An introduction to animal development

**Nature is always the same, and yet its appearance is always changing. It is our business as artists to convey the thrill of nature's permanence along with the elements and the appearances of all its changes.**

*Paul Cézanne (ca. 1900)*

*Happy is the person who is able to discern the causes of things.*

*Virgil (37 B.C.E.)*

---

The concept of an embryo is a staggering one, and forming an embryo is the hardest thing you will ever have to do. To become an embryo, you had to build yourself from a single cell. You had to respire before you had lungs, digest before you had a gut, build bones when you were pulpy, and form orderly arrays of neurons before you knew how to think. One of the critical differences between you and a machine is that the machine is never called on to function until after it is built. Every animal has to function as it builds itself.

**The scope of developmental biology**

For animals and plants, the sole way of getting from egg to adult is by developing an embryo. The embryo mediates between genotype and phenotype, between the inherited genes and the adult organism. Whereas most of biology studies *adult* structure and function, developmental biology finds the transient stages more interesting. Developmental biology is a science of becoming, a science of process. To say that a mayfly lives but one day is meaningless to a developmental biologist. It may only be an adult for a day, but it spends the other 364 days as an embryo and larva.

The questions asked by developmental biologists are often questions about becoming rather than about being. To say that XX mammals are usually females and XY mammals are usually males does not explain sex determination to a developmental biologist. The developmental biologist wants to know *how* the XX genotype produces a female and *how* the XY genotype produces a male. Similarly, a geneticist may ask how globin genes are transmitted from one generation to the other, and a physiologist might ask about the function of globin in the body. But the developmental biologist asks how it is that the globin genes become expressed only in red blood cells and how they become active only at certain times in development. (We do not know the answers yet.)

Developmental biology is a great science for people who want to integrate different levels of biology. We can take a problem and study it on the molecular and chemical levels (e.g., How are globin genes transcribed, and how do the factors activating their transcription interact with one another on
the DNA?), on the cellular and tissue levels (e.g., Which cells are able to make globin, and how does globin mRNA leave the nucleus?), on the organ and organ system levels (e.g., How do the capillaries form in each tissue, and how are they instructed to branch and connect?), and even at the ecological and evolutionary levels (e.g., How do differences in globin gene activation enable oxygen to flow from mother to fetus, and how do environmental factors trigger the differentiation of more red blood cells?). Developmental biologists can study any organism and any cell type.

Developmental biology is one of the fastest growing and most exciting fields in biology. Part of the excitement comes from its subject matter, for we are just beginning to understand the molecular mechanisms of animal development. Another part of the excitement comes from the unifying role that developmental biology is assuming in the biological sciences. Developmental biology is creating a framework that integrates molecular biology, physiology, cell biology, anatomy, cancer research, neurobiology, immunology, ecology, and evolutionary biology. The study of development has become essential for understanding any other area of biology.

The problems of developmental biology

Development accomplishes two major functions: it generates cellular diversity and order within each generation, and it ensures the continuity of life from one generation to the next. Thus, there are two fundamental questions in developmental biology: How does the fertilized egg give rise to the adult body, and how does that adult body produce yet another body? Each species has its own answers, but some generalizations can be made. Traditionally, these questions have been subdivided into four general problems of developmental biology:

- **The problem of differentiation.** A single cell, the fertilized egg, gives rise to hundreds of different cell types—muscle cells, epidermal cells, neurons, lymphocytes, blood cells, fat cells, and so on. This generation of cellular diversity is called differentiation. Since each cell of the body contains the same set of genes, we need to understand how this same set of genetic instructions can produce different types of cells.

- **The problem of morphogenesis.** Our differentiated cells are not randomly distributed. Rather, they are organized into intricate tissues and organs. These organs are arranged in a given way: the fingers at the tip of our hands, not in the middle; the eyes in our heads, not in our toes or gut. This creation of ordered form is called morphogenesis. How do cells organize themselves and form the correct arrangements?

- **The problem of growth.** We are bigger than the egg, but how did the cells know when to stop dividing? If each cell in our face were to undergo just one more cell division, we would be considered horribly malformed. If each cell of our arms underwent just one more round of cell division, we could tie our shoelaces without bending over.

- **The problem of reproduction.** The sperm and egg are very specialized cells. Only they can transmit the instructions to make an organism from one generation to the next. How are these cells set apart to form the next generation, and what are the instructions in the nucleus and cytoplasm that allow them to function this way?

Recently, a fifth problem has been reemphasized:

- **The problem of evolution.** Evolution involves inherited changes in development. When we say that today's one-toed horse had a five-toed ancestor, we are saying that changes in the development of cartilage
and muscles occurred over many generations in the embryos of the horse's ancestors. How do changes in development create new body forms? Which heritable changes are possible, given the constraints imposed by the necessity of the organism to survive as it develops?

The stages of animal development

According to Aristotle, the first major embryologist known to history, science begins with wonder: "It is owing to wonder that people began to philosophize, and wonder remains the beginning of knowledge." The development of an animal from an egg has been a source of wonder throughout human history. The simple procedure of cracking open a chick egg on each successive day of its three-week incubation provides a remarkable experience as a thin band of cells is seen to give rise to an entire bird. Aristotle performed this procedure and noted the formation of the major organs. Anyone can wonder at this remarkable—yet commonplace—phenomenon, but it is the scientist who seeks to discover how development actually occurs. And rather than dissipating wonder, new understanding increases it.

Multicellular organisms do not spring forth fully formed. Rather, they arise by a relatively slow process of progressive change that we call development. In nearly all cases, the development of a multicellular organism begins with a single cell—the fertilized egg, or zygote, which divides mitotically to produce all the cells of the body. The study of animal development has traditionally been called embryology, referring to the fact that between fertilization and birth the developing organism is known as an embryo. But development does not stop at birth, or even at adulthood. Most organisms never stop developing. Each day we replace over a gram of skin cells (the older cells being sloughed off as we move), and our bone marrow sustains the development of millions of new erythrocytes every minute of our lives. Therefore, in recent years it has become customary to speak of developmental biology as the discipline that studies embryonic and other developmental processes.

The major features of animal development are illustrated in Figure 1.1. The life of a new individual is initiated by the fusion of genetic material from the two gametes—the sperm and the egg. This fusion, called fertilization, stimulates the egg to begin development. The subsequent stages of development are collectively called embryogenesis. Throughout the animal kingdom an incredible variety of embryonic types exist, but most patterns of embryogenesis comprise variations on four themes:

1. Immediately following fertilization, cleavage occurs. Cleavage is a series of extremely rapid mitotic divisions wherein the enormous volume of zygote cytoplasm is divided into numerous smaller cells. These cells are called blastomeres, and by the end of cleavage, they generally form a sphere known as a blastula.

2. After the rate of mitotic division has slowed down, the blastomeres undergo dramatic movements wherein they change their positions relative to one another. This series of extensive cell rearrangements is called gastrulation. As a result of gastrulation, the typical embryo contains three cell regions, called germ layers.* The outer layer, the ectoderm, produces the cells of the epidermis and the nervous sys-

*From the Latin germ, meaning "bud" or "sprout" (the same root as in the word germination). The names of the three germ layers are from the Greek: ectoderm from ektos ("outside") plus derma ("skin"); mesoderm from mesos ("middle"); and endoderm from endon ("within").
Figure 1.1
Developmental history of a representative animal, a frog. The stages from fertilization through hatching (birth) are known collectively as embryogenesis. The region set aside for producing germ cells is shown in color. Gametogenesis, which is complete in the sexually mature adult, begins at different times during development, depending on the species.

1. The outer layer, the **ectoderm**, gives rise to the nervous system, dermis, and epidermis. The nervous system is the control center of the body, responsible for regulating various physiological processes.

2. The middle layer, the **mesoderm**, gives rise to the muscle, connective tissue, and bone. These structures are essential for movement and support.

3. The inner layer, the **endoderm**, produces the lining of the digestive tube and its associated organs (pancreas, liver, lungs, etc.).

The process of **organogenesis** involves the differentiation of these germ layers into specific organs. For example:

- The nervous system differentiates into the brain and spinal cord.
- The muscle layer forms muscles, tendons, and ligaments.
- The endoderm develops into the lining of the digestive tract, including the gut, liver, and pancreas.

