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The *Dictyostelium* Life Cycle: Variations within Variations

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. 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 reaches an illuminated area, migration ceases, and the culmination stages of the life cycle take place as 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 cellulose 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 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.

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

<Barresi13e, Further Development_c25_05_02>

formerly independent cells. Nevertheless, many of the principles of development demonstrated by this “simple” organism also appear in the 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 crucial for cell migration and morphogenesis during animal development. Moreover, the role of cell surface proteins in cell cohesion is seen throughout the animal kingdom, and differentiation-inducing molecules are now being isolated in metazoan organisms.

*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.

WEBSITE 2.5 Slime mold life cycle. Check out this website to see digitized videos of the *Dictyostelium* life cycle.

VADE MECUM^2 Slime mold life cycle. 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. The Slime Mold segment in Vade Mecum^2 contains a remarkable series of videos. [Click on Slime Mold]

In addition to this asexual cycle, there is a possibility of sex for *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 differentiate into 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, a process 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.

<Barresi13e, Further Development_c25_05_02>

AGGREGATION OF *DICTYOSTELIUM* CELLS. The first of these questions is, What causes the myxamoebae to aggregate? Time-lapse videomicroscopy 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 mm/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 a result of **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 2A**.

Aggregation is initiated as each of the myxamoebae 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 the 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 for about a minute, and they release cAMP of their own (Robertson et al. 1972; Shaffer 1975). The movement of each myxamoeba is caused by the change in cytoskeletal polarity brought about by the cAMP (Parent et al., 1998; Iijima et al., 2002) . After this happens, the cell is unresponsive to further cAMP pulses for several minutes. During this time, an extracellular membrane-associated phosphodiesterase then cleaves the remaining camp from the environment, allowing the receptors to get ready to receive another pulse. The result is a rotating spiral wave of cAMP that is propagated throughout the population of cells (**Figure 2. B–D**). As each wave arrives, the cells take another step toward the center.*

*The biochemistry of this reaction involves a receptor that binds cAMP. This binding activates a small G-protein that regulates the polymerization of the actin portion of the cytoskeleton. Furthermore, the immediate exposure of the front of the cell to cAMP causes the polarity of certain enzymes to shift, as well. 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

<Barresi13e, Further Development_c25_05_02>

cAMP and migration seen in Figure 2. 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).

Recent studies (Kriebel et al 2008, 2018) suggest that some fraction of the cAMP is released from the migrating amoeba within extracellular vesicles. These vesicles form within each cell and are released upon their migration. These microvesicles contain cAMP as well as the channels that export the cAMP and the enzymes that synthesize cAMP. The secreted vesicles appear to be making and secreting cAMP, thereby maintaining a trail chemoattractant during migration.

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, posterior cells are usually destined to form spores. However, surgically removing the anterior part of a 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 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 adhesiveness about 8 hours after starvation. The initial cell-cell adhesion is mediated by a 24-kilodalton glycoprotein (gp24; DdCad1) that is absent in myxamoebae but appears shortly after mitotic division ceases (Figure 2.3; Knecht et al. 1987; Wong et al. 1996).

<Barresi13e, Further Development_c25_05_02>

This protein is synthesized from newly transcribed mRNA and becomes localized in the cell membranes of the myxamoebae. Like many important mammalian cell-cell adhesion proteins, gp24 needs calcium to become active. Moreover, if myxamoebae are treated with antibodies that bind to and mask this protein, the cells will not stick to one another, and all subsequent development ceases.

SCIENTISTS SPEAK 25.2 Dr. John Tyler Bonner discusses his pioneering work demonstrating how the environment alter development to turn a single cell organism multicellular.

Once this initial aggregation has occurred, it becomes stabilized by a second cell adhesion molecule. This 80-kDa glycoprotein (gp80; CsaA) 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). During late aggregation, the levels of gp80 decrease, and its role is taken over by a third cell adhesion protein, a 150-kDa protein (gp150; LagC) whose synthesis becomes apparent just prior to aggregation and which stays on the cell surface during grex migration (Wang et al 2000; **Figure 3**). If *Dictyostelium* cells lack functional genes for gp150, development is arrested at the loose aggregate stage, and the prespore and prestalk cells fail to sort out into their respective regions. 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.

