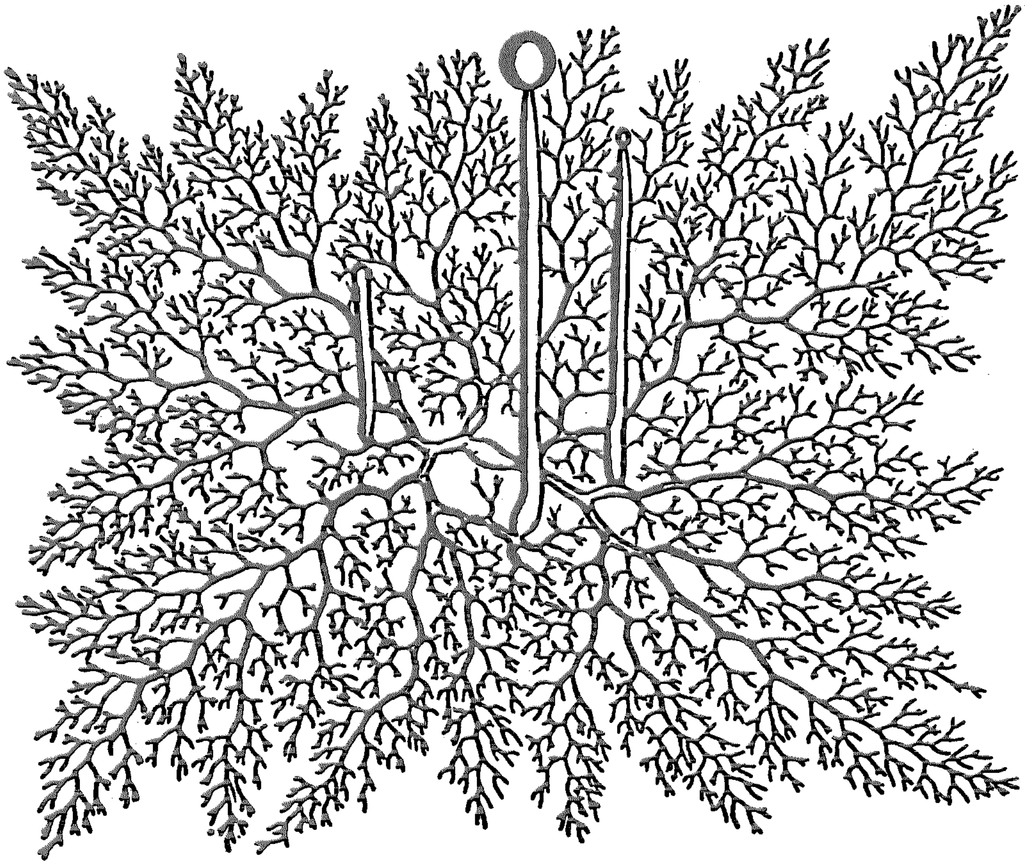


The humble fungus Phycomyces—*a valuable research tool for Caltech's molecular biologists.*

Molecular Biology—The Next Phase

By MAX DELBRÜCK

Some years ago R. P. Feynman gave a speech rather puzzlingly entitled "There's Plenty of Room at the Bottom." As it turned out, his comments were concerned with the revelations of molecular genetics showing that living nature had evolved degrees of miniaturization of devices for the storage, replication, and readout of information which far surpassed anything that engineering science has developed so far. As is now well known even to children in elementary school, the principal device employed here is the famous *double helix*. The molecular biology revolving around this helix might be classified as one-dimensional molecular biology, since, from the point of view of physics, the DNA molecule is a one-dimensional solid state object.

It stands to reason that nature, operating in three dimensions, long ago figured out that two-dimensional structures may also have their special virtues. Indeed, ultrastructural work done with the electron microscope during the last decades has amply revealed that the cells of all organisms employ two-dimensional structures not only on the outer boundary but also as parts of intracellular organelles. These structures are membranes with very characteristic general features—a thickness of about 70 angstroms and a composition always involving two classes of compounds, polar lipids and proteins. These membranes serve many functions. We find them functioning as parts of chemical factories—as floor space for the organized arrangement of systems of enzymes. We find them as phase separators, creating and maintaining volume phases of different chemical composition. We find them as surface structures of nerve fibers capable of transmit-

To subdivide space for concentration and seclusion, to localize function and partition structure, cells use membranes. Membranes are particularly conspicuous in many sense organs. What is their role therein?

ting signals along the length of the fiber.

