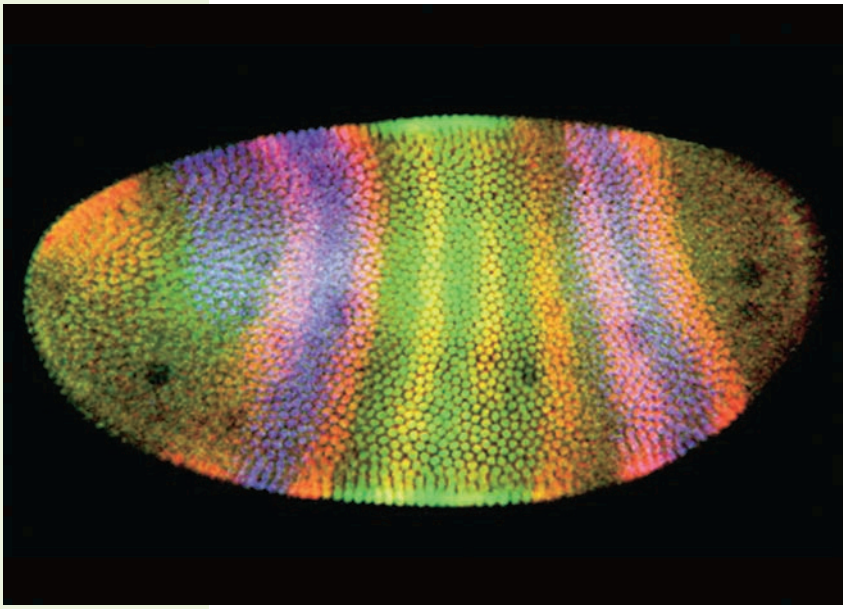
17 Developmental Genetics



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Differential gene expression in the model organism *Drosophila melanogaster.*

KEY CONCEPTS

17.1 Differential gene expression controls the development of specialized tissues and organs.

17.2 Many genes that regulate development are quite similar in a wide range of organisms, from fruit flies to humans. Mutations in genes that regulate development have provided insights into how those genes function.

17.3 Mutations in oncogenes and tumor suppressor genes may lead to cancer.

Developmental genetics is the study of the genes involved in cell differentiation and development of an organism. Until the late 1970s, biologists knew little about how genes interact to control development. Unraveling the genetic interactions that take place during development was an intractable problem using traditional methods. However, rapid progress in recombinant DNA research led scientists to search for developmental mutants and to apply sophisticated techniques to study them. (A *mutant* is an individual with an abnormal phenotype caused by a gene mutation.)

The organism in the photograph is a developing embryo of the fruit fly *Drosophila melanogaster.* Geneticists used **immunofluorescence,** in which a fluorescent dye is joined to an antibody that binds to a specific protein to localize the protein. In this case researchers bound three different antibodies—one red, one blue, and one yellow—to three specific proteins. The patterns of colored bands indicate that different cells of the embryo have *differential gene expression*—that is, different genes are active at the same time.

Work with *Drosophila* and other organisms has profound implications for understanding both normal human development (including aging) and malfunctions that lead to birth defects and cancer. Striking similarities among genes that govern development in widely different species suggest that developmentally important genetic mechanisms are deeply rooted in the evolutionary history of multicellular organisms. There are

also differences in species' developmental patterns that reflect their separate evolutionary paths. As you will learn in Chapter 18, developmental genes have played a role in reshaping organisms during the course of their evolution.

Biologists are now studying how genes are activated, inactivated, and modified to control development. Eventually, scientists expect to understand how a single cell—a fertilized egg—develops into a multicellular organism as complex as a human.

17.1 CELL DIFFERENTIATION AND NUCLEAR EQUIVALENCE

LEARNING OBJECTIVES

1. Distinguish between cell determination and cell differentiation, and between nuclear equivalence and totipotency.
2. Describe the classic experiments of Steward, Gurdon, and Wilmut.
3. Define *stem cells*, distinguish between embryonic stem cells and pluripotent stem cells, and describe some of the promising areas of research involving stem cells.

