells, the complicated process of development is further protected from fluctuations and perturbations. If each cell had a predetermined destiny, then a possible loss of any given cell could lead to dramatic patterning defects. In contrast, if cells have a wide range

of fates they can assume, other cells can replace the loss of a given cell. This characteristic is similar to the declaration that there is no person who cannot be replaced.

As dramatic as Spemann and Mangold's experiment was (it is hard to be more dramatic than growing a second head on a newt embryo), its conclusions were left hanging for more than five decades. This gap occurred because, although the existence of cell communication whereby cells of one type instruct their neighbors (induction) was recognized, the agents that mediate this interaction remained unknown. The molecular components of cell communication could not be isolated biochemically. Despite the biological importance of these agents, many of them could be present in minute amounts, and the biological assays for their activity are not straightforward. In other words, without any clues as to their nature, it is impossible to fish out the specific components of cell communication from a mixture of all the elements of the cell. We can compare the situation to grinding up a cell phone in a blender. Without any previous knowledge, would you be able to identify the critical memory chip in the final mixture?

Using genetic tools, scientists eventually uncovered the pathways by which cells communicate. The genetic makeup of an organism is called the genotype, and it dictates the organism's final shape, or phenotype. Genetic analyses depend on the ability to generate and study mutations in genes. Because the sequence of DNA dictates the structure of the proteins that will form, it follows that a change in the sequence of a gene, arising from a mutation, should lead to a parallel modification in the structure of the protein. Depending on the nature of the change, this alteration can be subtle (like a single misspelled letter in a sentence) or drastic, leading to the formation of a nonfunctional protein. When mutations in the DNA modify a critical protein, an alteration in the appearance or functionality of the organism (leading to a new phenotype) is likely to follow. The classic example is the first mutation isolated by Thomas H. Morgan in 1910 in the fruit fly, *Drosophila*, in which an alteration in a single gene converted the color of the eye from red to white.

The isolation of this mutation, termed "*white*" after the phenotype it generates, exemplifies the logic of uncovering the genetic basic for biological processes. This mutation appeared randomly in a large population of flies and could be readily followed genetically over subsequent generations of

Figure 9. Genetic mutations reveal the hidden function of genes
When genes are removed, the defects that arise make the missing gene function apparent. Similarly, after a leaf imprints wet cement, its missing presence is recorded (B. Shilo, Hampi, India)

flies by virtue of the distinct eye color phenotype. To this day hundreds of research groups use this mutation in their research. The phenotype indicates that the gene plays a crucial role in eye pigmentation, since in its absence, no pigmentation is observed. The subsequent analysis of the *white* gene indeed uncovered the molecular basis for the production and transport of pigments to the eye. In other words, the phenotype arising from loss of a gene marks the gene as a central player in that process (fig. 9).

Consider, for example, the game we used to play as children in which one person initiates motions and the other people in the room imitate this person. If you step into the room, you do not know who initiated the motions you are observing. So, at random, you ask one person to leave the room. If the others continue their motions, obviously the person you selected was not the leader, and you go on to ask another person to leave the room. Only when the initiator leaves the room will the motions stop, and this will indicate that person's role.

But if we don't know beforehand which genes and proteins are relevant for the process under study, how do we generate mutations in these genes? The answer is that from the outset we don't target the mutations to distinct genes. Instead, we use the phenotypes that arise from the loss of or from defects in genes as the basis for identifying the genetic circuitry that dictates a particular biological process. The tissue that we study (for example, the coloration of the fruit fly eye or the shape of its wings) will define the genes that we will eventually select for further analysis. We can thus inactivate genes randomly in what we call a "genetic screen" and then follow up on the consequences by choosing the tissue or stage where we want to look for the arising phenotypes.

How is a genetic screen carried out? We can introduce mutations into the genome of an organism by randomly exposing it to ionizing radiation or chemicals that alter the fidelity of the DNA during replication. This "insult" to the genome will also alter the DNA that is carried by sperm and eggs. If we take the offspring of the mutated animals, we are stably transferring a given random mutation to all cells of its progeny, through the mutagenized DNA of sperm or egg. Such mutations can then be maintained in a stable manner, since they will always be transmitted to the next generation.

As you'll recall, the genome of multicellular organisms contains two complete copies of each chromosome, one derived from the sperm and the other from the egg. Thus, even if an essential gene is mutated and inactivated, in most cases its normal counterpart will remain active and "fill in" for the missing gene. In other words, cells and organisms can appear perfectly normal even when they carry deleterious mutations in one of their genes.

