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A Brief Introduction to *Dictyostelium discoideum* and its Relatives

Dictyostelium discoideum is the most studied species of the social amoebae, which are also known as the cellular slime molds. All of these organisms live in the soil and feed on bacteria, living a solitary life until the bacteria are consumed. The onset of starvation forces a major revision in the life cycle, and the amoebae respond by collecting into aggregates which transform into an organism that undergoes cell differentiation and morphogenesis. The result is a fruiting body consisting of a ball of resistant spores suspended on a stalk. *D. discoideum* and similar species have evolved strategies to survive in the harsh environment of the soil. A close examination of these strategies raises questions at all levels of biology: How do the amoebae sense starvation and other stresses, and how do they respond? How do they communicate with each other and how do they move? What mechanisms of signal transduction do they use, and how do those resemble the mechanisms of more complex organisms? How did the extraordinary cooperativity of development evolve? Rather than forcing the reader who has no experience with these organisms into details immediately, this chapter will provide a short glossary of terms and an overview of development, first in *D. discoideum*, and then in a few related species.

The developmental cycle begins when the amoebae consume all of their prey. If they do nothing to protect themselves, they will die from starvation. In response to this pressure, three distinct responses can occur – the amoebae can form microcysts, macrocysts, or fruiting bodies. The last is by far the most studied because it exhibits the fundamentals of all developing organisms. The cells signal each other to insure their correct proportion and pattern and they regulate – creating two full organisms from the two halves of a severed one. Development in *D. discoideum*, and numerous organisms like it, is composed of two phases: an aggregative period during which cells assemble in response to a

chemotactic signal, and a complex fruiting body stage in which cells differentiate and rearrange themselves to form a mass of spores supported by a stalk. The Dictyostelia have strictly separated growth and developmental stages. A cell either consumes bacteria or other nutrients and divides mitotically, or it develops in response to starvation. One of the useful consequences of this separation is that genes that are induced during development are usually not needed for mitotic growth and so they can be mutated without affecting the viability of the growing organism. Starvation induces a variety of new genes whose products are necessary for chemotaxis toward cAMP (or other chemoattractants). These will be presented in detail in future chapters, but for the moment it is sufficient to realize that cAMP is the molecule that the amoebae recognize during chemotaxis. Their ability to synthesize, release, detect, and degrade cAMP is critical for aggregation and none of these capacities exists in the growing amoebae – all are induced as the cells starve.

The amoebae are grown on lawns of bacteria or in a sterile liquid medium. To begin development in the laboratory we remove the source of nutrients and put the cells on a moist solid substratum. The substrates can be agar or filter paper, as long as it is sufficiently moist. No nutrients are provided during development – the amoebae aggregate and make their fruiting structures entirely on metabolic reserves accumulated during the trophic phase. After the washed amoebae are deposited on the substrate, there is a period of apparent inactivity as many of the genes required during growth are down-regulated, or their proteins are degraded and new genes – whose products are essential for aggregation – are induced. Gradually, after a period of hours, occasional amoebae begin to release cAMP into the population and as the macromolecules that produce, detect, and modulate the cAMP signal are made in increasing amounts, the propagation of the signal becomes stronger and stronger.

The signals that *D. discoideum* amoebae use are relayed, which allows the organism to collect cells from a wide area, so that an aggregate of 100,000 cells can result. The relay system is shown diagrammatically in Fig. 1.1. A single cell in a field of starving cells releases a pulse of cAMP, and other cells detect the cAMP as it binds to their cell surface receptors. First the cells change their shape and then respond in two ways – by releasing another pulse of cAMP, and by moving up the gradient of cAMP released by the first cell. Thus there is an outwardly propagated wave of cAMP and an inward movement of cells. The cAMP propagation and the cell movement happen in steps – the central cells release a pulse of cAMP about every 6 minutes and inwardly moving cells only move as long as the slope of the gradient is positive, so that when the wave decays, the amoebae stop. With dark-field illumination we can see the moving cells because they appear lighter than non-moving cells that are awaiting another wave of cAMP. Such results can be seen in Fig. 1.2. Amoebae can move into aggregation centers from aggregation territories as much as 1 cm across. In the soil, movement of cells into an aggregate would be from three dimensions and over much tougher terrain. The circular patterns of aggregation often evolve into spirals and eventually, as aggregation progresses, the concentric rings break down into long streams of cells, which still move in a