The study of **development,** broadly defined as all the changes that occur in the life of an individual, encompasses some of the most fascinating and difficult problems in biology today. Of particular interest is the process by which cells specialize and organize into a complex organism. During the many cell divisions required for a single cell to develop into a multicellular organism, groups of cells become gradually committed to specific patterns of gene activity through the process of **cell determination.** As cell determination proceeds, it restricts an embryonic cell's developmental pathway so that its fate becomes more and more limited. The final step leading to cell specialization is **cell differentiation.** A differentiated cell, which has a characteristic appearance and characteristic activities, appears to be irreversibly committed to its fate.

Another intriguing part of the developmental puzzle is the building of the body. In **morphogenesis,** the development of form, cells in specific locations differentiate and become spatially organized into recognizable structures. Morphogenesis proceeds through the multistep process of **pattern formation,** the organization of cells into three-dimensional structures. Pattern formation includes signaling between cells, changes in cell shapes, and cell migrations. Depending on their location, cells are exposed to different concentrations of signaling molecules that specify positional information. Thus, *where* a given cell is located often determines *what* it will become when it matures.

The human body, like that of other vertebrates, contains about 250 recognizably different types of cells (**FIG. 17-1**). Combinations of these specialized cells, known as **differentiated cells,** are organized into diverse and complex structures—such as the eye, hand, and brain—each capable of carrying out many sophisticated activities. Most remarkable of all is the fact that all the structures of the body and the different cells within them descend from a unicellular **zygote,** a fertilized egg.

All multicellular organisms undergo complex patterns of development. The root cells of plants, for example, have structures and functions very different from those of the various types of cells located in leaves. Diversity is also found at the molecular level; most strikingly, each type of plant or animal cell makes a highly specific set of proteins. In some cases, such as the protein hemoglobin in red blood cells, one cell-specific protein may make up more than 90% of the cell's total mass of protein. Other cells may have a complement of many cell-specific proteins, each of which is present in small amounts but still plays an essential role. However, because certain proteins are required in every type of cell (all cells, for example, require the same enzymes for glycolysis), cell-specific proteins usually make up only a fraction of the total number of different kinds of proteins.

When researchers first discovered that each type of differentiated cell makes a unique set of proteins, some scientists hypothesized that each group of cells loses the genes it does not need and retains only those required. However, this does not generally seem true. According to the principle of **nuclear equivalence,** the nuclei of essentially all differentiated adult cells of an individual are genetically (though not necessarily metabolically) identical to one another and to the nucleus of the zygote from which they descended. This means that virtually all *somatic cells* in an adult have the same genes. However, different cells express different subsets of these genes.

Somatic cells are all the cells of the body other than **germ line cells,** which ultimately give rise to a new generation. In animals, germ line cells—whose descendants ultimately undergo meiosis and differentiate into gametes—are generally set aside early in development. In plants, the difference between somatic cells and germ line cells is not as distinct, and the determination that certain cells undergo meiosis is made much later in development.

The evidence for nuclear equivalence comes from cases in which differentiated cells or their nuclei have been found to retain the potential of directing the development of the entire organism. Such cells or nuclei are said to exhibit **totipotency.**

Most cell differences are due to differential gene expression

Because genes do not seem to be lost regularly during development (and thus nuclear equivalence is present in different cell types), differences in the molecular composition of cells must be regulated by the activities of different genes. The process of developmental gene regulation is often referred to as **differential gene expression.**

As discussed in Chapter 14, the expression of eukaryotic genes is regulated in many ways and at many levels. For example, a particular enzyme may be produced in an inactive form and then be activated later. However, much of the regulation that is important in development occurs at the transcriptional level. The transcription of certain sets of genes is repressed, whereas that of other sets is activated. Even the expression of genes that are *constitutive*—that is, constantly transcribed—is regulated during development so that the *quantity* of each product varies from one tissue type to another.