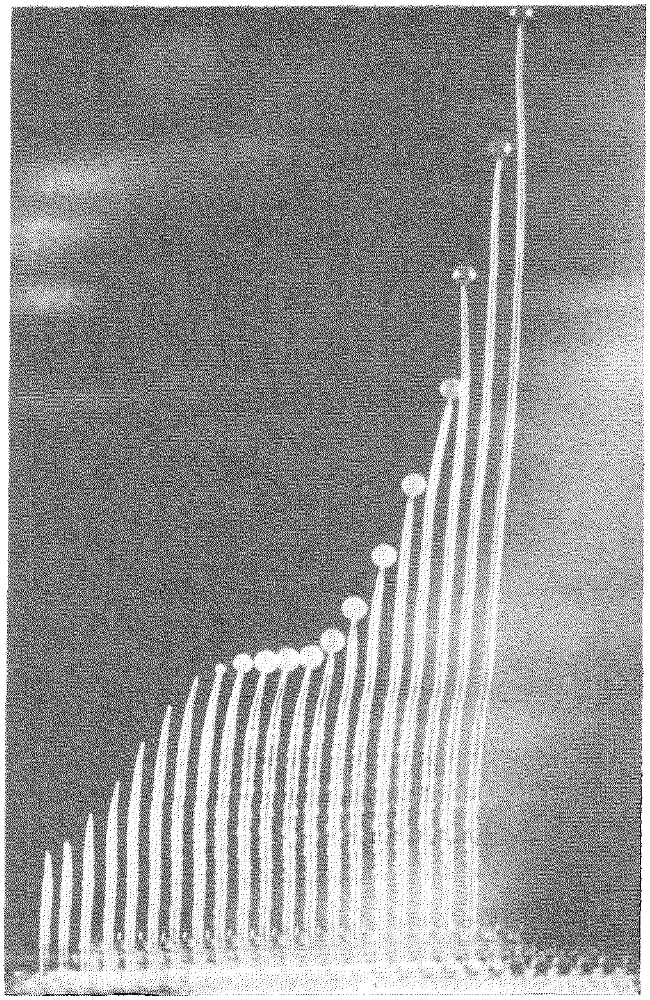
In all of these situations the functions of membranes are sensitively controlled by environmental physical and chemical factors. This control is driven to its ultimate degree of discrimination in the display of surface specificity involved in development, and reaches its ultimate degree of sensitivity in the devices used to process incoming signals such as light, touch, or smell—devices used to adjust the behavior of organisms to the external environment. Through modification of membranes the behavior of the whole cell is then profoundly influenced. On the molecular level these transducer mechanisms are not understood and will constitute the principal challenge for the next phase of molecular biology.

The depth of our ignorance in this area may be compared with the depth of our ignorance with respect to the molecular basis of genetics 30 or 40 years ago. We knew then that there were genes, and we knew that the genes were located in chromosomes, and we knew that they were arranged in a linear order. We also knew that the chromosomes contained proteins and nucleic acids, but for several decades we thought that the proteins represented information storage (specificity, as it was then called) and that the nucleic acids represented a structural backbone. We now know that the reverse is true. Similarly, with respect to membranes, there exists now an extraordinary degree of uncertainty as to the relative roles of protein and lipid: Which one determines structure and which one function?

It must not be thought that sense organs are a specialization limited to animals, though it is true that animals have developed a greater degree of organ specialization for the various sense qualities. But plants, too, respond very sensitively to light, touch, various gases, and gravity, as do microorganisms. It has been known for almost 100 years that some bacteria which use light as an energy source also adjust their motions so that, when they are exposed to a spectrum of light projected onto a microscopic slide, they will congregate at those wavelengths which they can utilize for photosynthesis. Bacteria will also move toward higher concentrations of oxygen, sugars, and amino acids. Wonderful work has been going on in the laboratory of Julius Adler at the University of Wisconsin in recent years, analyzing this sensitivity of bacteria to various stimuli. These studies are making use of the powerful methods of microbial genetics.