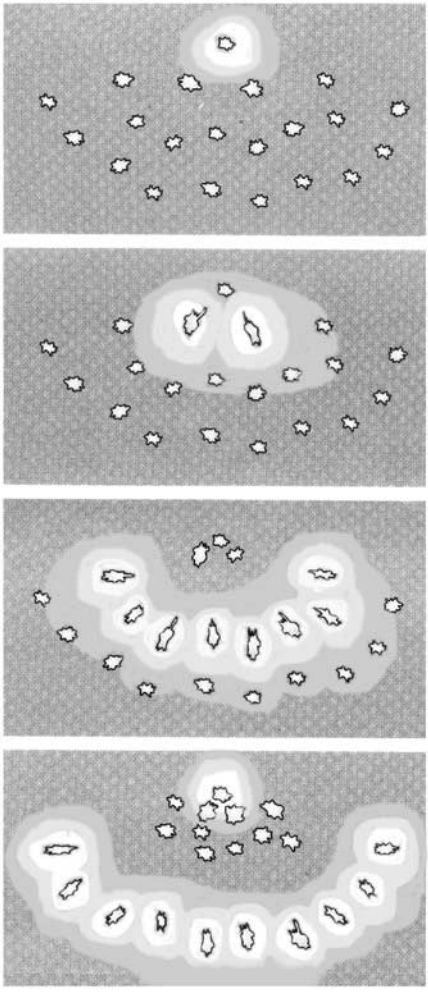

Figure 1.1 During starvation cells develop the capacity to synthesize, detect and destroy cAMP. When one cell releases a pulse of cAMP, neighboring cells detect it, move up its gradient toward higher concentrations and after a minute or so, release cAMP of their own. This attracts more outlying cells. The process is repeated about every 6 minutes under laboratory conditions. (Reprinted by permisson of *American Scientist*, magazine of Sigma Xi, The Scientific Research Society (Kessin and van Lookeren Campagne, 1992).)

periodic fashion into the center. As the cells move into the aggregate they become adhesive and capable of constructing a three-dimensional structure.

Once the cells have collected into a central point, a process that takes about 8 hours under most laboratory conditions, a stage is attained which is called the loose aggregate. This is shown in the scanning electron microscope figure produced by Grimson and Blanton (Fig. 1.3). The loose aggregate is relatively flat, with indistinct borders, but over the next few hours it changes to a

Figure 1.2 The patterns of aggregation can be seen by dark-field illumination because moving and stationary cells reflect light differently. In the region of the immobilized cells (dark bands) there is no longer a cAMP gradient, a situation that will change when the next wave of cAMP is propagated from the center. (Courtesy of Peter C. Newell, University of Oxford.)

hemispherical shape, shown as the second structure in Fig. 1.3, the tight aggregate, or mound. It is covered with a layer of mucopolysaccharide and cellulose that is called the sheath. The third structure in the figure, moving from lower right to left, has formed a critical new element, called the tip. The tip is the source of signals that organize the behavior of cells that are behind it. The tip is

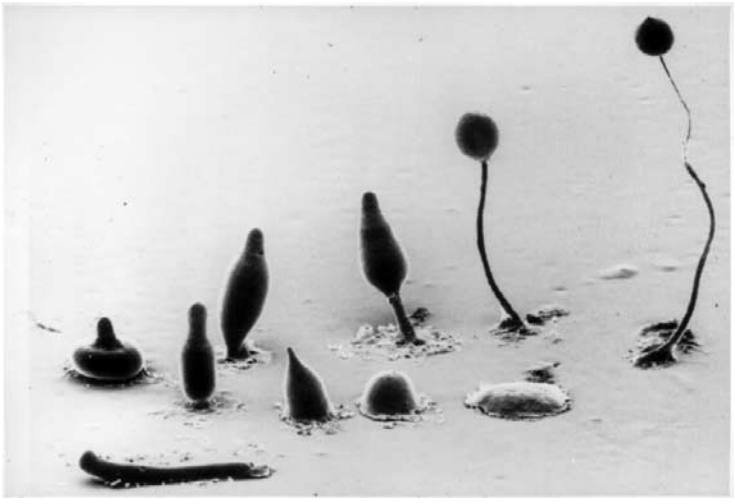

Figure 1.3 The post-aggregation stages of *Dictyostelium* development. Moving counter-clockwise, the stages encountered are the loose aggregate, the tight aggregate, the tipped aggregate, an elongated form called the finger, a slug, and then the stages of culmination leading from the Mexican hat stage to the fruiting body. (This scanning electron micrograph is by R. Lawrence Blanton and Mark Grimson, Texas Tech University.)