KEY POINT As development proceeds, somatic cells that previously had the potential to develop into a variety of cells become increasingly committed to a specific fate.

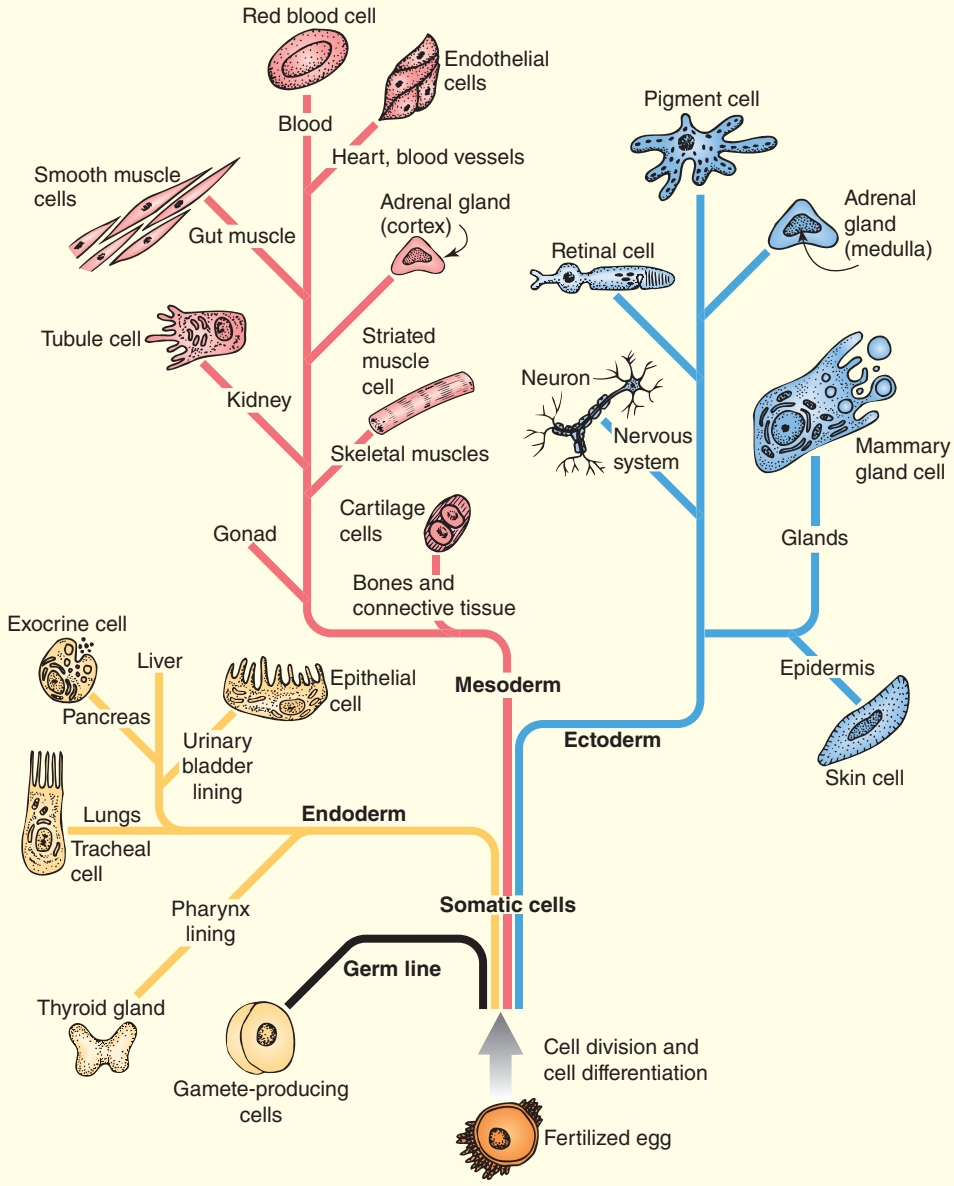


FIGURE 17-1 Vertebrate cell lineages

Repeated divisions of the fertilized egg (*bottom*) result in the establishment of tissues containing groups of specialized cells. Germ line cells (cells that produce the gametes) are set aside early in development. Somatic cells progress along various developmental pathways undergoing a series of commitments that progressively determine their fates.

