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Problems in the Rate of Evolution in Biological Systems [and Discussion]

F. R. Whatley, W. H. McCrea and N. W. Pirie

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Problems in the rate of evolution in biological systems

By F. R. Whatley, F.R.S.
Botany School, South Parks Road, Oxford OX1 3RA, U.K.

The earliest fossils date from 1.5 Ga ago. They are tentatively identified as prokaryotic blue-green algae and seem to represent organisms that are already metabolically complicated. We have little idea of the timescale associated with the stages of evolution that preceded them. Presumably they were antedated by bacteria, for which there are no fossil remains.

The first bacteria must have been fermentative. Only after the establishment of oxygen-evolving photosynthesis in the blue-green algae could aerobic (respiring) bacteria evolve. The simplest eukaryotes presumably arose by uptake of certain aerobic bacteria into primitive nucleated cells, themselves evolved from a prokaryote, now lost. This line subsequently evolved into the multicellular animal line for which the more recent fossil record is good and enables us to identify eras in which there were successively predominant a series of invertebrates (e.g. corals, insects and molluscs) and vertebrates (e.g. fishes, amphibians, reptiles, birds and mammals).

Evolution of the plant kingdom required the uptake by primitive eukaryotes of photosynthetic symbionts. The successive uptake of various photosynthetic prokaryotic and eukaryotic algae into different eukaryotic hosts provided the basis for the multiphyletic origin of the present day plant kingdom. The land plants, derived from the ‘green’ line, first appeared 350 Ma ago; the fossil record indicates a steady progression represented successively by the age of the lycopods and seed ferns, of gymnosperms and of angiosperms.

From this record one can recognize the enormous conservatism of certain genera (e.g. *Nautilus*; sharks, blue-green algae) an obvious onwards progression with others (e.g. *Eohippus* to the modern horse) and abrupt changes with many others.

It is this apparent inequality in rates of evolution of individual species that makes the temporal evaluation of the evolutionary record so difficult.

Introduction

It is tempting to imagine that evolution took place in an orderly fashion against an even timescale. While one can find in the fossil records sequences suggesting such an even progression of evolution over a limited timespan, what is just as characteristic is the sudden appearance of apparently novel forms with a new capability, which undergo rapid adaptive radiation and enable the novel form to exploit many of the existing ecological niches. The largest discontinuity is perhaps represented by the appearance of the eukaryotic cell, with its nucleus and characteristic populations of organelles, as a completely new form in succession to the prokaryotic (bacterial) level of organization. The attainment of the eukaryotic level led to the exploitation of new areas of the environment, thus extending the realm of living things, and the new level of cellular organization led additionally to the evolution of multicellular systems and further discontinuities in the evolutionary timescale. Equally there must have been discontinuities in the appearance of the starting materials (the organic molecules) on which the organization of living systems depends, as well as in the types of biochemical apparatus necessary to process and ultimately produce them.

The earliest organization

The origin of the primeval soup is not known with certainty! Assuming its existence, we may get clues to its origin from modern experiments, such as those done by Miller & Urey (Miller 1953), in which mixtures of methane, ammonia, water and hydrogen gases were sparked and a number of small carbon compounds (up to C_5 and C_6) were accumulated. The gas mixture chosen was supposed to represent the primeval atmosphere, but we have no evidence by which we can confirm the nature of this atmosphere and the biochemistry of life has certainly altered it. The study of molecules in interstellar Space may be more rewarding in clues about primitive chemistry, for there is no going back once life begins. Electrical discharges were chosen as the energy source in the Miller experiments, as such discharges are expected in the violent thermal circulation of the primitive atmosphere. A selection of the amino acids, compounds of potential biochemical interest, obtained in Miller-type experiments is given in table 1. A modern biochemist is much interested by the production of so many compounds that are familiar to him in modern metabolic pathways. The prebiotic soup might alternatively have been formed in outer Space. Table 1 also shows a selection of nitrogen-containing organic compounds identified in the Murchison meteorite and this is characteristic of many meteorites that have been examined.

Table 1. Amino acids of the prebiotic soup?

Miller–Urey	*Murchison meteorite*
glycine	glycine
sarcosine	sarcosine
alanine	alanine
β-alanine	β-alanine
α-aminobutyric acid	α-aminobutyric acid
aspartic acid	α-aminoisobutyric acid
glutamic acid	aspartic acid
urea	glutamic acid
	γ-aminobutyric acid
	proline
	valine
	norvaline
	N-ethylglycine
	N-methylalanine

The recognition of a number of radicals presumably derived from parent molecules in the solid nucleus of a few comets, including CH, CN, CO and CS, and perhaps H_2O, has led to the suggestion that intermittent heating of comets may have given rise to complex organic molecules from such core materials and that these might from time to time, like meteorites, have fallen to Earth to give areas rich in components of the prebiotic environment (Hoyle & Wickramasinghe 1977). The whole matter of the cometary contribution to the origin of life has recently been reviewed by Irvine *et al.* (1981), who conclude that the likelihood that comets may have contributed to life on Earth is small, though the possibility can never be discounted that we all originated extraterrestrially. As Pirie has pointed out (this symposium), this is no different from comments on earlier hypotheses, but merely moves the question away from the terrestrial origin of life to the origin of life elsewhere and is not helpful.

