CELLULAR DIFFERENTIATION IN THE PROCESS OF GENERATION OF THE EUKARYOTIC CELL

HAKOBU NAKAMURA

Biological Institute, Faculty of Science Konan University, Kobe 658, Japan

and

ATSUSHI HASE

Osaka City Institute of Public Health and Environmental Sciences, Osaka 543, Japan

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Abstract. Primitive atmosphere of the earth did not contain oxygen gas (O_2) when the proto-cells were generated successfully as the resut of chemical evolution and then evolved. Therefore, they first had acquired anaerobic energy metabolism, fermentation. The cellular metabolisms have often been formed by reorganizing to combine or recombinate between pre-existing metabolisms and newly born bioreactions. Photosynthetic metabolism in eukaryotic chloroplast consists of an electron-transfer photosystem and a fermentative reductive pentose phosphate cycle. On the other hand, O_2-respiration of eukaryotic mitochondrion is made of Embden-Meyerhof (EM) pathway and tricarboxylic acid cycle, which originate from a connection of fermentative metabolisms, and an electron-transfer respiratory chain, which has been derived from the photosystem. These metabolisms already are completed in some evolved prokaryotes, for example the cyanobacterium *Chlorogloea fritschii* and aerobic photosynthetic bacteria *Rhodospirillum rubrum* and *Erythrobacter* sp. Therefore, it can be reasonably presumed that the eukaryotic chloroplast and mitochondrion have once been formed as the result of metabolic (and genetic) differentiations in most evolved cyanobacterium. Symbiotic theory has explained the origin of eukaryotic cell as that in which the mitochondrion and chloroplast have been derived from endosymbionts of aerobic bacterium and cyanobacterium, respectively, and has mentioned as one of the most potent supportive evidences that amino acid sequences of the photosynthetic and O_2 -respiratory enzymes show similarities to corresponding prokaryotic enzymes. However, as will be shown in this discussion, many examples have shown currently that prokaryotic sequences of informative molecules are conserved well not only in those of the mitochondrial and chloroplast molecules but also in the nuclear molecules. In fact, the similarities in sequence of informative molecules are preserved well among the organisms not only in phylogenetically close relationships but also under highly selective pressure, that is under a physiological constraint for the species in their habitats. Therefore, the similarities in amino acid sequences of proteins between the prokaryotes and the organelles are not necessarily direct evidence for their phylogenetical closeness: it gives still less evidence for a symbiotic relationship between the prokaryotes and the organelles. The metabolic compartmentalization of the membranes is an important tendency in cellular evolution to guarantee high specificity and rate of the metabolisms. It is suggested from the data that the intracellular membranes are not static but undergo dynamic turnover. Furthermore, these facts strongly support the Membrane Evolution Theory which was proposed by one of the authors in 1975.

Introduction

A concept has been generally accepted on the basis of current fossil records that the proto-cells originated 3.8 (to 4.0) billion years ago as a result of the chemical evolution, and that the eukaryotic cells first appeared about 1.5 billion years ago. Therefore, the long time span of 2.3 (to 2.5) billion years since the origin of life was an era dominated by the prokaryotic world. In this period, the prokaryotes evolved and succeeded in developing highly organized metabolic systems, which

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have found their way into today's eukaryotic world. The metabolisms include fermentation, photosynthesis, respiratory metabolism, and syntheses of macromolecules such as DNA, RNA and proteins. Therefore, it should be concluded that the cellular evolution from prokaryotes to eukaryotes was an essential step in metabolic compartmentalization to promote efficiency of life's activity, and the membranes played a very important role in the evolution.

The purpose of this paper is to review the metabolic and molecular evolution which has been abundantly accumulated by a number of investigators and to discuss the significance of membranous differentiation to compartmentalize the metabolisms during the process of generating eukaryotic cells. Our laboratory has also studied and published many papers on metabolic and molecular mechanisms of O_2-respiratory photosynthetic bacteria including purple non-sulfur bacteria and cyanobacteria and O_2-respiratory non-photosynthetic bacterium *Escherichia coli* K-12 over the past thirty years. Therefore, the present discussion will include these data. This communication also contains unpublished data of ours, but the detailed methods and materials for the experiments are not given here. The reader may write for further information.

Since Margulis (1970, 1981) re-emphasized the (endo-)symbiotic theory on the origin of eukaryotic cells, it has become fashionable. It has surely been explained in a simple manner and thus it gives an understandable account of the process. However, the content of the theory is too crude and rather morphological. It is generally said that the simpler the 'theory', the better. However, simplicity of illustration is independent of the facts. Anyway, we have to give an extensive analysis of the cyanobacterial world because the cells have completed the same respiratory and photosynthetic metabolisms as the eukaryotic organelles, mitochondria and chloroplasts, and because the cells have evolved a membranous organization to the level of the eukaryotic one (Seckbach, 1989). Therefore, we believe that eukaryotic cells originated as a result of cellular differentiation of highly developed prokaryotes and that this process was essential in cellular evolution.

It is a fact that base sequences of the mitochondrial and chloroplast DNAs resemble those of bacterium *Escherichia coli*. Symbiotic theory considers this fact to the important evidence to explain that those organelles were derived from the corresponding symbiotic prokaryotes. However, the nuclear DNA also had to be derived from a certain prokaryotic genome; otherwise, the nuclear DNA must be lined from an ancestral eukaryote which originated independently from the prokaryotic world. Further, the symbiotic theory states that chloroplast is an offspring of a cyanobacterial symbiont. If this is the case, the organelle DNA must be largely different in base sequence from that of the aerobic eubacterium, which was an ancestor of mitochondrion according to symbiotic theory. As will be discussed in the following, the theory contains an important discrepancy in itself.

