ARTICLES

The Origins of Multicellularity

JOHN TYLER BONNER

There is great interest in the invention of multicellularity because it is one of the major transitions during the course of early evolution.[1] Most of the emphasis has been on why it occurred. For instance, recently Gerhart and Kirschner[2] have argued that a multicellular organism has gained the advantage of a unicellular ancestor because it can more effectively shield itself from the vagaries of the environment by producing its own internal environment. In broader terms, this is Dawkins'[3] argument that a competitively effective way of carrying the genes from one generation to the next is by building a complex soma that safely sees to it that the germ plasm survives.

KEY WORDS: multicellularity, cell differentiation, early evolution

It has been my contention for some years that while these reasons for the appearance of multicellularity are convincing, they are achieved at the most fundamental level by increased size and thus the key step is a transition to larger size.[4–7] Becoming larger makes it possible to be isolated from the outside world and to be able to protect the genes for the next generation. Furthermore, size increase will be accompanied by a division of labor in the form of cell differentiation, which adds to the selective advantages of the organism under some circumstances. My line of reasoning, which will be developed further presently, is that the first step in the evolution of multicellularity was a size increase due to an accident, e.g., a mutation that prevents the daughter cells from separating. If the larger cell mass has any advantages, such as ensuring the safety of the germ line by producing a protected internal environment, then natural selection will see to it that the novelty is retained. I would argue then that the size increase came first, and the possible advantages that this change might provide would follow.

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Another well-worn point must be stressed from the outset. One assumes the reason for the non-stop selection for organisms of increased size is that the top of the size scale is an ever-present open niche, and has been open during the entire course of organic evolution. Among the many lines of evidence to support this contention, perhaps the most compelling is the simple observation that there exists a continuum—a complete array of sizes among organisms, from the minutest prokaryotes to the largest mammals and angiosperms. The only place for further expansion is into the unfilled niche at the top of the spectrum. For any one population of an organism there can equally likely be a selection for a size decrease, should there be an open niche for smaller forms. In other words, the direction of the selection for size change depends solely on the ecological opportunities, and there is always room at the top. When the earth was populated with nothing but single cells, selection opportunities for multicellularity must have been inexhaustible.

Many of the recent discussion of the appearance of multicellularity are confined to animal evolution. It is usually inferred that this was a unique event that did not spawn appreciable diversity until the great proliferation of body type in the Precambrian era. This view has arisen partly because of the fossil record, which is notably sparse until the rich findings in the Burgess Shale and similar deposits. But as someone who was raised as a cryptogamic botanist, it has always seemed to me that this stress on animal evolution is rather anthropocentric. If one thinks in terms of all organisms known to exist today, or to have existed in the past, then one can only be impressed by the diversity of the beginnings of multicellularity itself.

BRIEF DESCRIPTION OF THE MAJOR ORIGINS OF MULTICELLULARITY

The danger in describing all the different steps toward multicellularity is that the

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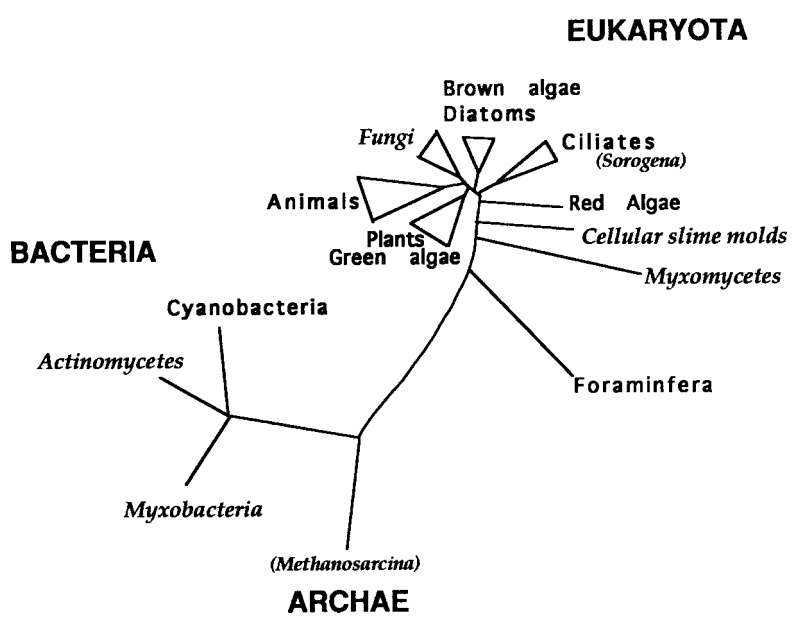

Figure 1.

reader could well become overwhelmed by the detail. My other difficulty is that it is a subject I have discussed at some length previously.[4–8] Here I want to do something different. To begin, I will adapt one of the current molecular phylogenies of the major groups of organisms as proposed by Sogin[9] (based on a single gene of a small subunit of rRNA), and modify his figure by including only those groups that are multicellular (Fig. 1, Drawings by Hannah Bonner). Thirteen separate inventions of multicellularity are indicated in the figure, but this is far below the actual number because some of those eight are well known to be polyphyletic, as I will illustrate. My descriptions will begin with green algae and plants and move clockwise around the figure.

