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A Multiple Origin for Plastids and Mitochondria

Many independent symbiotic events may have been involved in the origin of these cellular organelles.

Peter H. Raven

In view of much accumulating evidence, it now seems almost certain that the plastids and mitochondria in eucaryotic cells originated as free-living procaryotes which found shelter within primitive eucaryotic cells and eventually were stabilized as permanent symbiotic elements within them (*1*). This evidence, however, has been largely ignored in the construction of phylogenetic classifications of living organisms, such as the excellent one recently presented by Whittaker (*2*). What implications for the phylogenetic relationships between groups of organisms does a symbiotic origin of plastids and mitochondria have?

Evidence for Symbiotic Origin

The evidence for a symbiotic origin of mitochondria and plastids will be presented in two parts. First, we shall review the properties of these cellular organelles themselves, and then we shall consider the nature and occur-

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rence of analogous symbioses that occur at the present day.

Both mitochondria and plastids have the capacity for semiautonomous growth and division which is only partially controlled by the nuclear DNA. They arise only from preexisting mitochondria (*3*) and plastids, respectively. Both contain unique base compositions and configurations of DNA and RNA (*4, 5*), both of which can be synthesized and replicated within the organelle. The DNA is known in the mitochondria of a number of multicellular animals to be present in the form of double-stranded circles, each with a molecular weight of about 9 to 10×10^6 (*5*). In other organisms, such as the higher plants and *Neurospora*, the mitochondrial DNA has a much higher molecular weight and has not yet been shown to exist in a circular form (*5*).

In yeast, circular DNA appears to be only a minor component of mitochondrial DNA (*6*). The amount of DNA in a mitochondrion may amount to about 0.01 of that present in a cell of *Escherichia coli*, or sometimes considerably more (*5*). In chloroplasts, there is nearly a hundred times as much, almost as much as in *Escherichia coli* (*7*). It is not known whether chloroplast DNA is divided into molecules or exists as one continuous piece (*8*). In bacteria, mitochondria, and chloroplasts, the DNA is histone-free and bound to membranes; in eucaryotic chromosomes, it is associated with histones and not bound to membranes (*5, 9*).

The extent to which plastids and mitochondria of DNA act as templates for transcription of specific messenger RNA's is currently the subject of investigation in a number of laboratories. Both chloroplasts and mitochondria are able to incorporate amino acids into proteins in vitro (*5, 10*). In the cell, chloroplast DNA plays a role in the synthesis of at least some of the characteristic proteins of chloroplasts, but nuclear DNA likewise participates to a large extent in these syntheses (*11*). One of the most significant findings has been that mutant *Euglena* strains which lack the ability to synthesize chloroplasts also lack DNA of characteristic low guanosine-cytosine content (*12*). In mitochondria, a few, but certainly not all (*5*), of the characteristic proteins are produced within the mitochondria from a template of mitochondrial DNA (*13*). For example, Woodward and Munkres (*14*) have found differences in at least one amino acid residue in mitochondrial structural protein in two "cytoplasmic" mutants of *Neurospora*. In mitochondria, the replication of DNA seems to occur independently of that in the nucleus (*15*). Many species of transfer RNA are found only in the mitochondria of rat livers and not elsewhere in the same cells (*16*). The DNA polymerase which has been found in mitochondria (*17*) may therefore also be produced within these organelles. Furthermore, *n*-formylmethionyl transfer RNA is known to be present only in the mitochondria of eucaryotes and in bacteria (*18*).

Chloroplast ribosomes from higher plants resemble bacterial ribosomes in their sedimentation behavior and the sizes of their RNA components (*19*), and the fact that their ability to incorporate amino acids into proteins is inhibited by chloramphenicol (*20*), as it is in mitochondria (*21*). In contrast, cytoplasmic ribosomes are larger than chloroplast ribosomes (*22*) and are insensitive to chloramphenicol both in vitro (*23*) and in vivo (*24*). Hybridization studies have shown that ribosomal RNA probably originates from organelle DNA, not from nuclear DNA as in eucaryotic systems (*25*).