The theory also emphasizes that almost all genomic DNA of the symbionts, aerobic bacterium and cyanobacterium, was transferred into nuclear genomes of so-called eukaryotic host cells during symbiosis, and that there occurred sharing

of DNAs among the organelles, mitochondrion, chloroplast and nucleus. This is an explanation for the fact that, in the extant eukaryotic cells, more than 90% of the genetic information about function and structure of the mitochondrion and chloroplast are kept in the nuclear DNA. However, there is no evidence to demonstrate that many parts of DNAs of the symbionts were transferred into and recombined with the nuclear DNA, and that either of the sequences common to the symbionts and nuclear DNAs was effectively eliminated.

Furthermore, genetics has evidenced that the genetic recombination does not occur basically between different species, to say nothing of that between different phylla of bacterium modified to mitochondrion, cyanobacterium modified to chloroplast and mycoplasma as host (after Margulis, 1970). Each of the organisms must surely possess species-specific endonucleases (restriction enzymes), as will be discussed later.

Shinozaki *et al.* (1986), Ohyama *et al.* (1980), and Hiratsuka *et al.* (1989) have determined the entire base sequences of chloroplast DNA in tobacco, liverwort, and rice plants, respectively. According to their data, the chloroplast DNA contains only limited numbers of genes for specific tRNAs, rRNAs, and proteins. Interestingly, the DNA has introns in the genes, which are specific for the eukaryotes but not for the prokaryotes, and has no sequences for 3′-terminal CCA of the tRNA. Further, some sequences homologous to the human mitochondrial genes of the respiratory chain have been found in the chloroplast DNA of tobacco (Anderson *et al.*, 1981, Matsubayashi *et al.*, 1987). These facts may support the idea that chloroplast is a product of eukaryotic differentiation and evolution of the genes, as suggested in the following section of metabolic differentiation.

Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is a major fraction of stromal protein of chloroplast and is composed of 8 identical large subunits (LS) and 8 identical small subunits, (SS), which are encoded by the chloroplast DNA and the nuclear DNA, respectively. Symbiont theory has emphasized that a cyanobacterial endosymbiont, cyanelle, of protozoa (also classified as Cryptophyta of Plantae) *Cyanophora paradoxa* is a proto-chloroplast because the cyanelle's DNA content is of a similar level to that of chloroplast. However, the sequence analysis has shown clearly that the cyanelle's DNA contains both the genes responsible for LS and SS of RuBisCo, and thus is identical in genetic composition to that of cyanobacterium (Nakamura, 1987c).

It is difficult, after all, to explain reasonably the process of generating eukaryotic organelles with the help of symbiotic theory. Therefore, we will try to elucidate the process by using another theory, called Membrane Evolution Theory, in the following.

Metabolic Evolution

1. Fermentation

Fermentation is the most basic metabolism for life, which developed in the primitive era in a non-oxygenic atmosphere. The second half of the EM pathway, that is a series of reactions from glyceraldehyde phosphate to pyruvate, is known to occur throughout biological world: this suggests that it is one of the most primitive metabolisms in the cell (Battley, 1987; Broda, 1978: Holland *et al.*, 1987). That part of the EM pathway seems to have been used to generate more complex fermentative metabolisms, such as the pentose phosphate cycle, which is a very important metabolism that produces ribose for RNA and deoxyribose for DNA (de Witt, 1977; Nakamura, 1983, 1987a). The pentose phosphate cycle is also known to occur with some (or without) exceptions throughout the biological world. Interestingly, the pentose phosphate cycle had reversed to result in a reductive pentose phosphate cycle, which became the so-called dark reaction, Calvin cycle, for CO_2 fixation in photosynthesis and chemosynthesis in anaerobic (primitive) photosynthetic and chemosynthetic bacteria, respectively (de Witt, 1977; Nakamura, 1987a, 1988a). It has been demonstrated that a green sulfur bacterium *Chlorobium thiosulphatophilum* and a purple non-sulfur bacterium *Rhodospirillum rubrum*, which belong to photosynthetic bacteria, carry a reductive tricarboxylic acid (TCA) cycle to fix CO_2, which consists of the final reactions of the EM pathway and the whole

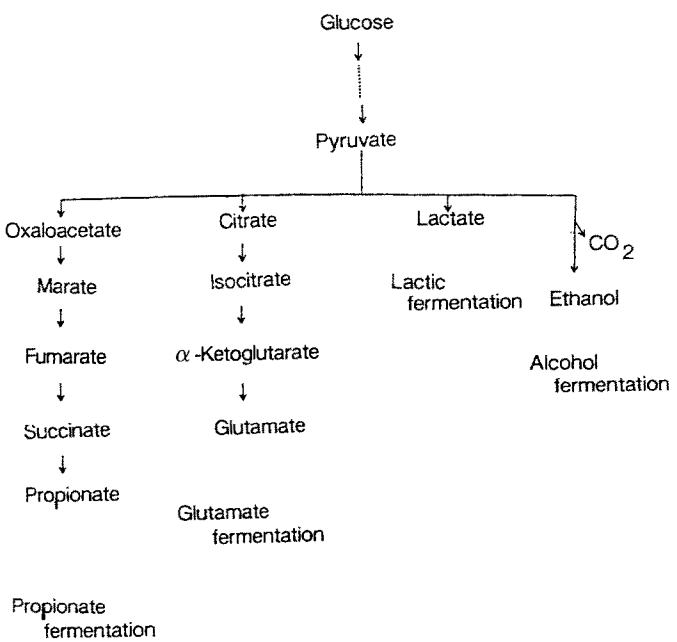

Fig. 1. Propionate and glutamate fermentations that branched from EM pathway.