In vertebrates, the analysis of sensory transducer processes has made remarkably slow progress in spite of the sustained efforts of vast numbers of physiologists. The reason lies largely in the complexity of the sense organs, the smallness of the individual units, and in many cases in the inaccessibility of the sensory cells from the outside of the living organism. Situations exceptionally favorable for research are presented by the chemical senses of insects where the sense organs are often single cells, external and susceptible to a great deal of manipulation. A marvelous example is the silkworm moth. The male is attracted from great distances to the female by a sex lure emitted by the female in microgram quantities over several days. The receptor organs are hair cells, 10,000 of them, on the antennae of the male. Each of these hair cells is about 2 microns thick and 100 microns long. The cells are arranged in a basket form so as to create a sieve for the oncoming air to pass through. The dimensions are such that molecules of the sex lure are likely to strike one of these hairs while passing through the sieve. Each hair cell has a relatively thick cuticle quite impenetrable to the odorant, but this cuticle is perforated at distances of a few thousand angstroms by little pores about 150 angstroms in diameter. One must imagine that the molecules of the odorant—a C_{16} alcohol—when they strike the hair cell anywhere on its surface, move to these pores by surface diffusion and, having reached a pore, somehow trigger nerve impulses in the two or three fibers that innervate the hair all the way up the length of the hair cell.

The whole arrangement presents a system of



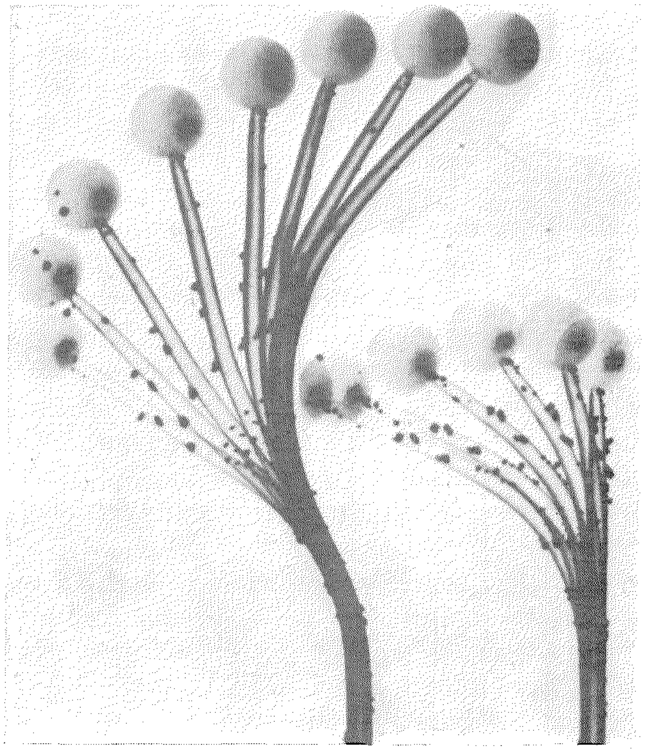
Twenty photos of a sporangiophore, taken at one-hour intervals, show how it grows from a simple cylindrical cell with a conical top to a height of one or two centimeters—at which point it stops growing and forms the sporangium. In a few hours, elongation resumes and quickly reaches a steady rate of about three millimeters an hour until the sporangiophore is about ten centimeters high.

molecular counters far beyond anything engineers have produced with respect to miniaturization, just as the DNA storage of information is far beyond current computer devices. The manner in which the molecules of the odorant trigger the nerve cells is unknown.

Our own work at Caltech has for a number of years been concerned with a microorganism, a fungus of a kind that the taxonomists place very low on the tree of evolution—lower than *Neurospora, Aspergillus,* or yeast, whose genetics have been studied so extensively. But experience with molecular genetics has shown that humbleness can be very profitable. Molecular genetics got its biggest boost from studies with bacteria and bacterial viruses, so why not stoop to a lowly fungus in the hope of here finding relatively simple answers to very deep questions? Let me hasten to add that we have *not* found the answers to these deep questions and that my presentation of what we are doing should be considered as an invitation to join an expedition rather than as a travelogue of adventures of the past.

Our creature, *Phycomyces,* forms a branching mycelium, which grows on almost anything. From this mycelium, sporangiophores, or stalks, are sent up into the air, carrying at their top a little ball—the sporangium—from which eventually 100,000 spores will be dispersed, each capable of initiating a new mycelium.