Therefore, mitochondria and plastids clearly resemble entire procaryotic cells more closely than other components of the eucaryotic cells in which they occur (*26*). They have a higher degree of autonomy than any other cellular component. They likewise resemble procaryotic cells in size (typically 1 to 5 micrometers).

Mitochondria and plastids differ from all other cellular organelles in being bound by a double membrane. The inner membrane is convoluted into a series of folds, greatly increasing the "working area" of the cell on which enzymes are located. Similarly, in bacterial cells, the plasma membrane is often extensively folded into the interior of the cell. The outer cell wall of bacteria appears to be a specialized structure, whereas the outer layer of mitochondria, at least, may be in effect an extension of the endoplasmic reticulum (*26*). Photosynthetic bacteria and the blue-green algae have membranous vesicles and lamellae upon which many of the photosynthetic pigments are located, and these are presumably homologous with the inner membranes of mitochondria and chloroplasts.

A Common Origin for Mitochondria and Plastids?

DuPraw (*27*) has pointed out that there are remarkable similarities between mitochondria and chloroplasts, many of which have been stressed in the preceding discussion. The structural proteins of mitochondria and chloroplasts are similar—both may contain an actomyosin-like contractile protein, and both show contraction dependent on adenosine triphosphate. Both mitochondria and chloroplasts carry out the phosphorylation of adenosine diphosphate coupled to electron transport phenomena, with similar electron carriers. These considerations have led to the hypothesis that mitochondria and chloroplasts may have had a common origin.

In any case, it is virtually certain that procaryotes with the properties of chloroplasts evolved before those with the properties of mitochondria, even though mitochondria are found in all eucaryotic cells, chloroplasts only in some. Free oxygen is utilized by mitochondria as an acceptor for electrons, but chloroplasts can carry out their functions anaerobically. It now seems likely that photosynthesis had already evolved by the time of deposition of the first known fossils, at least 3.2×10^9 years ago (*28*). Blue-green algae appear to be at least 2.7×10^9 years old as a group. On the other hand, eucaryotic cells probably did not evolve much more than 1.1×10^9 years ago (*29*). Other evidence strongly suggests that all the oxygen in the atmosphere has been derived from the process of photosynthesis. The achievement of current concentrations of oxygen in the atmosphere is often coupled with the invasion of the land by plants, animals, and fungi, an event that seems to have taken place about 4.5×10^8 years ago (*30*). At any rate, it is likely that there was insufficient oxygen in the atmosphere to allow the evolution of cells with the properties of mitochondria until perhaps 1.5×10^9 years ago, and perhaps much more recently than that.

If plastids and mitochondria did have a common origin, then, it appears almost certain that mitochondria are derived from plastids. In that case, an autotrophic procaryote presumably became a symbiote in the cells of a larger anaerobe maintaining itself with energy from glycolysis. This symbiote was then functionally equivalent to a chloroplast in the cells of the anaerobic host. Later, modification of its properties in the course of evolution led to its acquiring the oxidative capabilities associated with mitochondria.

It appears more likely, however, that, although mitochondria and plastids did have a common origin in the sense that both are derived from procaryotic symbiotes that became stabile elements in eucaryotic cells, they originated from separate symbiotic events. First, there are impressive differences between them. Chloroplasts are larger than mitochondria, have a different characteristic shape, contain nearly a hundred times as much DNA, and differ greatly biochemically. Second, mitochondria occur in all eucaryotic cells, including protists, plants; animals, and fungi. Plastids, on the other hand, are found only in plants. It appears simplest to visualize the acquisition of mitochondria by the common ancestor of all eucaryotes and the subsequent acquisition of plastids by one or more lines of eucaryotes as separate events. The simplicity with which intracellular symbioses apparently become established and are maintained argues in favor of a separate symbiotic origin of mitochondria and chloroplasts.

A Multiple Origin for Mitochondria?