TCA cycle (Nakamura, 1987a; 1988a). The EM pathway was further elongated and branched to produce various organic acids, being referred to as propionate fermentation, glutamate fermentation and others via pyruvate (Holland *et al.*, 1987).

The TCA cycle is a central metabolism for respiration, glyoxylic acid cycle, and syntheses and degradations of amino acids, fatty acids and other intermediates in the cells. Metabolic construction of the TCA cycle shows that it has been derived from a connection of both the pathways of propionate and glutamate fermentations (Figure 1). However, in this case, the metabolic flow of the propionate fermentation had to be reversed for the TCA cycle and to be connected enzymatically between α-ketoglutarate and succinyl-CoA. In fact, almost all species of the anaerobic bacteria and some of the cyanobacteria have an imperfect TCA cycle which is not connected at the reaction from α-ketoglutarate to succinyl-CoA (Tandlau de Marsac and Houmard, 1987: Carr and Whitton, (1982), and the so-called glyoxylate pathway, which has been found in many species of eukaryotic plants and micro-organisms, is also blocked in that reaction (Bryant, 1980; Douce, 1985).

There is little doubt that the respiratory chain was derived from the photosystem of bacterial photosynthesis (Nakamura, 1987), because photosynthesis can be considered to be older in origin than O_2 – respiration, and a high similarity of amino acid sequence has been demonstrated between the cytochromes corresponding to the respiratory chain of mitochondrion and the photosystem of chloroplast (Nakamura, 1987b). After all, we can deduce that the photosynthesis has been constructed by the photosystem and the fermentative reductive pentose phosphate cycle, and that the respiratory metabolism has been constructed by the fermentative EM pathway, the TCA cycle, and a derivative of the photosystem. Therefore, it can be concluded that the cellular metabolisms have been constructed by means of repeated reconstruction of the pre-existing metabolisms so as to make them adapt to circumstances encountered.

2. Metabolic differentiation

Our laboratory has studied the molecular and subcellular biology of the photosynthetic bacterium *R. rubrum*, cyanobacterium *Synecosistis* sp., and the non-photosynthetic bacterium *E. coli*. The following are some considerations about metabolic differentiations and their compartmentalization by the membranes during the evolution from prokaryote to eukaryote on the basis of these studies. The photosynthetic bacteria have been grouped into five classes: green sulfur bacterium, purple sulfur bacterium, purple non-sulfur bacterium, *Erthrobacter*, and cyanobacterium. The latter three possess O_2 respiration-ability. As shown in Figure 2, the *R. rubrum* cells can grow not only photosynthetically under anaerobic light conditions but also O_2-respiratory under aerobic dark conditions. Further, they can divide actively when cultured even under anaerobic dark conditions. For the photosynthetic growth of the bacterial cells, a specific synthetic medium was filled up in the culture bottles to make the conditions anaerobic and the cultures were incubated in a lighted (1000 lux) incubator at 30 °C. For the aerobic growth, the bacterial cultures

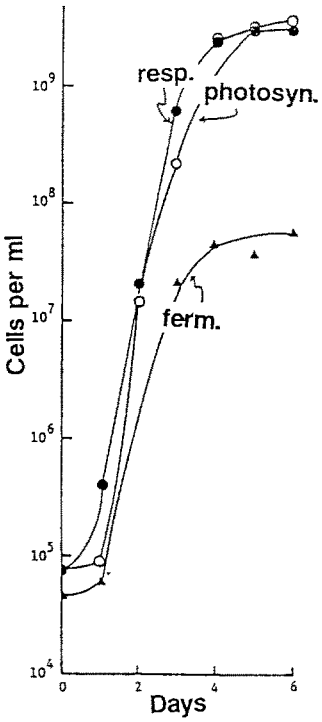

Fig. 2. Adaptive growth of purple non-sulfur bacterium *Rhodospirillum rubrum* using three energy-metabolisms, photosynthesis (abbreviated to photosyn. on the figure) under anaerobic light conditions, O_2-respiration (abbreviated to resp.) under aerobic dark condition, and fermentation (abbreviated to ferm.) under anaerobic dark condition.

with a broth medium were shaken vigorously in a water bath at 30 °C, and for the anaerobic growth, the broth medium was filled up in the culture bottles to make conditions rather anaerobic and the bacterial cultures stood without shaking in the water bath. The data show clearly that the bacteria have accumulated historic energy metabolisms of fermentations, photosynthesis, and respirations, and thereby adapted to various environments encountered. This is the reason why we have selected the photosynthetic and respiratory bacterial species, such as *R. rubrum*, to study metabolic differentiation.

The purple non-sulfur bacterium has an electron transfer system of flow from ubiquinone to cytochrome *c* via cytochrome *b*, which is commonly used by both the metabolisms of photosynthesis and respiration (Nakamura, 1983, 1987b). Therefore, when the photosynthesis is blocked conditionally, for example by darkness, or mutationally, the bacterial cells acquire the living energy from respiration only. Contrarily, if the respiration is inhibited conditionally, for example by anaerobiosis, they can grow using photosynthesis.