The descriptions themselves will be brief, and to make it easier to fix them in one's mind, some of the more important forays into multicellularity will be accompanied by drawings. The reader may want to skim over this section just sufficiently to appreciate the variety of separate ways single cells have evolved into multicellular organisms.

Green Algae and Green Plants

The green algae began their multicellularity in water. They provide some splendid separate examples of aquatic origins in the form of what is traditionally known as colonies. It is assumed that all higher plants came from green algae in which the cells had moderately rigid walls and the division products of an asexual spore or a zygote remained glued together. This is well illustrated in the sea lettuce *Ulva* (Fig. 2) and its smaller relatives, where one can trace the transition from a simple filament to a thickened thallus.

A somewhat different mode of becoming multicellular is seen in the Volvocales, in which the division products are surrounded and held together by jelly and the colonies they form may be flat, for more often are hollow spheres typified by *Volvox*, (Fig. 3) the largest member of the group.

The Chlorococcales become multicellular in a radically different way. For example, in *Pediastrum* (Fig. 4) the products of cell division are confined within the mother cell (in a vesicle that lines the mother cell). While the daughter cells become detached and swim about using their flagella, they are initially imprisoned within the vesicle and eventually, they lose their ability to move, they become cemented into a flat plate that will burst free from the vesicle as they grow.

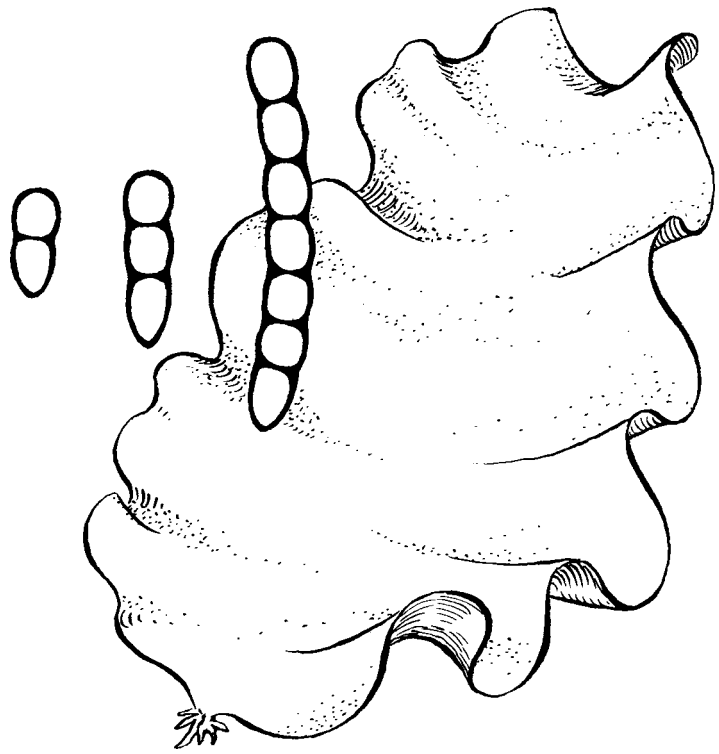

Figure 2.

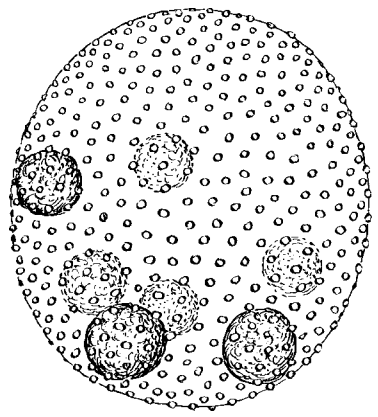

Figure 3.

Hydrodictyon is closely related to *Pediastrum*, but differs in that it produces, by much the same development, a huge colony. Finally, I should mention the coenocytic and multinucleate green algae such as *Caulerpa*, a large marine form that is attached to the ocean floor by a holdfast. Inside there are no cross-walls, but merely a large vacuole surrounded by streaming cytoplasm containing vast numbers of nuclei.

The question why there is such a variety of shapes in these algae was addressed many years ago by Baker,[10] who pointed out that photosynthetic algae did not need to develop an elaborate feeding mechanism to catch particulate food; all they needed was to be able to catch the sun, making it possible to invent a great variety of different shapes, which is exactly what they have done.

Animals

In the case of animals, we know little about the transition stages and are left with a huge gap between the sponges and the ancestral single cells. Either all the known invertebrates, living and fossil, came from one multicellular ancestor, or possibly there was more than one ancestor, sponges having had a separate origin.

Fungi

Fungi are a heterogeneous group and the possibility that they are invented multicellularity more than once is a reasonable hypothesis. All fungi are filamentous in nature, and they are to varying degrees syncytial or multinucleate, especially in the growth phases of their life cycle. One very common aspect of their multicellularity is that when they go into their reproductive, or fruiting phase (Fig. 5), all the nuclei and cytoplasm suddenly flow through the hyphae to central collection points and rapidly produce spore-bearing fruiting bodies. These can be quite small, as in the case of simple molds, or they can be very large mushrooms.