We can think of differentiation as a series of pathways leading from a single cell to cells in each of the different specialized tissues, arranged in an appropriate pattern. At times a cell makes genetic commitments to the developmental path its descendants will follow. These commitments gradually restrict the development of the descendants to a limited set of final tissue types. Determination, then, is a progressive fixation of the fate of a cell's descendants.

As the development of a cell becomes determined along a differentiation pathway, its physical appearance may or may not change significantly. Nevertheless, when a stage of determination is complete, the changes in the cell usually become self-perpetuating and are not easily reversed. Cell differentiation is usually the last stage in the developmental process. At this stage, a precursor cell becomes structurally and functionally recognizable as a bone cell,

for example, and its pattern of gene activity differs from that of a neuron (nerve cell) or any other cell type.

A totipotent nucleus contains all the instructions for development

In plants, some differentiated cells can be induced to become the equivalent of embryonic cells. Biologists use *tissue culture techniques* to isolate individual cells from certain plants and to allow them to grow in a nutrient medium.

In the 1950s, F. C. Steward and his coworkers at Cornell University conducted some of the first experiments investigating cell totipotency in plants (**FIG. 17-2**). **Totipotent cells** have the potential to give rise to all parts of an organism because they contain a complete set of genetic instructions required to direct the normal development of an entire organism. Steward and his colleagues induced root cells from a carrot to divide in a liquid nutrient medium, forming groups of cells called *embryoid* (embryo-like) *bodies.* These clumps of dividing cells were then transferred to an agar medium, which provided nutrients and a solid supporting structure for the developing plant cells. Some of the cells of the embryoid bodies gave rise to roots, stems, and leaves. The resulting small plants, called *plantlets* to distinguish them from true seedlings, were then transplanted to soil, where they ultimately developed into adult plants capable of producing flowers and viable seeds.

Because these plants are all derived from the same parent plant, they are genetically alike and therefore constitute a clone. As mentioned in Chapter 15, a **clone** consists of individual organisms, cells, or DNA molecules that are genetically identical to another individual, cell, or DNA molecule, from which it was derived. The methods of plant tissue culture are now extensively used to produce genetically engineered plants because they enable researchers to regenerate whole plants from individual cells that have incorporated recombinant DNA molecules (see Chapter 38).

In the 1950s, researchers began testing whether steps in the process of determination are reversible in animal cells by transplanting the *nucleus* of a cell in a relatively late stage of development into an egg cell that had been *enucleated* (that is, its own nucleus had been destroyed). Robert Briggs and Thomas J. King of the Institute for Cancer Research in Pennsylvania pioneered *nuclear transplantation experiments.* They transplanted nuclei from frog cells at different stages of development into egg cells whose nuclei had been removed. Some of the transplants proceeded normally through several developmental stages, and a few even developed into normal tadpoles. As a rule, the nuclei transplanted from cells at earlier stages were most likely to support development to the tadpole stage. As the fate of the cells became more and more determined, the probability quickly declined that a transplanted nucleus could control normal development.

British biologist John B. Gurdon carried out experiments on nuclear transplantation in frogs during the 1960s. In a few cases he demonstrated that nuclei isolated from the intestinal epithelial cells of a tadpole directed development up to the tadpole stage (**FIG. 17-3**). This result occurred infrequently (about 1.5% of the time); however, in these kinds of experiments success counts more than failure. Therefore, he could safely conclude that at least some nuclei of differentiated animal cells are in fact totipotent.