Once the three germ layers are established, the cells interact with one another and rearrange themselves to produce tissues and organs. This process is called **organogenesis**. In vertebrates, organogenesis is initiated when a series of cellular interactions causes the mid-dorsal ectodermal cells to form the neural tube. This tube will become the brain and spinal cord.) Many organs contain cells from more than one germ layer, and it is not unusual for the outside of an organ to be derived from one layer and the inside from another. Also during
organogenesis, certain cells undergo long migrations from their place of origin to their final location. These migrating cells include the precursors of blood cells, lymph cells, pigment cells, and gametes. Most of the bones of our face are derived from cells that have migrated ventrally from the dorsal region of the head.

4. As seen in Figure 1.1, in many species, a specialized portion of egg cytoplasm gives rise to cells that are the precursors of the gametes. These cells are called germ cells, and they are set aside for their reproductive function. All the other cells of the body are called somatic cells. This separation of somatic cells (which give rise to the individual body) and germ cells (which contribute to the formation of a new generation) is often one of the first differentiations to occur during animal development. The germ cells eventually migrate to the gonads, where they differentiate into gametes. The development of gametes, called gametogenesis, is usually not completed until the organism has become physically mature. At maturity, the gametes may be released and participate in fertilization to begin a new embryo. The adult organism eventually undergoes senescence and dies.

Our eukaryotic heritage

Organisms are divided into two major groups, depending on whether their cells possess a nuclear envelope. The prokaryotes (from the Greek karyon, meaning “nucleus”), which include the archaeabacteria and the eubacteria, lack a true nucleus. The eukaryotes, which include protists, animals, plants, and fungi, have a well-formed nuclear envelope surrounding their chromosomes. This fundamental difference between eukaryotes and prokaryotes influences how these two groups arrange and utilize their genetic material. In both groups, the inherited information needed for development and metabolism is encoded in the DNA sequences of the chromosomes. The prokaryotic chromosome is generally a small, circular double helix of DNA consisting of approximately 1 million base pairs. The eukaryotic cell usually has several chromosomes, and the simplest eukaryotic protists have over 10 times the amount of DNA found in the most complex prokaryotes. Moreover, the structure of a eukaryotic gene is more complex than that of a prokaryotic gene. The amino acid sequence of a prokaryotic protein is a direct reflection of the DNA sequence in the chromosome. The protein-coding DNA of a eukaryotic gene, however, is usually divided up such that the complete amino acid sequence of a protein is derived from discontinuous segments of DNA (Figure 1.2). The intervening DNA often contains sequences that are involved with regulating the time and place that the gene is activated.

Eukaryotic chromosomes also are very different from prokaryotic chromosomes. Eukaryotic DNA is wrapped around specific protein complexes called nucleosomes that are composed of histone proteins. These nucleosomes organize the DNA into compact structures and are important in regulating which genes become expressed in which cells. In bacteria, there are no histones. Moreover, eukaryotic cells undergo mitosis, wherein the nuclear envelope breaks down and the replicated chromosomes are equally divided between the daughter cells (Figure 1.3). In prokaryotes, cell division is not mitotic; no mitotic spindle develops, and there is no nuclear envelope to break down. Rather, the daughter chromosomes remain attached to adjacent points on the cell membrane. These attachment points are separated by the growth of the cell membrane between them, eventually placing the chromosomes into different daughter cells.
Prokaryotes and eukaryotes have different mechanisms of gene regulation. In both prokaryotes and eukaryotes, DNA is transcribed by enzymes called RNA polymerases to make RNA. When messenger RNA (mRNA) is produced in prokaryotes, it is immediately translated into a protein while the other end of it is still being transcribed from the DNA (Figure 1.4). Thus, in prokaryotes, transcription and translation are simultaneous and coordinated events. But the existence of the nuclear envelope in eukaryotes provides the opportunity for an entirely new type of cell regulation. The ribosomes, which are responsible for translation, are on one side of the nuclear envelope, and the DNA and the RNA polymerases needed for transcription are on the other side. In between transcription and translation, the transcribed RNA must be processed so that it can pass through the nuclear envelope. By regulating which mRNAs can pass into the cytoplasm, the cell is able to select which of the newly synthesized messages will be translated. Thus, a new level of complexity has been added, one that is extremely important for the developing organism.

**Development among the unicellular eukaryotes**

All multicellular eukaryotic organisms have evolved from unicellular protists. It is in these protists that the basic features of development first appeared. Simple eukaryotes give us our first examples of the nucleus directing morphogenesis, the use of the cell surface to mediate cooperation between individual cells, and the first occurrences of sexual reproduction.

**Control of Developmental Morphogenesis in Acetabularia**

A century ago, it had not yet been proved that the nucleus contained hereditary or developmental information. Some of the best evidence for this theory came from studies in which unicellular organisms were fragmented into nu-
cleft and anucleate pieces (reviewed in Wilson, 1896). When various protists were cut into fragments, nearly all the pieces died. However, the fragments containing nuclei were able to live and to regenerate entire complex cellular structures (Figure 1.5).

Nuclear control of cell morphogenesis and the interaction of nucleus and cytoplasm are beautifully demonstrated in studies of *Acetabularia*. This enormous single cell (2–4 cm long) consists of three parts: a cap, a stalk, and a rhizoid (Figure 1.6A). The rhizoid is located at the base of the cell and holds it to the substrate. The single nucleus of the cell resides within the rhizoid. The size of *Acetabularia* and the location of its nucleus allow investiga-
tors to remove the nucleus from one cell and replace it with a nucleus from another cell. In the 1930s, J. Hämmerling took advantage of these unique features and exchanged nuclei between two morphologically distinct species, *A. mediterranea* and *A. crenulata*. As the photographs show, these two species have very different cap structures. Hämmerling found that when the nucleus from one species was transplanted into the stalk of another species, the newly formed cap eventually assumed the form associated with the donor nucleus (Figure 1.6B). Thus, the nucleus was seen to control *Acetabularia* development.

The formation of a cap is a complex morphogenetic event involving the synthesis of numerous proteins, the products of which must be accumulated in a certain portion of the cell and then assembled into complex, species-specific structures. The transplanted nucleus does indeed direct the synthesis of its species-specific cap, but it takes several weeks to do so. Moreover, if the nucleus is removed from an *Acetabularia* cell early in development, before it first forms a cap, a normal cap is formed weeks later, even though the organism will eventually die. These studies suggest that (1) the nucleus contains information specifying the type of cap produced (i.e., it contains the genetic information that specifies the proteins required for the production of a certain type of cap), and (2) material containing this information enters the cytoplasm long before cap production occurs. This information in the cytoplasm is not used for several weeks.

Figure 1.5
Regeneration of the anucleate fragment of the unicellular protist *Stylonychia*. The anucleate fragments survive for a time but finally die.
One current hypothesis proposed to explain these observations is that the nucleus synthesizes a stable mRNA that lies dormant in the cytoplasm until the time of cap formation. This hypothesis is supported by an observation that Hämerling published in 1934. Hämerling fractionated young Acetabularia into several parts (Figure 1.7). The portion with the nucleus eventually formed a new cap as expected; so did the apical tip of the stalk. However, the intermediate portion of the stalk did not form a cap. Thus, Hämerling postulated (nearly 30 years before the existence of mRNA was known) that the instructions for cap formation originated in the nucleus and were somehow stored in a dormant form near the tip of the stalk. Many years later, Kloppstech and Schweiger (1975) established that nucleus-derived mRNA does accumulate in this region. Ribonuclease, an enzyme that cleaves RNA, completely inhibits cap formation when added to the seawater in which Acetabularia is growing. In enucleated cells, this effect is permanent; once the RNA is destroyed, no cap formation can occur. In nucleated cells, however, a new cap can form after the ribonuclease is washed away, presumably because new mRNA is then made by the nucleus. Garcia and Dazy (1986) have also shown that protein synthesis is especially active in the apex of Acetabularia.

It is clear from the preceding discussion that nuclear transcription plays an important role in the formation of the Acetabularia cap. But note that the
cytoplasm also plays an essential role in cap formation. The mRNAs are not translated for weeks, even though they are in the cytoplasm. Something in the cytoplasm controls when the message is utilized. Hence, the expression of the cap is controlled not only by nuclear transcription but also by the translational control of the cytoplasmic RNA. In this unicellular organism, "development" is controlled at both the transcriptional and translational levels.