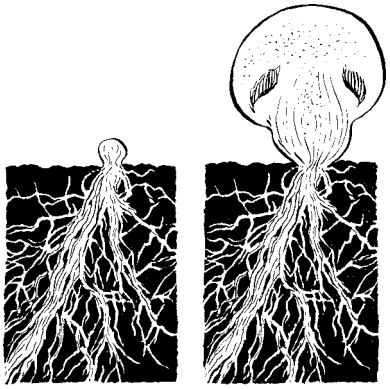

Figure 5.

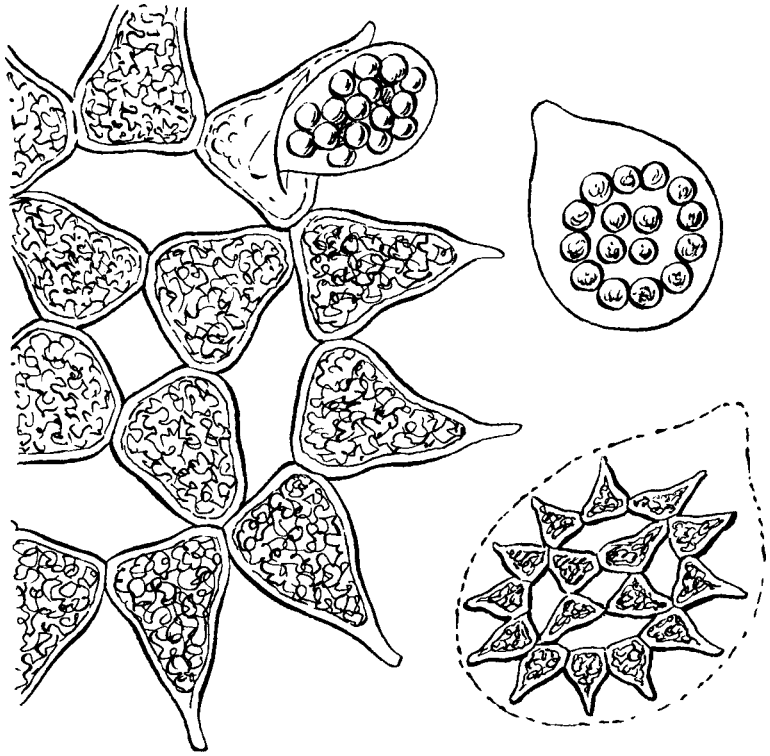

Figure 4.

Brown Algae and Diatoms

The brown algae are largely marine and are notable for having some forms, such as *Macrocystis*, which are over 100 ft in length. It is assumed that their method of initially becoming multicellular was much the same as I have already described for the green alga *Ulva*; that is, they started as cells with rigid walls that failed to separate upon division.

Diatoms, which are related to the brown algae, would seem to be quintessential unicellular forms; each cell is encased in a hard silica shell. There are, in fact, two minor but interesting exceptions within the group. There are a few species in which the dividing cells remain attached at one end to form sessile colonies. The other exception is particularly odd: all the motile cells secrete a tube that surrounds them and expands as they multiply. This tube, which branches, is anchored to the ocean floor. The secreted house may be a centimeter or more in height, and the separate cells actively move about inside it (Fig. 6).

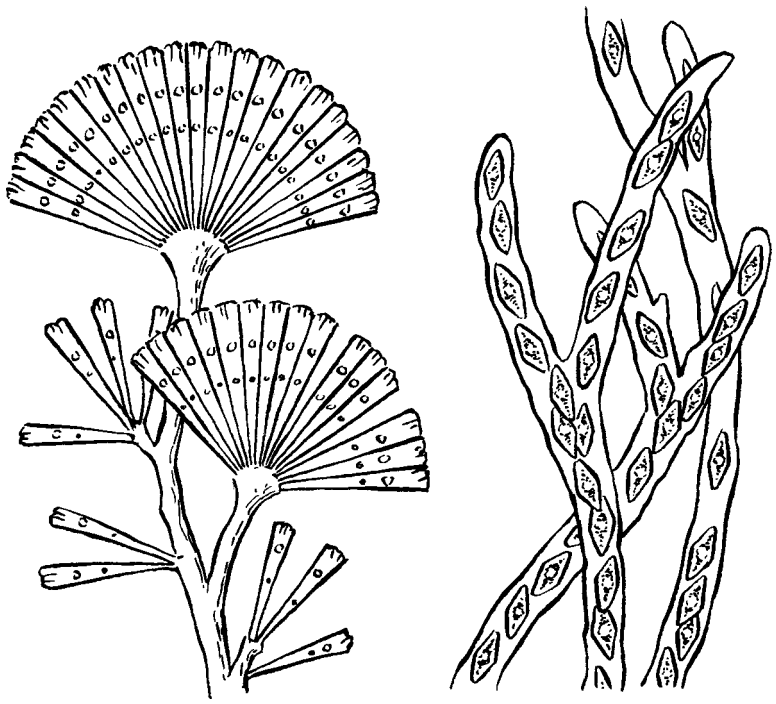

Figure 6.

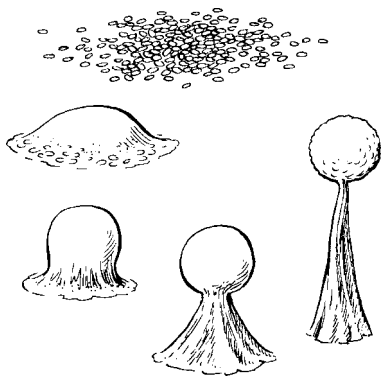

Figure 8.

