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THE ROLE OF NATURAL SELECTION IN THE ENDOSYMBIOTIC ORIGIN OF ORGANELLES

Alexis Harington

A dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science.

Johannesburg, 1985.

DECLARATION

I declare that this dissertation is my own unaided work and that the technical assistance which I have received is detailed in the Acknowledgements.

No part of this dissertation has been submitted in the past, or is being submitted, or is to be submitted for a degree at any other university.



A Harington

ABSTRACT

The subject of this thesis forms an analysis of hypothesised evolutionary events associated with the origin of the DNA-containing organelles such as mitochondria and plastids.

In the first part of the thesis, evidence for the Serial Endosymbiosis Theory was reviewed. In particular, the use of cladistic methodology was discussed as a possible aid towards proving the endosymbiotic origin of mitochondria and plastids.

Following upon this, and assuming an endosymbiotic origin of the organelles in question, the process of gene transfer was then dealt with. The reasons for, and effects of genic integration, were next evaluated and it was concluded that selective advantages are associated with the process. Possible reasons for the continued existence of mitochondrial and plastid DNAs were given.

Thereafter, on the assumption of successful gene transfer, the importation of transferred gene encoded proteins into proto-organelles was analysed. Spontaneous importation was suggested to have rendered the origin of import-receptors possible without affecting the course of the endosymbiosis.

The effects of continued cytoplasmically-located genomes were discussed in terms of group selection and 'selfish DNA' theory. A simulation was devised to illustrate the elimination of 'selfish' cytobionts in an evolving host-endosymbiont system.

Finally, the interrelationships between natural selection and passive effects were placed into context with respect to hypothesised events discussed in earlier parts of the thesis. Certain weaknesses of the Autogenous Theories were discussed and it was concluded that chance effects and pre-adaptations in the context of endosymbiosis had a far-reaching influence on the evolution of the organelles.

ACKNOWLEDGEMENTS

I wish to thank Professor A L Thornley, Department of Zoology, University of the Witwatersrand, for suggesting the subject of this thesis and for considerable encouragement during the course of the work. Professor Thornley's invaluable assistance in the preparation of publications emanating from the work is highly appreciated, as is his editorial assistance given and criticism made during the course of the study. These contributions were invaluable towards the completion of the work.

Mr M R Centner, Department of Zoology, is thanked for many interesting discussions on group selection, for designing the framework of the computer model and for his assistance during programming.

I would like to acknowledge a stimulating discussion on cladistics with Dr E S Vrba, Transvaal Museum, Pretoria.

As regards the preparation of the manuscript, typing and proofreading, I am greatly indebted to Mrs P Thornley for her careful and critical assistance. Also, Mrs D M Fitzgerald is thanked for her efforts in typing the first draft. Dr J S Harington is thanked for editorial assistance and encouragement during the progress of the work.

The award of the Bradlow Scholarship and CSIR bursaries is gratefully acknowledged.

PUBLICATIONS

The following papers, based on the study undertaken in this dissertation, have already been published or presented at scientific meetings:

Harington A, *Thornley A (1980) Endosymbiosis, gene transfer and the* mitochondrial genetic code. S Afr J Sci 76: 162-165

Harington A, Thornley AL (1981) Interpretation of enzyme sequence homologies in the light of the theory of Serial Endosymbiosis. South African Biochemical Society sixth congress, Abstract, p P1-B2

Thornley AL, Harington A (1981) Diploidy and sex as selective advantages for retaining genes transferred from mitochondrial and plastid ancestors in the nuclear genome. J theor Biol 91: 515-523

Harington A, Centner M (1982) Group selection and the endosymbiotic origin of cellular organelles (Abstract). S Afr Genet Soc, Eighth Congress, Johannesburg, South Africa

Harington A, Thornley AL (1982) Biochemical and genetic consequences of gene transfer from endosymbiont to host genome. J Mol Evol 18: 287-292

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CHAPTER ONE

INTRODUCTION

The eukaryote is usually characterised by the presence of two or three distinct genomes within the cell. These genomes are surrounded by two membranes, the nuclear, mitochondrial or plastid envelopes. The nuclear genome is in communication with the rest of the cell (including its organelles) but the genomes of the latter generally confine their influence to their own respective compartments although this is not always the case. Current knowledge suggests that the compartments form a single interdependent unit.

The evolution of a multigenomic system from a unigenomic one would conceivably take one of two pathways, each of which are dealt with below.

