yields 21.7 hours for the length of day at that time. Wells's evidence provides a remarkable demonstration of the average constancy of the phase lag, if, indeed, the coral structures are indicators of the diurnal cycle.

Alterations of both the length of day and the obliquity of the earth are certain to have had major geologic consequences. Accompanying the change in the rate of rotation is a change in the figure of the earth; this alteration produces stresses within the earth and provides one source of energy for tectonic processes. Changes in obliquity and rate of rotation undoubtedly produce changes in the climate of the earth, the nature of which are still uncertain. The length of day determines, in part, the range of day-night temperatures. In the past, this range must have been smaller, and in the future it will be greater. The obliquity of the equator to the ecliptic is responsible for the different seasons, and it determines, in part, the temperature differences between the equator and the poles. If all other factors remain constant, a decrease in the obliquity results in a decrease in the seasonal variation and an increase in the temperature difference between pole and equator; these conditions held in the past. In the future, the increasing obliquity will increase the maximum difference between summer and winter and decrease the temperature difference between equator and poles. Increasing obliquity results in a more uniform distribution of insolation over the globe. Indeed, if the obliquity were about 35 degrees, then all latitudes would, on the average, receive approximately the same amount of solar energy. Provided insolation is the controlling factor, this obliquity would result in a subtropical climate over much of the world. The changes in the rotational parameter of the earth raise important questions regarding the climates of the past and the relation of these climates to the evolution of life on the earth.

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Plastids and Mitochondria: Inheritable Systems

Do plastids and mitochondria contain a chromosome which controls their multiplication and development?

A. Gibor and S. Granick

Two important types of cytoplasmic organelles are the plastids of plant cells, which function in photosynthesis, and the mitochondria of both plant and animal cells, which function in oxidative respiration. Within the last few years new information has become available which supports the hypothesis that these organelles do not arise *de novo* but that plastids arise from preexisting plastids and mitochondria arise from preexisting mitochondria. The original evidence reviewed by Granick (*1*) included observations on the division of plastids in algae and genetic studies of chloroplast inheritance in variegated plants and *Oenothera*. Recent studies indicate that (i) the plastids and mitochondria contain DNA and RNA; (ii) the organelles are self-duplicating bodies that do not arise *de novo*; (iii) the DNA represents a multigenic hereditary system which is not derived from the nucleus; (iv) the multigenic system of an organelle is responsible, in part, for the specific biochemical properties of the organelle; and (v) the organelles are controlled by an adaptive mechanism which in the case of plastids is responsive to light and in the case of mitochondria is responsive to O_2.

In this article we review the evidence for the foregoing statements. We consider especially the data provided by the genetic systems of the plastids of *Euglena* and the mitochondria of yeast. A comparison of the properties of these two genetic systems illustrates their fundamental similarities (Table 1). For reasons not known, these systems are exceptionally mutable, a property, however, which has made possible the recognition of their multigenic components.

Evidence for Nucleic Acids in Plastids

In order to establish that plastids are semiautonomous units with their own hereditary apparatus, it is necessary to show that (i) they contain DNA and RNA; (ii) the DNA replicates in the organelle; (iii) the DNA functions to make messenger RNA; and (iv) the messenger RNA codes specifically for certain proteins or enzymes of the organelle.

Experiments to test for these properties are of various kinds, and some of the tests are more conclusive than others. However, taken together, the data strongly support the view that these organelles contain an autonomous DNA genetic apparatus.

DNA content. Reported findings of

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traces of DNA in plastids have been questioned because of the possibility of contamination with nuclear DNA. Gibor and Izawa (*2*) overcame this difficulty in analyses of *Acetabularia* chloroplasts. They succeeded in growing this large unicellular alga free from bacterial contamination. At the right stage of growth the single large nucleus at the base of the plant was cut off. The chloroplasts were squeezed out from the enucleated portion of the cell and purified by differential centrifugation, and their DNA content was determined before and after treatment with deoxyribonuclease. The chloroplasts were found to contain 0.2 microgram of DNA per milligram of protein, or approximately 1×10^{-16} gram of DNA per plastid. This DNA content is similar to that found in the vaccinia virus and in the even-numbered T bacteriophages (*3*) and would be sufficient for the coding of several hundred genes. Baltus and Brachet (*4*) also recently reported the presence of DNA in chloroplasts of enucleated *Acetabularia* which were apparently free of bacteria.

By density-gradient ultracentrifugation the DNA from the green plants was found to be composed of a major and minor (or satellite) component, by Chun, Vaughan, and Rich (*5*), Sager and Ishida (*6*), and Leff, Mandel, Epstein, and Schiff (*7*). It has been suggested by a number of workers that the satellite DNA of plants is actually the DNA of the chloroplasts. When purified chloroplast preparations were examined, as much as 50 percent of their DNA was found to be of the satellite type. Such analyses suggested that the satellite DNA was derived from the chloroplasts. A second satellite DNA which was sometimes seen may have been derived from the mitochondria.