Red Algae

Although red algae have many multicellular forms that are shaped like some of the green and brown algae, their cytological and biochemical characteristics are quite distinct and set them apart. Nevertheless, it is assumed that they achieved their multicellularity in the same fashion previously described for the other algae. Because they differ in so many details of their cell structure, they must have made the step to multicellularity independently.

Ciliates

The cell structure of ciliates is highly specialized and different from all other organisms. Ciliates are unique in having a separation of the germ line micronucleus, and a huge macronucleus that controls the morphogenetic events of the complex cortex. It could be argued that this is another way of becoming larger—one that avoids multicellularity. However, there are a number of genuine multicellular, or colonial, forms which exude a supporting adhesive thread at one end of the dividing cells, ultimately building a sessile colony of individual but connected cells. In *Zoothamnium* (Fig. 7) the cells are also linked by a muscle thread so that if one cells is touched the whole colony will contract to avoid danger.

One particularly curious form of multicellularity is found in *Sorogena* (Fig. 8) a ciliate that lives in the soil. When its food has been depleted, the separate cells aggregate to form a small fruiting body that sticks up in the air.

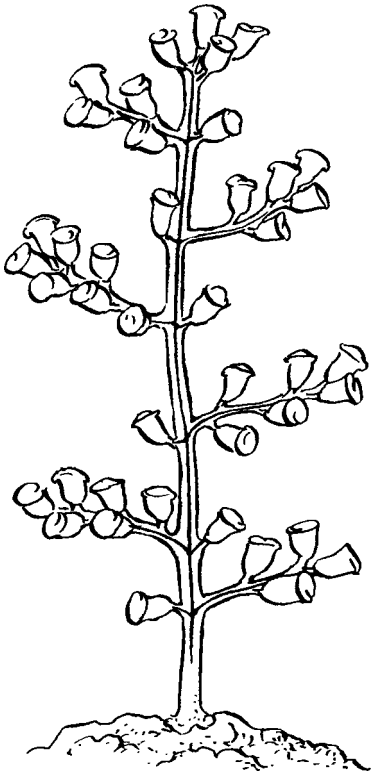

Figure 7.

Cellular Slime Molds

These organisms are characterized by an asexual life cycle in which they feed as separate amoebae. When their food supply is consumed they aggregate into collections of cells that form small, multicellular fruiting bodies bearing resistant spores (Fig. 9).

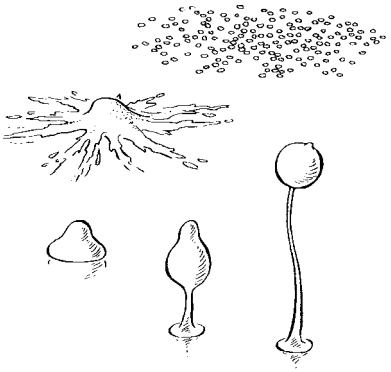

Figure 9.

The cellular slime molds consists of two major groups, the Acrasids and the Dictyolstelids. They clearly have separate origins for their differ in their cell structure and are quite far removed in a molecular phylogeny.[11,12]

Myxomycetes

True slime molds are quite different than the cellular slime molds. They have a sexual cycle in which the zygote begins as an uninucleate amoeba, but as it feeds and becomes larger only the nuclei divide and it develops into a large, multinucleate mass of naked protoplasm—a plasmodium. When the conditions favor fruiting, the plasmodium breaks up into a single aggregate, and more often many small ones, each of which forms a fruiting body bearing haploid spores that give rise to the gametes of the next generation (Fig. 10).

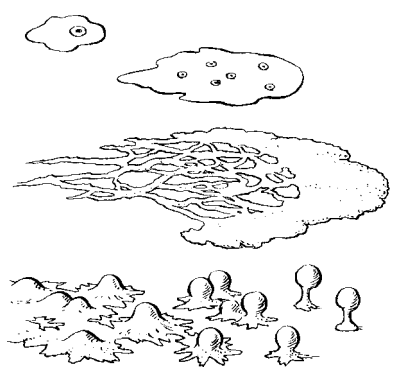

Figure 10.

Foraminifera and Radiolaria

These are amoebae with beautiful shells made with calcium or silica. They mostly float free in the ocean—a few are sessile. As they grow they become multinucleate and in the foraminiferans they secrete additional chambers to their shells with size increase.

Archaebacteria

Archaebacteria are a newly identified group of prokaryotes that are quite distinct in many of their biochemical characteristics from eubacteria. These ancient organisms are notable in that they can exist in many extreme environments. There are reports that some species of *Methanosarcina* form groups of compacted cells that seem to adhere closely to one another after division. It has been pointed to me by Prof. Karl Stetter that this organism is an obligate anaerobe and that these compact colonies might serve as a mechanism to keep the internal cells protected from a sudden influx of oxygen in the environment.

Eubacteria: Myxobacteria

When these motile rod-shaped bacteria grow and divide the daughter cells remain close to one another, forming a wandering swarm that steadily increases in size. When the conditions are right these masses of rods come together in central collection points to fruit—to form cysts or microspores. In some species, such as *Chondromyces* (Fig. 11), the cysts are lifted up into the air on a stalk.