Did mitochondria themselves arise from a series of separate symbiotic events? Symbioses that are observed at the present time invariably involve autotrophs and are thus analogous to the events that presumably led to the evolution of chloroplasts. All eucaryotic cells already have mitochondria, and a symbiosis involving additional mitochondrion-like particles would appear to have no selective value. In the past, this may not have been the case. If eucaryotic cells acquired characteristic mitochondria after their pattern of nuclear organization was already established, and they were in a sense already eucaryotic, one could visualize a series of symbiotic events involving mitochondrion-like organisms analogous to those involving autotrophs we see at the present day. It seems likely, however, that only one sort of procaryote was involved, because of the impressive similarities between mitochondria in all eucaryotic cells that have been investigated. In this connection, it is interesting to note that the ciliate *Paramecium aurelia*, for example, harbors a number of endosymbiotic gram-negative bacteria of uncertain adaptive value in nature, among them kappa, mu, lambda, and sigma particles (*31*). In any event, it is difficult to evaluate the question of a common or multiple origin for mitochondria by studying contemporary organisms.

Contemporary Symbioses and the Origin of Plastids

Photosynthetic algae are, at the present time, symbiotic in a very wide variety of organisms. Symbiotic relationships involving the procaryotic blue-green algae will be reviewed below, but first a brief survey of the symbiotic relationships of eucaryotic algae will be presented here to illustrate the diversity of these interactions.

In addition to the symbioses involving eucaryotic algae with higher plants, other algae, and vertebrates, the relationships between the algal and fungal components of lichens have been much discussed and studied experimentally. In addition to their participation in all of these kinds of systems, autotrophic algae are known to occur as symbiotes in more than 150 genera of invertebrates, representing eight phyla (*32, 33*). In the vast majority, if not all, of these instances, the symbiotic relationships arose independently. The symbiotes include three orders of green algae as well as the dinoflagellates and diatoms.

All stages in reduction of the cell wall in the symbiotic algae are represented among these relationships. For instance, in the dinoflagellate *Platymonas convolutae*, symbiotic in the marine acoelous turbellarian *Convoluta roscoffensis*, the symbionts lack the cell wall, capsule, flagella, and stigma of the free-living forms of the same species (*31*). They likewise have finger-like extensions 1 to 2 micrometers long that greatly increase the area of contact with the host cytoplasm.

A number of opisthobranch gastropods feed on siphonaceous green algae and have symbiotic chloroplasts, apparently derived from these algae, which are confined to the cells that form the hepatic tubules (*32*). These functional and clearly autonomous chloroplasts play an important role in the nutrition of their hosts.

The diversity of contemporary symbiotic relations attests both to the high selective value of such relations for heterotrophs and to the ease with which they are established. Thousands of such symbioses of varying age exist at the present day, and have been established even in the face of competition from established autotrophs, both unicellular and multicellular.

Symbiotic Origin of Mitochondria and Plastids

In view of the similarities between existing mitochondria and plastids, on the one hand, and procaryotic cells, on the other, it would be very difficult to conclude that they had originated separately. When the array of demonstrable symbiotic relationships between eucaryotic and, as we shall see shortly, procaryotic autotrophs and various kinds of heterotrophic organisms is taken into account, the case becomes overwhelming. Some symbiotic blue-green algae can at the present day be distinguished only with the greatest difficulty from chloroplasts—and, indeed, the distinction seems to be a false one. The case for the symbiotic origin of plastids appears overwhelming, and some of the implications of this for a phylogenetic understanding of the relationship between organisms will now be considered.

Mitochondria, like 9-plus-2 flagella and a differentiated nucleus (but see *34*), appear to be characteristic of all eucaryotic cells, but they may well have had a multiple origin, as suggested above. They have apparently lost most of their DNA content subsequently. Whether the spindle apparatus of eucaryotic cells likewise had a symbiotic origin, as suggested by Sagan (*35*), is a separate question that will not be considered in this article.