DIFFERENTIATION OF DICTYOSTELIUM CELLS

Differentiation into stalk cell or spore cell reflects another major phenomenon of embryogenesis: the cell's selection of a developmental pathway. In *Dictyostelium*, as in *Volvox*, we see a simple dichotomous decision, because only two final cell types are possible. How is it that a given cell becomes a stalk cell (somatic) or a spore (germline) cell? There

appears to be a progressive commitment to one of the two alternative pathways (**Figure 4**). At first there is a *bias* toward one path or another. Then, there is a *labile specification*, a time when the cell will normally become either a spore cell or a stalk cell, but when it can still change its fate if placed in a different position in the organism. The third and fourth stages are a *firm commitment* to a specific fate, followed by the cell's *differentiation* into a particular cell type, either a stalk cell or a spore cell.

BIAS. Although the details are not fully known, a cell's fate appears to be regulated by both internal and external agents. Pre-aggregation myxamoebae are not all the same; they can differ in several ways. The internal factors distinguishing individual myxamoebae include nutritional status, cell size, cell cycle phase at starvation, and intracellular calcium levels (Nanjundiah 1997; Azhar et al. 2001). Each of these factors can act to bias the cell toward a prespore or a prestalk pathway. For instance, cells starved in the S and early G2 phases of the cell cycle have relatively high levels of calcium and display a tendency to become stalk cells, while those starved in mid- or late G2 have lower calcium levels and tend to become spore cells.

LABILE SPECIFICATION. Several factors are important in specifying cells as stalk or spore. Cyclic AMP, after functioning as an aggregation factor, is still needed to form the prestalk and prespore cells. However, due to the biases in these cells, cAMP is used in different ways by the prespore and prestalk cells (see Kimmel and Firtel, 2004). In the pre-spore cells of the grex, extracellular cAMP initiates the expression of spore-specific mRNAs. It does this by inducing a protein called β-catenin, which enters the nucleus to activate certain spore-specific genes (Ginsburg and Kimmel 1997; Plyte et al., 1999; Kim et al., 2002). In the prestalk cells that are in the anterior tip of the grex, cAMP suppresses this pathway and causes these cells to become prestalk cells. Another group of prestalk cells are formed by a secreted chlorinated lipid, DIF-1, which is made by the prespore cells (Fukuzawa et al., 2003; Thompson et al., 2004),

COMMITMENT AND DIFFERENTIATION. Cell migration continues even while the cells are in the grex. Cell movement within the slug is also mediated by chemotaxis to the source of cAMP. In the mound and slug, waves of cAMP start in the apex, the uppermost cells. Prestalk cells move more rapidly than the prespore cells, and this results in the most of the

prestalk cells being in the anterior of the migrating grex (Clow et al., 2000; Dormann and Weijer, 2001). Some of the same cell adhesion systems that were responsible for the aggregation of myxamoebae also appear to be functioning for the correct orientation of the cells in the grex (Wong et al., 2002).

Two secreted proteins, spore differentiation factors SDF1 and SDF2, appear to be important in the final differentiation of the prespore cells into encapsulated spores (Anjard et al. 1998a,b). SDF1 is important in initiating culmination, while SDF2 seems to cause the prespore cells (but not prestalk cells) to become spores. The prespore cells appear to have a receptor that enables them to respond to SDF2, while the prestalk cells lack this receptor (Wang et al. 1999). Culmination is also brought about by declining ammonia concentrations (Follstaedt et al., 2003). Ammonia is released preferentially in the anterior portion of the slug, and it appears to help regulate chemotaxis of the prestalk cells as well as aid in the production of spore cells (Oyama and Blumberg 1986; Feit et al., 2001). The formation of stalk cells from prestalk cells is similarly complicated and may involve several factors working synergistically (Early 1999). Indeed, prestalk cells from different parts of the grex pass through different intermediary cell types before reaching the final stage of stalk cell. Thus, the stalk cells that cover the spores have a slightly different history than those stalk cells that hold the sorus above the ground. The differentiation of stalk cells appears to need a signal from the intracellular enzyme PKA, and at least one type of stalk cell is induced by the DIF-1 lipid (Thompson and Kay 2000; Fukuzawa et al. 2001).