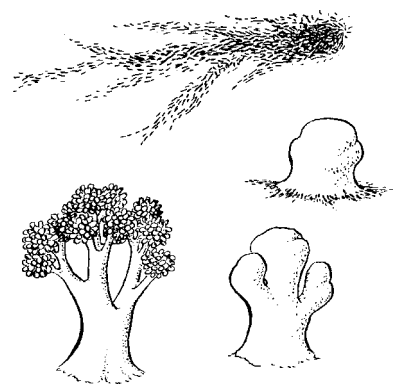

Figure 11.

Actinomycetes

These soil bacterial form small, branching thread-like filaments. Some of those filaments will reach up into the and produce spores. Often the spores bud off a linear series, although in some species there is a spherical mass of spores at the tip of a filament. They are a large and diverse group; perhaps the best known is *Streptomyces* (Fig. 12), which produces the antibiotic streptomycin.

Cyanobacteria

The cells of these eubacteria are large—as large as many eukaryotic cells. They are mostly multicellular, the usual form being linear filaments; however, there are a number of species, with branching filaments. Although they are primarily aquatic, they are extraordinarily hardy and can withstand considerable exposure to air. They do form resistant spores and since they are photosynthetic they also have specially differentiated cells (heterocysts) to fix nitrogen from the air (Fig. 13).

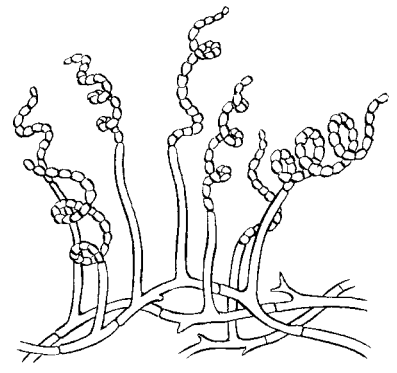

Figure 12.

Sexuality vs. Asexuality

Let me add a comment here concerning all of these different explorations into multicellularity. Unlike so many advances in evolution, sex does not seem to have been a prerequisite for the invention of multicellularity. Clearly sexuality exists among unicellular organisms; in other words, sex undoubtedly antedated multicellularity. However, there is no obvious correlation between inventing multicellularity and sexuality. The primitive multicellular organisms may produce either asexual spores or gametes. Among the fungi and the green algae are species in which there is an alternation of sexual and asexual cycles, both of which will produce spores or some form of unicellular propagules.

MECHANICAL WAYS OF BECOMING MULTICELLULAR

If we now look at these various experiments in inventing multicellularity it is intriguing to ask if some general statements about their different mechanisms can be made. There is one particularly important point that I

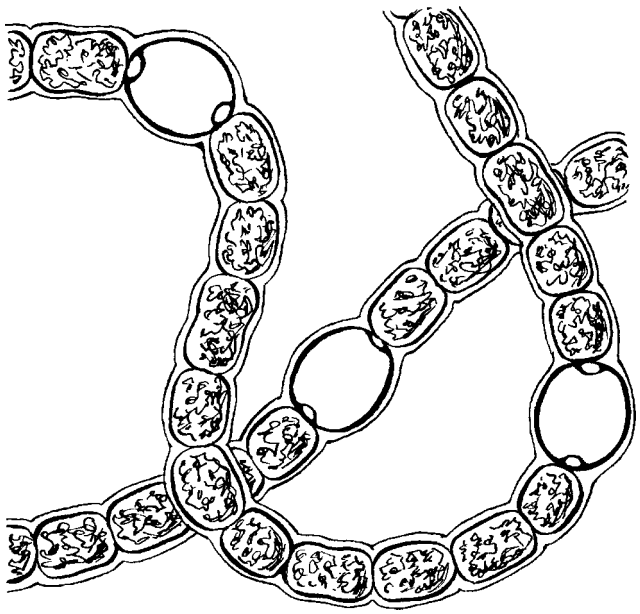

Figure 13.

have made earlier.[8] One can identify a sharp distinction between those organisms that became multicellular under water and those that did so on land. All the aquatic organisms began their multicellularity by the products of cell division failing to separate, while most terrestrial microorganisms involve some form of motile aggregation of cells or nuclei in a multinucleate syncytium. There are some apparent exceptions such as the actinomycetes and a few cyanobacteria, e.g., some species of *Stigonema*, which live in air in moist environments. However, it is most likely that the ancestors of these particular forms were aquatic. The terrestrial origins are indicated in italics and the aquatic origins in regular type (Fig. 1).

Aquatic Origins

In the case of the aquatic origins there is great variety and this variation seems to be correlated largely with the type of cell, and especially the type of cell surface. For those organisms with cell walls one of the most prevalent methods is to have the cells fail to separate after division. The hard polysaccharides of the cell walls found in green, brown, and red algae have permitted the rise of filaments and ultimately the branching and thickening of those filaments to form solid tissues and produce large plants. The same mechanism in its filamentous form is found in the cyanobacteria and some other eubacteria. All of these forms use their cells as building blocks once they fail to separate.

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Animal cells do not have a cell wall and they stick together because of adhesion molecules on their surface membranes. That they are covered only by a membrane is no doubt related to the fact that originally the cells were amoebae and they had to retain a pliable membrane so that they could engulf bacteria and other food particles. Consistent with Baker's[10] point mentioned earlier, the next big mechanical step must have been devising a feeding mechanism for a group of cells, but any clue as to how this was achieved initially has been long lost.