**Differentiation in the Amoeboflagellate Naegleria**

One of the most remarkable cases of protist "differentiation" is that of *Naegleria gruberi*. This organism occupies a special place in protist taxonomy because it can change its form from that of an amoeba to that of a flagellate (Figure 1.8). During most of its life cycle, *N. gruberi* is a typical amoeba, feeding on soil bacteria and dividing by fission. However, when the bacteria are diluted (either by rainwater or by water added in an experiment), each *N. gruberi* rapidly develops a streamlined body shape and two long anterior flagella, which it uses to find regions of more abundant bacteria. Thus, instead of having several differentiated cell types in one organism, this one cell has different cell structures and biochemistry at different times of its life.

Differentiation into the flagellate form occurs in about an hour (Figure 1.9). During this time, the amoeba has to create centrioles to serve as the basal bodies (microtubule-organizing centers) of the flagella, as well as to create the flagella themselves. Basal bodies and flagella are made from many proteins, the most abundant of which is tubulin. The tubulin molecules are organized into microtubules, and the microtubules are further organized into an arrangement that permits flagellar movement. Fulton and Walsh (1980) showed that the tubulin for the *Naegleria* flagella does not exist in the amoeba stage. It is made de novo ("from scratch"), starting with new tran-
scription from the nucleus. To show this, the investigators manipulated transcription at various stages with actinomycin D, an antibiotic drug that selectively inhibits RNA synthesis. When added before the dilution of the food supply, this antibiotic prevents tubulin synthesis. However, if the actinomycin D is added 20 minutes after dilution, tubulin is still made at the normal time (about 30 minutes later). Therefore, it appears that the mRNA for tubulin is made during the first 20 minutes after dilution and is used shortly thereafter. This interpretation was confirmed when it was shown that mRNA extracted from amoebae does not contain any detectable messages for flagellar tubulin, whereas mRNA extracted from differentiating cells contains a great many such messages (Walsh, 1984).

Here, then, is an excellent example of the transcriptional control of a development process: the Naegleria nucleus responds to environmental changes by synthesizing the mRNA for flagellar tubulin. We also see another process that remains extremely important in the development of all other animals and plants, namely, the assembly of tubulin molecules to produce a flagellum. This arrangement, whereby tubulin is polymerized into microtubules and the microtubules assembled into an ordered array, is seen throughout nature. In mammals, it is evident in the sperm flagellum and in the cilia of the spinal cord and respiratory tract. Moreover, tubulin alone does not make a flagellum. There are around 300 other proteins in each flagellum, and flagellar movement depends on the proper orientation of these proteins with respect to each other. So even cellular processes have their own “morphogenesis” based on molecular interactions between the parts of the proteins. Such posttranslational control, whereby a protein is not functional until it is linked with other molecules, will be discussed more fully later. We see, then, that development in unicellular eukaryotes can be controlled at the transcriptional, translational, and posttranslational levels.
The Origins of Sexual Reproduction

Sexual reproduction is another invention of the protists that has had a profound effect on more complex organisms. It should be noted that sex and reproduction are two distinct and separable processes. Reproduction involves the creation of new individuals. Sex involves the combining of genes from two different individuals into new arrangements. Reproduction in the absence of sex is characteristic of organisms that reproduce by fission; there is no sorting of genes when an amoeba divides or when a hydra buds off cells to form a new colony. Sex without reproduction is also common among unicellular organisms. Bacteria are able to transmit genes from one individual to another by means of sex pili (Figure 1.10). This transmission is separate from reproduction. Protists are also able to reinsert genes without reproduction. Paramecia, for instance, reproduce by fission, but sex is accomplished by conjugation. When two paramecia join together, they link their oral apparatuses and form a cytoplasmic connection through which they can exchange genetic material (Figure 1.11). Each macronucleus (which controls the metabolism of the organism) degenerates while each micronucleus undergoes meiosis to produce eight haploid micronuclei, of which all but one degenerate. The remaining micronucleus divides once more to form a stationary micronucleus and a migratory micronucleus. Each migratory micronucleus crosses the cytoplasmic bridge and fuses with ("fertilizes") the stationary micronucleus, thereby creating a new diploid nucleus in each cell. This diploid nucleus then divides mitotically to give rise to a new micronucleus and a new macronucleus as the two partners disengage. Therefore, no reproduction has occurred, only sex.
The union of these two distinct processes, sex and reproduction, into sexual reproduction is seen in unicellular eukaryotes. Figure 1.12 shows the life cycle of *Chlamydomonas*. This organism is usually haploid, having just one copy of each chromosome (like a mammalian gamete). The individuals of each species, however, are divided into two mating groups: *plus* and *minus*. When these meet, they join their cytoplasms, and their nuclei fuse to form a diploid zygote. This zygote is the only diploid cell in the life cycle, and it eventually undergoes meiosis to form four new *Chlamydomonas* cells. Here is sexual reproduction, for chromosomes are reassorted during the meiotic divisions and more individuals are formed. Note that in this protist type of sexual reproduction, the gametes are morphologically identical; the distinction between sperm and egg has not yet been made.

In evolving sexual reproduction, two important advances had to be achieved. The first is the mechanism of meiosis (Figure 1.13), whereby the diploid complement of chromosomes is reduced to the haploid state (discussed in detail in Chapter 22.) The other advance is the mechanism whereby the two different mating types recognize each other. In *Chlamydomonas*, recognition occurs first on the flagellar membranes (Figure 1.14; Bergman et al., 1975; Goodenough and Weiss, 1975). The agglutination of flagella enables specific regions of the cell membranes to come together. These specialized sectors contain mating-type-specific components that enable the cytoplasms to fuse. Following agglutination, the *plus* individuals initiate the
Figure 1.12
Sexual reproduction in Closterium. Two strains, both haploid, can reproduce asexually when separate. Under certain conditions, the two strains can unite to produce a diploid cell that can undergo meiosis to form four new haploid organisms. (After Strickberger, 1985.)

Figure 1.13
Summary of meiosis. The DNA and associated proteins replicate during interphase. During prophase, the nuclear envelope breaks down and homologous chromosomes (each chromosome being double, with the chromatids joined at the centromere) align in pairs. Chromosomal rearrangements can occur between the four homologous chromatids at this time. After the first metaphase, the two original homologous chromosomes are segregated into different cells. During the second division the centromere splits, thereby leaving each new cell with one copy of each chromosome.

The nuclear envelope breaks down and homologous chromosomes (each chromosome being double, with the chromatids joined at the centromere) align in pairs. Chromosomal rearrangements can occur between the four homologous chromatids at this time.
fusion by extending a **fertilization tube**. This tube contacts and fuses with a specific site on the *minus* individual. Interestingly, the mechanism used to extend this tube—the polymerization of the protein **actin**—is also used to extend processes of sea urchin eggs and sperm. In Chapter 4, we will see that the recognition and fusion of sperm and egg occur in a manner amazingly similar to that of these protists.

Unicellular eukaryotes appear to have the basic elements of the developmental processes that characterize the more complex organisms: cellular synthesis is controlled by transcriptional, translational, and posttranslational regulation; there is a mechanism for processing RNA through the nuclear membrane; the structures of individual genes and chromosomes are as they will be throughout eukaryotic evolution; mitosis and meiosis are perfected; and sexual reproduction exists, involving cooperation between individual cells. Such intercellular cooperation becomes even more important with the evolution of multicellular organisms.

**Figure 1.14**

Two-step recognition in mating *Chlamydomonas*. (A) Scanning electron micrograph (7000×) of mating pair. The interacting flagella twist about each other, adhering at the tips (arrows). (B) Transmission electron micrograph (20,000×) of a cytoplasmic bridge connecting the two organisms. The microfilaments extend from the donor (lower) cell to the recipient (upper) cell. (From Goodenough and Weiss, 1975, and Bergman et al., 1975; by permission of U. Goodenough.)

---

**MEIOSIS II**

- **Anaphase I**
- **Telophase I**
- **Metaphase II**
- **Anaphase II**
- **Telophase II**

The two original homologous chromosomes are segregated into different cells.