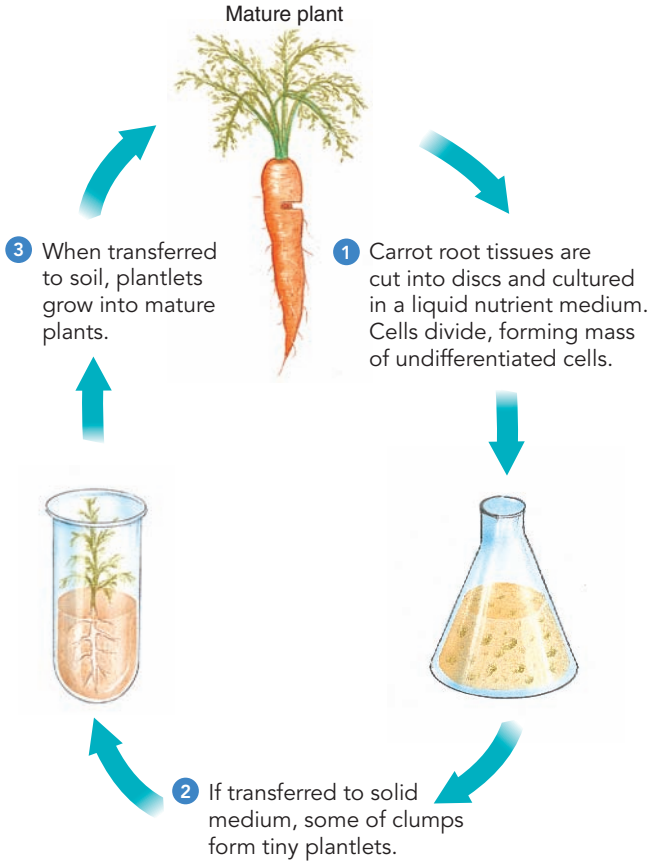
For many years, because these successes with frogs could not be repeated with mammalian embryos, many developmental biologists concluded that some fundamental feature of mammalian reproductive biology might be an impenetrable barrier to mammalian cloning. This perception changed markedly in 1996 and 1997 with the first reports of the birth of cloned mammals.

KEY EXPERIMENT

QUESTION: Are differentiated somatic plant cells totipotent?

HYPOTHESIS: Differentiated somatic carrot cells can be induced to develop into an entire plant.

EXPERIMENT: F. C. Steward and his coworkers cultured carrot root tissues in a liquid nutrient medium. These cells divided to form clumps of undifferentiated cells. The clumps were then transferred to a solid growth medium.



RESULTS AND CONCLUSION: The development of a complete carrot plant from differentiated somatic cells demonstrated the totipotency of these cells.

Source: Shantz, E. M., and F. C. Steward. "Investigations on Growth and Metabolism of Plant Cells VII: Sources of Nitrogen for Tissue Cultures under Optimal Conditions for Their Growth." *Annals of Botany,* Vol. 23, 371–390, 1959. By permission of Oxford University Press.

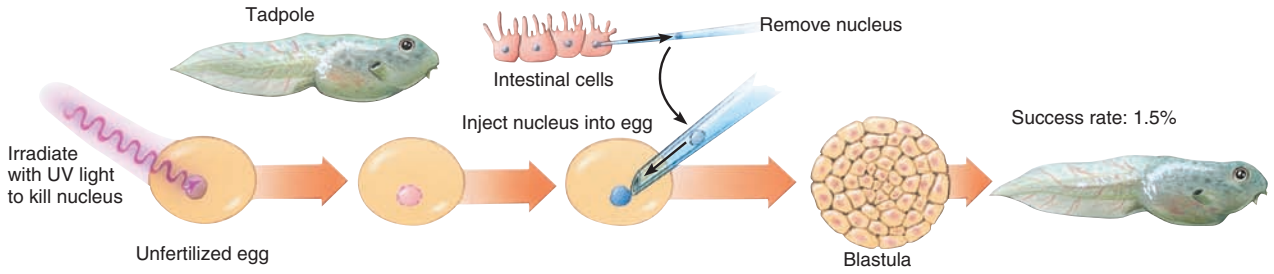
FIGURE 17-2 Steward's experiment on cell totipotency in carrots

KEY EXPERIMENT

QUESTION: Are nuclei in differentiated animal cells totipotent?