Diatoms and ciliates have highly specialized outside coverings that present another kind of problem. In the case of diatoms, it is the rigid silica shell, while in ciliates, it is the elaborate cortex and its cilia and other complex structures; in both cases, an external armor raises a challenge to cell adhesion. Yet a few species of both groups have found an identical solution. By exuding an adhesive stalk at one end of the elaborate cells they can divide without interference from the adhesive, and the daughter cells will be attached to one another at one end—by their roots, so to speak—and in this way form a branching colony.

Then there is a genuinely curious solution, also previously described, of the diatom that surrounds itself with a branched secreted tube as it multiplies. It is as though it were creating a miniature biosphere.

Terrestrial Origins

For microorganisms it is difficult to draw a sharp line between aquatic and terrestrial, because the latter need water too and will always exist in a thin layer of water covering particles of soil or humus. In other words, all terrestrial microbes are to some degree aquatic. This is especially true of their feeding phase, for almost none of the multicellular terrestrial microorganisms are photosynthetic and they all require a liquid film. If they are particle feeders they need this to move about and engulf their food. If they are saprophytes they must get the food itself in liquid form.

These terrestrial organisms send a spore-bearing body that pushes up through the water interface into the air for dispersal. This is certainly true for the fungi with their enormous array of aerial fruiting bodies (although the water molds have similar sporangia that form and liberate motile zoospores below the surface of water). It is equally the case for both kinds of cellular slime molds as well as the true slime molds, or myxomycetes. Aerial spore-bearing bodies are also characteristic of terrestrial multicellular prokaryotes: some myxobacteria product fruiting bodies with prominent stalks while others merely form mounds, and the actinomycetes have chains of spores sticking up into the air.

The mechanisms of aggregation, which is characteristic of most terrestrial microorganisms, takes two forms: it involves either the gathering of separate uninucleate cells or the gathering of nuclei and cytoplasm in a multinucleate syncytium.

In the case of aggregating single cells, we have a remarkable bit of convergence, for aggregation occurs independently in eubacteria, in two distinct kinds of cellular slime molds, and in ciliates; in other words, it has arisen independently at least four times. In addition, there are numerous variations in the mechanics of fruiting body formation within some of the groups. In the cellular slime molds, for example, the spores can be pushed up as the cells squirm past one another, rising into the air; by creating a stalk of dead cells made by adding live cells at the tip which become trapped and vacuolate; by rising on a slender, cell-free hair of cellulose also secreted by the cells at their apical end; and by secreting a non-cellular cone and using osmotic pressure to push the spores up to its narrow ends like an erupting volcano. This latter mechanism is interesting because it is somewhat similar to the method used by the totally unrelated ciliate, *Sorogena*; again a striking case of convergence (see Fig. 8).

In those instances where there is a multinucleate syncytium the aggregation stage is quite different; it involves the directed movement of a great mass of protoplasm toward central locations that are the incipient fruiting bodies as we saw in fungi and myxomycetes. The multinucleate mass in myxomycetes, which can be very large, is covered by only a thin membrane and therefore requires very moist conditions on the forest floor or on dead logs. This plasmodium is the feeding stage, which is quite separate in time from the fruiting or dispersal stage.

The very same separation of the feeding and fruiting phases is found among the great multitude of species of fungi. However, there is one major difference. The syncytium is always confined in a chitinous tube, the hypha. As these hyphae branch and spread into the mycelium, they will invade the soil or rotten wood, whatever might be its substrate, sopping up nutrients, and continuously expanding and enlarging. When the right conditions arise, all growth ceases, and the protoplasm surges back through the hyphae to a central collection point (see Fig. 5). It may be a modest surge and produce a simple spore-bearing mass held up in the air by a single hypha, as in *Mucor*, a bread mold, or it may involve the aggregation of a large amount of protoplasm in a vast mycelium to form a mushroom (which can be very large indeed, as in a giant puffball that may weigh pounds).

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WHEN MULTICELLULARITY OCCURRED IN EARTH HISTORY

There is a general point about the origins of multicellularity that needs emphasis. It is obvious that the moment in evolutionary history for each of the different organisms described here undoubtedly occurred not only as separate events but at different times. There is good evidence that multicellularity in cyanobacteria was invented at a very early stage, a good 3.5 billion years ago,[16] yet the first multicellular animals was probably a much more recent event. Unfortunately, we have no way of knowing the sequence in time of the origins of the various groups of organisms shown in Figure 1, but they must span an eon. Indeed there is nothing to rule out the possibility that at this very moment multicellularity is in the process of being invented by some single-cell form somewhere on our earth.

WHAT MIGHT BE THE SELECTION PRESSURES THAT PRODUCE MULTICELLULARITY?

Aquatic Origins

The fact that multicellularity arose independently so many times is the primary basis for believing that there has been a significant selection for it in the ancient unicellular world. Yet it is difficult to guess that the first advantages might have been. In the case of aquatic origins, it is easy to imagine that the failure of the daughter cells to separate might be the result of a simple mutation. It is the next step that is harder to picture: what advantages would clusters of cells have over single cells? Perhaps initially they had neither advantage nor disadvantage and survived by drift until some further mutational change endowed them with a skill that was not possible for their single-cell relatives. I will pursue this possibility in the next section; here I am only concerned with the initial step.