Symbiosis and the Origin of Plastids

The numerous symbiotic associations that can be observed in living organisms, together with their diversity, strongly suggest that such relations arise with relative ease. They would have had an even higher selective value when no eucaryotic cells were autotrophic, a fact that lends credence to the view that plastids arose not once, but many times. The implications of this view for the phylogenetic relationships of the major groups of autotrophic procaryotes will now be considered.

Blue-Green Algae as Symbionts

One group of living procaryotes is biochemically and structurally (*36*) similar to the chloroplasts of certain eucaryotes. These are the blue-green algae, which, in addition to chlorophyll *a*, contain phycobilins, two unusual porphyrins related to the bile pigments (*37*) in their cells (*38*). Outside of the cells of the blue-green algae, phycobilins occur in the red algae (*37*), in several genera of flagellated Cryptophyta (*39*), and in the anomalous hot-spring alga *Cyanidium caldarium* (*40*). They function as accessory pigments, and, because of their unusual structure, it is difficult to imagine that they evolved independently in these four groups, which are markedly dissimilar when judged on other criteria—so much so that it is extremely difficult to imagine a direct phylogenetic connection between them.

Symbiotic relationships involving blue-green algae are very common. These procaryotes are frequently found as symbiotic components in the cells of amoebae; flagellated protozoa; green algae such as *Gloeochaete, Glaucocystis,* and *Cyanoptyche* that lack chloroplasts; and diatoms (*41*). Some are even associated with fungi such as the phycomycete *Goesiphon pyriforme* (*42*). In all of these organisms, the blue-green algae play the role of chloroplasts, bringing the characteristic biochemistry of their procaryotic free-living relatives with them. In the symbiont of *Glaucocystis nostochinearum, Skujapelta nuda*, the cell walls are nearly or entirely lacking, a condition that Hall and Claus (*43*) consider an apparent adaptation to the intracellular habitat and symbiotic association.

These relationships suggest that the simplest hypothesis to account for the biochemical similarities between the groups of algae mentioned above would be that the chloroplasts of red algae, flagellated Cryptophyceae, and *Cyanidium* are in fact blue-green algae that entered into symbiotic relationships with the ancestors of these organisms. For each group, there is evidence for and against the proposed hypothesis.

In the blue-green algae, chlorophyll *a* occurs with several carotenoids and phycobilins. No other chlorophyll is present. The presence of a cell wall in the blue-green algae and its absence in chloroplasts poses no problem for, in at least two instances (*44*), known symbiotic blue-green algae lack a cell wall.

In red algae, chlorophyll *d* has been reported in many, but not all forms examined. Chlorophyll *d*, when present, is usually only a trace constituent; it has never been detected in the absorption spectra of living red algal cells or thalli; and it may merely be an oxidation product derived from chlorophyll *a* in vitro (*45*). This hypothesis has recently been strengthened by the detection of a pigment with the spectral qualities of chlorophyll *d* in extracts derived from the green alga *Chlorella pyrenoidosa* (*46*). The phycobilins of red algae are slightly different from those in blue-green algae, but, all in all, there seems to be no compelling biochemical reason not to regard the chloroplasts of red algae as ancient symbiotic blue-green algae. Moreover, the structure of the chloroplasts in red algae is extremely simple. These chloroplasts appear to contain a single thylakoid (*47*) and thus to be virtually identical to entire cells of the blue-green algae. The blue-green algae have been in existence for some 3 billion years (*48*), the red algae probably for less than 650 million. If the hypothesis presented here is accepted, the relationship to be considered is not that between the procaryotic blue-green algae and the eucaryotic red algae, but between the former and the chloroplasts of the latter. Aside from their chloroplasts, the cells of red algae have nothing in common with blue-green algae in organization or biochemistry (*49*).

Blue-green algae, lacking cell walls, are in fact the functional chloroplasts in one member of the Cryptophyta, *Cyanophora paradoxa* (*43*). The flagellated Cryptophyta—cryptomonads—are essentially Protozoa with a proteinaceous pellicle and a contractile vacuole. Some of them are heterotrophic and ingest food particles. They are not plantlike in any of their characteristics except for the biochemistry of their chloroplasts. The chloroplasts are relatively simple and similar in structure to those of the red algae (*47*) and to entire cells of the blue-green algae. Could these chloroplasts be ancient symbiotic blue-green algae?