Available data suggest that not only *Acetabularia* chloroplasts but also the chloroplasts of higher plants and of *Euglena* contain approximately 10^{-15} to 10^{-16} gram of DNA. Chun *et al.* (*5*) found that the DNA of the chloroplasts of spinach and beets was about 1 percent of the total DNA. If the number of plastids in a leaf parenchyma cell is assumed to be 100, the DNA per plastid would be 0.01 percent of the nuclear DNA. The amount of DNA in a diploid nucleus of a bean plant was estimated, by Rasch, Swift, and Klein (*8*), to be 18×10^{-12} gram; thus, a plastid of a higher plant would contain 10^{-15} to 10^{-16} gram of DNA. In *Euglena*, the total amount of DNA per cell is 2.5 to 4.3×10^{-12} gram (*9*). Leff *et al.* (*7*) estimated that in *Euglena* the satellite DNA comprises 4 percent of the total DNA. Lyman *et al.* (*10*) determined that a *Euglena* cell contains 30 ultraviolet-sensitive replicating units which control the development of chloroplasts. Thus, each replicating unit contains approximately 4×10^{-15} gram of DNA.

Table 1. Properties of the cytoplasmic mutations in *Euglena* and yeast.

Euglena	Yeast
Phenomenon	
Bleaching; impaired photosynthetic apparatus	"Petit" colony; impaired oxidative respiration
Anatomical changes	
Proplastids fail to differentiate on exposure to light; no chloroplast lamellae develop (*50–52*)	Promitochondria fail to differentiate on exposure to air; no cristae membranes develop (*49*)
Mutation rate (spontaneous)	
1%; higher in unstable clones (*50*)	1%; higher in unstable clones (*53*)
Mutation rate (induced)	
Up to 100% (*50, 54, 55*)	Up to 100% (*39, 53*)
Agents that induce the mutations	
1) Ultraviolet light, 260 mμ maximum, photoreactivable (*10*)	1) Ultraviolet light, 260 mμ maximum, photoreactivable (*41*)
2) Elevated temperature during growth (*55*)	2) Elevated temperature during growth (*53*)
3) Basic drugs (streptomycin) (*54*)	3) Basic drugs (acridines) (*53*)
Variability of mutants	
Differences in carotenoid content and in porphyrin-synthetic abilities	Suppressive and neutral types; differences in degree of suppressiveness
Less defective plastids are larger (*50, 51*)	Suppressives can give rise to neutrals (*56*)
Inheritance	
Cytoplasmic, by ultraviolet microbeam (*13*)	Cytoplasmic, by genetic analysis of crosses (*39*)

Electron microscope studies provide independent support for the conclusion that DNA is made in the plastids. Ris and Plaut (*11*) were the first to observe fibrils 25 to 30 angstroms thick in the chloroplasts of *Chlamydomonas*; the fibrils were removed by deoxyribonuclease digestion. Similar DNA fibrils have been found in Swiss chard proplastids by Kislev, Swift, and Bogorad (*12*).

Replication of DNA in the organelle. Evidence that DNA is made in the plastids, rather than derived from nuclear DNA, is indirect. It depends on showing that mutations of the plastids in *Euglena* can be produced by ultraviolet irradiation of the cytoplasm even though the nucleus is shielded from irradiation. The ultraviolet light causes irreversible bleaching of the plastids, and the irradiated *Euglena* individuals in successive generations never recover the ability to green. Lyman, Epstein, and Schiff (*10*) found that the action spectrum for bleaching had a maximum at 260 mμ, indicative of an effect of ultraviolet light on nucleic acids. To decide whether the nucleic acids responsible for these mutations were localized in the cytoplasm or nucleus, we (*13*) used a microbeam of ultraviolet light to irradiate the cytoplasm alone while shielding the nucleus, or to irradiate the nucleus alone while shielding the cytoplasm. Only irradiation of the cytoplasm caused irreversible mutation of the plastids. The affected plastids then multiplied as tiny proplastids from generation to generation. They had lost the ability to differentiate into chloroplasts. If the DNA units of the cytoplasm which were sensitive to ultraviolet light had been formed by the nucleus, then one might expect that new DNA units, formed by the nucleus, would replace the damaged DNA units of the cytoplasm and thus prevent bleaching. Because the nonirradiated nucleus did not "cure" the bleached cells, it is inferred that the DNA units of the cytoplasm, presumably in the plastids, did not originate from the nucleus. The experiments of Lyman *et al.* (*10*) indicated that about 30 hits on a *Euglena* cell were required for bleaching. Since each cell contains about ten plastids, this result would be compatible with the hypothesis that there are 30 replicate DNA units, three per plastid, each of which must undergo mutation before bleaching can occur. Brawerman (*14*) found that *Euglena* plastids contained DNA with a unique base ratio; the adenine and thymine pair accounted for 80 percent

Table 2. Enzyme systems in the differentiation of the organelles in *Euglena*: differentiation of proplastids to chloroplasts by exposure to light (*50, 58, 59*).

