Although all the volvocaceans, like their unicellular relative Chlamydomonas, reproduce predominantly by asexual means, they are also capable of sexual reproduction, which involves the production and fusion of haploid gametes. In many species of Chlamydomonas, including the one illustrated in Figure 2.10, sexual reproduction is isogamous ("the same gametes"), 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 pattern is called heterogamy ("different gametes"). 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 and Speculations). Here we see one type of gamete specialized for the retention of nutritional and developmental resources and the other type of 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, which is capable of surviving harsh environmental conditions. When conditions allow the zygote to germinate, it first undergoes meiosis to produce haploid offspring of the two different mating types in equal numbers.

**Differentiation and Morphogenesis in Dictyostelium: Cell Adhesion**

**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 2.17A. In its asexual 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 myxamoebae join together to form moving streams of cells that converge at a central point. Here they pile atop one another to produce a conical mound called a tight aggregate. Subsequently, a tip arises at the top of this mound, and the tight aggregate bends over to produce the migrating slug (with the tip at the front). The slug (often given the more dignified title of pseudoplasmodium or grex) is usually 2–4 mm long and is encased in a slimy sheath. The grex begins to migrate (if the environment is dark and moist) with its anterior tip slightly raised. When it

*Though colloquially called a "cellular slime mold," Dictyostelium is not a mold, nor is it consistently slimy. It is perhaps best to think of Dictyostelium as a social amoeba.
reaches an illuminated area, migration ceases, and the grex differentiates into a fruiting body composed of spore cells and a stalk. The anterior cells, representing 15–20% of the entire cellular population, form the tubed stalk. This process begins as some of the central anterior cells, the prestalk cells, begin secreting an extracellular coat and extending a tube through the grex. As the prestalk cells differentiate, they form vacuoles and enlarge, lifting up the mass of prespore cells that had made up the posterior four-fifths of the grex (Jermyn and Williams 1991). The stalk cells die, but the prespore 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 myxamoebae 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 myxamoebae into a single organism is an incredible feat of organization that invites experimentation to answer questions about the mechanisms involved.

Vade Mecum Slime mold: The life cycle of Dictyostelium—the remarkable aggregation of myxamoebae, the migration of the slug, and the truly awesome culmination of the stalk and fruiting body—can best be viewed through movies.

[Click on Slime Mold]

Aggregation of Dictyostelium Cells. The first question is, What causes the myxamoebae 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 can be 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 one another 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) (Konijn et al. 1967; Bonner et al. 1969), the chemical structure of which is shown in Figure 2.18A.
Figure 2.18
Chemotaxis of Dicystostelium myxamoebae due to spiral waves of cAMP. (A) Chemical structure of cAMP. (B) Visualization of several cAMP "waves." Central cells secrete cAMP at regular intervals, and each pulse 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 myxamoebae moving toward the initial source of cAMP: (C) This digitally processed dark-field photomicrograph shows about 16° cells. Because moving and non-moving 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; C, D from Siegert and Weijer 1989.)

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 myxamoebae (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 2.18B–D). As each wave arrives, the cells take another step toward the center.*

The differentiation of individual myxamoebae into either stalk (somatic) or spore (reproductive) cells is a complex matter. Raper (1940) and Bonner (1957) demonstrated that the anterior cells normally become stalk, while the remaining, pos-

*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 the cell's 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 should 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 (see Nanjundiah 1997, 1998). The result is the rotating spiral of CAMP and migration seen in Figure 2.18. 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).
terior cells are usually destined to form spores. However, surgically removing the anterior part of the slug does not abolish its ability to form a stalk. Rather, the cells that now find themselves at the anterior end (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 or 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 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 individual cells stick together to form a cohesive organism? This problem is the same one 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 myxamoebae but appears shortly after division ceases (Figure 2.19; 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 myxamoebae are treated with antibodies that bind to and mask this protein, they will not stick to one another, 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. This protein appears to be important in the movement of the prestalk cells to the apex of the mound (Ginger et al. 1998). 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 "simple" organism also appear in embryos of more complex phyla (see Loomis and Insall 1999). The ability of individual cells to sense a chemical gradient (as in the myxamoeba's response to cAMP) is very important for cell migration and morphogenesis during animal development. Moreover, the role of cell surface proteins in cell cohesiveness is seen throughout the animal kingdom, and differentiation-inducing molecules are beginning to be isolated in metazoan organisms.

