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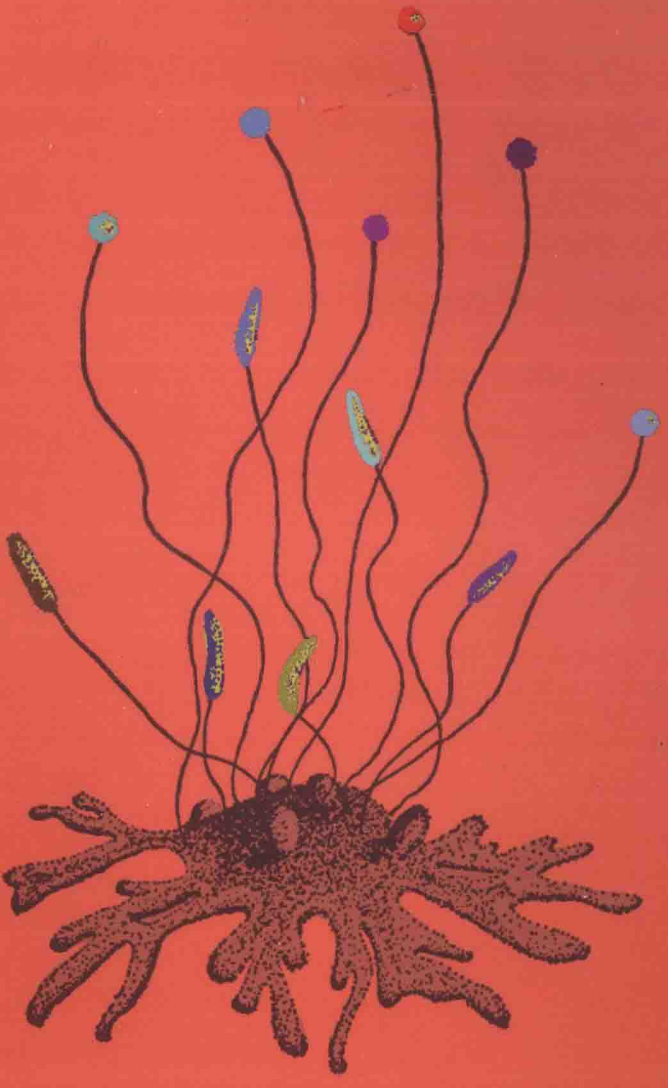

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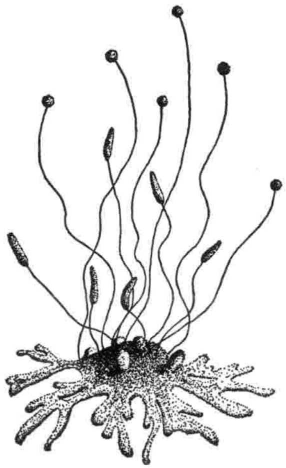

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Preface

I have lived with my beloved slime molds for a long time, and now suddenly I find myself quite overcome by the vast amount of new facts that have accumulated to account for every stage, every step (however small) of their life cycle. In the late 1940s and early 1950s (1945 to 1951) an average 3.4 papers on cellular slime molds were published a year; now, over the past seven years, there is an average of 224 papers a year! We are in danger of drowning in facts.

This is what stimulated me to write this book. It is an exercise to clarify my own thoughts. As I wandered into thinking about it, I decided to write something that is aimed at the curious layperson if I could explain the complete picture to him or her then I would understand it myself. I plan to shed all unnecessary jargon and concentrate on what I think are the main issues, all the while trying to give a rounded and complete picture of the biology of cellular slime molds. At the same time I will ask where slime molds fit into all of biology and even how they might illuminate that vast subject.

This will be done by concentrating on all of their biology, including their molecular biology. I plan to give equal time to their evolution, their ecology, their behavior, both as single amoeba and as cell masses, and their development. Some of these subjects have made great advances using molecular techniques; others, by their very nature, have been virtually untouched by molecular biology.

It must be said right from the beginning that this is a very personal book: it is my view of slime molds, and my view of biology. Others would no doubt write a very different book, but I hope that my individual slant does not interfere with the story I want to tell. My prejudices will affect the emphasis, and often the choice of topics, but my wish is not to obscure the significance of cellular slime molds and all the lessons they have for us.