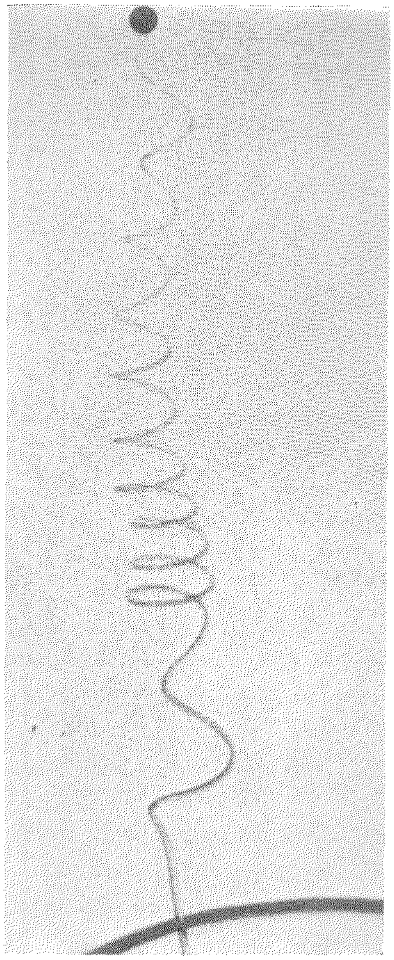
Of greatest interest to us is the bending response to light of the sporangiophore. How is this bending toward the light accomplished? It turns out that simple optics plays an important role. The receptive zone of the stalk, a portion about two millimeters long, is located immediately below the sporangium.



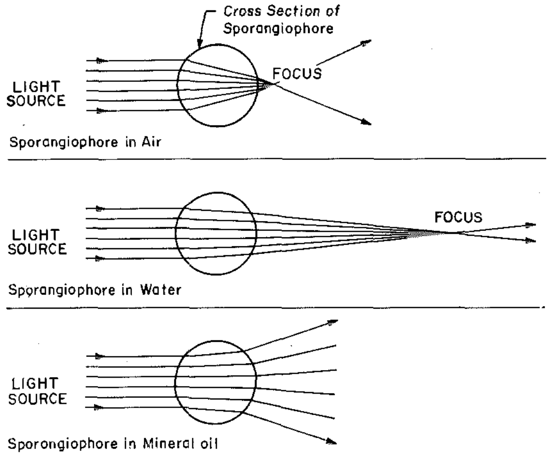
Pictures taken at five-minute intervals show how the sporangiophore (lower right), illuminated by a light source from the left, grows toward the light. When the light from the left is cut off and a light from the right turned on, the sporangiophore (left) quickly responds to the new light stimulus. The small dark specks are starch grains dusted onto the specimen. By following their position in successive pictures it can be seen where the sporangiophore stretches and how it twists during growth.

This is where elongation occurs, and this is also where the bending occurs. This stalk is transparent, and it acts like a cylindrical lens, focusing the impinging light near the distal side. We know that this focusing plays a determining role because if we immerse our specimen in mineral oil, which has a higher refractive index than the stalk, the converging lens becomes a diverging lens and the specimen, instead of growing towards the light, grows away from it (below). Counteracting this simple focusing effect, which in air favors the distal side, absorption and scattering subtract light on its passage through the stalk and favor the proximal side. These factors have been analyzed by finding the "balancing points," i.e., immersion fluids with refractive indices giving phototropic neutrality for a number of wavelengths and a number of mutants differing in their content of colored carotenes.

If our organism can respond to light, it must have a pigment absorbing the light, thereby initiating some mechanism controlling the growth rate. By measuring the relative effectiveness of various colors (the action spectrum), one can form an idea of the absorption spectrum of the receptor pigment. This action spectrum has some peaks close to those in the absorption spectrum of the principal pigment found in the organism. This pigment is β-carotene, and many people have guessed that β-carotene might be the receptor pigment. At Caltech, however, Martin Heisenberg recently obtained mutants which are practically free of colored carotenes or contain a different carotene—lycopene, the pigment which makes tomatoes red. It turns out that these carotene mutants respond to light just as well as the wild type, and we therefore do not think it likely that the receptor pigment is a carotene. Similarly, Gerhard Meissner, a research fellow in biology, was able to rule out retinal, the visual pigment of animals, found in trace amounts also in *Phycomyces.*



The phototropic reaction of a growing sporangiophore can be sustained for many hours if the sporangiophore is put on a turntable making one revolution every two hours, while being illuminated from only one side. Here, 11 full turns are made during 22 hours, and the growth is more than six centimeters.