Structure or function involved	Normal		Bleached	
	Dark	Light	Dark	Light
Organized lamellae	—	+	—	—
Chlorophylls	—	+	—	Porphyrins*
Photosynthetic enzymes	—	+	—	+†
Carotenoids	Trace	+	—	+*
Paramylum synthesis	+	+	+	+
Plastid multiplication	+	+	+	+

* In some strains (*50*). † In Astasia (*59*) the presence of ribulose-diphosphate-carboxylase, a photosynthetic enzyme, was demonstrated.

of the total bases. The high thymine content may explain in part the sensitivity of the plastids to ultraviolet irradiation, since the damaging effect of such irradiation on DNA may result from the dimerization of the thymine molecules (*15*).

A number of earlier studies also provide evidence that the inheritable factors, which we now equate with DNA, reside in the plastids. In the same cell—that is, under the influence of the same nucleus and cytoplasm—one may have chloroplasts that are different from each other. For example, van Wisselingh (*16*) observed that in *Spirogyra triformis* some cells contained normal chloroplasts with pyrenoids and, in addition, a chloroplast which lacked pyrenoids. In subsequent cell divisions the latter, abnormal chloroplast persisted in the cells in the presence of the normal chloroplasts. If a mutation that affected pyrenoid development had occurred in the nucleus or cytoplasm, it should have affected all the plastids alike.

Other examples include Bauer's (*17*) classic work on variegated *Pelargonium zonale* var. Albomarginata, Schwemmle's (*18*) interspecies crosses of *Oenothera,* and Michaelis's (*19*) studies on *Epilobium.* These are genetic studies in which advantage is taken of the fact of maternal inheritance—that is, of the fact that plastids of the male gamete are not transmitted to the zygote during fertilization. The results of a cross in which maternal inheritance is involved are as follows. If the female gamete contains only damaged or bleached, colorless plastids, the new plant will be colorless. If the female gamete contains normal plastids, the new plants will be green. The new cells that arise are the products of a female nucleus, male nucleus, female cytoplasm, male cytoplasm, and female plastids, but not of male plastids. Whether the new plant will be colorless or green thus depends on the inheritable units of the plastids themselves.

In a recent report, Bell and Muhlethaler (*20*) reach conclusions diametrically opposed to those cited above. These investigators propose that, in the fertilized egg of the fern *Pteridium equilinum*, the plastids and mitochondria originate *de novo* from outfoldings of the nuclear membrane. (This view is not in agreement with the genetic evidence of maternal inheritance of the defective plastids in higher plants.)

Evidence that the DNA in plastids replicates to make more DNA is also suggested by radioautographic studies. The plastids in *Spyrogyra* cells (*21*) and in *Euglena* (*22*) were found to incorporate tritiated thymidine. In *Euglena* the labeled thymidine was removed from the plastids only by treatment with deoxyribonuclease. Since, as discussed above, the DNA of the plastids appears not to be derived from the nucleus, its synthesis probably occurs within the plastids.

DNA as template for RNA. The presence of RNA in plastids is well supported by experiment (*1*). Here we cite only the most recent work. Biggins and Park (*23*) found that defatted spinach chloroplasts, which were isolated by Behren's nonaqueous method, contained 7 percent RNA. Lyttleton (*24*) isolated ribosomes which contained 45 percent RNA from spinach chloroplasts. With the electron microscope, ribosome-like granules, digestible by ribonuclease, were observed in corn proplastids by Jacobson, Swift, and Bogorad (*25*), and in spinach chloroplasts by Murakami (*26*).

Evidence that the DNA of the plastids serves as a template for RNA is indirect. Kirk (*27*) reported the incorporation of labeled precursors of RNA by isolated bean chloroplasts. Treatment with actinomycin D reduced the amount of the label incorporated. This drug blocks the synthesis of RNA which is dependent on a DNA template. Similarly, Gibor and Izawa (*28*) found that the rate of incorporation of labeled uridine into the RNA of isolated chloroplasts of *Acetabularia* was reduced in the presence of actinomycin D. This suggests that DNA-dependent RNA synthesis occurs in these plastids. Brawerman *et al.* (*29*) found that a new kind of RNA was synthesized when *Euglena* cells grown in the dark were exposed to light. The new RNA contained a lower concentration of guanosine and cytosine than the RNA of the dark-grown cells. The satellite DNA of green *Euglena* (*7*) also had a lower content of guanosine and cytosine relative to adenine and thymine than the DNA of the nucleus had. All these observations suggest that the RNA species which is synthesized in response to light is coded by the satellite DNA.

Although protein synthesis in isolated plastids is suggested by the incorporation of radioactive amino acids (*29, 30*), no net synthesis of specific protein of the plastids has been demonstrated as yet.

Evidence for Nucleic Acids in Mitochondria

The RNA content of mitochondria is low compared to that of the plastids. Lindberg and Ernster (*31*) suggested that a value of about 0.5 percent of the dry weight was a reasonable estimate for the RNA of mitochondria, and Rendi (*32*) obtained a value of 1.2 percent for the ratio (by weight) of RNA to protein in liver mitochondria.

Kroon (*33*) has reported that protein synthesis by isolated liver mitochondria was inhibited in the presence of actinomycin D. This finding suggests that there must be active synthesis of RNA on a DNA template for protein synthesis to occur in mitochondria.