It might be that the mutation that allowed the cells to adhere to one another also allowed them to stick to the substratum. Under some circumstances, where the cells in an ideal location for growth are likely to be swept away by currents, remaining fixed to one spot might be selectively advantageous. The same advantages would apply to the daughter cells, thereby giving rise to a multicellular sessile colony. Such an occurrence might be the origin of colonial stalked ciliates and diatoms. This could even apply to the diatom that is encased in a tube—instead of having the adhesive glue the cells to the substratum, they had a different set of mutations that allowed them to build a cocoon of stiff material around them, inside of which they could divide and grow. In these instances, becoming multicellular is the inevitable consequence of the advantage of remaining in one spot.

I have said very little about foraminiferans and radiolarians. They are mostly free in the ocean, or pelagic, and as they grow they become multinucleate. But what is the advantage in their doing so? One argument has been put forth by Bell[13] for *Volvox* that might apply here: the size increase prevents filter feeders from being able to eat them. The problem for this ingenious argument is that we are looking for the origins, and the "too big to eat"' idea requires that there are already large multicellular predators about. Perhaps initially pelagic multicellularity had no advantage—they just grew.

In the case of primitive flagellated or ciliated cells, it is conceivable that size increase is an advantage, because the larger the organism the faster it will swim. Again it is easy to see that in an aquatic world where there is a multitude of different-sized organisms, being large and fast helps to catch prey (or to escape), but again this does not help us explain the first step. There lurks the possibility that initially there was no advantage and it was only later that it was retained because of positive selection pressure.

Terrestrial Origins

The incredibly large number of different organisms existing today that have fruiting bodies suggests that there has been and still is an enormously strong selection pressure for the dispersal of spores, cysts, and even seeds in higher plants. At first glance it would seem that, as before, this development of a fruiting body must have been something that occurred well after the appearance of multicellularity, but let us examine the possibilities a bit more carefully.

In the case of cellular slime molds, there is a unicellular relative, *Protostelium* (Fig. 14), that makes its own stalk so that it rises up into the air to form a single spore.

Clearly, if a fruiting body could be made with numerous cells it might be even more effective in dispersal. For a soil amoeba, where feeing must be done as single cells by phagocytosis, the aggregation of cells is required to achieve multicellularity. The question of how this arose is more difficult, but a species of the distantly related soil amoeba *Hartmanella* forms resistant cysts in clusters.[14] There is an aggregation of the amoebae before encystment, yet no fruiting body. We do not know enough about dispersal mechanisms in the soil, and can only ask whether this primitive clustering of cysts might somehow enhance dispersal.

Becoming multicellular opens the gateway for all sorts of remarkable innovations that would be impossible for single cells.

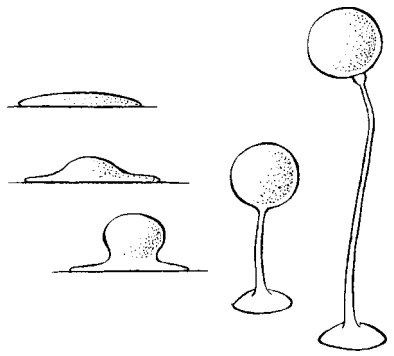

Figure 14.

In the case of syncytial forms, the multicellularity is more closely associated with feeding. In both the myxomycetes (a eukaryote) and the myxobacteria (a prokaryote), it is clear that by an increase in size of the feeding mass they can feed more effectively. They produce extracellular enzymes that digest large particulate food which they then absorb directly. In myxobacteria, Dworkin[15] has called this wolf-pack feeding. So in both of these cases we could guess that multicellularity arose as an advantage in feeding, and the formation of fruiting bodies was secondarily derived because of the advantage of effective dispersal. The very same arguments would apply to the fungi.

CAPITALIZING ON MULTICELLULARITY

Becoming multicellular opens the gateway for all sorts of remarkable innovations that would be impossible for single cells. By being larger and by being made up of numerous cells, organisms can have a division of labor and also respond to their environment in new and sensitive ways, all adaptations that have led to their success. There are many possible examples to illustrate this point; here I will give two.

Division of Labor in Cyanobacteria

The first example comes from the cyanobacteria, known from the fossil record to be a very ancient group. They have two biochemical functions that are immiscible, which has led to a division of labor. Cyanobacteria are photosynthetic organisms and therefore produce oxygen in the presence of sunlight, and at the same time they must fix the free nitrogen from their immediate environment, an absolute necessity to make their proteins and all the other component molecules in their body that contain nitrogen. Since nitrogen fixation can only take place in the total absence of oxygen, the two processes are essential

for their existence cannot occur simultaneously in the same place. Many species of cyanobacteria have solved the problem by doing their photosynthesis in the daytime and their nitrogen fixation at night, but here I am more interested in a second and more sophisticated method, where some of the cells in the filament become specialized for nitrogen fixation.