3. Genetic and membranous differentiation

Recently, we have found that, when the purple-colored wild type strain of *R. rubrum*

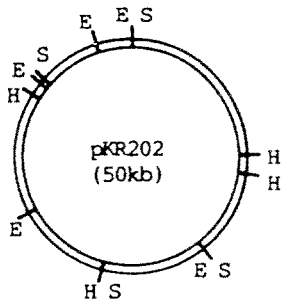

Fig. 3. 50 k Base-paired ring plasmid carried by the wild type *R. rubrum* cells and its restriction enzyme map.

was treated by acriflavine, one of the acridine dyes, some non-colored, white mutants were produced. Acriflavine was added into a broth medium with a rather alkaline pH (8.1) and the wild type cells were incubated in the medium for 3 to 5 hours at 30 °C and plated on the non-acriflavine broth agar medium. After several days, the white and weakly-colored mutants appeared with a frequency of 10^{-1} to 10^{-5} orders, depending on conditions used. We picked up and used typical white mutants as the standard for experiments. To eliminate a contamination of non-*R. rubrum* and white-colored bacteria, a streptomycin resistance mutation was introduced into the starting wild-type strain of *R. rubrum* and the maker was tested before every experiment for the wild type and the white mutant strains. Of course the white mutant can not grow by dependence on photosynthesis but on respiration. Kuhl *et al.* (1983) have isolated the same type mutant of *R. rubrum* by treatment with a mutagen, ethyl methanesulfonate. We demonstrated by molecular analysis that the wild type cells contain rather large circular plasmid of ca. 50 k base pairs long (Figure 3).

Acetone and methanol (7:2) extraction of pigment from the wild type cells showed clear absorbance for carotenoid at 459 and 525 nm and for bacteriochlorophyll at 775 nm, but that of the white mutant cells had no absorbance for both the pigments. Further, electron-micrographs of the wild type cells which were cultured under somewhat anaerobic condition showed that membranous photosynthetic organelles, chromatophores, are packed to full, whereas there were little or no chromatophore in the white mutant cells. Thus, it is apparant from these facts that the plasmid contains genes which are responsible for syntheses of the photosynthetic pigments and for formation of the chromatophores in the cells. On the other hand, we demonstrated that when the white mutant cells were transformed with the plasmid DNA prepared, the wild type pigments and the intracellular organelles can be regenerated. We are now doing genetic analysis of the plasmid. It is interesting that the genes responsible for photosynthesis and morphogenesis

in the photosynthetic bacteria are located on the plasmid DNA. Currently, it has been apparent that the bacterial DNAs, both of the main chromosomes and plasmids, bind to cell membrane and/or membranous organelles, such as mesosomes (Nakamura, 1975b, 1978; Kornberg, 1980), and the replicon theory of Jacob (1963) has supposed that their replications and divisions to both poles are controlled by the machinery which is located in the membrane system. Recently, we have found that when the chromatophores of *R. rubrum* cultured under the photosynthetic conditions were isolated and purified by repeated density-gradient centrifugations of the osmotically disrupted spheroplasts, the organelles bound small DNA fragments, which then were ascertained to be the 50 kbp plasmid by the restriction enzyme and hybridization analyses and to contaminate with little chromosomal DNA. At present, experiments are in progress to determine the binding site sequence of the plasmid with the organelle membrane and to observe by electron microscope the binding figure (to be published by the Japanese Society of Plant Science, 1990). Further, we are now studying cyanobacterium, which once evolved from a line of photosynthetic bacteria and which is known to carry the same metabolic systems of photosynthesis and respiration as the purple non-sulfur bacteria (Loomis, 1988; Pescheck, 1984, 1987) and to contain plasmids of various sizes (Tandeau de Marsec and Hownard, 1987).

4. Membrane development in *E. coli*

Our next approach was to observe membrane formation in *E. coli* cells. Intracellular membrane organelles seem to be few in the non-photosynthetic prokaryotes as compared to the photosynthetic ones. However, in Gram-positive bacterium *Bacillus subtilis* for example, we can observe rather complex organelles mesosomes, which have been supposed to contain redox-reactions, DNA replication machinery and others.

The membranous organelles, chromatophores and cell membranes of the photosynthetic bacteria contain electron-transfer system, photosystem and respiratory chain, respectively, and other enzymes. Thylakoid and mesosome lamellae of cyanobacterial cells are also the organelles containing the photosystem and respiratory chain (Carr and Whitton, 1982). In this way, the prokaryotic cells can develop membrane systems, in which many kinds of enzymes are integrated, during their active growth. However, the membrane differentiation is dynamic, rather than fixed, and is dependent on the physiological requirements of the cells.

On the other hand, it is not general in *E. coli* that the cells have membranous organelles except for the cell membrane. However, in the course of study on the action mechanism of acriflavine, which can effectively eliminate plasmids such as the sex (F) and drug resistance (R) factors from the *E. coli* cells, we found that the drug induces some intracellular membrane formation.

We have isolated an acriflavine-sensitive (*acrA*) mutant from the wild type ($acrA^+$) strain. The *acrA* gene is located min 10.6, just near gene *dnaZ* for the DNA synthesis, on the *E. coli* chromosome (Nakamura, 1965). The plasmids become quite unstable