Direct chemical evidence for the presence of DNA in mitochondria is still lacking, probably because of the small amounts of DNA present and the difficulty of avoiding contamination with nuclear DNA during isolation procedures. With the electron microscope, Nass and Nass (*34*) observed the presence of fibrils 25 to 100 angstroms thick which could be removed through digestion by deoxyribonuclease. The fibrils occupied a clear area in the fixed mitochondrion, similar to the clear area occupied by the DNA fibers of a bacterium. A long mitochondrion had two of these areas, as if the mitochondrion had not yet undergone fission. The studies of Chevremont (*35*) also suggested that DNA is formed by mitochondria. Chevremont found that chick fibroblasts, when grown in vitro for 1 day at 16°C or when treated with deoxyribonuclease

II, accumulated DNA in their mitochondria. The DNA was demonstrated by Feulgen staining, by deoxyribonuclease digestion, and by radioautography after the incorporation of tritiated thymidine.

The presence of DNA in mitochondria is also shown by studies on the kinetoplasts of hemoflagellate protozoa. Steinert, Firket, and Steinert (*36*) demonstrated that the kinetoplasts gave a positive Feulgen reaction which disappeared when cells were pretreated with deoxyribonuclease. They thus concluded that the kinetoplasts contained DNA. Rapid incorporation of tritium-labeled thymidine into the kinetoplasts suggested that DNA is synthesized within them (*36*). Electron microscope studies by Steinert on *Trypanosoma* (*37*) and by Rudzinska, D'Alesandro, and Trager on *Leishmania* (*38*) revealed that the kinetoplast is a specialized mitochondrion. It was found that the kinetoplast enlarges during metamorphosis from the parasitic leishmania form to the free living leptomonad form and develops into a single convoluted mitochondrion with typical cristae which carry out oxidative respiration. The kinetoplast may represent a special mitochondrion in which the DNA is condensed in one segment of the organelle. In the development of the large respiring kinetoplast, the DNA may or may not become distributed into the branching mitochondrial arms of this structure.

In support of the conclusion that DNA is present in mitochondria one must also consider the genetic evidence provided by the outstanding studies of Ephrussi and his co-workers (*39*). By genetic crosses of certain yeast strains, they showed that one kind of "respiratory deficiency" was the result of mutations in the inheritable factors which reside in the cytoplasm. These mutations were not "cured" by the normal nucleus, and therefore one could conclude that the inheritable factors for respiratory deficiency were not derived from the nucleus. By electron microscope studies, the respiratory deficiency was correlated with an absence of cristae in the mitochondria (*40*). Respiratory deficiency could be induced in yeast by ultraviolet irradiation (*41*). The action spectrum for this induction had a maximum at 260 mμ, suggesting that nucleic acids were the targets being irradiated. It is therefore inferred that the hereditary factors for respiratory deficiency are localized in the mitochondria and that these factors probably contain DNA.

In animals some instances have been found in which mitochondria are inherited only through the egg, not through the sperm. These examples of maternal inheritance may prove useful for investigating the genetics of mitochondrial DNA, perhaps in tissue culture. Bourne (*42*) has cited the following examples. When the fertilized egg of the bat divides, the male mitochondria sometimes pass into only one of the first two cells. In echinids the male mitochondria were traced into only one cell of a 32-cell embryo. In Nereis the middle piece of the sperm which carries the mitochondrial material does not enter the egg at all. The individual in this case contains its mother's and father's nuclear chromosomes, but only its mother's mitochondria.

Self-Duplication of Plastids and Mitochondria

Chloroplasts and mitochondria may be considered to represent fully differentiated stages, respectively, of proplastids and promitochondria. Both the proplastids and the promitochondria are characterized morphologically as vesicles, approximately 1 micron in diameter, surrounded by a double membrane. During differentiation the inner membrane invaginates at various points to give rise to tubules that become the cristae of the mitochondria; in the plastids, they are transformed to chloroplast discs. It has often been suggested that the proplastids and promitochondria are identical (*43*), but the concept of a particular DNA for each is incompatible with this hypothesis. The high sensitivity of the plastids of *Euglena* to various mutagens and the much lower sensitivity of the mitochondria of the same cells (Fig. 1) illustrate the difference between the DNA of the plastids and of the mitochondria.

The plastids and mitochondria, whether differentiated or not, divide by fission. One may expect that the DNA unit of the organelle will replicate before fission takes place, so that each daughter organelle will contain an identical DNA unit. Electron microscope studies of the organelles in fission may eventually provide visual evidence of such multiplication. The problem becomes complicated if organelles contain not single but, rather, multiple DNA units. For example, the organelle may increase in size but not undergo fission even if the DNA has replicated. An analogous case is seen in some bacteria where the pinching off of the cells is delayed and long bacterial threads are formed which contain many DNA units. It is possible that a situation of this kind may occur in algae, which have huge chloroplasts. For example, the long strap-shaped chloroplasts of *Oedogonium* continue to grow without separating into individual chloroplasts. The high DNA content of the single chloroplast of *Chlamydomonas* (*6*) may also represent multiple DNA units. Similarly, the high DNA content of the kinetoplast may represent a multiple DNA unit of the mitochondrion.