One explanation, the Serial Endosymbiosis Theory (Margulis 1970), or the Xenogenous Theory (Taylor 1974), deals with the concept of symbiosis between an ancestral host cell and one or more intracellular prokaryotes. The three present genomic lineages correspond to three distinct cellular lineages (Gray and Doolittle 1982). By contrast, the Autogenous alternatives (see Taylor 1976) depend on the idea of a single ancestral cellular line differentiating into three specialised genomes which are subsequently partitioned.

Variants of these theories (for example, that of Cavalier-Smith 1981) incorporate elements of both of the above explanations and are termed Partial Xenogenous Theories (Taylor 1976). Up to the present time, a major difficulty in deciding the validity of the two main explanations has been the fact that both could conceivably have reached a similar end point, that is, the partially-independent evolution of lineages within the context of cell fitness.

The Serial Endosymbiosis Theory (Margulis 1981) is in apparent opposition to several established patterns in evolution. Once lineages have split, their identity is generally maintained and they possess their own evolutionary tendencies and fate. This idea of 'identity', together

with the major predominance of divergent (or cladogenic) evolution, led to initial avoidance of the Serial Endosymbiosis Theory which implies that certain lineages have suffered partial loss of identity due to reticulate evolution (as opposed to divergent evolution). Previously lineages hitherto completely independent appear to have undergone a fusion process resulting in a new co-evolving lineage.

For a long time, the Autogenous Theories were acceptable because they did not 'violate' the ideas of identity and divergent evolution. The splitting of an ancestral genomic lineage into specialised functional genomes was seen as a feasible result of natural selection. Thus in this case, one is dealing with the generation of new evolutionary identities and fates rather than the compromising of these characteristics.

1.1 Impediments affecting the divergent evolution of intracellular genomes

Autogenous Theories rest on the assumption that a central genome is the origin of all genetic information which is found in the DNA-containing organelles. Using this idea as a basis, two different autogenous origins for the proto-organelle genomes have been proposed. One type of theory, such as that proposed by Raff and Mahler (1975) and by Cavalier-Smith (also in 1975), appeals to the idea of a preassembled plasmid, bearing genes for tRNAs, rRNAs, aminoacyl tRNA synthetases and respiratory or photosynthetic enzymes. This idea, however, has not proved attractive to supporters of both the Serial Endosymbiosis Theory and Autogenous Theories especially, for example, to Reijnders (1975) and Keyhani (1981). To avoid preassembly problems, these authors proposed a second type of origin for the organelle genome, namely, duplicated identical genomes.

The initial sharing of a translatory apparatus acts as a cohesive force between diverging genomes. If a proto-organelle genome (such as a plasmid) does not contain genes for all the necessary proteins, importation into the organelle will have to take place. The importation of amino-acyl tRNA synthetases, for example, would place definite restrictions on the divergence of co-adapted proto-organelle tRNA genes. When two genomes do occur within a single cell, such as phage T_4 genomes

and the bacterial host genome (see Cedergren et al. 1981), competitive effects can result in the origin of a significant 'driving force' though it is difficult to conceive how such competitively based trends can successfully provide a driving force for the production of metabolically-specialised plasmids, since they are normally associated with parasitism. Alternatively, if duplicated (unlinked) whole genomes are postulated, directed change towards three different end points is not easily envisaged. There is no reason to believe that (unlinked) genes for divergent functions acting together in a co-ordinated way on one genome would be conserved, while other genes on the same genome would be allowed to decay.

Faced with the difficulties alluded to above, Autogenous Theories rely heavily on the creative role of natural selection (see Gould and Lewontin 1979) to account for the presence of secondary genomes within the cell. This approach rests on the assumption that the current utility of a component, such as an organelle, or a biochemical pathway within the organelle, is directly associated with the reasons for its origin. These ad hoc explanations - in which adaptation is a main component - rely for acceptance on plausibility and the 'problem-solving capacity' of natural selection.

The Serial Endosymbiosis Theory avoids a great many of the problems described above and the ingestion of 'pre-evolved' metabolic units explains the origins of divergence and identity and does not depend totally on direct adaptation. In fact, a good many features of the present composite eukaryote cell can be viewed as incidental effects rather than as functions. The Serial Endosymbiosis Theory easily accounts for the divergent aspect of present-day organelle genomes, but more problematic is the phenomenon of nuclear-located 'prokaryotic' genes.