A number of quite complicated ring compounds can be synthesized by the polymerization of simple molecules, of the type already identified in interstellar Space. Ring compounds themselves are notably absent from lists of compounds identified in Space, although this may represent observational limitations. As indicated in the 'paper chemistry' scheme of figure 1, the stepwise polymerization of HCN with the help of absorbed light quanta to rearrange the structure at critical points can lead to the formation of the purine adenine. Adenine is of course synthesized in the chemical industry nowadays by a polymerization process! If we assume that the adenine (and other purines and pyrimidines) is capable of participating in the formation of polymers leading to primitive self-reproducing nucleic acids, a special problem in the organization of living systems immediately becomes apparent. If we start off with an accumulated prebiotic soup (which will have to be shaded from intense ultraviolet light if its components are to survive), under conditions where there can be little or no expectation of further additions of 'raw materials' to the accumulation, we soon realize that there is a considerable pressure for biochemical evolution. The organization (polymerization) of molecules will cause depletion of the starting material, and if it were to run out, evolution of life would cease. It has been suggested that basing living systems on a restricted number of compounds, initially present accidentally, may have brought about a curious backward evolution, as spoken of by Horowitz. He points out that the purine skeleton, supposedly formed by polymerization of HCN in primeval times, is now formed by a complex series of biochemical reactions. It is almost as if we have now reached the position where we need the purine and have evolved a complex mechanism to synthesize it in adequate quantities for our present usage. The basic skeleton of purine nowadays receives its carbon atoms from carbon dioxide, formate and glycine, and its nitrogen atoms from glycine, aspartate and glutamine (Conn & Stumpf 1972). It is tempting to suggest that there are other examples in which an original accidental choice has been perpetuated, and that the evolution of a biochemical method of obtaining the chosen compound has been 'forced'.

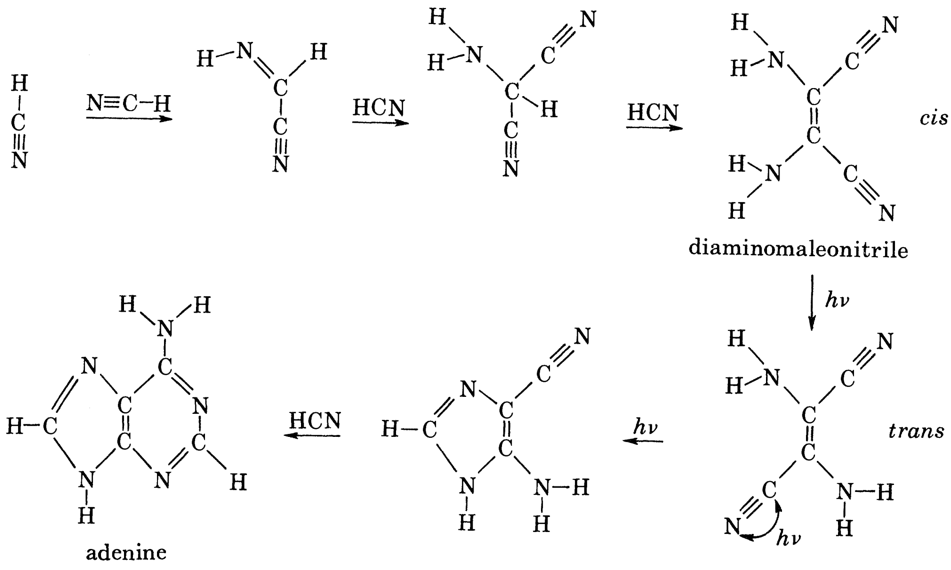

Figure 1

The adenine molecule appears also to have been chosen as a component of the energy carrier ATP, and it forms an integral part of many modern biochemical pathways involving energy transfer, as in respiration and many synthetic sequences. While the earliest 'energy-rich phosphate' compounds may well have been polyphosphates (significantly these are still found in some photosynthetic bacteria), their more effective utilization seems to have depended on the specificity and fine tuning brought about by combination with ribose and adenine or other purines. If ATP is a primitive substance central to energy metabolism, then we have a further reason for the evolution of an efficient adenine-synthesizing system.