an important group of cells that controls development – cut it off and development stops until a new tip is formed. The tip functions like the Mangold/Spemann organizer in vertebrates. Under the control of the tip, the aggregate elongates and makes a structure called a finger, or standing slug. At this point the organism may proceed directly to the production of fruiting bodies, or the finger may fall over and migrate in a structure called the slug or pseudoplasmodium. This is shown as the structure out of the progression in Fig. 1.3. The slug is wonderfully phototactic and is also capable of migrating up very shallow heat gradients. Once they have aggregated, *Dictyostelium* aggregates have many of the properties of an embryo – they have polarity, they have exquisite proportioning, they regulate, and they have an organizing center – the anterior tip.

The transition from migrating slug or standing slug to the fruiting body occurs by a process called culmination. The origins of cells that will make the prespore cells or the prestalk cells can be traced to the growing cells and depends to a certain extent, in a way we will describe later, on the position of a cell in the cell cycle when starvation is imposed. Prespore cells are those which have synthesized certain proteins in preparation for becoming spore cells, while prestalk cells are similarly identified by the expression of proteins that contribute to the stalk. As their names imply, each type is a progenitor of a terminally differentiated cell. Whether this separation into two precursor cell types occurs by a positional information mechanism or by a sorting mechanism is a question that we will defer. Neither of these cell types is fully differentiated

or committed. Until very late in development, one type can convert to the other and given food, each type will revert to an amoeboid state.

By the time of aggregation, genes that are essential to the formation of the spore or the stalk have been expressed in the two precursor populations, and by the time of the tipped aggregate these two cells types have nearly sorted out, so that the cells that will make the stalk are on top and the cells that will form the spores are on the bottom. The topological problem faced by the developing cells is how to get the spores on top and the stalk on the bottom. The stalk is formed by a movement of cells from the tip through a collar at the apex of the aggregate. A special class of prestalk cells migrates through and down toward the substratum. During this period they become vacuolated as they make cellulose and die. Gradually the prespore cells are lifted up, as shown in the four last structures of Fig. 1.3. The left most structure in Fig. 1.3 is called a Mexican hat, for obvious reasons. It is the earliest of the culminating forms. The next stages (left to right) are called early to late culminants. As the spore mass is pulled up the stalk, the prespore cells undergo encapsulation, in which the contents of internal vesicles are released by exocytosis to form a layered cell wall of mucopolysaccharide and cellulose around each spore. Encapsulation spreads in a wave from the top of the developing spore mass to the bottom. How this is regulated so that it only happens at the very end of culmination will be described in a future chapter. The final structure, the fruiting body, contains a spore mass, supported by a slender stalk. The ratio of stalk to spore cells is about 1:4 and varies little between large fruiting bodies and small ones.

In *D. discoideum*, no mature stalk cells are present in the slug. Some species have a variant on this theme in which the stalk forms in the center of a migrating slug, which appears to have a rod down the middle. Such is the case with *D. mucoroides*, the first species isolated by Brefeld in 1869. As the slug migrates, it extends along the stalk which is left behind and can provide tensile strength, so that migration is in the air as well as on a surface. There are a variety of other species, some much more common than *D. discoideum*. These include *D. purpureum, D. giganteum*, *D. lacteum* and many others (Cavender, 1990; Hagiwara, 1989; Raper, 1984).

There are other variations – in the species *Polysphondylium violaceum* and *Polysphondylium pallidum*, culminating fruiting bodies develop delicate whorls, such that a series of secondary stalks and spore masses are produced along the main axis (Byrne and Cox, 1986; Harper, 1929). How this occurs so precisely, so that the secondary spore masses are evenly spaced, is a fundamental question of pattern formation. The various mature fruiting bodies are shown in Fig. 1.4. There is another difference among species, particularly the two species of *Polysphondylium*. Although these are all aggregative organisms, they do not all use cAMP as a chemotactic molecule. *P. violaceum* uses a dipeptide called glorin, which was identified in a tour de force of chemical analysis by Shimomura, Suthers, and Bonner (1982).