The chief objection to this hypothesis appears to be the presence of chlorophyll *c*, together with carotenoids and phycobilins, in the chloroplasts of at least some cryptomonads (*50*). In structure, chlorophyll *c* differs widely from other chlorophylls (*51*). It occurs as an accessory pigment in the diatoms and some other Chrysophyta, in the dinoflagellates (Pyrrhophyta), and in the brown algae (Phaeophyta). These groups seem totally unrelated, and perhaps chlorophyll *c* evolved separately in the "chloroplasts" of cryptomonads. It may well be that the Cryptophyta are polyphyletic, as a number of phycologists have suggested. In a review of the algae (*52*), some are said to have "a cellulose membrane" and there are said to be two types of chloroplasts—"small, blue-green bodies" (symbiotic blue-green algae?) and "one or two parietal plates." As the rather numerous genera of cryptomonads are investigated further, it should be possible to determine which have chloroplasts of blue-green algal origin.

There appears to be no valid reason not to regard the large lobed chloroplast of *Cyanidium caldarium* as a symbiotic blue-green algal cell that has become stabilized in this role. These chloroplasts contain chlorophyll *a*, carotenoids, and phycobilins, thus closely approximating the biochemistry of living blue-green algae, to which they are likewise similar in structure. If this is the case, then biochemical evidence from the chloroplasts should not be taken into account in deciding where *Cyanidium* should be placed among the groups of organisms (*53*).

The Origin of Plastids in Other Algae

If similar reasoning is applied to the biochemistry of the chloroplasts in other groups of algae, certain relationships become evident (*54*). For example, the green algae (Chlorophyta) and euglenoids (Euglenophyta) have chloroplasts that are essentially identical biochemically. These chloroplasts resemble those of the land plants in containing chlorophyll *b* as an accessory pigment. In other respects, the green algae—which are "typical" plants with a cellulose cell wall—could not be more different from the euglenoids—which are "typical" flagellate protozoa with a proteinaceous pellicle and a contractile vacuole, and which at times ingest solid food particles (*55*). The simplest assumption to account for the similarity between the two groups is that both harbor the remnants of an ancient line of procaryotes, now presumably extinct, which had both chlorophyll *a* and *b*. These hypothetical procaryotes, then, would have been the group in which the photosynthetic apparatus characteristic of the land plants evolved (*56*).

The remaining algae—Phaeophyta, Chrystophyta, Xanthophyta, and Pyrrhophyta are the major groups—have chloroplasts in which chlorophylls *a* and *c* are associated with various carotenoids. If we can allow for the evolution and diversification of carotenoids after the establishment of symbiotic relationships in each case, then the biochemical similarities between the chloroplasts characteristic of these taxa might be accounted for by postulating a third group of procaryotes with chlorophyll *a*, in this case accompanied by chlorophyll *c*, which became symbiotic in cells ancestral to these groups (*34*, *57*).

To summarize, the simplest way to account for the similarities and differences between the biochemistry of the procaryotic blue-green algae on the one hand and the chloroplasts of the various algal divisions on the other is as follows. First, chlorophyll *a* and a system of photosynthesis that led to the evolution of oxygen evolved in one line of procaryotes. In these, chlorophyll *a* and probably certain carotenoids that served as accessory pigments were probably arranged on photosynthetic lamellae within their cells. This line gave rise to at least three biochemically distinct derivatives: (i) the living blue-green algae, in which evolved phycobilins; (ii) the "green procaryotes," in which evolved chlorophyll *b*; and (iii) the "yellow procaryotes," in which evolved chlorophyll *c*. All of these groups entered into symbiotic associations with primitive eucaryotic cells, probably more than once, and groups (ii) and (iii) no longer exist as free-living organisms. From symbiotes of group (i) were derived the chloroplasts of the Rhodophyta, Cryptophyceae, and the anomalous genus *Cyanidium*. From symbiotes of group (ii) were derived the chloroplasts of the Chlorophyta (and through them, the bryophytes and vascular plants) and Euglenophyta. From symbiotes of group (iii) were derived the chloroplasts of Phaeophyta, Chrysophyta, Xanthophyta, and Pyrrhophyta.