The question of the origin and individuality of the mitochondrion is less clear than the analogous question of the origin of the plastid. The small size of the mitochondrion, its plasticity, and its lack of pigmentation have led to diverse interpretations. A clear example of the origin of a mitochondrion from a preexisting one was discovered by Manton during her electron microscope studies of the tiny unicellular marine alga *Micromonas* (*44*). This organism contains one nucleus, one chloroplast, and one mitochondrion; the three divide synchronously at the time of cell division. Further evidence that mitochondria arise from preexisting ones is provided by the experiments of Luck (*45*) on a choline-deficient strain of *Neurospora*. By means of radioactive choline, the mitochondria of the cells were pulse-labeled in an early logarithmic phase of growth. The labeled lecithin formed was tightly bound to the mitochondria and did not turn over during this growth period. When the mitochondria increased in number, all were found to contain labeled lecithin. The results were compatible with the hypothesis that the new mitochondria did not arise *de novo* but arose through growth and division of the originally labeled organelles.

However, a number of observations on mitochondria are not readily explained. For example, both fission and fusion of mitochondria have been observed with the phase microscope (*46*), but it has not been established by electron microscopy whether the fusion between two mitochondria represents merely temporary adhesion or the disappearance of the four membranes at the point of contact. If fusion does occur, then the possibility of genetic

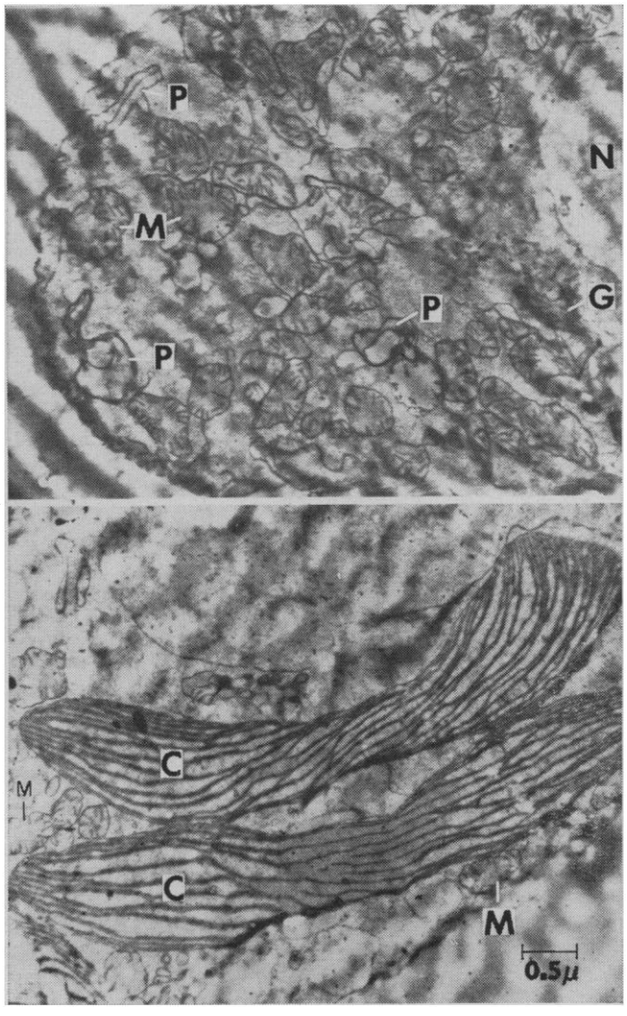
recombination should be investigated. The unexpected finding by Fletcher and Sanadi (*47*) that liver mitochondria have a half-life of about 10 days —an estimate based on the rate of disappearance of three incorporated isotopes—indicates that there is much to learn about the life history of these organelles.

The spontaneous origin of yeast mitochondria was suggested by Linnane, Vitols, and Nowland (*48*), on the basis of an electron microscope study of anaerobically grown yeast in which no mitochondria could be identified. Yotsuyanagi (*49*), however, found small, faintly staining promitochondria in anaerobic yeast. These structures developed into mitochondria within several hours after exposure to oxygen.

Evidence for a multigenic system in Euglena *plastids and yeast mitochondria.* The hereditary factors in *Euglena* plastids and yeast mitochondria are remarkably similar with respect to a number of properties (Tables 1 and 2). These similarities suggest the hypothesis that more than one hereditary factor or gene, including a regulator control mechanism, resides in each of these organelles, and that most of the mutations bring about a defective differentiation of proplastids in *Euglena* and of promitochondria in yeast. A discussion of the properties listed in Table 1 follows.