The significance of aggregation as a means of producing a mass of cells for differentiation is discussed in the next chapter, but the Dictyostelid species described above have more distant relatives (Cavender, 1990). These are

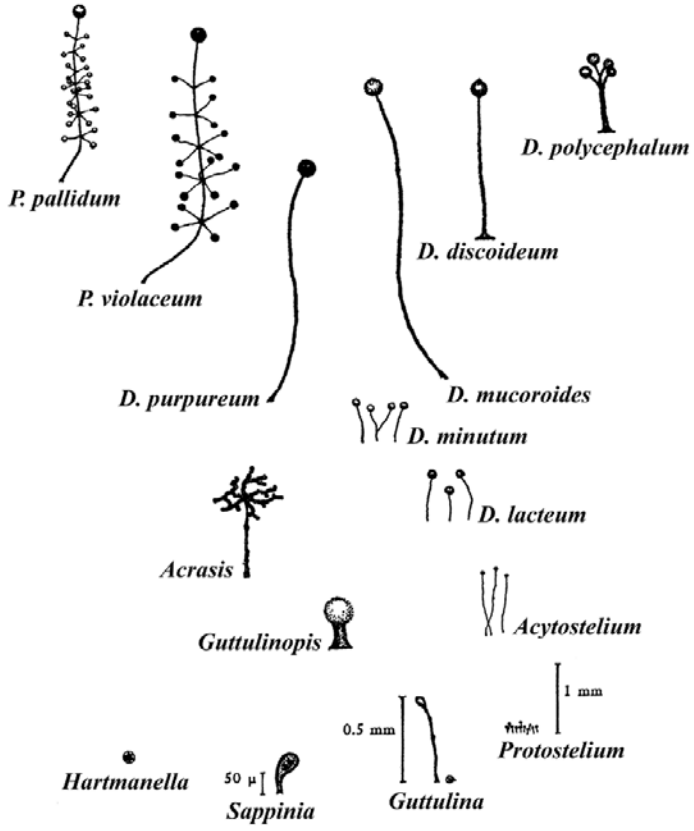

Figure 1.4 The various fruiting body structures of Dictyostelids. Note the distinction between *Dictyostelium* and *Polysphondylium* with its secondary stalks. Other much more distantly related organisms are also shown. These share an aggregative life style but may be of independent evolutionary origin. *Protostelium* forms a stalk and spore from a single cell (Bonner, 1967). (Reprinted by permission of Princeton University Press.)

grouped under the title of Acrasidae and Acytosteliaceae by Raper, whose 1984 book should be consulted for keys and other material for assigning unknown organisms to particular classes or species, as well as excellent descriptions (Raper, 1984). A series of aggregative organisms, all of which have an amoeboid trophic form and a fruiting structure that depends on aggregation, are known. These include species like *Acrasis rosea, Copromyxa protea*, and *Guttulina rosea* (Bonner, 1967; Raper, 1984). Most are little studied, especially from the cell biological or molecular point of view, but all share the capacity to assemble multiple cells to form a fruiting structure and all are inhabitants of the soil. Little is known about the details of their development or whether the chemotactic and developmental mechanisms they use resemble those of *D. discoideum*. There is no reason to believe that these organisms and *D. discoi-*

deum are monophyletic – the aggregative strategy for creating a larger organism could have evolved many times, and probably did (Blanton, 1990).

Since their discovery in 1869 by Brefeld, these organisms have been called slime molds. Phylogenetically they are not fungi, as we will learn in Chapter 3, nor are they slimy, as organisms go. For this reason, and because the title *slime mold* does not sound sufficiently elevated or convey an idea of the true evolutionary niche, many workers in the field have decided to use the designations *Dictyostelium* or social amoeba. Attempts to redirect a language are usually not successful, as the French Academy is no doubt aware, but in this book, we will use *Dictyostelium* or social amoeba.