Prospects for the Future

The relationships discussed here suggest several promising lines of investigation. First, the diversity of base-pair ratios in the blue-green algae (*58*) might provide a clue as to which might have been most likely to have given rise to chloroplasts in particular groups during the course of evolution, although the base ratios may of course have been altered subsequently by the operation of distinctive DNA polymerases. If we may take them more or less at face value, however, they may be useful; for example, the base ratios in the endosymbiote *Cyanocyte korschikoffiana*, which occurs in the cryptomonad *Cyanophora paradoxa*, strongly support the placement of *Cyanocyta* (*10*) in the order Chroococcales of the blue-green algae (*58*).

Second, in searching for homologies between contemporary blue-green algae and chloroplasts, it may not be appropriate to consider the vascular plants, in which the chloroplasts are very likely not homologous with blue-green algae. When DNA-hybridization experiments are more widely applied to the problem of the origin of mitochondria and plastids and their homologies, the hypotheses presented here invite several specific avenues of attack. For example, it would seem appropriate to compare certain contemporary blue-green algae with the chloroplasts of the red algae, preferably by DNA hybridization.

Even though it appears almost certain that mitochondria and plastids had independent, multiple, symbiotic origins, their functions are shared to varying degrees by nuclear DNA in contemporary eucaryotes. The hypothesis presented here suggests that the degree to which the nuclei have taken over what were presumably once independent functions may vary widely in different groups of eucaryotes. If DNA has actually been lost in the course of evolution from the symbiotes, perhaps becoming incorporated in the nucleus in some, this might have also altered radically the base-pair ratios in their mitochondria and plastids and might lead to unexpected results in DNA-hybridization experiments. It might eventually be possible in some systems to show that the nuclear cistrons responsible for mitochondrial or plastid functions (for example, cytochromes) might have base compositions similar to that of the organelle which they affect.

Summary

The impressive homologies between mitochondria and plastids, on the one hand, and procaryotic organisms, on the other, make it almost certain that these important cellular organelles had their origin as independent organisms. The vast number of symbiotic relationships of all degrees of evolutionary antiquity which have been found in contemporary organisms point to the ease with which such relationships can be established.

In view of this, the similarities between such totally different groups as blue-green algae and red algae, dinoflagellates and brown algae, and green algae and euglenoids can best be explained by postulating an independent, symbiotic origin of the plastids in each instance. A minimum of three groups of photosynthetic procaryotes appears to be necessary to explain the relationships among contemporary Protista and green plants: (i) the blue-green algae, which possess chlorophyll *a*, carotenoids, and phycobilins; (ii) the "green procaryotes," a hypothetical group characterized by chlorophylls *a* and *b* and a distinctive assemblage of carotenoid accessory pigments, but not phycobilins; and (iii) the "yellow procaryotes," a second hypothetical group whose members had chlorophylls *a* and *c* and various carotenoids but not phycobilins. There is, however, no reason to think that only three kinds of organisms were involved; numerous symbiotic events presumably occurred in each of these lines.

The "green procaryotes" and "yellow procaryotes" survive today only as chloroplasts from which the characteristics of the original, free-living forms can be deduced only in part. Hybridization between selected plastid DNA's may be helpful in unraveling this story, and is likewise suggested as the key to understanding the relationship between the blue-green algae and the chloroplasts of the red algae and cryptomonads.

It is postulated that the symbiotic organisms have lost various functions to the nucleus in the course of evolutionary time. If mitochondria and plastids have had a multiple origin, as suggested here, it will be necessary to examine the division of function between the two subsets of DNA for a wide variety of organisms before valid conclusions can be obtained.

References and Notes

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