The centromere splits. Each new cell has one copy of each chromosome.
Colonial eukaryotes: The evolution of differentiation

One of evolution's most important experiments was the creation of multicellular organisms. There appear to be several paths by which single cells evolved multicellular arrangements; we will discuss only two of them here (see Chapter 23 for a fuller discussion). The first path involves the orderly division of the reproductive cell and the subsequent differentiation of its progeny into different cell types. This path to multicellularity can be seen in a remarkable series of multicellular organisms collectively referred to as the family Volvocaceae, or the volvocaceans.

The Volvocaceans

The simpler organisms among the volvocaceans are ordered assemblies of numerous cells, each resembling the unicellular protist *Chlamydomonas*. A single organism of the volvocacean genus *Gonium* (Figure 1.15) for example, consists of a flat plate of 4 to 16 cells, each with its own flagellum. In a related genus, *Pandorina*, the 16 cells form a sphere; and in *Eudorina*, the sphere contains 32 or 64 cells arranged in a regular pattern. In these organisms, then, a very important developmental principle has been worked out: the ordered division of one cell to generate a number of cells that are organized in a predictable fashion. As occurs in most animal embryos, the cell divisions by which a single volvocacean cell produces an organism of 4 to 64 cells occur in very rapid sequence and in the absence of cell growth.

The next two genera of the volvocacean series exhibit another important principle of development: the differentiation of cell types within an individual organism. The reproductive cells become differentiated from the somatic cells. In all the genera mentioned earlier, every cell can, and normally does, produce a complete new organism by mitosis (Figure 1.16A, B). In the genera *Pleodorina* and *Volvox*, however, relatively few cells can reproduce. In *Pleodorina californica*, the cells in the anterior region are restricted to a somatic func-
tion; only those cells on the posterior side can reproduce. In *P. californica*, a colony usually has 128 or 64 cells, and the ratio of the number of somatic cells to the number of reproductive cells is usually 3:5. Thus, a 128-cell colony typically has 48 somatic cells, and a 64-cell colony has 24.

In *Volvox*, almost all the cells are somatic, and very few of the cells are able to produce new individuals. In some species of *Volvox*, reproductive cells, as in *Pleodorina*, are derived from cells that originally lack and function like somatic cells before they enlarge and divide to form new progeny. However, in other members of the genus, such as *V. carteri*, there is a complete division of labor: the reproductive cells that will create the next generation are set aside during the division of the reproductive cells that are forming a new individual. The reproductive cells never develop functional flagella and never contribute to motility or other somatic functions of the individual; they are entirely specialized for reproduction. Thus, although the simpler volvocaceans may be thought of as colonial organisms (because each cell is capable of independent existence and of perpetuating the species), in *V. carteri* we have a truly multicellular organism with two distinct and interdependent cell types (somatic and reproductive), both of which are required for perpetuation of the species (Figure 1.16C). Although not all animals set aside the reproductive cells from the somatic cells (and plants hardly ever do), this separation of germ cells from somatic cells early in development is characteristic of many animal phyla and will be discussed in more detail in Chapter 13.

Although all the volvocaceans, including their unicellular relative *Chlamydomonas*, reproduce predominantly by asexual means, they are also capable of sexual reproduction. This involves the production and fusion of haploid gametes. In many species of *Chlamydomonas*, including the one illustrated in Figure 1.12, sexual reproduction is isogamous, since the haploid gametes that meet are similar in size, structure, and motility. However, in other species of *Chlamydomonas*—as well as many species of colonial volvocaceans—swimming gametes of very different sizes are produced by the different mating types. This is called heterogamy. But the larger volvocaceans have evolved a specialized form of heterogamy, called oogamy, which involves the production of large, relatively immotile eggs by one mating type and small, motile sperm by the other (see Sidelights & Speculations). Here we see one gamete specialized for the retention of nutritional and developmental resources and the other gamete specialized for the transport of nuclei. Thus, the volvocaceans include the simplest organisms that have distinguishable male and female members of the species and that have distinct developmental pathways for the production of eggs or sperm. In all the volvocaceans, the fertilization reaction resembles that of *Chlamydomonas* in that it results in the production of a dormant diploid zygote that is capable of surviving harsh environmental conditions. When conditions allow the zygotes to germinate, they first undergo meiosis to produce haploid offspring of the two different mating types in equal numbers.

---

Figure 1.16

Asexual reproduction in volvocaceans.
(A) Mature colony of *Eudorina elegans*.
(B) Each of the *E. elegans* cells divides and produces a new colony. (C) Mature *Volvox carteri*. Most of the cells are incapable of reproduction. Germ cells (gonidia) have begun dividing into new organisms. (A and B after Hartmann, 1921; C from Kirk et al., 1982, courtesy of D. Kirk.)
Sex and Individuality in Volvox

Simple as it is, Volvox shares many features that characterize the life cycles and developmental histories of much more complex organisms, including ourselves. As already mentioned, Volvox is among the simplest organisms to exhibit a division of labor between two completely different cell types. As a consequence of this, it is among the simplest organisms to include death as a regular, genetically programmed part of its life history.

Death and Differentiation
Unicellular organisms that reproduce by simple cell division, such as amoebas, are potentially immortal. The amoeba you see today under the microscope has no dead ancestors! When an amoeba divides, neither of the two resulting cells can be considered either ancestor or offspring; they are siblings. Death comes to an amoeba only if it is eaten or meets with a fatal accident; and when it does, the dead cell leaves no offspring.

Death becomes an essential part of life, however, for any multicellular organism that establishes a division of labor between somatic (body) cells and germ (reproductive) cells. Consider the life history of Volvox carteri when it is reproducing asexually (Figure 1.17). Each asexual adult is a spheroid containing some 2000 small, biflagellated somatic cells along its periphery and about 16 large, asexual reproductive cells, called gonidia, toward one end of the interior. When mature, each gonidium divides rapidly 11 or 12 times. Certain of these divisions are asymmetrical and produce the 16 large cells that will become a new set of gonidia. At the end of cleavage, all the cells that will be present in an adult have been produced from each gonidium. But the embryo is “inside out”: its gonidia are on the outside and the flagella of its somatic cells are pointing toward the interior of the hollow sphere of cells. This predicament is corrected by a process called inversion, in which the embryo turns itself right side out by a set of cell movements that resemble the gastrulation movements of animal embryos (Figure 1.18). Clusters of bottle-shaped cells open a hole at one end of the embryo by producing tension on the interconnected cell sheet (Figure 1.19).

![Figure 1.17 Asexual reproduction in V. carteri. When reproductive cells (gonidia) are mature, they enter a cleavage-like stage of embryonic development to produce juveniles within the adult. Through a series of cell movements resembling gastrulation, the embryonic volvox inverts and is eventually released from the parent. The somatic cells of the parent, lacking the gonidia, undergo senescence and die, while the juvenile colonies mature. The entire sexual cycle takes two days. (After Kirk, 1986.)](image)
The embryo everts through this hole and then closes it up. About a day after this is done, the juvenile colonies are enzymatically released from the parent and swim away.

What happens to the somatic cells of the "parent" Volvox now that its young have "left home"? Having produced offspring and being incapable of further reproduction, these somatic cells die. Actually, they commit suicide, synthesizing a set of proteins that cause the death and dissolution of the cells that make these proteins (Pommerville and Kochert, 1982). Moreover, in this death, the cells release for the use of others, including their own offspring, all the nutrients that they had stowed during life. "Thus emerges," notes David Kirk, "one of the great themes of life on planet Earth: 'Some die that others may live.'"

In *V. carteri*, a specific gene* that plays a central role in regulating cell death has been identified (Kirk, 1988). In laboratory strains possessing mutations of this gene, somatic cells abandon their suicidal ways, gain the ability to reproduce asexually, and become potentially immortal (Figure 1.20). The fact that such mutants have never been found in

*This gene (rgA) has now been cloned and found to encode a protein that acts to repress (directly or indirectly) all the genes whose products are required for cells to develop as gonidia. Loss-of-function mutations would prevent the protein from acting, and the cells would be able to become gonidia (D. Kirk, personal communication).