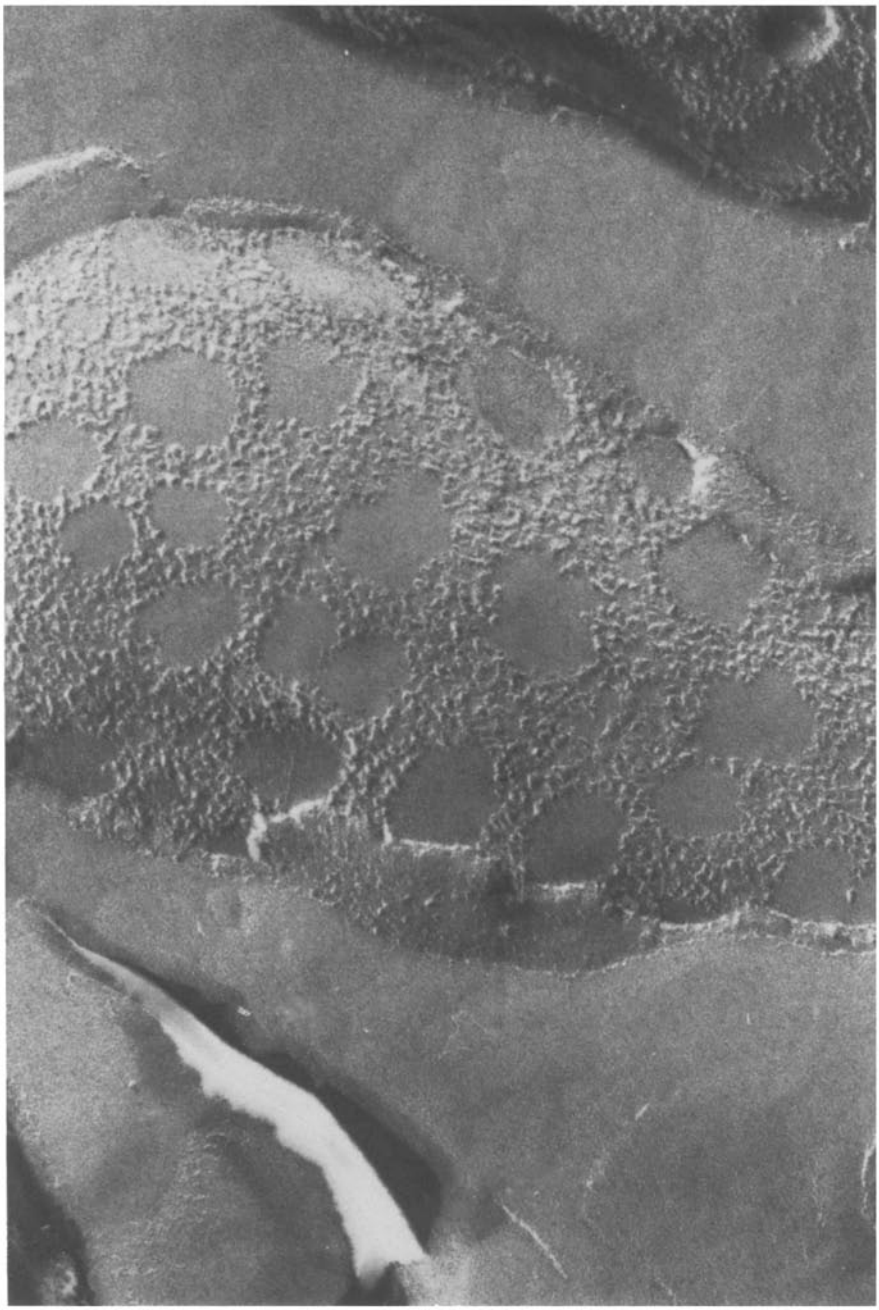

Fig. 4. Freeze-fractured micrographs of *Escherichia coli* cells show that formation of lamellar complex were induced from the cell membranes by the presence of acriflavine (100 μg mL^{-1}). A: *acrA* mutant cell; B (p. 508): its wild type cell.