I will look at everything through the lens of a full-time biologist. That will include the contributions that molecular biology has made that illuminate basic living processes. There is an enormous difference between what was known when I published the second edition of my *The Cellular Slime Molds* in 1967 and what we know now: it is quite overwhelming. One need only look at Richard Kessin's splendid book *Dictyostelium,* published in 2001, to again see in a dramatic way how rapidly the subject has advanced since 1967. And there have been many more new discoveries since his book was published.

The advances have been largely in the new molecular facts, and this is the reason there has been such a rapid turnover. It is my hope that, by looking at everything from a more generalized biological point of view, the lessons we have learned will last for some time.

One other difficulty in writing such a book is that there are scores of first-rate contributions that will not receive the credit, or even the mention, they deserve. This book is not an encyclopedia, a textbook, or a monograph; it covers only a fraction of all we know about slime molds. Rather, it is an essay on the big lessons we have learned, with a few unconventional and new insights. The details have been shed, fascinating though many of them are, and only the main plot is bared. Because of this I apologize to the many workers in the field whose fine work I have not mentioned, and some of them are—or at least were—good friends!

None of the facts in this book are new: many are common knowledge and some may lie unnoticed under a rock. If there is any novelty it is in the way they are put together.

A number of kind friends were good enough to read earlier versions of this book and their corrections of errors and advice for improvements were of incalculable help to me. In particular I would like to thank Leo Buss, Edward Cox, Vidyanand Nanjundiah, and Pauline Schaap. I would also like to thank Mary Jane West-Eberhard for looking at one section of the book. Throughout the preparation of this book Slawa Lamont has sustained me with her encouragement and her support. Finally, I have been lucky enough to get the tremendous, skilled help from a number of individuals at the Princeton University Press; my special thanks to Alice Calaprice, Alison Kalett, Dimitri Karetnikov, and Deborah Tegarden.

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1 Introduction

Evolution, cell biology, biochemistry, and developmental biology have made extraordinary progress in the last hundred years—much of it since I was weaned on schoolboy biology in the 1930s. Most striking of all is the sudden eruption of molecular biology starting in the 1950s. I will make a reckless generalization that each one of these surges was due to a collision with genetics. Perhaps it would be more accurate to say that they fused rather than just collided, because in each case an extraordinary fruitful symbiosis was the result. First, at the beginning of this century, genetics fused with nineteenth-century cytology, which gave us an understanding of how the genetic material was handled in the chromosomes in mitosis, and particularly in meiosis. Next, genetics fused with Darwinian evolution to give rise to population genetics, a signal advance at the time. Then, with the revolution started by Watson and Crick on the molecular structure of the gene, it was possible, through molecular biology to (1) have a second fusion of genetics with cell biology, making it possible to dissect out the biochemical or molecular events within a cell; (2) to devise a new way of attacking phylogenetic problems in the study of evolution using molecular-genetic techniques; and finally (3), these new approaches made it possible to dissect out the sequence of molecular steps in development.

I am not done with my generalizations! During all the events I have described, there has been a strong tendency to concentrate on "model" organisms. In the past century there was a tremendous emphasis on *E. coli,* the fruit fly *Drosophila*, and the nematode worm *Caenorhabditis*, but beginning back into the nineteenth century there have been many others that have played a similar role. To mention a few, there was Mendel with his garden peas, followed by other organisms such as maize, amphibian, chick and sea urchin embryos, yeast, myxobacteria, zebra fish, the small plant *Aribidopsis*, and the cellular slime molds. One could add a few more and the list would still be incomplete: for instance, ciliate protozoa (such as *Paramecium*), *Hydra* and other hydroids, sponges, *Volvox* and other algae, myxomycetes or true slime molds, *Phycomyces* and other fungi, mice and other mammals. The degree to which these various examples have been directly affected by genetics and molecular biology varies, but even in those cases where the influence has been small (due to the lack of attention) this is beginning to change. In fact, one can say that it is inconceivable to study the biology today on any organism without genetics and molecular biology. For completeness it should be added that there is now renewed interest in another collision: the realization that evolution and developmental biology are inseparable, something that was already recognized by Darwin.

My reason for using cellular slime molds as an example is that they (along with myself) went through the same evolution from a pregenetic period to one deeply involved in molecular genetics. I have been there to watch every step.