These so-called heterocysts are clear cells, lacking chlorophyll, and have thick walls that prevent the oxygen from neighboring cells from getting into their inner machinery. By this cellular division of labor cyanobacteria can take in energy from the sun and use it to make nitrogen compounds at the same time they are pulling in nitrogen from their surroundings. The nitrogen products are passed along to the photosynthesizing cells through the pores at the end walls of the heterocysts; all the cells therefore benefit from their work, and they in turn receive the nutrients they need from the neighboring green cells.

Over and above these remarkable facts, it is known that in some species the heterocysts are perfectly spaced along a filament of cells. Wilcox et al.[17] have shown that they give off an inhibitor that diffuses along the filament which prevents any cell for the limit of its effective diffusion from turning into a heterocyst. So despite the fact that they are simple prokaryotes they have differentiation into two cell types (in fact three, because some cells become resistant spores for surviving hard times), and that differentiation is organized into a regularly spaced multicellular pattern. None of these achievements could have arisen in a unicellular cyanobacterium.

Behavior in Cellular Slime Molds

In the cellular slime molds, spore dispersal appears to be of great importance for their reproductive success. So far I have said only that sticking up into the air is the key—in this way the spores will more effectively spread. There is reason to suspect that near the soil surface and in the humus where these organisms, passing animals such as mites, worms of various kinds, and other motile invertebrates that brush against the spore mass, pick up the sticky spores and carry them to some virgin patch of bacterial food. Now I want to show that these simple bags of aggregated amoebae do much more than just stick their spores up into the air to be tagged by passing beasts: they go to quite extraordinary lengths to see that the spores end up in the optimal place for dispersal. I will not give all the experimental evidence here for the basis of this assertion; I have reviewed it elsewhere.[8,18] Here I will only give the results and describe the skills of the aggregated slime mold cell mass.

These simple bags of aggregated amoebae do much more than just stick their spores up into the air to be tagged by passing beasts: they go to quite extraordinary lengths to see that the spores end up in the optimal place for dispersal.

It would appear that most of their abilities center around getting the spores from the deeper moist feeding area nearer to the surface of the soil where apparently there is a better chance of catching a ride. The migrating cell masses are extremely sensitive to light and will go toward light of surprisingly low intensities. Since daylight or moonlight will always be from above, this is one powerful way to orient them upward in the soil.

There is more. They are also highly sensitive to heat gradients and will orient in gradients as small as 0.5°C/cm. We first thought orientation only occurred toward warmer temperatures, but Whitaker and Poff[19] showed that this was only true if the slugs are migrating in a temperature range above that in which they had been raised; if the gradient was in a colder range they would go toward the colder side, i.e., they are negatively thermotactic. Whitaker and Poff[19] gave a neat and convincing explanation to this reversal of orientation: in daytime the sun would make the air and the surface of the soil generally warm so the migrating slugs would crawl toward the surface, but at night the heat gradient is reversed and the soil will be warmer than the cool night air; yet since in a cooler environment they are negatively thermotactic they will still go upward. By this intriguing mechanism they orient correctly toward the surface both night and day.

It is also known that cellular slime molds orient by exuding a gas which not only orients slugs, but more importantly positions the rising fruiting body. This gas, which we now know is ammonia, speeds up the cells, so if two fruiting bodies arise close to one another they will lean away from each other. This is because the ammonia that they give off is more concentrated between them, and the cells on the inner side of each will move faster and cause the rising cells masses to move away from each other. One can also show that they not only avoid other fruiting bodies but for the same reason they will move away from a wall—in fact, it is this mechanism that makes them rise at right angles from the substratum. If they are in a small cavity in the soil they will, by this gas orientation, position themselves in the dead centre of the cavity, and all these sensitive orientations contribute to putting the spore in the ideal pace for dispersal. These are remarkable feats for a bag of amoebae.

From all this we see that during the long course of evolution the numerous experiments in becoming multicellular were really just small steps compared to the wonders that followed.

CONCLUSIONS

What we see in this examination of the large array of experiments in multicellularity is that in early evolution becom-

ing larger took on many forms; in fact, there are no doubt others we do not even know about which have gone extinct. As I have argued, the most reasonable guess is that originally they arose by chance mutation, and subsequently were selected because of some advantage that they might accidentally have occurred. This initial success was often greatly improved upon, as we saw for cyanobacteria and cellular slime molds, but the raw materials for natural selection had been laid down by cells accidently clumping together.

These days we have become beguiled with diversity: how animals, such as insects, and plants, such as angiosperms, have produced so incredibly many species. In the origins of multicellularity we see a most primitive example of diversification. In some ways, it is almost an ideal case because we can make an argument for its basis: size increase is the common cause of all the small successes that I have described in this essay.

It is this very cellular diversification that will be so interesting to examine in our future inquiries. What are the biochemical differences and similarities among the adhesives that are used in the various aquatic forms that invented multicellularity? Is there a genetic connection between the separate ones, or are some or all of them unique? The same questions can be asked about chemotaxis in terrestrial forms: what are the molecular connections, if any, between the different chemoattractants? Adhesion plays a role in aggregation too. Finally, there is an especially fascinating question: in the first signs of cell differentiation there is both a mechanism to alter the fate of an individual cell and a mechanism to place the differentiated cells in a regulated, controlled pattern. What are the similarities and differences in the molecular mechanisms of all the independent inventions of these remarkable phenomena? I think the study of the origins of multicellularity has a bright future.

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