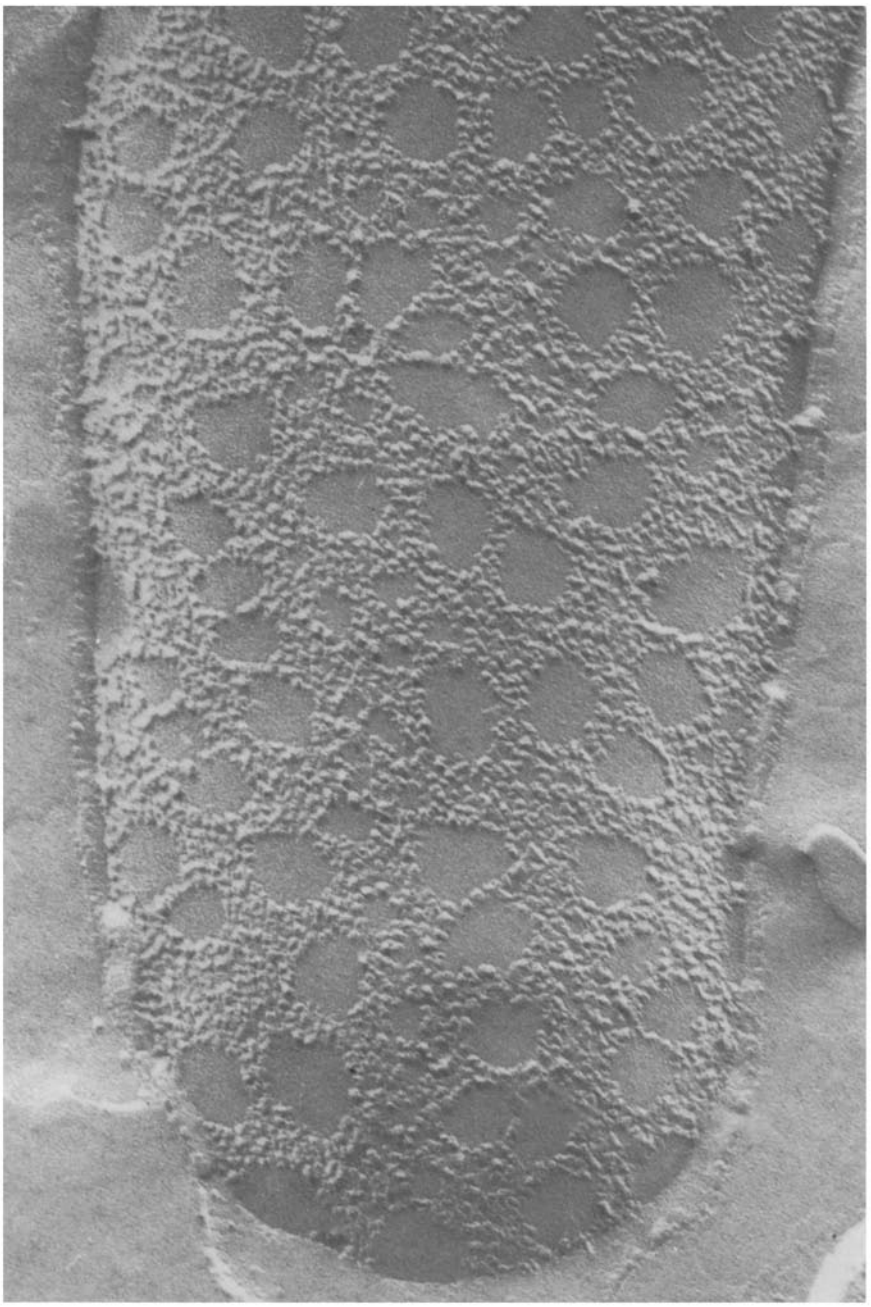

TABLE I

Phospholipid synthesis of the *E. coli* cells in the presence of acriflavine (analyzed by T. Sakaki). Phosphatidyl ethanolamine and cardiolipin are accumulated preferentially

		Phospholipid content (n mol 10^{-10} cells)				
	AF	PE	PG	CL	PS	PA
acrA	−	398.4	57.5	39.9	+	±
	+	693.4	46.7	85.2	+	±
acrA$^+$	−	199.5	89.2	10.6	+	±
	+	390.9	158.8	20.7	+	±

AF: acriflavine, PE: phosphatidyl ethanolamine, CL: cardiolipin, PG: phosphatidyl glycerol, PS: phosphatidyl serine, PA: phosphatidic acid.

in the mutant cells and are eliminated easily by the presence of low concentrations of acriflavine and other membrane-attackable agents and by heating at at least 42 °C, while the plasmids are stable in the wild type cells. (Nakamura, 1975c). Furthermore, we found recently that when the *acrA* and *acrA*$^+$ cells were treated with proper concentrations, for example 20 and 100 μg mL^{-1}, respectively, of acriflavine, lamellar structures develop from cell membranes and the membrane complexes are formed especially at the ends of the cells (Nakamura, 1983, 1988b). Figure 4 shows freeze-fractured electron-micrographs of the acriflavine-treated cells and Table I also indicates that the contents of phospholipids, particularly fractions of phosphatidyl ethanolamine and caldiolipine, increase significantly in the presence of acriflavine (100 μg mL^{-1}). From these data, we can conclude that the formation of the intracellular membrane is induced according to physiological conditions.

5. Preservation of Amino Acid Sequences of Proteins During the Evolution from Prokaryote to Eukaryote

According to studies on molecular evolution, mutations have been continuously accumulating in the genomic DNA over the geologic era. However, the rate of accumulation is gene-specific, because each of the genes in the genome has been suffering a certain physiological restriction under particular selective pressure. Furthermore, the accumulation of mutations on the base sequence of DNA with the constant rate is mainly a result of continuous errors in the DNA replication, in which mispairing of complementary bases occurs spontaneously at the rate of 10^{-8} to 10^{-9} mutations/cell/division. However, there are arguments as to whether the rate is absolutely constant or changeable dependent on the physiological significance of the gene, that is protein, during the life of the cells. In other words, the accumulation rate of mutations must be influenced by the significance of the functioning of each protein under selective pressure. This means that an organism will disappear from the population if an important protein (or enzyme)) loses or reduces its activity by a mutation, and thus the extant organism has relatively few mutations in the gene.

The physiological significance of amino acid sequences is different even within a single protein molecule. The sequence of active sites including the prothetic group-binding region, which are functionally most important for the protein, have been well conserved through prokaryote to eukaryote. It is the case in molecular evolution from the prokaryotic DNA not only to the nuclear DNA but also to the mitochondrial and chloroplast DNAs. The symbiotic theory says that the mitochondrion and chloroplast are symbiotic derivates of aerobic bacterium and cyanobacterium and considers as evidence supportive that the DNA of these organelles resembles in base sequence the prokaryotes, respectively. However, the prokaryotic sequences in DNA or protein have also been preserved in nuclear DNA, i.e. in the cytoplasmic proteins. This is quite natural because the nuclear genome is also derived from the prokaryotic one. For example, glyceraldehyde phosphate dehydrogenase, which works in a mid reaction of the EM pathway and is synthesized in cytoplasm under a control of nuclear genome, has amino acid sequences in mononucleotide-binding domains as shown in Figure 5. The domain is coded for by five exons which seem likely to have evolved from common precursor enzyme. The amino acid sequences of the enzymes isolated from *E. coli, Bacillus stearothermophilus*, yeast, lobster, chick, pig, and man, from prokaryotes to eukaryotes, appear to be conserved to a surprising extent, over 50%. The sequences shown are the first 50 amino acids which form a part of the NADH-binding portion (Loomis, 1988).