Figure 1.18 Inversion of asexually produced embryos of *V. carteri*. A–E are scanning electron micrographs of whole embryos. F–J are sagittal sections through the center of the embryo, visualized by differential interference microscopy. Before inversion, the embryo is a hollow sphere of connected cells. When cells change their shape, a hole (the phialopore) opens at the apex of the embryo (A, B, F, G). Cells then curl around and regain at the bottom (C–E, H–J). (From Kirk et al., 1982, courtesy of D. Kirk.)

Figure 1.19 "Bottle cells" near the opening of the phialopore. These cells remain tightly interconnected through cytoplasmic bridges near their elongated spines, thereby creating the tension that causes the curvature of the interconnected cell sheet. (From Kirk et al., 1982, courtesy of D. Kirk.)
nature indicates that cell death most likely plays an important role in the survival of *V. carteri* under natural conditions.

**Enter Sex**

Although *V. carteri* reproduces asexually much of the time, in nature it reproduces sexually once each year. When it does, one generation of individuals passes away, and a new and genetically different generation is produced. The naturalist Joseph Wood Krutch (1956) put it more poetically:

>The amoebae and the paramecia are potentially immortal... But for Volvox, death seems to be as inevitable as it is in a mouse or in a man. Volvox must die as Leeuwenhoek said it die because it had children and is no longer needed. When its time comes it drops quietly to the bottom and joins its ancestors. As Hegner, the Johns Hopkins zoologist, once wrote, "This is the first advent of inevitable natural death in the animal kingdom and all for the sake of sex." And he asked: "Is it worth it?"

For *Volvox carteri*, it most assuredly is worth it. *V. carteri* lives in shallow temporary ponds that fill with spring rains but dry out in the heat of late summer. During most of that time, *V. carteri* swims about, reproducing asexually. These asexual volvoxes would die in minutes once the pond dried out, but *V. carteri* is able to survive by turning sexual shortly before the pond dries up, producing dormant zygotes that survive the heat and drought of late summer and the cold of winter. When rain fills the ponds in spring, the zygotes break their dormancy and hatch out a new generation of individuals to reproduce sexually until the pond is about to dry up once more. How do these simple organisms predict the coming of adverse conditions with sufficient accuracy to produce a sexual generation just in time, year after year?

The stimulus for switching from the asexual to the sexual mode of reproduction in *V. carteri* is known to be a 30-kDa sexual inducer protein. This protein is so powerful that concentrations as low as $6 \times 10^{-17}$ cause gonidia to undergo a modified pattern of embryonic development that results in the production of eggs or sperm, depending on the genetic sex of the individual (Sumper et al., 1993). The sperm are released and swim to a female,

**Figure 1.20** Mutation of a single gene (called somatic regenerator A) abolishes programmed cell death in *V. carteri*. The newly hatched volvox carrying this mutation (A) is indistinguishable from the wild-type spheroid. However, shortly before the time when the somatic cells of the wild-type spheroids begin to die, the somatic cells of this mutant redifferentiate as gonidia (B). Eventually, every cell of the mutant will divide to form (regenerate) a new spheroid that will repeat this potentially immortal developmental cycle.

**Figure 1.21** Sexual reproduction in *V. carteri*. Males and females are indistinguishable in their asexual phase. When the sexual inducer protein is present, the gonidia of both mating types undergo a modified embryogenesis that leads to the formation of eggs in the females and sperm in the males. When the gametes are mature, sperm packets (containing 64 or 128 sperm each) are released and swim to the females. Upon reaching the female, the sperm packet breaks up into individual sperm, which can fertilize the eggs. The resulting zygote has tough cell walls that can resist drying, heat, and cold. When spring rains cause the zygote to germinate, it undergoes meiosis to produce haploid males and females that reproduce asexually until heat induces the sexual cycle again.
where they fertilize eggs to produce the dormant zygotes (Figure 1.21).

What is the source of this sexual inducer protein? Kirk and Kirk (1986) discovered that the sexual cycle could be initiated by heating dishes of *V. carteri* to temperatures that might be expected in a shallow pond in late summer. When this was done, the somatic cells of the asexual volvocines produced the sexual inducer protein. Since the amount of sexual inducer protein secreted by one individual is sufficient to initiate sexual development in over 500 million asexual volvocines, a single inducing volvox can convert the entire pond to sexuality. This discovery explained an observation made nearly 90 years ago that “in the full blaze of Nebraska sunlight, Volvox is able to appear, multiply, and riot in sexual reproduction in pools of rain water of scarcely a fortnight’s duration” (Powers, 1908). Thus, in temporary ponds formed by spring rains and dried up by summer’s heat, *Volvox* has found a means of survival: it uses the heat to induce the formation of sexual individuals whose mating produces zygotes capable of surviving conditions that kill the adult organism. We see, too, that development is critically linked to the ecosystem in which the organism has adapted to survive.

---

**Differentiation and Morphogenesis in Dictyostelium**

**The Life Cycle of Dictyostelium.** Another type of multicellular organization derived from unicellular organisms is found in *Dictyostelium discoideum.* The life cycle of this fascinating organism is illustrated in Figure 1.22. In its vegetative cycle, solitary haploid amoebae (called myxamoebae or “social amoebae”) to distinguish them from amoeba species that always remain solitary) live on decaying logs, eating bacteria and reproducing by binary fission. When they have exhausted their food supply, tens of thousands of these amoebae join together to form moving streams of cells that converge at a central point. Here they pile atop another to produce a conical mound called the tight aggregate. Subsequently, a tip arises at the top of this mound, and the mound bends over to produce the migrating slug (with the tip at the front). The slug (often given the more dignified title of pseudoplasmodium or greg) is usually 2–4 mm long and is encased in a slimy sheath. The greg begins to migrate (if the environment is dark and moist) with its anterior tip slightly raised. When the greg reaches an illuminated area, migration ceases, and the greg differentiates into a fruiting body composed of spore cells and a stalk. The anterior cells, representing 15–20 percent of the entire cellular population, form the tubed stalk. The stalk begins as some of the central anterior cells, the prestalk cells, begin secreting an extracellular coat and extending a tube through the greg. As the prestalk cells differentiate, they form vacuoles and enlarge, lifting up the mass of prespore cells that had been in the posterior four-fifths of the greg (Jermyn and Williams, 1991). The stalk cells die, but the posterior cells, elevated above the stalk, become spore cells. These spore cells disperse, each one becoming a new myxamoeba.

In addition to this asexual cycle, there is a possibility for sex in *Dictyostelium*. Two amoebae can fuse to create a giant cell, which digests all the other cells of the aggregate. When it has eaten all its neighbors, it encysts itself in a thick wall and undergoes meiotic and mitotic divisions; eventually, new myxamoebae are liberated.

*Dictyostelium* has been a wonderful experimental organism for developmental biologists, because initially identical cells are differentiated into one of two alternative cell types, spore and stalk. It is also an organism wherein individual cells come together to form a cohesive structure composed of differentiated cell types, akin to tissue formation in more complex organisms. The aggregation of thousands of amoebae into a single organism is an interesting phenomenon. *Dictyostelium* is not a mold (such as *Neurospora*), nor is it consistently slimy. It is perhaps best to think of it as a social amoeba.
credible feat of organization and invites experimentation to answer questions about the mechanisms involved.

**AGGREGATION OF DICTYOSTELIUM CELLS.** The first question is, What causes the amoebae to aggregate? Time-lapse microcinematography has shown that no directed movement occurs during the first 4–5 hours following nutrient starvation. During the next 5 hours, however, the cells are seen moving at about 20 μm/min for 100 seconds. This movement ceases for about 4 minutes, then resumes. Although the movement is directed toward a central point, it is not a simple radial movement. Rather, cells join with each other to form streams: the streams converge into larger streams, and eventually all streams merge at the center. Bonner (1947) and Shaffer (1953) showed that this movement is due to chemotaxis: the cells are guided to aggregation centers by a soluble substance. This substance was later identified as cyclic adenosine 3’,5’-monophosphate (cAMP) (Kornjän et al., 1967; Bonner et al., 1969), the chemical structure of which is shown in Figure 1.23A.