Evidence for proplastids and promitochondria in mutated cells. In the bleached *Euglena* cell, green chloroplasts do not develop and photosynthesis cannot take place. In respiratory deficiency of yeast, oxidative respiration, a process associated with mitochondria, does not occur. It has been suggested that these metabolic changes result from a permanent loss of the respective organelles; this, however, is not the case (Figs. 1 and 2). In *Euglena*, it has been established cytochemically (*50*) and with the electron microscope (*51*, *52*) that the plastids do not disappear but are present as tiny proplastids. These cannot differentiate to form organized chlorophyll-bearing lamellae, nor can they form the associated enzymes required for photosynthesis. Likewise, in the respiratory deficiency of yeast (Fig. 2), Yotsuyanagi (*40*) has found that mitochondria do not disappear but are present in a "promitochondrial" state —that is, a state in which they lack cristae membranes and some of the enzymes of the electron transport chain and are incapable of using O_2 for oxidative respiration.

Mutation rate. The rate of mutation to the bleached or to the respiratory-deficient state, respectively, was found to be very high. The frequency of occurrence of spontaneous mutations



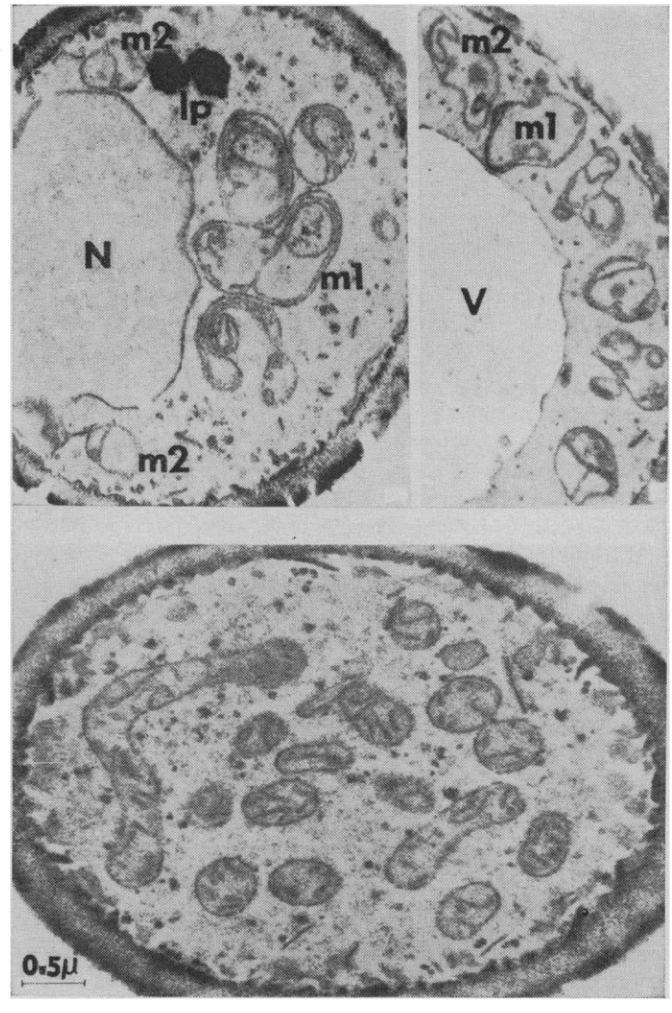


Fig. 1 (left). (Top) Normal mitochondria and mutated plastids of an ultraviolet-bleached *Euglena;* (bottom) Normal mitochondria and chloroplasts of a green *Euglena*. *C*, Chloroplast; *G*, golgi body; *M*, mitochondria; *N*, nucleus; *P*, plastid. [From Granick *et al.* (*51*)] Fig. 2 (right). Mitochondria of (top) a "petit" mutant of yeast, and of (bottom) a normal yeast. *m1* and *m2*, Mitochondria; *N*, nucleus; *V*, vacuole. [From Yotsuyanagi (*40*)]

Table 3. Enzyme systems in the differentiation of promitochondria to mitochondria by exposure to oxygen (*40, 53, 60*).

Structure of function involved	Normal		Mutated	
	Anaerobic	Aerobic	Anaerobic	Aerobic
Organized cristae	−	+	−	−
Cytochrome oxidase	−	+	−	−
Succinic cytochrome *c*-reductase	−	+	−	−
DPN cytochrome *c*-reductase	−	+	−	−
Cytochrome *c*	Trace	+	Trace	+
Cytochrome *c*-peroxidase	−	+	−	+
Catalase	Trace	+	Trace	+

could be as high as 1 percent. This frequency could be increased to 100 percent by feeble irradiation with ultraviolet light. The action-spectrum maximum in both cases was at 260 mμ, which is the absorption maximum for nucleic acids (*10, 41*). The mutation-inducing effect of ultraviolet light could be reversed by light of longer wavelength—that is, by photoreactivation. The mutation rate could also be increased by growing these organisms at elevated temperatures or by treatment with basic drugs such as streptomycin or acridine dyes (*53–55*). Such a high mutation rate has not been observed for nuclear genes.

Variability of mutants. The plastids of different bleached strains of *Euglena* were found to vary in their biochemical properties (*50*). Colonies derived from single cells bred true. The mutant strains differed in their ability to form carotenoids and in the enzymes of the porphyrin biosynthetic chain. Morphologic differences were also observed with the electron microscope; the less defective plastids usually appeared to be larger in size. These results, together with the results of the irradiation experiments described above, suggested that a number of cytoplasmic genes, presumably in the plastids, had undergone mutation.