Many of the pathways used by *Dictyostelium* to create its two cell types and multicellularity will also be seen to be used by animals. The ability to sense a gradient and respond to it by chemotaxis, the ability to receive an external signal and transduce that signal into the nucleus so that it can change gene expression, and the importance of calcium-dependent adhesion molecules will each be seen as important themes in animal and plant development.

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<Barresi13e, Further Development_c25_05_02>

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<Barresi13e, Further Development_c25_05_02>

<Barresi13e, Further Development_c25_05_02>

Figure 1 **{Figure 2.16 from 7th ed. P. 39}**

Life cycle of *Dictyostelium discoideum*. Haploid spores give rise to myxamoebae, which can reproduce asexually to form more haploid myxamoebae. As the food supply diminishes, aggregation occurs and a migrating slug is formed. The slug culminates in a fruiting body that releases more spores. Times refer to hours since the onset of nutrient starvation. Prestalk cells are indicated in yellow. (Photographs courtesy of R. Blanton and M. Grimson.)

Figure 2 **{Figure 2.17 from 6th ed., p. 40}**

Chemotaxis of *Dictyostelium* myxamoebae is a result of 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. The waves were 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) Spiral waves of myxamoebae moving toward the initial source of cAMP. 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. As cells form streams, the spiral of movement can still be seen moving toward the center. (D) Computer simulation of cAMP wave spreading across migrating *Dictyostelium* cells. The model takes into account the reception and release of cAMP, and changes in cell density due to the movement of the cells. The cAMP wave is plotted in dark blue. The population of amoebae goes from green (low) to red (high). Compare with the actual culture shown in (C). (B from Tomchick and Devreotes 1981; C from Siegert and Weijer 1989; D from Dallon and Othmer 1997.)

Figure 3 {Figure 2.18 from 7th ed.p. 40}

The three cell adhesion molecules of *Dictyostelium*. (A) *Dictyostelium* cells synthesize an adhesive 24-kDa glycoprotein (gp24) shortly after nutrient starvation. These *Dictyostelium* cells were stained with a fluorescently labeled (green) antibody that binds to gp24 and were then

observed under ultraviolet light. This protein is not seen on myxamoebae that have just stopped dividing. However, as shown here—10 hours after cell division has ceased—individual myxamoebae have this protein in their cell membranes and are capable of adhering to one another. (B) The gp80 protein, stained by specific antibodies (green), is present at the cell membranes of streaming amoebae. (C) The gp150 protein (green) is present in the cells of the migrating grex (cross-sectioned). Photographs are not at the same magnification. (Photographs courtesy of W. Loomis.)

Figure 4 **{Figure 2.19 from 7th ed.P. 43}**
Alternative cell fates in *Dictyostelium discoideum*. (A–C) Progressive commitment of cells to become either spore or stalk cells. (A) Myxamoebae may have biases toward stalk or spore formation due to the stage of the cell cycle they were in when starved. (B) As the grex migrates, most prestalk cells are in the anterior third of the grex, while most of the posterior two-thirds are prespore cells. Some prestalk cells are also seen in the posterior, and these cells will contribute to the cups of the spore sac and to the basal disc at the bottom of the stalk. The cell fates are not yet fixed, however, and if the stalk-forming anterior is cut off, the anteriormost cells remaining will convert from stem to stalk. (C) At culmination, the spore-forming cells are massed together in the spore sac. The stalk cells form the cups of the spore sac, as well as the stalk and basal disc. (D, E) Grex and culminant stained with dye that recognizes the extracellular matrix of the prestalk and stalk cells. (F, G) Grex and culminant stained with a dye that recognizes the extracellular matrix of prespore and spore cells. (After Escalante and Vicente 2000. Photographs courtesy of R. Escalante.)