The end points of both the Autogenous and Xenogenous Theories are partially independent organelle genomes. Because of two major difficulties, it has proved extremely difficult to prove rigorously the origin of organelle DNA. For one thing, most genes specifying organelle proteins are located in the nucleus. Thus, if significant sequence similarities between such genes (or their products) and their prokaryotic

counterparts are found, a further untested assumption of gene transfer has to be invoked in order to link these genes to the ancestry of the organelle. This gene-transfer assumption has been the basis of a major criticism of the Serial Endosymbiosis Theory. Because Autogenous Theories generally avoid the complication of gene transfer, they tend to regard organelle genes in the nucleus as being aboriginal.

A further more serious objection to organelle macromolecule sequence comparison centres around the way in which the ancestry of the eukaryote is viewed. If the traditional view that eukaryotes evolved from prokaryotic organisms is accepted (see Doolittle 1980), it follows that conserved 'prokaryotic' genes could be present in the eukaryote. Such genes could be nuclear- or organelle-located. Thus, genes thought to be of more recent prokaryote origin (via symbiosis) could alternatively date back to the ancestry of the host cell. As noted by Birky (1982), such organelle-bacterial sequence similarities cannot corroborate the Serial Endosymbiosis Theory because they (only) show phenetic relationships.

The second chapter of this thesis thus sets out to examine the question of how the Xenogenous Theory could be proved by the use of cladistic techniques. In the past the main body of evidence pointing towards an endosymbiotic origin for the organelles has been of a phenetic nature, that is, based on overall similarity. Lately the validity of this procedure as an indicator of relationship has been criticised and cladistic methodology as developed by Hennig (1965, 1966) has been called for. It is also argued that the discovery of the archaebacteria may provide crucial data which could be employed to prove the Serial Endosymbiosis Theory more rigorously.

1.2 Problems regarding information flow between nuclear and organelle genomes

Given that two or three different genomes exist within a cell, certain possibilities for information flow between the units would exist. Autogenous Theories do not usually appeal to the concept of information movement as an explanatory principle, although exceptions such as

Bogorad's Cluster Clone Theory do exist (Bogorad 1975; Bogorad et al. 1975). By contrast, movement of DNA from prokaryotic cytobionts to the host nucleus is an integral feature of the Serial Endosymbiosis Theory. Initially, the primary source of information (DNA) was endosymbiont coded, but found itself in the nucleus following gene transfer. Because the integrity of the organelles is maintained, the final form of the information (as protein) would return to a particular site wherever the genes may be.

The third chapter in the present thesis examines the question of gene transfer, assuming an endosymbiotic origin for the organelles. In at least five to six cases in eukaryote evolution, corresponding to three types of chloroplasts, cyanelles and the mitochondrion (which may have a biphyletic origin (Gray et al. 1984)), gene transfer seems to have taken place. There seem to be distinct advantages associated with gene transfer in terms of host and cytobiont fitness. It is suggested that the eukaryotic sexual system provides more scope for the evolution of cytobiont (= endosymbiont) genes than would be the case if they remained in the endosymbionts themselves. Although the advantage of sexual reproduction is a controversial issue (see Maynard Smith 1978), most workers agree that benefits of some sort or another are associated with the process. It is therefore not surprising that many symbiotic organisms may have become extinct via the process of gene transfer (see McLaughlin and Cain 1983), that is, they undergo a process of genetic absorption. Interestingly, in the cases of the chloroplasts, cyanelles and the mitochondrion, assimilation of cytobiont DNA has not been complete and presumed substantial gene transfer has left a very small coding responsibility on the organelle genome, particularly in the case of the mitochondrion. This reduced coding capacity seems to have resulted in a host of secondary changes, such as drift in the mitochondrial genetic code, variations in tRNA decoding patterns and elevated mutation rates.

The section on gene transfer in the present thesis also considers possible reasons why gene transfer ceased. It would appear that the essential compartmental and double-membraned nature of the organelles has required a specialised biogenesis which demands on-site synthesis of certain proteins.

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If a gene transfer is to be successful, the final representative of the information, the protein, must return to the precise location in the organelle. This would remove stabilising selection from endosymbiont genes and allow degeneration of the genes in question so as to lead to fixation of the nuclear-located form. Assuming that a gene transfer takes place, the protein must penetrate one or more cytobiont membranes and return to its functional site.