When a sufficiently large number of independent mutations are examined, the screen should identify most genes that are involved in the process at hand. We define such as screen as saturated. Let's return to the example I gave earlier of identifying the person who is the initiator of motions. Suppose there are twenty people in the room. If you ask one or two to leave, your chances of identifying the key person are slim. But if you ask all twenty people to leave, one at a time, you are bound to identify the initiator. You have "saturated" your screen because you tested all possible options.

It is important to highlight the open state-of-mind in which a researcher initiates such a genetic screen, especially when a complicated biological pro-

cess is being examined. The process is too complex for us to have a preconceived notion that is likely to bear any resemblance to how the real mechanism actually operates. So the scientists carrying out a genetic screen should be unbiased and allow the mutations they isolate to reveal the story of how a given process is executed.

In the late 1970s, Christiane Nüsslein-Volhard and Eric Wieschaus carried out such a mutagenic screen using fruit flies. They generated a collection of about eighteen thousand fly strains, each harboring one or more mutations in its genome. We now know that flies have about thirteen thousand genes, so this collection most likely included representative mutations in most of these genes. Using this collection, they then carried out a genetic screen. The goal Nüsslein-Volhard and Wieschaus set for themselves was extremely ambitious, because they wanted to identify the genes responsible for generating the embryonic pattern. Fruit flies represent a simpler model organism, and their process of embryonic development takes only twenty-four hours. Yet by the time embryogenesis is completed, the embryo contains fifty thousand cells, representing the full complement of differentiated tissues. In recognition of their seminal findings, they were awarded the Nobel Prize in 1995.

Within the library of mutants, every strain carries one normal and one mutated copy of the gene that was randomly modified. But when males and females from the same line are mated with each other, some of their progeny will contain two copies of the defective gene. In cases where this gene is essential, no viable progeny will emerge from this combination. Genes can be indispensable because they mediate a host of processes, such as metabolism or structural roles within the cell. But in the context of the genetic screen for developmental mutants, the researchers' focus was on genes involved in the determination of embryonic patterns.

In normal fruit fly embryo development, the skin cells of the embryo are patterned so that they outline structurally distinct domains, or segments. Each of these segments displays unique structures, according to its position within the embryo, in both head-tail and belly-back axes. The skin cells secrete a hard external protective layer, or cuticle, which we can think of as the outer skeleton of the embryo. The cuticle is composed predominantly of the nonliving substance chitin and is thus highly resilient and stable. Each cuticle's structure manifests the distinct properties of the cells that were re-

sponsible for its secretion. And it was these stable cuticle "shells" that helped crack the puzzle of embryonic development (fig. 10).

Even when mutant embryos die and disintegrate, their external cuticles remain stable and resilient. The researchers could collect the cuticles and analyze them to reveal the processes that went awry in the absence of a given gene, such as a missing tail or a duplicated head.

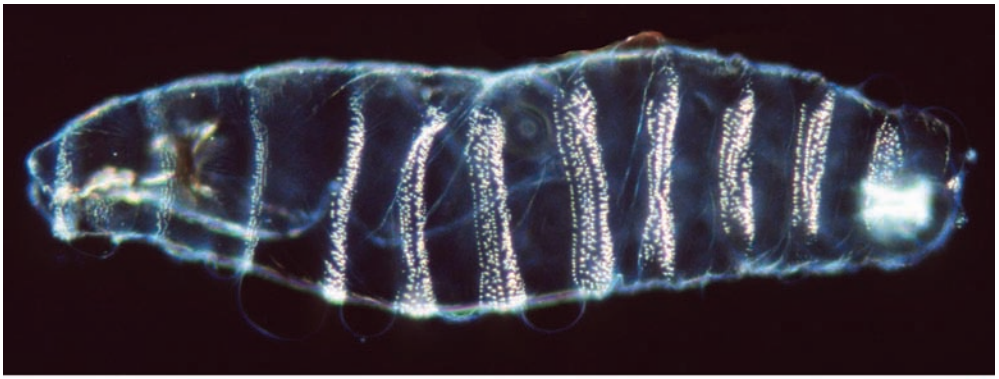
At the outset, it was not clear how many of the thirteen thousand genes in the genome of the fly are dedicated to determining the intricate embryonic pattern. The astonishing revelation was that mutations in only about two hundred genes gave rise to patterning defects. Since most of the genome had been screened, the obvious conclusion was that only a small number of genes is responsible for determining the complex structure and shape of the embryo. Furthermore, in many cases, mutations in different genes resulted in very similar defects, allowing this class of mutants to be grouped as players in the same process. In other words, the implication was that they act sequentially to carry out the same process.