Aggregation is initiated as each of the cells begins to synthesize cAMP. There are no “dominant” cells that begin the secretion or control the others. Rather, the sites of aggregation are determined by the distribution of amoebae (Keller and Segal, 1970; Tyson and Murray, 1989). Neighboring cells respond to cAMP in two ways: they initiate a movement toward the cAMP pulse, and they release cAMP of their own (Robertson et al., 1972; Shaffer,
1975). After this, the cell is unresponsive to further cAMP pulses for several minutes. The result is a rotating spiral wave of cAMP that is propagated throughout the population of cells (Figure 1.23B–D). As each wave arrives, the cells take another step toward the center.*

The differentiation of individual amoebae into either stalk (somatic) or spore (reproductive) cells is a complex matter. Raper (1940) and Bonner (1957) have demonstrated that the anterior cells normally become stalk, while the remaining, posterior, cells are usually destined to form spores. However, surgically removing the anterior part of the slug does not abolish the ability of the grex to form a stalk. Rather, the cells that now find themselves at the anterior end following the surgery (and which originally had been destined to produce spores) now form the stalk (Raper, 1940). Somehow a decision is made so that whichever cells are anterior become stalk cells and whichever are posterior become spores. This ability of cells to change their developmental fates according to their location within the whole organism and thereby

*The biochemistry of this reaction involves a receptor that binds cAMP. When this binding occurs, specific gene transcription takes place, motility toward the source of the cAMP is initiated, and adenyl cyclase enzymes (which synthesize cAMP from ATP) are activated. The newly formed cAMP activates its own receptors as well as those of its neighbors. The cells in the area remain insensitive to new waves of cAMP until the bound cAMP is removed from the receptors by another cell-surface enzyme, phosphodiesterase (Johnson et al., 1989). The mathematics of such oscillation reactions predict that the diffusion of cAMP would initially be circular. However, as cAMP interacts with the cells that receive and propagate the signal, the cells that receive the front part of the wave begin to migrate at a different rate than the cells behind them. The result is a rotating spiral of cAMP and migration as seen in Figure 1.23. Interestingly, the same mathematical formulas predict the behavior of certain chemical reactions and the formation of new stars in rotating spiral galaxies (Tyson and Murray, 1989).

Figure 1.23
Chemotaxis of Dictyostelium amoebae due to spiral waves of cAMP
(A) Chemical structure of cAMP.
(B) Visualization of several cAMP "waves" in the medium. Central cells secrete cAMP at regular intervals, and each secretion diffuses outward as a concentric wave. Waves are charted by saturating filter paper with radioactive cAMP and placing it on an aggregating colony. The cAMP from the secreting cells dilutes the radioactive cAMP.
When the radioactivity on the paper is recorded (by placing it over X-ray film), the regions of high cAMP concentration in the culture appear lighter than those of low cAMP concentration.
(C, D) Spiral waves of amoebae moving toward the initial source of cAMP. (C) This digitally processed dark-field photomicrograph shows about 10³ cells. Because moving and nonmoving cells scatter light differently, the photograph reflects cell movement. The bright bands are composed of elongated migrating cells; the dark bands are cells that have stopped moving and have rounded up. (D) As cells form streams, the spiral of movement can still be seen moving toward the center. (B from Tomchick and Devreotes, 1981, courtesy of F. Devreotes; C and D from Siegert and Weijer, 1989, courtesy of F. Siegert.)
**Figure 1.24**

*Dictyostelium* cells synthesize an adhesive, 24-kDa glycoprotein shortly after nutrient starvation. *Dictyostelium* cells were stained with a fluorescent antibody that binds to the 24-kDa glycoprotein and were then observed under ultraviolet light. This protein is not seen on amoeoeae that have just stopped dividing. However, as shown here—10 hours after cell division has ceased—individual amoeoeae are seen to have this protein in their cell membranes and are capable of adhering together. (Courtesy of W. Loomis.)

compensate for missing parts is called regulation. We will see this phenomenon in many embryos, including those of mammals.

**CELL ADHESION MOLECULES IN *DICTYOSTELIUM***. How do these individual cells stick together to form a cohesive organism? This is the same problem that embryonic cells face, and the solution that evolved in the protists is the same one used by embryos: developmentally regulated cell adhesion molecules.

While growing mitotically on bacteria, *Dictyostelium* cells do not adhere to one another. However, once cell division stops, the cells become increasingly adhesive, reaching a plateau of maximum cohesiveness around 8 hours after starvation. The initial cell-cell adhesion is mediated by a 24,000-Da (24-kDa) glycoprotein that is absent in growing cells but is seen shortly thereafter (Figure 1.24; Knecht et al., 1987; Loomis, 1988). This protein is synthesized from newly transcribed mRNA and becomes localized in the cell membranes of the myxamoebae. If these cells are treated with antibodies that bind to and mask this protein, the cells will not stick to each other and all subsequent development ceases.

Once this initial aggregation has occurred, it is stabilized by a second cell adhesion molecule. This 80-kDa glycoprotein is also synthesized during the aggregation phase. If it is defective or absent in the cells, small slugs will form, and their fruiting bodies will be only about one-third the normal size. Thus, the second cell adhesion system seems to be needed for retaining a large enough number of cells to form large fruiting bodies (Müller and Gerisch, 1978; Loomis, 1988). In addition, a third cell adhesion system is activated late in development, while the slug is migrating. The protein or group of proteins that mediates the third system may exist only on prespore cells and may be responsible for separating the prespore cells from the prespores (Loomis, personal communication). Thus, *Dictyostelium* has evolved three developmentally regulated systems of cell-cell adhesion that are necessary for the morphogenesis of individual cells into a coherent organism. As we will see in subsequent chapters, metazoan cells also use cell adhesion molecules to form the tissues and organs of the embryo.

*Dictyostelium* is a "part-time multicellular organism" that does not form many cell types (Kay et al., 1989), and the more complex multicellular organisms do not form by the aggregation of formerly independent cells. Nevertheless, many of the principles of development demonstrated by this "sim-
Evidence and Antibodies

Biology, like any other science, does not deal with facts; rather, it deals with evidence. Several types of evidence will be presented in this book, and they are not equivalent in strength. As an example, we will use the analysis of cell adhesion in Dicystostelium. The first, and weakest, type of evidence is correlative evidence. Here, correlations are made between two or more events, and there is an inference that one event causes the other. As we have seen, fluorescently labeled antibodies to a certain 24-kDa glycoprotein do not label dividing vegetative cells, but they do find this protein in myxamoeba cell membranes soon after the cells stop dividing and become competent to aggregate (see Figure 1.24). Thus, there is a correlation between the presence of this cell membrane glycoprotein and the ability to aggregate.

Correlative evidence gives a starting point to investigations, but one cannot say with certainty that one event causes the other based solely on correlations. Although one might infer that the synthesis of this protein caused the adhesion of the cells, it is also possible that cell adhesion caused the cells to synthesize this new glycoprotein or that cell adhesion and the synthesis of the 24-kDa glycoprotein are separate events initiated by the same underlying cause. The simultaneous occurrence of the two events could even be coincidental, the events having no relationship to each other.*

How, then, does one get beyond mere correlation? In the study of cell adhesion in Dicystostelium, the next step was to use those same antibodies to block the adhesion of myxamoebae. Using a technique pioneered by Gerisch's laboratory (Beug et al., 1970), Knecht and co-workers (1987) took the antibodies that bound this 24-kDa glycoprotein and isolated their antigen-binding sites (the portions of the antibody molecule that actually recognize the antigen). This was necessary because the whole antibody molecule contains two antigen-binding sites and would therefore artificially crosslink and agglutinate the myxamoebae. When these antigen-binding fragments (called Fab Fragments) were added to the aggregation-competent cells, the cells could not aggregate. The antibody fragments inhibited the cells' adhering together, presumably by binding to the 24-kDa glycoprotein and blocking its function. This type of evidence is called loss-of-function evidence. While stronger than correlative evidence, it still does not make other inferences impossible. For instance, perhaps the antibodies killed the cell (as might have been the case if the 24-kDa glycoprotein were a critical transport channel). This would also stop the cells from adhering. Or perhaps the 24-kDa glycoprotein has nothing to do with adhesion itself but is necessary for the real adhesive molecule to function (such as by stabilizing membrane proteins in general). In this case, blocking the glycoprotein would similarly cause the inhibition of cell aggregation. Thus, loss-of-function evidence must be bolstered by many controls demonstrating that the agents causing the loss of function specifically knock out the particular function and nothing else.