When, as an undergraduate, I began experiments on these slime molds in 1940, only one other person, Kenneth Raper, was working on them at that time. In fact, he discovered the "model" species *Dictyostelium discoideum*, which is the species used in the majority of the experimental work today. His early experiments were in the classic mold of the embryology of that time and are still recognized today as being at the root of all subsequent work.

As a young student at Harvard I developed two great interests. One was the fungi and other lower plants which were the province of my professor, William H. Weston. He was a charismatic teacher who exuded excitement for the possibility that lower (cryptogamic) plants, such as algae and fungi, made ideal subjects for experimental studies. He had many distinguished students, and when I started with him as an undergraduate, one of his finishing graduate students was John Raper, who was making the pioneer discovery of sex hormones in the water mold *Achlya*. Ralph Emerson had made similar significant advances with another water mold, *Allomyces*. While surrounded by these older students and Weston himself, I knew I wanted to be a cryptogamic botanist. But then I took a course in animal embryology with Professor Leigh Hoadley and was suddenly confronted with all the wonderful work of Hans Driesch, Wilhelm Roux, Hans Spemann, Edwin Grant Conklin, Ross Harrison, and many others who had advanced experimental embryology in the nineteenth and early twentieth centuries. I became entrapped all over again—I wanted to become two people. Then one day it dawned on me: why not work on the embryology (or developmental biology as it became to be known later) of lower plants!

To do this I had to find the ideal organism. Because I was surrounded by water mold enthusiasts it was very tempting to choose their area of interest. However, all that changed when one day I found the Ph.D. thesis of Kenneth Raper's (John's older brother), who had done his graduate work with Professor Weston a few years earlier. It described his discovery of *D. discoideum* and those wonderful experiments I mentioned. Here was exactly what I was looking for: the ideal non-animal embryo. I immediately wrote to Kenneth Raper, and he sent me cultures with some gracious encouragement that has kept me going for almost seventy years.

The question of how an egg develops into an adult was a matter of wonder and concern going back to Aristotle, and it blossomed at the end of the nineteenth century and into the twentieth: one could experiment on embryos and discover the causes of the developmental steps—why one stage produced the next. In the 1940s and 1950s it was realized that it was not just the embryos of higher animals and plants that developed, but development was a property of all organisms, from fungi to algae and other lower forms. Now the cellular slime molds showed themselves to be ideally suited for the study of experimental developmental biology. So first, following the tradition of Raper, an interest began not only in the description of the phases of their development, but in experimental studies parallel to those of causal embryology.

At first there was a difficulty because there was no known sexual system for the cellular slime molds. Later, when it was discovered, it turned out to be intractable and did not allow any way to do simple crossing experiments, unlike Mendel's model organism, the garden pea. In the beginning of the attack there were moderately successful ways of getting around this difficulty, and later, with the arrival of more and more clever molecular techniques, the disadvantage of the lack of ability to do crossing experiments virtually disappeared. The molecular genetics of the developmental biology of *D. discoideum* (which was now simply called *Dictyostelium*, reflecting its newfound status as a model organism) became central. The most recent high point in this program has been the sequencing of its entire genome: now it is possible to find out how many genes we share with a slime mold. The result is that our insights and understanding of the development of *Dictyostelium* have vastly increased.

In this joyous molecular roller-coaster ride there are many things about these slime molds that have to some degree been neglected, therefore stimulating me to write this book. Besides the central role of their development, many other aspects of their biology are equally fascinating, and here I would like to give the whole picture. As we shall see, they have not been ignored but simply overshadowed by the number of workers and publications in molecular developmental biology. Here I want to give all aspects of cellular slime mold biology equal time.

This means I want to give something closer to equal time to their evolution, their ecology, and their behavior, as well as their development. Within these big categories, starting with evolution, I will include discussions of their history on Earth, the taxonomy of the whole group and how they are related to one another, the origin of their multicellularity, and the interesting aspects of their peculiar sociobiology. The discussion of ecology will also involve their distribution in the soil, which is their natural habitat, their geographic distribution, and their mechanisms of dispersal. Concerning their behavior, besides the mechanism of how individual amoebae come together in the aggregation chemotaxis, two other matters are of major interest: one is the mechanism of locomotion of the migrating slugs, and the other is how slugs orient—what is the mechanism that makes them turn in towards light and sense heat and chemical gradients. More work has been done on aggregation chemotaxis than on any other aspect of slime mold biology; there is a rich literature. Turning to development, one aspect that has been studied in detail is the mechanism of differentiation of stalk cells and spores and how their proportions are controlled. Also there is the interesting question of the ways in which different species differ in their patterns of differentiation. As in all of biology, comparative studies showing differences among species are often helpful for a better understanding of the basic mechanisms; with all its advantages, there is a danger of clinging exclusively to one model organism. Finally, considering the great volume of work these days on the molecular analysis of development, I want to explore in what ways it has shed light on fundamental issues. It has done so, but some of the successes stand out as particularly significant.