BIOCHEMICAL EVOLUTION

If we can imagine a primitive self-reproducing system, it is clear that its continuing success depends on harvesting an available source of energy. The simplest energy-yielding systems are of the type that we now recognize as fermentations, which involve the rearrangement of molecules so that they go to a lower energy state and make available 'free energy' that can be harvested for metabolic purposes. Given the limited amount of primeval soup, the life time of any successful self-reproducing species (molecule or organism) is in the long term limited by lack of a continuously available energy source, and there is an enormous pressure in favour of an organism that can supplement its energy source in some way. A successful jump is by the use of tetrapyrrole molecules to form bacteriochlorophyll and its incorporation into lamellae to allow the capture of light energy for use in a primitive bacterial photosynthesis. This had the immediate advantage that the carbon compounds available in the soup could now be used for structural and synthetic purposes and were no longer primarily necessary as an energy source. The photosynthetic bacteria would have the advantage of surviving on organic compounds that were of no further value to the fermenting organisms. A problem for the photosynthetic bacteria is their necessary dependence on a reducing agent (e.g. H_2S) for CO_2 reduction (leading to sugar formation) since the supply of reducing compounds is in itself limited. The replacement of H_2S by H_2O as the final reducing agent, an invention brought about by a second photochemical reaction, which results in the discarding of oxygen as a waste product of photosynthesis, effectively removes the constraint from which the photosynthetic bacteria suffered. An organism capable of oxygen-evolving photosynthesis is effectively free to continue since it is no longer dependent on the prebiotic soup for its energy supply. The whole of this biochemical sequence of events occurred at the prokaryotic level of organization; the basic evolution of the energetic systems must of necessity have been completed at an early stage. Although this sequence is reasonable it is very difficult to put a real timescale on it. My particular point is that once a system having a prokaryotic type of organization was evolved, there was evolutionary pressure favouring the steps that led eventually to freedom from the primeval soup to take place relatively quickly. But in addition, the successful acquisition of each new feature represents a discontinuity and, in a biochemical sense, evolution clearly proceeds by a series of inspired jumps.

The consequence of the use of water as reductant was the throwing out of oxygen and the formation of an aerobic atmosphere. This made possible the appearance of respiring prokaryotes capable of using organic compounds either from the primeval soup or from the activities of the photosynthetic organisms for complete oxidation with oxygen as a terminal oxidant. It is, I think, significant that a number of the central components of the electron transport chain found today in a number of photosynthetic bacteria, e.g. *Rhodopseudomonas*, may be used either in a photosynthetic sequence or in an oxidative sequence. They operate from various donors on the one hand and bacteriochlorophyll or cytochrome *c* on the other. This is an example of the conservation of the biochemical apparatus that is used in more than one sequence: a very economical feature.

The synthesis of tetrapyrroles appears to be a primitive capability; both linear and cyclic tetrapyrroles appear early. The incorporation of a metal at the centre of the cyclic tetrapyrrole plate has led to the utilization of this central molecule either for light capture, as when magnesium is incorporated, or as a redox catalyst, as when iron is incorporated. The photochemical reaction involved for the conversion of light into chemical energy requires photoactivation of an electron and its secondary acceptance by an organic compound, so that the 'activated' electron becomes a 'reducing' electron. The successful completion of such a photochemical reaction requires that the central metal should be unable to undergo redox changes. A tetrapyrrole capable of the ferrous–ferric transformations would be useless in a light-capturing system.

Living systems based on proteins or nucleic acids

It has been argued that the most primitive 'living' systems could have been based solely on proteins and the following reasons have been put forward in support of this argument.

(*a*) Amino acids might well have been easier to make non-biologically than nitrogen bases and there might have been more of them available in the primeval soup.

(*b*) Sugars such as ribose and deoxyribose, though presumably capable of formation as polymers from formaldehyde, are likely to be less stable compounds and it might have been difficult to accumulate them in useful quantities.

(*c*) Purine synthesis, although possible on the basis of paper chemistry, is not likely to be favoured in the pre-biotic environment.

(*d*) If peptide bond formation (a dehydration) occurs between two amino acids, it is only possible in one way for most amino acids, as is seen in the polypeptide chain characteristic of present-day proteins. Clearly amino acids with R groups having basic or acidic features bring with them the chance of potential branching of such chains, but in general it is thought that the advantage of a single mode of combination in peptide bond formation must outweigh the difficulties with the acidic and basic amino acids, and is far more favourable to the continuity of a protein than the multiple branching arrangements of nucleotides which could have been possible in nucleic acid formation.