The strongest type of evidence is gain-of-function evidence. Here, the initiation of the first event causes the second event to happen even in instances where neither event usually occurs. Recently, da Silva and Klein (1990) and Fai and co-workers (1990) have obtained such evidence to show that the 30-kDa glycoprotein is an adhesive molecule. They isolated the gene for the 30-kDa protein and modified the gene in a way that would cause it to be expressed all the time. They then placed it back into well-fed, vegetatively growing myxamoebae that do not usually express this protein and that are not usually able to adhere to each other. The presence of this protein on the cell membrane of these dividing cells was confirmed by antibody labeling. Moreover, such cells now adhered to one another even in the vegetative stages, when they normally do not. Thus, they had gained an adhesive function solely upon expressing this particular glycoprotein on their cell surfaces. This gain-of-function evidence is more convincing than other types of analysis. Similar experiments have recently been performed on mammalian cells (see Chapter 3) to demonstrate the presence of particular cell adhesion molecules in the developing embryo.

*In a tongue-in-cheek letter spoofing such correlative inferences, Sies (1988) demonstrated a remarkably good correlation between the number of storks seen in West Germany from 1965 to 1980 and the number of babies born during those same years.
DIFFERENTIATION IN DICTYOSTELIUM. Differentiation into stalk cell or spore cell reflects one of the major phenomena of embryogenesis: the cell’s selection of a developmental pathway. Cells often select a particular developmental fate when alternatives are available. A particular cell in a vertebrate embryo, for instance, can become either an epidermal skin cell or a neuron. In *Dictyostelium*, we see a simple dichotomous decision, because only two cell types are possible. How is it that a given cell becomes a stalk cell or a spore cell? Although the details are not fully known, a cell’s fate appears to be regulated by certain diffusible molecules. The two major candidates are differentiation-inducing factor (DIF) and cAMP. DIF appears to be necessary for stalk cell differentiation. This factor, like the sex-inducing factor of *Volvox*, is effective at very low concentrations (10^{-10} M); and, like the Volvox protein, it appears to induce the differentiation of a particular type of cell. When added to isolated amoebae or even to prespore (posterior) cells, it causes them to form stalk cells. The synthesis of this low-molecular-weight lipid is genetically regulated, for there are mutant strains of *Dictyostelium* that form only spore precursors and no stalk cells. When DIF is added to these mutant cultures, stalk cells are able to differentiate (Kay and Jermy, 1983; Morris et al., 1987), and new stalk-specific mRNAs are seen in the cell cytoplasm (Williams et al., 1987). While the mechanisms by which DIF induces 20 percent of the grex cells to become stalk tissue are still controversial (see Early et al., 1995), DIF may act by releasing calcium ions from intracellular compartments within the cell (Shaulsky and Loomis, 1995).

Although DIF stimulates amoebae to become prestalk cells, the differentiation of prespore cells is most likely controlled by the continuing pulses of cAMP. High concentrations of cAMP initiate the expression of prespore-specific mRNAs in aggregated amoebae. Moreover, when slugs are placed in a medium containing an enzyme that destroys extracellular cAMP, the prespore cells lose their differentiated characteristics (Figure 1.25; Schaap and van Driel, 1985; Wang et al., 1988a,b).

**Figure 1.25**
Chemicals controlling differentiation in *Dictyostelium*. (A) and (B) show the effects of placing *Dictyostelium* slugs into a medium containing enzymes that destroy extracellular cAMP. (A) Control grex stained for the presence of a prespore-specific protein (white regions). (B) Similar grex stained after the treatment with cAMP-degrading enzymes. No prespore-specific product is seen. (C) Higher magnification of a slug treated with DIF (in the absence of ammonia). The stain used here binds to the cellulose wall of the stalk cells. All cells of the grex have become stalk cells. (A and B from Wang et al., 1988a; C from Wang and Schaap, 1989; courtesy of the authors.)
How the Grex Knows Which End Is Up

If all the amoebae of the grex start out equal, how can cells in the posterior four-fifths of the slug differentiate into spore cells while equivalent cells in the anterior fifth become stalk cells? The answer may lie in the observation that all the original cells are not equal. Amoebae that become starved during the early portion of their cell cycle tend to move to the anterior portion of the slug, while amoebae starved toward the end of their cell cycle tend to remain in the posterior (McDonald and Durston, 1984; Weijer et al., 1984). This work has been confirmed and extended by Ohmori and Maeda (1987), who showed that cells starved in the latter part of the cell cycle respond differently to cAMP and show much higher adhesivity than cells starved just after mitosis. Williams and co-workers (1989) have found that prespore and prestalk cells can be distinguished in early aggregates and that they are randomly distributed throughout these hemispherical mounds. Thus, the tendencies toward certain fates have been established even before the grex starts migrating. Within each aggregate, most of the prestalk cells actively migrate to the anterior, while prespore cells remain in what becomes the posterior region of the grex. This migration is probably due to repeated pulses of cAMP that are still emanating from the apical tip of the aggregate. These pulses are chemotactic for prestalk cells but not for prespore cells, so they draw the prestalk cells to the tip of the aggregate (Matsukuma and Durston, 1979; Mee et al., 1986; Siegert and Weijer, 1991; Takeuchi, 1991). Cyclic AMP, then, is seen to play several different roles in Dictyostelium development. It aggregates the cells together, it induces prespore cell differentiation, and it directs the migration of prestalk cells to the anterior of the aggregate.

Once the aggregate is complete, it tops over onto its side and forms the migrating grex. Most of the prestalk cells are in the anterior 20 percent of the grex, but there are also some scattered prespore cells throughout the posterior. Prestalk cells can be distinguished by their secretion of extracellular matrix protein A into the spaces between their cells. Within the center of the anterior portion of the grex, another group of prestalk cells begin secreting a second new protein (extracellular matrix protein B) into their extracellular matrix. These central cells are called prestalk B (pstB) cells, while the majority of the prestalk cells are denoted as prestalk A (pstA) cells (Figure 1.26). Another group of prestalk cells, the pstO cells, are scattered sparsely throughout the prespore cells, and they migrate more slowly toward the anterior. When the grex finds itself in sunlight, it ceases migrating and undergoes the final differentiation into spores and a stalk. During this process (called culmination), the grex sits on one end so that the rearward cells become its base. Some pstA cells migrate onto the central tube of pstB cells, and as they contact the central tube, they differentiate into pstB cells by synthesizing new extracellular matrix components. The new cells are added to the anterior region of the tube and force the tube farther down into the culminating structure. This tube differentiates to become the stalk. At the same time, the pstA cells that had been in the posterior region of the grex migrate to the boundaries of the prespore region and differentiate into the spore case and the basal disc (Williams and Jerryn, 1991; Harwood et al., 1992). Eventually, the spores are lifted 2 mm off the ground, from which point they can be dispersed by the wind or by a passing animal.

The trigger for culmination appears to be sunlight or low humidity. Recent experiments suggest that these two factors cause the diffusion of ammonia from the slug. Ammonia is copiously produced by

---

[Diagram showing cell differentiation stages]

---

Figure 1.26 Regulation of stalk cell differentiation during the culmination phase of Dictyostelium growth. Schematic representation of cell migration shows that prespore and prestalk cells are usually mixed in the early aggregate stage, but sort out so that most of the prestalk cells are at the anterior of the grex. The prestalk A cells constitute most of the anterior of the grex, with some similar cells in the posterior. Prestalk B cells are seen in the center of the anterior portion of the grex. In the early culmination stages, the prestalk cells in the posterior migrate to form the basal disc and cups of the spore sac; and the anterior prestalk A cells migrate toward the center and become prestalk B cells. This extends the stalk until it lifts the spore case off the surface. (After Harwood et al., 1992.)
migrating slugs and represses culmination. Whenever ammonia is depleted (either naturally or experimentally), culmination begins (Schindler and Sussman, 1977; Newell and Ross, 1982; Bonner et al., 1985). Ammonia inhibits the conversion of the pstA cells into pstB cells and prevents further stalk formation (Gross et al., 1983; Wang et al., 1990). Bonner and co-workers (1985) have suggested that because light causes more rapid diffusion of ammonia, it removes the inhibitor and thereby allows culmination to proceed.