Imagine the intricate process by which oranges are grown, harvested, and reach the supermarket shelf. Any defect along the way, from lack of adequate water and other growing conditions to mechanical problems in the truck transporting the oranges to the store will result in the same outcome: No oranges on the shelf! Each step thus defines an essential link in the same global process. Genes whose absence results in similar defects represent different steps in the same biological process. When these genes were subsequently isolated, the structure of the proteins they encode could be readily

Figure 10. The genetics of the fruit fly uncovered the molecular language of cells

The outermost cell layer of the fruit fly embryo secretes a hard external cover or "shell" that is patterned according to the fate of the cells that secreted it. By screening eighteen thousand fly strains carrying random mutations in their genome, researchers identified mutations that give rise to patterning abnormalities. These mutations defined the genes encoding the proteins that mediate cell communication during development. Although the outer layer is only a "shadow" of the real embryo, it provided the clues for most of the components that comprise the blueprint for making the embryo.

Top: External "shell" of a fruit fly embryo (R. Schweitzer and B. Shilo, Weizmann Institute); *bottom:* Shadows can reveal the deeper underlying story (B. Shilo, Israel Museum, Jerusalem)



decoded. In many cases, these structures were extremely revealing, because they provided clues to the location of a given protein within the cell and how it might function at the molecular level. It indicated that these proteins may represent the molecular means by which cells communicate with one another. The mechanism Spemann postulated had now been validated and dissected. The "language" of cell communication was at hand.

How do we envisage this communication at the molecular level? Every cell is surrounded by a membrane, which is composed of multiple units of fatty acids, each consisting of a long chain of carbon atoms. The separation between the fatty acids and the aqueous environment of the cell defines the membrane as a distinct border between the cell and its surroundings. The membrane allows the cell to maintain a unique intracellular environment by selectively concentrating a host of substances within the cell and excreting others out of the cell.

Specific proteins are embedded within this membrane and fulfill a wide range of functions. The class of molecules relevant for our discussion is termed receptors. These receptors are responsible for the transmission of information across the cell membrane. The receptors are embedded within the membrane and protrude into the extracellular environment, where they contact hormone-like proteins that were secreted by other cells. Once contact between the receptor and the protein is established, the receptor's structure is altered. This happens not only in the external part where it associates with the hormone but also in the internal part that extends into the cell.

How is this change now transmitted all the way to the cell nucleus? Proteins can bind together, to alter their structure, stability, or location within the cell to form signaling pathways. In "relay race" fashion, these chains of interacting proteins transmit to the nucleus that the receptor was activated. In other words, a chain of interacting proteins transfers information from the membrane to the cytoplasm and then to the nucleus (fig. 11).

Although each signaling pathway has a similar goal, each uses a different set of proteins and distinct "tactics" to achieve it. In the signaling process many levels of modulation and regulation exist. Signals can be dampened as they are transmitted within the cell, or conversely they can be amplified when one protein activates many other proteins. One way of picturing the transmission and alteration of signals is Norman Rockwell's humorous and

Figure 11. The mechanism of cell communication
Cells receive signals from neighboring cells through their external environment. A chain of proteins transmits the information through the cell membrane all the way to the cell nucleus to induce expression of critical target genes. Such protein cascades function as a "bucket brigade," here illustrated by a Playback improvisational theater group, where actors transfer an imaginary object from one to another, modifying it in the process (B. Shilo, Cambridge, Massachusetts)

folksy illustration *The Gossips*. Rockwell depicts a rumor being whispered and transmitted from one person in town to the next. By the time it comes back to the initiator of the rumor, she (in this case) can no longer recognize the original message, which has been altered and modified along the way.

Having discovered signaling pathways through the genetic screens, the researchers had two additional surprises in store. First, they learned that only a handful of signaling pathways by which cells communicate with one another operate throughout the embryo's development. Five of them are very prevalent and are used repeatedly at consecutive decision junctions; three others are less prominent. Each pathway has distinct components and uses different molecular modes. Yet they all relay information across the membrane and into the nucleus in order to alter the pattern of gene expression.

Second, they found that these pathways are highly conserved in evolution, most being shared among both simple and complex organisms. This means that cells in all multicellular organisms communicate using the same language and that this is also the language used by their common ancestors throughout evolution. The discovery of shared signaling pathways used by such a diverse array of organisms, from nematode worms and fruit flies to humans, changed the basic culture of scientific research. From segregated communities of scientists, each focusing on a different organism, it suddenly became apparent that everyone is actually studying the same molecular system. Processes as diverse as eye development in flies and human cancer are actually using exactly the same machinery. The universality of signaling fostered a broader, more global approach that scientists are now applying to biology.