DIFFERENTIATION IN DICTYOSTELIUM. Differentiation into stalk cell or spore cell reflects another major phenomenon 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 ($10^{-11} M$); and, like the Volvox protein, it appears to induce differentiation into a particular type of cell. When added to isolated myxamoebae 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 prestalk-specific mRNAs are seen in the cell cytoplasm (Williams et al. 1987). While the mechanisms by which DIF induces 20% 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).
Evidence and Antibodies

How, then, does one get beyond mere correlation? In the study of cell adhesion in Dictyostelium, the next step was to use the antibodies that bound to the 24-kDa glycoprotein to block the adhesion of myxamoebae. Using a technique pioneered by Gerisch's laboratory (Beug et al. 1970), Knecht and co-workers (1987) isolated the antibodies' 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 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 cells (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 (perhaps, for example, it stabilizes 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. For instance, da Silva and Klein (1990) and Faix and co-workers (1990) obtained such evidence to show that the 80-kDa glycoprotein of Dictyostelium is an adhesive molecule. They isolated the gene for the 80-kDa protein and modified it in a way that would cause it to be expressed all the time. They then placed it back into well-fed, dividing myxamoebae, which do not usually express this protein and are not usually able to adhere to one another. The presence of this protein on the cell membrane of these dividing cells was confirmed by antibody labeling. Moreover, the treated cells now adhered to one another even in the proliferative 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 evidence. Similar experiments have recently been performed on mammalian cells to demonstrate the presence of particular cell adhesion molecules in the developing embryo.

Evidence must also be taken together. "Every scientist," writes Fleck (1979), "knows just how little a single experiment can prove or convince. To establish proof, an entire system of experiments and controls is needed." Science is a communal endeavor, and it is doubtful that any great discovery is the achievement of a single experiment, or of any individual. Correlative, loss-of-function, and gain-of-function evidence must consistently support each other to establish and solidify a conclusion.

Although DIF stimulates myxamoebae 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 myxamoebae. Moreover, when slugs are placed in a medium containing an enzyme that destroys extracellular cAMP, the prespore cells lose their differentiated characteristics (Figure 2.20; Schaap and van Driel 1985; Wang et al. 1988a,b).

W E B S I T E 2.5 The control of Dictyostelium cell type.
Whether a myxamoeba becomes a spore cell or a stalk cell depends on the stage in the cell cycle at which it stops moving as well as on what chemical cues are in its immediate environment.
Sponges develop in a manner so different from that of any other animal group that some taxonomists do not consider them metazoans at all, and call them "parazoans." A sponge has three major types of somatic cells, but one of these, the archaeocyte, can differentiate into all the other cell types in the body. Individual cells of a sponge passed through a sieve can reaggregate to form new sponges. 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 archaeocytes collect cells from their own species and not from others (Turner 1978). Sponges contain no mesoderm, so the Porifera have no true organ systems; 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 (Fell 1997). However, sponges do share many features of development (including gene regulatory proteins and signaling cascades) with all the other animal phyla, suggesting that they share a common origin (Coutinho et al. 1998).

**Diploblasts**

Diploblastic animals are those who have ectoderm and endoderm, but no true mesoderm. These include the cnidarians (jellyfish and hydras) and the ctenophores (comb jellies).

Cnidarians and ctenophores constitute the Radiata, so called because they have radial symmetry, like that of a tube or a wheel. In these animals, the mesoderm is rudimentary, consisting of sparsely scattered cells in a gelatinous matrix.

**Protostomes and deuterostomes**

Most metazoans have bilateral symmetry and three germ layers. The animals of these phyla, known collectively as the Bilateria, are classified as either 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 may have resembled the larvae of certain contemporary coelenterates.

There are two divisions of bilaterian phyla, the protostomes and the deuterostomes. **Protostomes** (Greek, "mouth first"), which include the mollusca, 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 **coelom**, or body cavity, of these animals forms from the hollowing out of a previously solid cord of mesodermal cells.

There are two major branches of the protostomes. The **ecdysozoa** includes those animals that molt. Its major constituent is Arthropoda, a phylum containing insects, arachnids, mites, crustaceans, and millipedes. The second major group of protostomes are the **lophotrochozoa**. They are char-