The growing zone of a sporangiophore is a section two millimeters long, located just below the sporangium. This drawing of a cross section shows how the single cylindrical cell forms a converging lens which concentrates light on its distal side, resulting in a faster stretch on that side—thus a growth toward the light. In mineral oil the cylindrical lens is a diverging one, and the specimens bend away from the light. In water the lens is very weakly converging; the lens effect is overpowered by losses of light due to scattering and absorptions during passage through the sporangiophore so that a slow bending away from the light results.

In the early stages before it has formed the sporangium, the stalk looks somewhat like a miniature centrifuge tube. In a sense it *is* a centrifuge tube. It is a single cell containing protoplasm and a vacuole and, in the protoplasm, a great variety of organelles. It can also be used as a centrifuge tube by floating it inside a capillary tube in a medium that buoys it up and then by putting the whole contraption into a high-speed centrifuge.

This technique was invented and worked out at

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Caltech by Marko Zalokar. It shows how the various organelles inside the cell are sorted out into layers. The nuclear layer, for instance, can be obtained in high purity, and the nuclei in this layer can be shown to be functional by injecting nuclei from one mutant into the stalk of another mutant, thus forming functional heterokaryons. We were in great hopes that one of these layers would show the receptor pigment in such high concentration that we would be able to see it by direct microspectrophotometry. Indeed, a narrow yellow band was found, but closer study showed that this narrow band did not have an absorption spectrum in any way related to that of the receptor pigment. Electron microscopic studies by Dr. Zalokar showed that this layer contains *ferritin,* a protein enclosing a micelle of several thousand units of ferric hydroxide. It is a very interesting molecule in itself and has long been known to occur in animals and higher plants, but it is certainly not related to the light responses.

Another facet of our work concerns the fact that *Phycomyces* resembles the human eye in its ability to cope with an immense range of intensities covering a factor of about 10^9, from bright sunlight to the dimmest the human eye can perceive. The kinetics of this dark/light adaptation are quite similar in *Phycomyces* and in man. Our hope is that, however distant our relation to *Phycomyces* might seem, in this important respect nature may employ a similar device. We hope to analyze this device more deeply by utilizing another class of mutants, the "night-blind" ones, which seem to operate only in the upper range of light intensities.

There are other aspects which recommend the sporangiophore of *Phycomyces* as a model case for the study of sensory transducer processes. It turns out that the growing zone exhibits not only sensitivity to light but also to mechanical stretching, to gravity, and to smell. In regard to stretch sensitivity, which has been studied in some detail by David S. Dennison and Carol Roth at Dartmouth, the basic observation is this: A pull of about one milligram causes a transient slowdown of the growth rate, and a release of that pull causes a transient speedup. Now it must be understood that mechanical tension belongs to a class of stimuli fundamentally different from light or olfaction. In the latter cases the input is molecular. The product of a photochemical reaction or the olfactant molecule attacks the sensor at a defined point in space and time. In contrast, tension, like temperature or electric potential, is a continuous "variable of state," attacking a macroscopic structure as a whole. The type of instability leading to responses in the continuous and in the molecular case, respectively, may be quite different. We suspect, however, that in any of these cases the instability is an expression of highly cooperative phenomena, i.e., phenomena involving very large numbers of interacting subunits (thus giving rise to phase transitions, phase boundaries, and dislocations), and that the structures concerned are membranes of the type alluded to at the beginning of this article.

Characterizing the next phase of molecular biology as a step-up from one to two dimensions may create apprehension that 20 years from now we may be heralding a transition to three-dimensional molecular biology as the next phase. I consider this very unlikely. There exist sound mathematical reasons for believing that nature has found it expedient to reduce all three-dimensional processes of transport, such as tracking and control, to one- and two-dimensional ones. These reasons are related to an interesting mathematical discovery made in 1921 by George Polya. Polya showed that the attempt to reach a given destination by a random walk in unbounded space is certain to meet with success when it is carried out in one or two dimensions but not so in three dimensions. In bounded space the counterpart to this theorem is: If one wants a molecule to reach a specified site by diffusion in three-dimensional space, it is economical to embed the target site in a membrane to which the molecule in question will stick sufficiently tightly to stay adsorbed when it hits the membrane anywhere, and yet sufficiently loosely to enable it to perform two-dimensional diffusion on the membrane. It stands to reason that nature started exploiting the implications of this theorem a few billion years before Polya discovered it.