Presumably the original amino acids formed prebiotically were synthesized as D- and L-amino acids (racemic mixture). It may be accidental that the L-amino acids were chosen for living systems, since it is difficult to think of a disadvantage for the D-amino acids. The use of polypeptide chains to direct the synthesis of new polypeptide chains (living systems) present more of a difficulty.

Living systems based on nucleic acids

These might depend on the capability of certain purines and pyridines to form pairs by hydrogen bonding. Thus adenine pairs with uracil, and guanidine with cytosine, and these pairs form the basis of RNA molecules. The pairs adenine and methyluracil and guanidine and cytosine form the basis of DNA molecules. The formation of polymers from single nucleotides (nitrogen–base–sugar-phosphate) readily gives polymers of single nucleotides, e.g. polyuridine. It is observed that a mixture of polyuridine and, say, polyadenosine readily combines to give a triple helix of, say, poly(U) + poly(U) + poly(A); similarly, poly(U) + poly(U) + poly(G) can form a triple helix. These polymers have characteristic hydrogen bonding capacities at the surface that could direct their self-duplication by a mechanism of template synthesis.

It is suggested that nucleotide polymers might: (*a*) increase the rate of template replication of other polynucleotides ('a primitive nucleotide polymerase'); (*b*) increase the supply of the activated precursors leading to polynucleotides (primitive synthetic enzymes); (*c*) destroy unrelated polynucleotides, thus controlling the number of variants present (primitive nucleases). Such nucleotide polymers, according to modern information, might well form the basis of a genetic code.

Living systems based on nucleic acids and proteins

These would have considerable potential advantages. The nucleic acids might give the possibility of genetic continuity, and the proteins might offer the possibility of controlling the environment by their catalytic activities.

Protein synthesis in modern systems requires loading of tRNA with activated amino acids. Each tRNA (a polynucleotide) is composed of characteristic bases, and in the primitive state each is specific for an individual amino acid. The synthesis of proteins then involves a template provided by the RNA at the site of protein synthesis (the ribosomes), the RNA sequence itself being derived from a DNA template. Arrangement of individual nucleotides in the RNA template causes the attraction and ordering by hydrogen binding of specific tRNA molecules bearing their characteristic amino acids. When the amino acids are correctly lined up on the template, they are knitted together by an amino acid polymerase, to form a polypeptide chain. The earliest catalytic activities might have been found on clay particles and the basic dehydration reaction leading to proteins may have been catalysed on clay particles (Bernal 1967). There is the question of virus molecules arriving from interstellar Space and triggering the initial formation of living systems in the prebiotic soup. Modern viruses operate by redirecting the metabolism of existing organisms to their own ends, i.e. reproduction of the virus, which is synthesized by its host's diverted biochemistry. Such viruses are uniformly susceptible to ultraviolet irradiation and it is difficult to say how primitive viruses could have effectively entered the Earth after exposure to the fierce ultraviolet environment of Space. Secondly, if they, like modern viruses, depended on a functional biochemical system ready to take over, an impasse would result, as no such system would have existed when they arrived.

THE ORIGIN OF ENZYMATIC SYSTEMS

By heating mixtures of amino acids in the dry state and subsequently adding them to water, Fox (1964) has produced proteinoid microspheres by a process of self-assembly. These microspheres have the curious feature of having a double membrane structure. The droplets so produced appear to be positively charged internally and negatively externally and to have the capacity for acid–base catalysis. Among other new activities that have been detected in the Fox microspheres are the inherent activities of glucose breakdown, esterase activity and peroxidase activity, and these putative enzymatic activities arise *de novo* in these artificial microsphere systems. What we see here is characteristic of many biological systems: the phenomenon of self-assembly. Oparin (1968) has also produced coacervate droplets in which the enzyme RNA polymerase has been trapped. When appropriate substrates, e.g. ATP, are added to the external medium these particles take up the substrates and, using RNA polymerase, produce RNA polymers internally; these eventually cause an accumulation of excess materials inside. When the excess becomes large enough the vesicle divides by fission into two daughter vesicles and when the materials from which they are composed are available in the substrate these daughter vesicles grow and again show the phenomenon of self-assembly.

A modern example of the self-assembly of a catalytic protein from its constituent parts can be seen in the enzyme ferredoxin. This protein consists of a prosthetic group in which two ferric atoms are combined with two sulphur atoms at the level of H_2S embedded in a protein chain containing four cysteine residues bound together in a covalent fashion to the two irons.