Variations in the genetic properties of different mitochondrial-gene mutants have been observed in yeast. Ephrussi *et al.* (*56*) found two types of respiratory-deficient cells, suppressive and neutral. When neutral respiratory-deficient cells were crossed with cells containing normal mitochondria the progeny had normal respiration, as if the normal mitochondria had replaced or perhaps "cured" the defective neutral promitochondria. Strains derived from crossing suppressive respiratory-deficient cells and cells containing normal mitochondria had inhibited respiration. The suppressive cells could also mutate to the neutral form. No simple interpretation is possible as yet to explain the apparently "dominant" and "recessive" types of the mitochondrial mutations. Electron microscope studies on the fate of the different types of mitochondria in a zygote should help to clarify these points.

Agents of Environmental Control over Genes of the Organelles

In both types of organelles, differentiation is controlled by specific environmental agents. For the *Euglena* plastids, the agent is light; for the yeast mitochondria it is O_2.

Differentiation in Euglena *plastids.* When grown in the dark the cell contains tiny proplastids 1 to 2 microns in diameter, faintly yellow, and associated with paramylum grains (the carbohydrate-reserve polymer of euglenoids). The proplastids reproduce by fission; synchronized division of proplastids as teardrop-shaped bodies pulling apart was observed at the time of cell division (*51*). When the cells are exposed to light the proplastids enlarge, develop chloroplast lamellae and the associated pigments and enzymes of photosynthesis, and become functional chloroplasts. The location in the cell of the light-absorbing pigment system which triggers the differentiation is not known. It has been suggested that, in higher plants, phytochrome is the pigment (*57*).

The undifferentiated proplastids of normal cells grown in the absence of light are similar to the plastids found in bleached *Euglena* mutants (Table 2). The mutated plastids, however, cannot differentiate even when they are exposed to light. Any mutation that inhibits either adequate protein synthesis for the formation of lamellae or adequate pigment synthesis will prevent chloroplast formation. A number of bleached *Euglena* mutants have been observed to respond to light by synthesizing more carotenoids or by increasing the rate at which they convert δ-aminolevulinic acid to porphyrins. This suggests that in such mutants the mutation is probably not in the control mechanism that initiates the differentiation.

Differentiation in yeast mitochondria. When normal yeast cells are grown in the absence of O_2 they contain a few tiny promitochondria, less than 1 micron in diameter. These promitochondria possess few internal lamellae or membranes, have only a trace of cytochrome *c*, lack cytochrome oxidase and cytochrome *b*, and cannot respire O_2. When such cells are transferred to an atmosphere containing O_2 (see Table 3), the mitochondria become numerous by the end of the logarithmic phase of growth, have well-organized cristae, contain the enzymes of the electron transport system, and respire O_2. Evidently there is a mechanism triggered by O_2 that induces the differentiation of mitochondria to a form that can use the O_2 for oxidative metabolism. The nature of the triggering mechanism and its location in the cell are not yet known.

The promitochondria in the normal yeast cell grown in the absence of O_2 resemble those found in the respiratory-deficient mutants of yeast. The control mechanism for differentiation need not be damaged to cause respiratory deficiency. For example, when respiratory-deficient cytoplasmic mutants, grown in the absence of O_2, were then grown in air, it was observed that increased amounts of cytochrome *c*, cytochrome *c*-peroxidase, and catalase were synthesized. Morphologically, the exposure of respiratory-deficient cells to O_2 caused an increase in density of the outer membranes, together with enlargement of the promitochondria (*49*).

Significance of Organelles in Function and Heredity

What is the significance of each cell having multiple numbers of organelles, each with its own DNA unit? One possibility is that the mitochondrial energy-releasing factory and the photosynthesizing factory are thus maintained inviolate, through having many replicates with their own specialized protein-synthesizing equipment. Multiple numbers of organelles would therefore provide greater phylogenetic stability. On the other hand, certain mutations could occur independently in each organelle DNA unit, and these mutations could be carried along so that, when drastic environmental

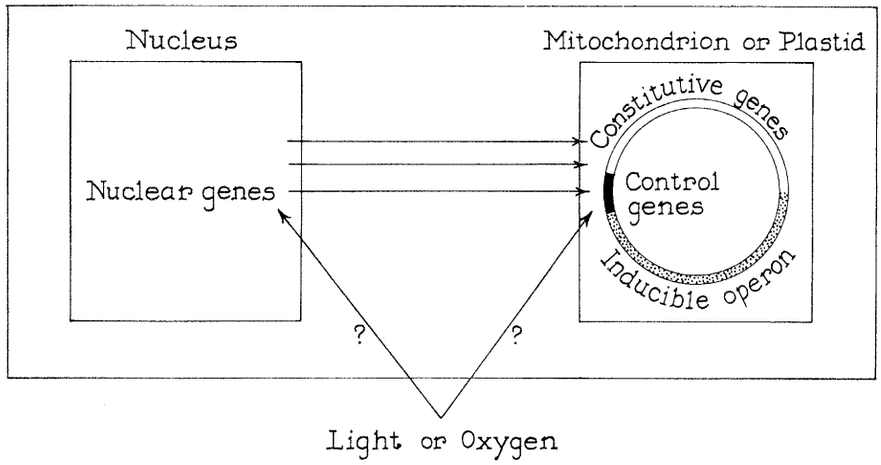


Fig. 3. Diagram of the hypothetical spherical chromosome of a cytoplasmic organelle and factors controlling its activity.

changes occurred, there could be selection for the most suitable organelles. Thus, a multiple number of mutated organelles per cell could provide for more rapid evolutionary change.