Maltose phosphorylase gene of bacteria has been recruited in eukaryote to form glycogen phosphorylase gene. There is over 60% similarity in the amino acid sequence of these phosphorylases in *E. coli*, yeast and rabbit muscle cells. Further, we can find many examples to show high similarty in amino acid sequences of proteins between prokaryotes and eukaryotic cytoplasms.

Here, we can conclude from data of the molecular evolution from prokaryotes to eukaryotes that the similarities in sequences of informative molecules are determined by various parameters which have affected the organisms during evolution over the geologic era, and thus it is difficult to determine whether the data can cite as evidence for the morphological evolution of the cells, and also as evidence for the symbiotic origins of mitochondrion and chloroplast (Nakamura, 1990).

Membrane evolution and origin of eukaryotic cell

As mentioned in the above sections, we have doubted whether chloroplasts and mitochondria of the eukaryotic cells were evolutionally derived from endosymbionts such as cyanobacterium and aerobic bacterium, respectively (c.f. Margulis, 1970). So we proposed a 'Membrane Evolution Theory' in 1975 instead of the symbiotic theory of Margulis (1970, 1981). The following is a discussion of our new theory. It has been established that the purple non-sulfur bacterium including *R. rubrum* contains both metabolisms of the ancestral photosynthesis and O_2-respiration (Nakamura, 1983, 1987b). Similarly, cyanobacteria, for example, *Chlorogloea fritschii* which was derived from the photosynthetic bacteria, and is believed to link

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Man(cytoplasm)      GlyLysValLysValGlyValAspGlyPhe GlyArgIleGlyArgLeuValThrArgAla
Pig(cytoplasm)       -  - ValLysValGlyValAsp - Phe GlyArgIleGlyArgLeuValThrArgAla
Chick(cytoplasm)     -  - ValLysValGlyValAsnGlyPhe GlyArgIleGlyArgLeuValThrArgAla
Lobster(cytoplasm)   -  - SerLysIleGlyIleAspGlyPhe GlyArgIleGlyArgLeuValLeuArgAla
Yeast(cytoplasm)     -  - ValArgValAlaIleAsnGlyPhe GlyArgIleGlyArgLeuValMetArgAla
E. coli             MetIleThrLysTyrGlyIleAsnGlyPhe GlyArgIleGlyArgIleValPheArgAla
B. stearo.           - AlaValLysValGlyIleAsnGlyPhe GlyArgIleGlyArgAsnValPheArgAla

Man(cytoplasm)      AlaPheAsnSerGlyLysValAspIleVal AlaIleAsnAspProPheIleAspLeuHis
Pig(cytoplasm)      AlaPheAsnSerGlyLysValAspIleVal AlaIleAsnAspProPheIleAspLeuHis
Chick(cytoplasm)    AlaValLeuSerGlyLysValGlnValVal AlaIleAsnAspProPheIleAspLeuAsn
Lobster(cytoplasm)  AlaSerCysGlyAlaGlnValValAlaVal  -  - AsnAspProPheIleAlaLeuGlu
Yeast(cytoplasm)    AlaLeuSerArgProAsnValGluValVal AlaLeuAsnAspProPheIleThrAsnAsp
E. coli             AlaGlnLysArgSerAspThrGluIleVal AlaIleAsnAsp - LeuLeuAspAlaAsp
B. stearo.          AlaLeuLysAsnProAspIleGluValVal AlaValAsnAsp - LeuThrAsnAlaAsp

Man(cytoplasm)      TyrMetValTyrMetPhe - TyrAspSer ThrHis
Pig(cytoplasm)      TyrMetValTyrMetPhe - TyrAspSer ThrHis
Chick(cytoplasm)    TyrMetValTyrMetPheLysTyrAspSer ThrHis
Lobster(cytoplasm)  TyrMetValTyrMetPheLysTyrAspSer ThrHis
Yeast(cytoplasm)    TyrAlaAlaTyrMetPheLysTyrAspSer ThrHis
E. coli             TyrMetAlaTyrMetLeuLysTyrAspSer ThrHis
B. stearo.          GlyLeuAlaHisLeuLeuLysTyrAspSer ValHis
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Fig. 5. Amino acid sequences of glyceraldehyde-phosphate dehydrogenases in the EM pathway of various prokaryotes and eukaryotes, which are synthesized in the cytoplasms under the nuclear controls.

phylogenetically between unicellular and simple filamentous forms (Fogget *et al.*, 1973), provides more evolved photosynthesis which consists of serial photosystem, I and II, evolving O_2 gas. Furthermore, the species has the complete O_2-respiration metabolism which is made of EM pathway, TCA cycle, and respiratory chain (Ragan and Champman, 1978). In cyanobacteria, DNA contents of the cells have been demonstrated to increase duplicatively from unicellular to filamentous forms (Herdman *et al.*, 1979). Some biologists have pointed out that, generally, the cyanobacterial cells are low in respiratory activity. However, this comes from a problem of so-called metabolic regulation and the cyanobacterial chromosome does carry a whole set of the genes responsible for O_2-respiration. Even in the higher plants, during the day, the ATP needed is provided mainly by photosynthesis in chloroplast, rather than by mitochondrial respiration.

In other words, the evolved species of cyanobacteria have already been endowed with genes coding for the eukaryotic photosynthesis and respiration. Therefore, it is quite reasonable to consider that the DNA-carrying organelles, such as chloroplast, mitochondrion and nucleus, have been generated as the result of genetic and membranous differentiation from the evolved cyanobacterium, when endoplasmic reticulum, Golgi apparatus and other membranous organelles in the pro-eukaryotic cells have differentiated.