HYPOTHESIS: Nuclei from differentiated cells contain the information required for normal development.

EXPERIMENT: John Gurdon injected the nuclei of differentiated cells (tadpole intestinal cells) into eggs whose own nuclei were destroyed by ultraviolet radiation.



RESULTS AND CONCLUSION: Normal development proceeded to the tadpole stage in about 1.5% of trials, indicating that the genes for programming development up to that point were still present and could be appropriately activated.

Source: Gurdon, J. B. "The Developmental Capacity of Nuclei Taken from Intestinal Epithelium Cells of Feeding Tadpoles." *Journal of Embryology and Experimental Morphology* Vol. 10, Dec. 1962.

FIGURE 17-3 Gurdon's experiment on nuclear totipotency in frogs

The first cloned mammal was a sheep

In 1996, Ian Wilmut, Keith Campbell, and their coworkers at the Roslin Institute in Edinburgh, Scotland, reported that they had succeeded in cloning sheep by using nuclei from an early stage of sheep embryos (the *blastocyst* stage; see Chapter 51). These scientists received worldwide attention in early 1997 when they announced the birth of a lamb named Dolly (after the singer Dolly Parton). Dolly's genetic material was derived from a cultured sheep mammary gland cell that was fused with an enucleated sheep's egg. The resulting cell divided and developed into an embryo that was then cultured in vitro until it reached a stage at which it could be transferred to a host mother (**FIG. 17-4**). Not surprisingly, the overall success rate was low: of 277 fused cells, only 29 developed into embryos that could be transferred, and Dolly was the only live lamb produced.

Why did Wilmut's team succeed when so many other researchers had failed? Applying the basic principles of cell biology, they recognized that the **cell cycles** (see Chapter 10) of the egg cytoplasm and the donor nucleus were not synchronous. The egg cell is arrested at metaphase II of meiosis, whereas the actively growing donor somatic cell is usually in the DNA synthesis phase (S), or in G_2. By withholding certain nutrients from the mammary gland cells used as donors, the researchers caused these cells to enter a nondividing state referred to as G_0. This had the effect of synchronizing the cell cycles of the donor nucleus and the egg. They then used an electric shock to fuse the donor cell with the egg and initiate embryo development.

Although an extremely high level of technical expertise is required, these and other researchers have modified and extended these techniques to produce cloned calves, pigs, horses, rats, mice, dogs, and cats, among others. The list of mammalian species successfully cloned continues to grow. However, the success rate for each set of trials is low, around 1% to 2%, and the incidence of genetic defects is high. Dolly was euthanized at age 6 because she was suffering from a virus-induced lung cancer that infected several sheep where she was housed. However, she developed arthritis at 5½ years, which is relatively young for a sheep to have this degenerative disease. Some biologists speculate that using adult genetic material to produce a clone might produce an animal with prematurely old cells (see discussion of telomeres and cell aging in Chapter 12). Further research may provide some answers to this potential problem.

The main focus of cloning research is the production of **transgenic** organisms, in which foreign genes have been incorporated (see Chapter 15). Researchers are actively pursuing new techniques to improve the efficiency of the cloning process. Only then will it be possible to produce large numbers of cloned transgenic animals for a variety of uses, such as increasing the populations of endangered species. For example, the first healthy clone of an endangered species, a wild relative of cattle known as a *banteng*, was born in 2003. The nucleus for this clone came from a frozen skin cell of a banteng that died in 1980 at the San Diego Zoo.

Stem cells divide and give rise to differentiated cells

Stem cells are undifferentiated cells that can divide to produce differentiated descendants yet retain the ability to divide to maintain the stem cell population. When a stem cell divides, its daughter cells can remain stem cells or differentiate into specialized cells such as muscle cells, neurons, or blood cells. What happens depends on the presence or absence of an array of biochemical signals. One of