Ammonia appears to inhibit stalk production in at least two ways. First, it inhibits the action of DIF (Wang and Schaap, 1989). Second, it inhibits the production of cAMP in the prestalk cells (Schindler and Sussman, 1977; Harwood et al., 1992). This cAMP is needed to activate the enzyme cAMP-dependent protein kinase (PKA). Prestalk cells carrying nonfunctional PKA cannot phosphorylate certain proteins. These cells do not migrate into the central anterior region, nor do they differentiate into stalk cells (Firtel and Chapman, 1990; Harwood et al., 1992). The data suggest that when PKA is activated, it phosphorylates a repressor that had been inhibiting the stalk differentiation genes from being expressed. In its phosphorylated state, the repressor is inactive. Therefore, once cAMP levels are elevated (by the removal of ammonia), PKA can inactivate the inhibitor of the stalk-forming genes (Figure 1.27).

![Diagram of developmental patterns among the metazoans]

**Developmental patterns among the metazoans**

Since the remainder of this book concerns the development of metazoans—multicellular animals that pass through embryonic stages of development—we will present an overview of their developmental patterns.* The final chapter of the text will discuss these patterns in more detail. Figure 1.28 illustrates the major evolutionary trends of metazoan development. The most striking observation is that life has not evolved in a straight line; rather, there are several branching evolutionary paths. We can see that most of the species of metazoans belong to one of two major branches of animals: protostomes and deuterostomes.

*Plants undergo equally complex and fascinating patterns of embryonic and post-embryonic development. However, plant development differs significantly from that of animals, and to have included a comprehensive treatment of plant development would have doubled the length of this text. Therefore, the decision was made to focus this text on the development of animals. For an overview, see Singer, 1997.
Figure 1.28
Major evolutionary divergences in extant animals. (Other models are possible, but the general schemes are all similar to the one shown here.)

The Porifera
The colonial protists are thought to have given rise to at least two groups of metazoa, both of which pass through embryonic stages of development. One of these groups is the Porifera (sponges). These animals develop in a manner so different from that of any other animal group that some taxonomists do not consider them metazoa at all (and call them "parazoans"). A sponge has three major types of somatic cells, but one of these, the archeocyte, can differentiate into all the other cell types in the body. The cells of a
sponge passed through a sieve can regenerate new sponges from individual cells. Moreover, in some instances, such reaggregation is species-specific: if individual sponge cells from two different species are mixed together, each of the sponges that re-forms contains cells from only one species (Wilson, 1907). In these cases, it is thought that the motile archeocytes collect cells from their own species and not from others (Turner, 1978). Sponges contain no mesoderm, so there are no true organ systems in the Porifera; nor do they have a digestive tube or circulatory system, nerves, or muscles. Thus, even though they pass through an embryonic and a larval stage, sponges are very unlike most metazoans (see Fell, 1997).

**Protostomes and Deuterostomes**

The other group of metazoans arising from the colonial protists is characterized by the presence of three germ layers during development. Some members of this group constitute the Radiata, so called because they have radial symmetry, like that of a tube or a wheel. The Radiata include the cnidarians (jellyfish, corals, and hydras) and ctenophores (comb jellies). In these animals, the mesoderm is rudimentary, consisting of sparsely scattered cells in a gelatinous matrix. Most metazoans, however, have bilateral symmetry and thus constitute the Bilateria. These bilateral phyla are classified as either flatworms, protostomes, or deuterostomes. All Bilateria are thought to have descended from a primitive type of flatworm. These flatworms were the first to have a true mesoderm (although it was not hollowed out to form a body cavity), and they are thought to have resembled the larvae of certain contemporary coelenterates. While the flatworms are coelolate (having no body cavity), the roundworms (and rotifers) have a body cavity distinctive from all other animals since it is not lined with mesoderm. The majority of phyla are coelolate, that is, they possess a mesoderm-lined body cavity.

The differences in the two coelomate divisions of the Bilateria are illustrated in Figure 1.29. **Protostomes** (from the Greek, meaning “mouth first”), which include the mollusc, arthropod, and worm phyla, are so called because the mouth is formed first, at or near the opening to the gut, which is produced during gastrulation. The anus forms later at another location. The body cavity of these animals forms from the hollowing out of a previously solid cord of mesodermal cells. The other great division of the Bilateria is the **deuterostome** lineage. Phyla in this division include chordates and echinoderms. Although it may seem strange to classify humans and horses in the same group as starfish and sea urchins, certain embryological features stress this kinship. First, in deuterostomes (from the Greek, meaning “mouth second”), the mouth opening is formed after the anal opening. Also, whereas protostomes generally form their body cavities by hollowing out a solid mesodermal block (schizocoelous formation of the body cavity), most deuterostomes form their body cavities from mesodermal pouches extending from the gut (enterocoelous formation of the body cavity). It should be mentioned that there are many exceptions to these generalizations.

Protostomes and deuterostomes differ in the way they undergo cleavage. In most deuterostomes, the blastomeres are perpendicular or parallel to each other. This is called radial cleavage. Protostomes, on the other hand, have a wide variety of cleavage types. Many species form blastulae composed of cells that are at acute angles to the polar axis of the embryo. Thus, they are said to undergo spiral cleavage. Furthermore, the cleavage-stage blastomeres of most deuterostomes have a greater ability to regulate development than do those of protostomes. If a single blastomere is removed from a four-cell sea urchin or mouse embryo, that blastomere will develop
into an entire organism, and the remaining three-quarters of the embryo will also develop normally. However, if the same operation were performed on a snail or worm embryo, both the single blastomere and the remaining ones develop into partial embryos—each lacking what was formed from the other.

The evolution of organisms depends on inherited changes in their development. One of the greatest evolutionary advances—the amniote egg—occurred among the deuterostomes. This type of egg, exemplified by that of a chicken (Figure 1.30), is thought to have originated in the amphibian ancestors of reptiles about 255 million years ago. The amniote egg allowed vertebrates to roam on land, far from existing ponds. Whereas most amphibians must return to water to breed and enable their eggs to develop, the amniote egg carries its own water and food supplies. The egg is fertilized internally and contains yolk to nourish the developing embryo. Moreover, it contains four sacs: the yolk sac, which stores the nutritive proteins, the amnion, which contains the fluid bathing the embryo; the allantois, in which waste materials from embryonic metabolism collect; and the chorion, which interacts with the outside environment, selectively allowing materials to reach the embryo. The entire structure is encased in a shell that allows the diffusion of oxygen but is hard enough to protect the embryo from environmen-
Figure 1.30
Diagram of the amniote egg of the chick, showing the development of membranes enfold ing the embryo. (A) Three-day incubation. The extraembryonic mesoderm extends from the embryo to provide blood vessels to and from the various regions outside the embryo. (B) Seven-day incubation. The origin of the membranes will be detailed in Chapter 9. The yolk is eventually surrounded by the yolk sac, which allows the entry of nutrients into the blood vessels. The chorion is derived in part from the ectoderm and extends from the embryo to the shell (where it will exchange oxygen and carbon dioxide and obtain calcium from the shell). The amnion provides the fluid medium in which the embryo grows, and the allantois collects nitrogenous wastes that would be dangerous to the embryo. Eventually, the endoderm becomes the gut and encircles the yolk. The evolution of the amnion and the other extraembryonic membranes formed a great dividing line between those vertebrates whose reproduction is tied to water (amniotes) and those that can reproduce on dry land (amniotes).

Developmental biology provides an endless assortment of fascinating animals and problems. In this text, we will encounter but a tiny sample of them to illustrate the major principles of animal development. (For a more comprehensive survey of the diversity of animal development across the phyla, see Gilbert and Raunio, 1997.) We are merely observing the tide pool within our reach while the whole ocean of developmental phenomena lies before us. After a brief outline of the genetic and cellular principles relevant to developmental biology, we will investigate the early stages of animal embryogenesis: fertilization, cleavage, gastrulation, and the establishment of the vertebrate body plan. Later chapters will concentrate on the genetic and cellular mechanisms by which animal bodies are constructed. Although an attempt has been made to survey the important variations throughout the animal kingdom, a certain deuterostome chauvinism may be apparent.

LITERATURE CITED