Here, it is unnecessary to suppose that the genes of photosynthesis and respiration were derived from some separate symbionts which once invaded into unknown prokaryotic host. Originally, the cell does not accept foreign DNA and does not allow recombination between DNAs of different species. Species-specific restriction enzymes and other nucleases are primarily so-called arms which defend the cell against the invader DNAs. Therefore, all biological species must have specific enzymes which digest the foreign DNA. It is apparent that such careful mechanisms have preserved the phylogenecity in the biological world. In fact, no natural hybridization occurs without a single, or near, taxonomic species. On the other hand, it natural that the symbiosis, or parasitism, is popular beyond the taxonomy or even between animal and plant kingdoms.

Taken together with other biological phenomena, we have proposed as shown in Figure 6. A long DNA fiber in the evolved cyanobacterium was broken into at least three parts: two small fragments and one large one. Then, these were wrapped by the intracellular membranes, such as thylakoids and mesosomes. A small amount of DNA containing some genes relating to respiratory chain, t- and r-RNAs, and others was organized as the mitochondrion, and some other DNA containing genes relating to photosystem, t- and r-RNAs and others was organized as the chloroplast. Furthermore, the remainder of this DNA is comprised in the nucleus. We believe that the t- and r-RNAs coded for by the mitochondrial and chloroplast DNAs are duplicative offsprings which have an ancestral nucleotide sequence common with the nuclear t- and r-RNAs, but they became diverged phylogenetically since the cellular differentiation. The break and reunion of the DNA strands in a (clonal) cell are usually observed in both the prokaryote and the eukaryote. When the

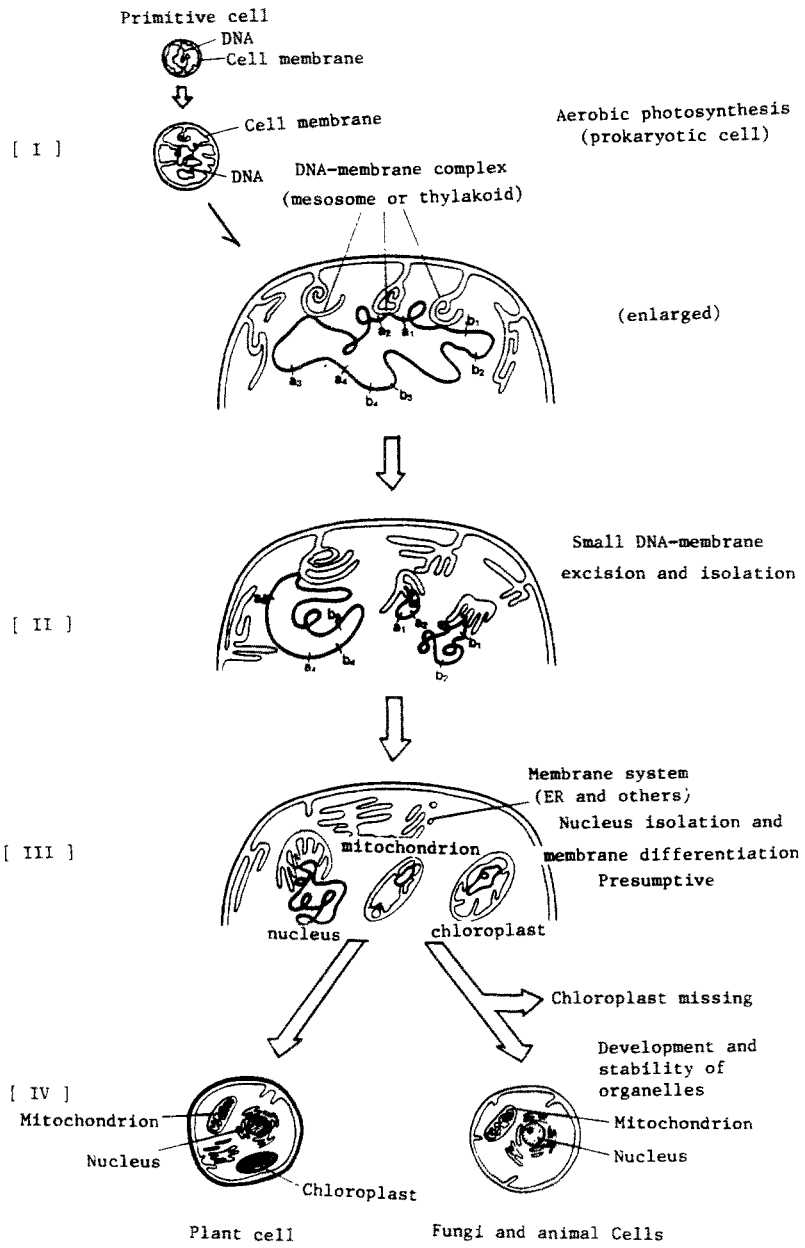

Fig. 6. Membrane evolution theory. DNA and membranous system of the evolved cyanobacterium once differentiated to form presumptive mitochondrion, chloroplast, and nucleus. Non-photosynthetic animal and fungal cells were generated as a result of spontaneous elimination of the chloroplast. Their regulatory mechanism of genetic expression among the DNA-organelles have to be inherited from the prokaryotic age.

differentiated cell lost the presumptive chloroplast, they became non-photosynthetic cells as animal and fungus. Spontaneous or induced elimination of the chloroplast from green algae have often been observed in the laboratory.

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