2

A History of Research on *Dictyostelium discoideum*

The first description of *Dictyostelium*, by the mycologist Oskar Brefeld, is 130 years old (Brefeld, 1869). Many of the features of these organisms that modern workers assume to be obvious – the phagocytic nature of the amoebae, the separation of growth and developmental cycles, the absence of cell fusion in the aggregate – were not apparent to early workers. Culture systems had not been developed and the ease of manipulation that now makes these organisms so attractive would not be used until the 1930s by Kenneth Raper (Raper, 1937).

Brefeld (1869) first observed *Dictyostelium mucoroides* while examining the fungal flora in horse dung, and then grew purer cultures in rabbit dung. Even with this difficult culture method, Brefeld realized that the amoebae were the trophic (feeding) form, and that they aggregated to give rise to fructifications. He named the species *Dictyostelium* (Dicty means net-like and stelium means tower) because the aggregation territories he observed looked like nets (Fig. 2.1) and the fruiting bodies like towers (Fig. 2.2). He added the qualifier *mucoroides* because the new organism resembled the fungus *Mucor*. This was a misnomer because closer examination by Brefeld (1884) established that his new species did not have the same sporangial walls as the fungus, but instead the spores were suspended in a drop of liquid. The germination of the spores led not to hyphae and a mycelium but to distinctly amoeboid cells, which the microscopes of the time were quite capable of resolving. Brefeld correctly determined that the walls of stalk and spores contained cellulose.

It was left to Van Tieghem to show in 1880 that, far from fusing in the manner of *Physarum*, the cells of *D. mucoroides* and several other species retain their individual, if cooperative, character in the aggregate (Van Tieghem, 1880). Brefeld, realizing his earlier mistake, published a paper in 1884 which discerned the individual nature of the cells in the pseudoplasmodium (Brefeld,

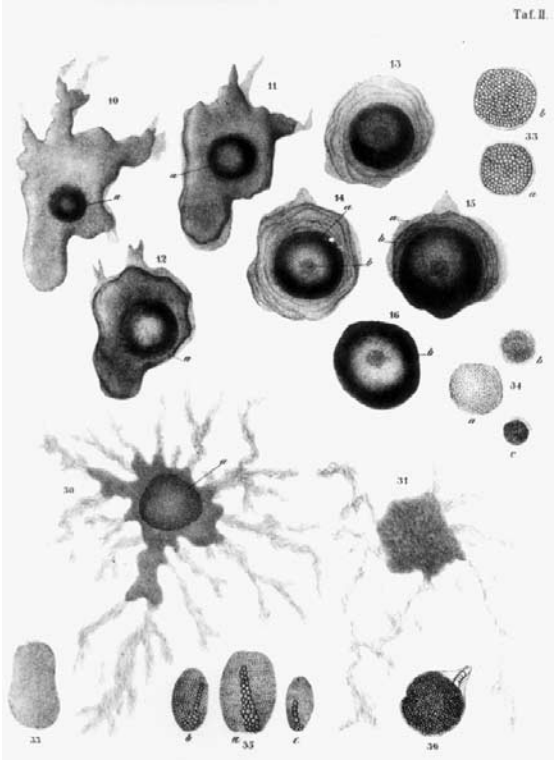

Figure 2.1 The original drawings of Brefeld show the aggregation fields of *D. mucoroides* and the aggregates. Stages that we now call tight aggregates are also shown. In 1869, Brefeld thought these were syncytial. On the upper right are drawings of what are probably macrocysts (Raper, 1984). (I am grateful to Thomas Winckler of the Johann Wolfgang Goethe University for finding the original paper and preparing these photographs.)

1884). Brefeld's 1884 paper was richly illustrated, which the Van Tieghem papers were not.

The nineteenth century investigators were constrained by difficult growth conditions based on concoctions of dung, sometimes stiffened with agar, sometimes not, but in all cases contaminated with bacteria upon which, we now know, the amoebae feed. Prior to the work of the great immunologist Elie Metchnikoff, phagocytosis was poorly recognized and there was no reason for early workers to believe that the amoebae obtained nourishment in a way different from that of the fungi – by extracellular digestion. That cells could surround and digest bacteria internally was a revolutionary idea. So it is not surprising that in 1899, when G. A. Nadson reported that *D. mucoroides* grew with a known species of bacteria – *Bacillus fluorescens liquifaciens* – he assumed that the two organisms were symbionts, rather than predator and prey (Nadson, 1899/1900). In 1902, Potts developed media more sophisticated than