2 The Life Cycle

One of the most striking things about cellular slime molds is their life cycle; it is so different. I remember the times when I first gave lectures on my experiments in the 1940s, and at question time the audiences of biologists seemed to show no interest in all the clever things I thought I had done, but they wanted to know more of what was then an unheard-of life cycle. Now that cycle can be found in every elementary biology textbook.

All the organisms with which we are most familiar, both animal and plant, including ourselves, start as a single cell, usually a fertilized egg, that takes in nutriment from a yolk (or a yolklike substance as in the cotyledons of a higher plant), and the egg begins to grow through repeated cell divisions, in that way achieving a large, multicellular state. In the case of animals, by the time the yolk is used up the embryo must have some other means of taking in energy: it must eat. This means that the yolk must sustain it long enough to allow time for the construction of a gut with a mouth to take in food and a tube that not only produces enzymes to break down the larger molecules to smaller sugars and amino acids, but also makes it possible for them to be absorbed through the gut wall. Animal alimentary systems are complex, and their invention must have had a long evolutionary history. Once achieved, it clearly was not reconstructed *de novo* for each animal group; all used the same basic food-processing machinery and the variations are later adaptations for the kind of food taken in. Some time ago, John R. Baker[1] wrote a short note in *Nature* pointing out that small photosynthetic organisms, compared to primitive animals, produced a great variety of shapes simply because they did not need a feeding device; they could take in all the energy they required and be any old shape: all they had to do is catch the Sun's rays. However, in both animals and plants they become large by growth made possible by a constant influx of energy.

The cellular slime molds acquire their energy in an entirely different way. They feed first as independent soil amoebae. Each individual amoeba surrounds a bacterium with its pseudopods, encases it in a food vacuole, and extracts the needed nutrients. Once they have cleaned an area of bacteria, they then come together; they aggregate to form a multicellular organism. Unlike animals and plants, they eat first; they grow by simply producing an increasing number of separate amoebae, and then when all the food is gone they stream together to become multicellular. It means that once they form their fruiting bodies they can no longer do anything that requires an intake of energy: they are static. The only part of them that is alive are the dormant spores. So we do have certain advantages in our kind of life cycle: we can keep going and enjoy life; we are not permanently frozen

[1] Baker, J. R. (1948).

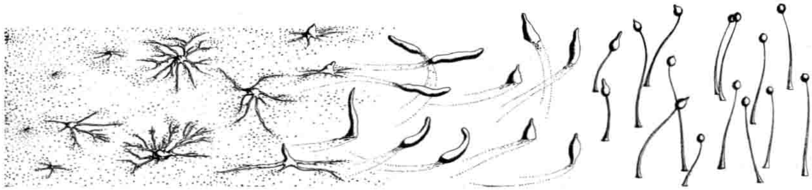

Fig. 1. The life cycle of *Dictyostelium discoideum* from the feeding stage (*left*), through aggregation, migration, and the final fruiting (*right*). (Drawing by Patricia Collins, *Scientific American* 1969)

once mature. (It should be noted that in general annual vascular plants do something very similar to slime molds. For example, a wheat plant will shoot up into the air in late summer, produce a bunch of dormant seeds at the top, and then the rest of the plant dies, turning a beautiful golden yellow.)

There is another group of organisms that do their feeding as single cells. Sponges are lined with flagellated chambers that sweep in currents of water from their submerged environment, and each of the flagellated cells will feed on incoming food particles. The difference between sponges and cellular slime molds is that sponges develop and eat at the same time, while slime molds separate the two processes in time and do one after the other.

Let me now describe in some detail the life cycle of one species of slime mold. I will choose the most familiar, *Dictyostelium discoideum*, but all are essentially similar (fig. 1). They normally grow in the soil where they are impossible to see, and therefore in the laboratory we cultivate them on a clear agar gel surface making it possible to follow all the steps of their progress as though they were living in a glass house. First they are supplied with bacterial fodder. This can be