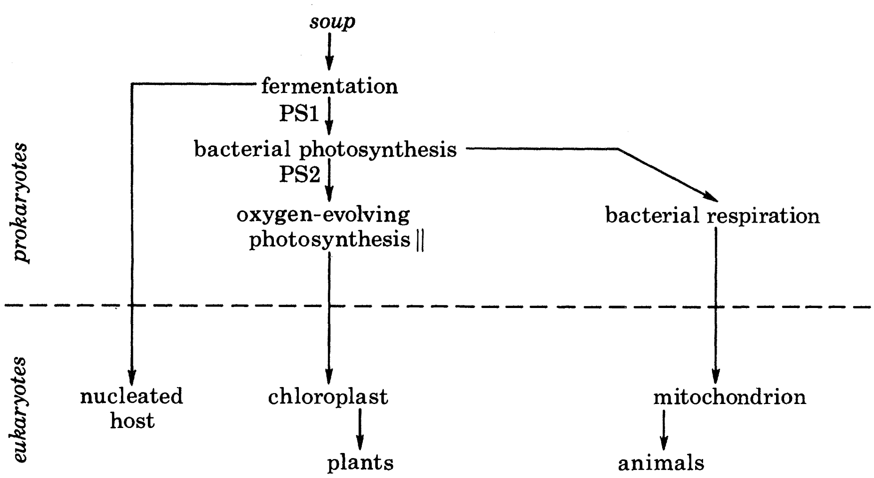

Figure 2

The cysteines are incorporated into a rather small polypeptide chain synthesized by modern organisms before making ferredoxin (the apoprotein). If we imagine a similar protein chain synthesized early on in evolution, we can demonstrate how the synthesis of ferredoxin could occur by a purely chemical synthesis. If the apoprotein is allowed to react with H_2S in the presence of ferrous iron kept reduced with dithiothreitol, the iron and sulphur atoms are incorporated into the molecule through the sulphurs of cysteine, and the newly formed new protein, ferredoxin, is fully active as a catalyst. It now has a redox potential of -420 mV, equivalent to that of hydrogen at physiological pH. On their own the constituent parts have little catalytic activity; in chemical combination they form an enzyme that is a very powerful hydrogen carrier.

The endosymbiotic jump

I have indicated that the potential for the establishment of polymers and their self-reproduction may well have existed at the time of the primeval soup. Biochemical evolution to free the new life forms from the constraint of a limiting amount of soup, in terms both of energetics and of organic carbon compounds, was necessarily a process with a limited time-scale. The major essential metabolic pathways can be seen to have evolved already at the simplest level of cellular organizations, the bacterial, or prokaryotic, level (figure 2). There remain large areas of the world inaccessible to prokaryotic organisms because of their environmental requirements. The greatest single discontinuity in the evolution of living systems is thought (e.g. by Stanier 1970) to be the attainment of the eukaryotic state of organization.

According to the theory of serial endosymbiosis, reviewed recently by Margulis (1981), the characteristic eukaryotic organelles, mitochondria and chloroplasts and perhaps flagella and parts of the nuclear organization, are derived from unicellular symbionts taken up by endocytosis and subsequently modified into fully integrated parts of the new system (instead of merely serving as a food source). Figure 3 is a representation of serial endosymbiosis and shows mitochondria as preceding chloroplasts.