Working Hypothesis

On the basis of the facts and inferences that have been presented, and in analogy with schemes that have been used in bacterial genetics, we suggest the following model (Fig. 3) and working hypothesis for the genetic mechanisms of plastids and mitochondria.

Each organelle contains a DNA unit, or multiples of these units. A unit is sufficiently long to code for several hundred different proteins. The DNA unit may perhaps be a circular chromosome, analogous to the circular chromosome of some phages and bacteria. Some of the genes function as constitutive genes, while others are inducible. The constitutive genes are responsible for duplication of the organelles as well as for other biochemical processes, such as starch synthesis in the plastids.

Differentiation from proplastids to chloroplasts or from promitochondria to mitochondria is inducible and is controlled by a regulator-type gene of an operon unit. In *Euglena* and yeast it is the genes within this operon which easily undergo mutation, thus blocking the normal differentiation of the organelles. It is not yet known whether the induction by light or O_2 occurs by way of a direct effect on the genes of the organelle or whether the effect is mediated by activation of nuclear genes.

In addition to the control of the inducible genes of the organelle by environmental factors, products of nuclear genes are known to affect, directly or indirectly, the expression of the genes of the organelle. There are numerous nuclear genes in higher plants which affect the normal development of the plastids. The mutation of certain nuclear genes of yeast cause the suppression of mitochondrial differentiation. Nuclear genes could affect the organelles, for example, by limiting the supply of essential metabolites such as the pyridine nucleotides, or they could, more specifically, affect the organelles by the production of specific inhibitors or activators that would act on certain key genes of the organelle.

Summary and Conclusion

Evidence has been cited which indicates that RNA and DNA are present in plastids and mitochondria. A multigenic apparatus in the plastid is deduced from the properties of bleached *Euglena* strains. Control mechanisms are present for the differentiation of proplastids to chloroplasts in *Euglena* and in higher plants, and for the differentiation of promitochondria to mitochondria in yeast. An operon-regulator mechanism for this control is suggested. A comparison of the hereditary cytoplasmic units of *Euglena* plastids and yeast mitochondria indicates great similarities in their properties. Because of these similarities in two unrelated organisms, we suggest that a DNA unit which is self-duplicating and which serves as a code for RNA is the basic hereditary unit of each plastid and mitochondrion. Much work must be done if this reasonable hypothesis is to be converted into well-founded theory.

Some pressing problems await solution. We do not understand the nonrandom distribution of plastids in the mitotic divisions of variegated plants. A related unresolved problem is that of maternal inheritance, in which nonrandom segregation of cytoplasmic organelles occurs after fertilization, causing elimination of the organelles which are contributed by the male parent. How different are the gene components of one plastid in a cell from the gene components of other plastids in the same cell, and how do we test for these differences? Can gene exchange or recombination occur between organelles within the same cell? The answers to these questions may have to await development of more sophisticated techniques, such as the ability to transplant these organelles between different cells or to culture cellular organelles in vitro.

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Stimulation of Health Research

Catalytic activities of Public Health Service study sections from 1946 to 1964 are discussed.

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The Public Health Service is well known as the channel through which flows a large part of the funds appropriated by Congress for health-related research. The scientific community is especially familiar with this agency's extensive extramural program of grants and other awards aimed at supporting and strengthening research. In contrast, relatively few scientists are aware of the extent and nature of other kinds of assistance which the Public Health Service, particularly through the institutes and divisions of the National Institutes of Health and their advisory groups, has given to the development of research. This article describes one aspect of this assistance—the work of the advisory groups known as study sections. These bodies, organized in NIH's Division of Research Grants and working in close collaboration with the institutes, have paid much attention to problems of research development of specific scientific disciplines (*1*).

The 21 study sections which the Public Health Service organized in 1946 had grown in number to 51 by 1964. These bodies are composed of eminent scientists from universities, hospitals, foundations, and other research institutions. Since their memberships change partially every year, they serve as constantly renewed sources of independent viewpoints for the Public Health Service.

Study sections have two responsibilities. The first is to evaluate the scientific merit of research grant applications. This is in itself a demanding task which requires members to spend a great deal of time both in studying applications and in preparing preliminary review comments, as well as in actual participation, on visits to project sites and in study section meetings, in the discussions leading to ultimate recommendations. The second responsibility of these scientists is to survey the status of research in their fields in order to determine where research activities should

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