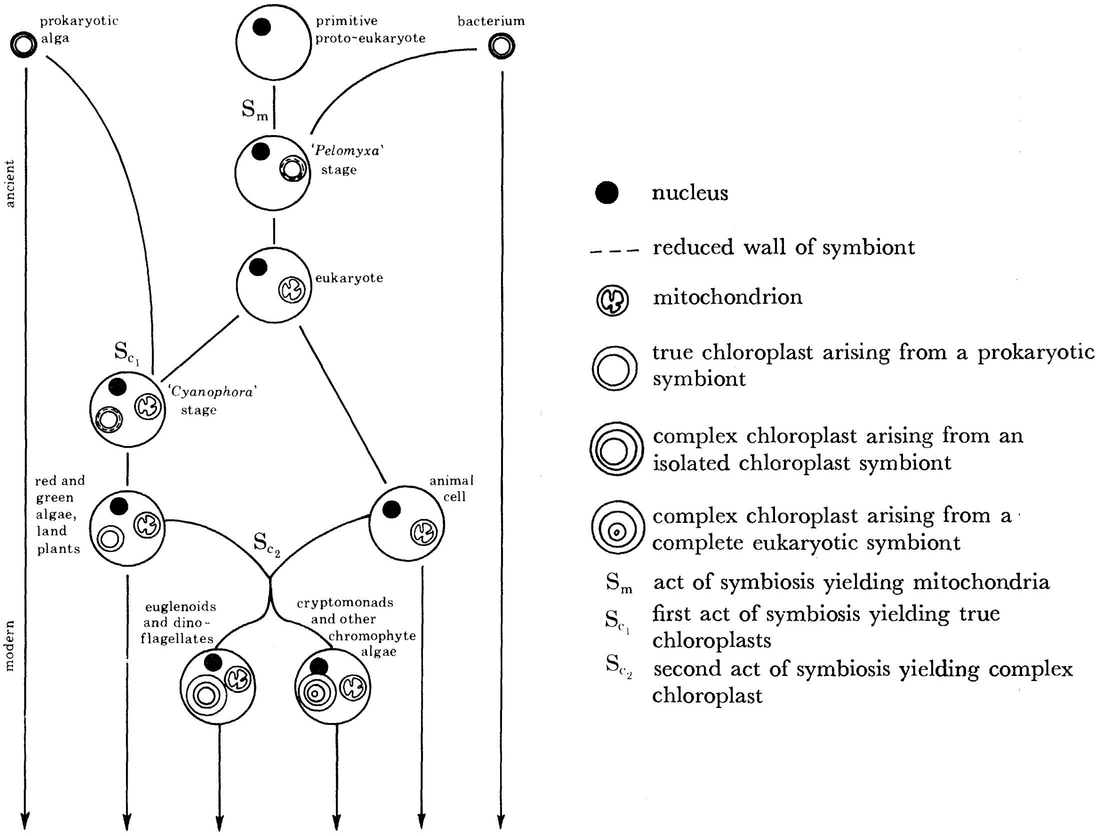

FIGURE 3. Serial endosymbiosis.

The initial proto-eukaryote of figure 3, a fermenting nucleated organism, which has presumably been lost, is perhaps largely mimicked by the present-day archebacterium *Thermoplasma acidophilium* (Searcy *et al.* 1981), which may be protected as a living fossil by its requirement to grow at 60 °C at pH 1–2, thereby escaping competition. This unusual organism has no rigid cell wall, is of large size, has a labile internal cytoskeleton and, although still at the prokaryotic level of organization, resembles modern eukaryotes in having much redundant DNA, which is condensed with histones (a nuclear chromosome feature). The metabolism is essentially fermentative but the membranes have a rudimentary respiratory chain, confined to cytochrome *b* and a quinone, apparently concerned with rejecting protons that soak in from the acid environment.

The multinucleate giant amoeba *Pelomyxa palustris* (figure 3), which is very primitive and lacks mitochondria, contains as symbionts its own population of aerobic bacteria (J. M. Whatley *et al.* 1979); these are never found free-living and appear to be well integrated with their host. *Pelomyxa* with its bacteria represents a modern parallel to an intermediate stage in the evolution of a eukaryotic cell with mitochondria. The *Pelomyxa* bacteria are contained in close-fitting vacuoles provided by the host, the vacuolar membrane; the bacterial plasma membrane, the site of respiration, is thought to correspond to the inner mitochondrial membrane.

A modern aerobic bacterium with many of the characters that would be expected in the ancestral bacterium that was taken up and evolved into the mitochondrion is *Paracoccus denitrificans* (figure 3). This organism contains in its plasma membrane an electron transport chain and a phosphorylating mechanism that very closely resemble these systems in the inner membrane of the modern mitochondrion. The similarities have been discussed in detail elsewhere (J. M. Whatley *et al.* 1979). In addition to possessing these constitutive features, *Paracoccus* can adapt to the use of nitrate or nitrite as alternative electron acceptors when the cells are grown anaerobically in the presence of nitrate, and to the use of an impressive array of individual carbon compounds as electron donors.

The biochemical changes that an organism like *Paracoccus* would need to undergo when it changed from a free-living bacterium into an organelle would involve (1) the retention of the constitutive features of the electron transport chain and of oxidative phosphorylation, (2) the loss of the adaptive capabilities of the aerobic bacterium, (3) alteration of the mechanism of uptake of substrates from a strict proton symport to an exchange mechanism, (4) the loss of the bacterial cell wall, and (5) the acquisition of an adenylate translocase, whereby an exchange of ADP for ATP between the organelle and the cytoplasm of the host would be achieved. Only when it has acquired the adenylate translocase can the symbiotic bacterium be deemed to have attained the full status of a mitochondrion (John & Whatley 1975).

ORIGIN OF CHLOROPLASTS

The theory of serial endosymbiosis as shown in figure 1 indicates that red and green algal chloroplasts may have arisen by the uptake as symbionts of the prokaryotic algae. This is analogous to the origin of mitochondria from prokaryotic aerobic bacteria. However, detailed evidence similar to that described above for the origin of mitochondria is not available. By combining the available information one can put forward a sequence of events (figure 3) describing the possible evolution of red and green algal chloroplasts from two different types of prokaryotic symbionts, the blue-green algae and the prochlorons respectively (J. M. Whatley *et al.* 1979).

When the blue-green symbiont entered the eukaryotic host it entered a new and very different environment. In order to integrate with the metabolism of the host the prokaryote presumably became leaky with respect to specific carbohydrates. There are already clear indications that many modern prokaryotes and eukaryotes acquire a greatly enhanced and altered ability to release carbon substrates into their host cytoplasm when they exchange a free-living for a symbiotic mode of life (Smith 1979).

Like the uptake of a blue-green prokaryote to yield red algal chloroplasts, the uptake of a *Prochloron*-like prokaryotic symbiont and its alteration into a 'green' chloroplast must have necessitated the integration of the metabolism of the organelle with that of the host. Just as there is a traffic in ATP and carbon compounds developed between host and mitochondria there is traffic in only a small number of carbon compounds between chloroplast and host.

The chloroplast membrane is largely impermeable to most of the intermediates of the reductive pentose cycle, and as a consequence, the working concentrations of these in the stroma is easily maintained. Export of carbon compounds resulting from CO_2 fixation is mainly restricted to the three C_3 compounds glyceraldehyde-3-phosphate, dihydroxyacetone phosphate and phosphoglyceric acid. Their loss from the chloroplast stroma into the host cytoplasm is controlled by having them pass only through specific sites in the chloroplast membrane where they must exchange with inorganic phosphate or a phosphorylated C_3 intermediate (Heber 1974). The export of C_3 compounds can yield carbon skeletons for use directly by the host, and inorganic phosphate is released to act in the exchange reaction, which allows more C_3 to be exported under control, or the C_3 compounds may yield ATP by further metabolism in the mitochondria.

Photosynthetically active chloroplasts make ATP on their thylakoids by light reactions. Since ATP is needed for the operation of the reductive pentose cycle for CO_2 fixation, the chloroplast cannot afford to lose ATP into the host cytoplasm. As one might expect, fully differentiated chloroplasts are not permeable to ATP or ADP and have no translocase for the adenine nucleotides. But during chloroplast differentiation, before the production of photochemically competent thylakoids, the plastids can only obtain their supply of ATP from the host's cytoplasm, where the ATP is constantly replenished by the mitochondria. When the thylakoid apparatus is first being synthesized in proplastids there is a large demand for ATP but there is no internal photosynthetic source of ATP. It is therefore not surprising to find that differentiating plastids are permeable to ATP, but that this permeability becomes lost after greening.

The conventional theory of endosymbiosis envisages the evolution of mitochondria and chloroplasts from free-living prokaryotes. This theory adequately accounts for the origin of mitochondria and of the chloroplasts of red algae and of green algae and land plants; in all of these the photosynthetic organelles are surrounded by two membranes. However, the theory is inadequate to explain the origin of those chloroplasts in other groups of algae which are surrounded by three membranes (euglenoids with chlorophylls *a* and *b*, and dinoflagellates with chlorophylls *a* and *c*) or by four membranes (the remaining algae, the Chromophyta of Christensen (1964), all of which have chlorophylls *a* and *c*). The presence in these chloroplasts of more than two surrounding membranes implies an origin involving a second act of endosymbiosis of eukaryotic rather than prokaryotic photosynthetic organisms. The suggested mode of formation of these more complex chloroplasts is by the uptake of 'naked' chloroplasts from a eukaryotic donor (three membranes) or by the uptake and simplification of a complete eukaryotic alga (four membranes). The rationale for this proposal of a polyphyletic origin of chloroplasts has been discussed in some detail elsewhere (J. M. Whatley 1981). Suffice it to say that it is consistent with patterns of change in the photosynthetic pigments, in the thylakoid system, in the location of the glucose polymer synthesizing system and in the progression from predominantly α–1,4 to β–1,3 glucan storage products. The distribution of these features within the diverse chloroplasts of the several groups of algae and land plants is more easily rationalized through the idea of the uptake of a complete package of characters with each new act of endosymbiosis than by way of the repeated and independent evolution of individual characters.

The new endosymbiotically produced eukaryotic combination is invested with additional capabilities not formerly present in either participant alone and can now exploit new niches.

It is tempting to suggest that the sudden emergence in the early Palaeozoic of many forms of marine algae is due to the attainment of eukaryotic states at that time. The evolution of a basic multicellular organization and its subsequent modification allowed permanent colonization of the land, first by plants and then by animals.

Some idea of the timescale of evolution is indicated in table 2. The Proterozoic rocks contain fossils characterized as prokaryotic blue-green algae and bacteria, together with fossil traces of simple eukaryotic organisms, which are in general poorly preserved. It is only much later that 'complete' eukaryotic organisms can be recognized. The early forms were 'quickly' followed by a succession of plants and animals. The formation of each new group involves a characteristic jump in organization and we may imagine a discontinuous series of new forms, each of which underwent adaptive radiation in its turn.

Table 2. A timescale for evolution

time ago/Ma				
2	Quaternary			
	Cainozoic			man
			grasses	
60	Tertiary			
				mammals
125	*Mesozoic*	late	angiosperms	
				birds
185		early	gymnosperms	
				reptiles
310	*Palaeozoic*	late	lycopods; seed ferns	
				amphibians
380		middle	land plants (*Rhynia*)	
553		early	marine algae	
				fishes
				invertebrates
1500	*Proterozoic*		bacteria; blue-green algae	

It should be emphasized that any conclusions about evolution must always be tempered by the thought that the species preserved in the fossil record represent only a small proportion of those that have existed. Nevertheless, a general view indicates that evolution, as seen in hindsight, represents an orderly sequence of changes, but that the continuous series of small events leading to overall change is commonly interrupted by a small number of large discontinuities that have led to the variety of organisms and diverse metabolic patterns that we see today. The biggest single discontinuity is the borderline between the prokaryotic and eukaryotic levels of organization.

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Discussion

W. H. McCrea, F.R.S. (*Astronomy Centre, University of Sussex, Brighton, U.K.*). Professor Whatley has described clever things – and some apparently not so clever – that may occur on Earth, given the existence of replicating organisms. The replication presumably requires the presence of complete specific molecules of a highly complex character. Yesterday we heard how molecules actually identified in interstellar Space make it plausible to suppose that amino acids also exist there, but it seems that there is no positive evidence for any molecules more complicated than these. Where and how might the enormously more complicated molecules required for Professor Whatley's processes come to be constructed?

F. R. Whatley. I do not believe that Professor McCrea's question can be answered! Bernal (1967) has speculated that some clay lattices may catalyse the polymerization of amino acids to polypeptides and bases to 'nucleic acids', and suggests that self-replicating molecules could have arisen in this way. Fox (1964) suggests that heating amino acid mixtures may have led to polypeptide formation by dehydration reaction. The self-replicating molecules may direct their own resynthesis by using hydrogen bonding to arrange the building bricks. Complicated assemblages may self-assemble if the resultant polymer is at a lower energy level.

It is possible that more complicated molecules exist in Space but cannot be detected by present techniques: they are likely to have much more diffuse spectra than the simple radicals so far detected.

N. W. Pirie, F.R.S. (*Rothamsted Experimental Station, Harpenden, U.K.*). Professor Whatley, like many other writers on the origins and development of life, used the concept of fermentation. In spite of my respect for Haldane's many contributions to discussion on the subject, I have never liked his suggestion that there was a stage between macromolecular synthesis and photosynthesis in which life was maintained by fermentation. Fermentation is the restoration of greater stability to a system made chemically metastable by the activities of an organism. The canonical example is glucose, made by photosynthesis, and then degraded exergonically to more stable molecules. I cannot envisage the accumulation of anything comparable to glucose at or near the surface of probiotic Earth. Such substances would be too unstable to wait around at any significant concentration; they would soon undergo, by inorganic catalysis and illumination, those 'fermentative' reactions of which they were capable. It seems to me much more likely that in the beginning, as now, life depended on photosynthesis – or at any rate photoactivation. The model I have in mind is an enzyme system that brings about rapid reactions between light-activated molecules that would, in the absence of the enzyme(s), have regained equilibrium more slowly. Essentially what I suggest is that fermentation depends on a large accumulation of capital, whereas the essential reactions in my model depend on transient income.

F. R. WHATLEY. Mr Pirie has neatly stated the problem of the early occurrence of fermentation as we know it. Today all organisms depend on photosynthesis, directly or indirectly, and glucose produced in excess by green plants is a principal substrate for fermentation. But it is by no means the only one. Proteolytic bacteria like *Clostridium* use amino acids in fermentation reactions (Strickland reaction), with glycine as a common oxidant and a range of other amino acids as reductants. I support the proposal of Clarke & Elsden (1980) that the earliest catabolic pathways coupled with substrate phosphorylation could have been of the Strickland reaction type; this would be in accord with the conclusion of Miller & Orgel (1974) that the primeval soup was rich in amino acids.

Mr Pirie's model substitutes photoreactions for enzymes in early fermentations: he could have a point. However, it is clear that there would have been enormous pressure early in evolution to supplement the energy income of the early forms with photochemical reactions that *store* energy. Incidentally the archebacterium *Halobacterium* appears to conserve energy by direct light-driven proton pumping without a respiratory chain (? a primitive trait), but it has a sophisticated ATPase-like aerobic bacteria.

References

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Miller, S. L. & Orgel, L. E. 1974 *The origins of life on Earth*. New York: Prentice-Hall.