The main point here is that a delicate balance between extremes of permeability of the endosymbiont envelope had to exist. If the permeability is too high, contamination of the organelles with host and organelle macromolecules would result in ambiguous translation and impaired metabolism (Ninio 1975). If permeability is too low, evolution of the organelle by host take-over is prevented. In other words, gene transfer is contingent upon the importation and accurate localisation of transferred gene-encoded proteins.

The fourth chapter in the thesis deals with possible mechanisms that allowed transferred nuclear-located genes to replace their cytoplasmic counterparts. A spontaneous importation of transferred gene-encoded proteins, followed by the evolution of specific organelle receptors, has been suggested. The chapter also considers the secondary incorporation of host proteins into the cytobionts. Once the cytobiont DNA has served its role as a 'back-up' function allowing receptors to evolve, it degenerates, thus completing and finalising the gene transfer process.

1.3 Selection of plasmons

Since the presumed gene transfer has not been complete (at least in the cases of the plastids, cyanelles and mitochondria), secondary genomes are present in the cytoplasm. An important consequence of this situation is that evolution, which can be viewed as a change in gene frequency, occurs by somewhat different mechanisms in the nucleus and the cytoplasmic population of genomes. The total gene set of an organelle is partitioned into two different cellular locations. In spite of the fact that these two positions are subject to slightly different selective mechanisms, all genes encoding the organelles have to evolve in concert.

Three distinct levels of selection can in fact be distinguished to act on a host and cytobionts: individual selection of host cells (corresponding to intercellular selection); group selection of populations of cytobionts (a consequence of intercellular selection), and individual selection of cytobionts (intracellular selection). A consequence of this is that an increase of cytobiont fitness need not be in concert with an increase in host fitness. It is conceivable that 'selfish' replicators among the endosymbionts could endanger the stability of the host-cytobiont relationship.

In the fifth chapter of the thesis, the question of how group selection can counteract the spread of 'parasitically-replicating' cytobionts is addressed. The conclusion reached is that the short-term advantage for the endosymbionts is outweighed by long-term symbiotic stability. In this way the fitness of the symbionts is maximised by favouring the spread of the host.

1.4 Incidental effects of natural selection

Throughout this theris the relationship between incidental effects and natural selection is emphasised. In spite of the fact that such pre- and non-adaptations seem to have had far-reaching effects on organelle evolution, their role has not been fully appreciated. It is argued that these factors have played important roles since the initial establishment of the endosymbiotic relationship and have continued up to the present time. Furthermore, the most important period in organelle evolution was the modification of bona fide prokaryotes to their present specialised state. Examples of how incidental effects contributed to the emergence of the DNA-containing organelles are given and their consequences discussed.

CHAPTER TWO

CLADISTICS AND THE ENDOSYMBIOTIC THEORY

In spite of the crucial role systematics can, and ought to, play in the elucidation of the origin of mitochondria and plastids, it has largely been ignored. In more recent times, some authors such as Uzzel and Spolsky (1974, 1981), Phillips and Carr (1977, 1981), Cavalier-Smith (1980), Doolittle and Bonen (1981), and Gray and Doolittle (1982) have started to integrate systematics into the problem.

2.1 The question of similarity

A great deal of circumstantial evidence for the Xenogenous Theory rests on the similarities between bacteria and cyanobacteria on the one side, and mitochondria and plastids on the other. Such similarities include overall morphology (or ultrastructure)(Margulis 1970); metabolism (Whatley 1981a, 1981b; Shimakata and Stumpf 1982a, 1982b); pigments (see Margulis 1976); similarities in DNA and translatory apparatus structure (see Gillham 1978, and review by Gray and Doolittle 1982) and similarities in amino acid and nucleic acid sequences (Phillips and Carr 1977; Schwartz and Dayhoff 1978, 1981, for example).

As Cavalier-Smith (1980) and Gray and Doolittle (1982) have pointed out, such correspondence cannot be regarded as proof for the Serial Endosymbiosis Theory. It has been clearly stated by Hennig (1965, 1966) and accepted by many, that overall similarity is an unsatisfactory criterion with which to gauge relationship. This is also accepted by 'evolutionary' systematicists such as Mayr (1974). Hennig (1965) maintained that resemblance is a composite concept and consist of three fac ts, namely, plesiomorphy (primitive features), apomorphy (derived features) and convergent characters. Plesiomorphies remain unchanged in spite of cladogenesis and accordingly cannot be used as evidence of the close relationship of their possessors. Only the demonstration of synapomorphies (shared derived features) between two taxa can indicate

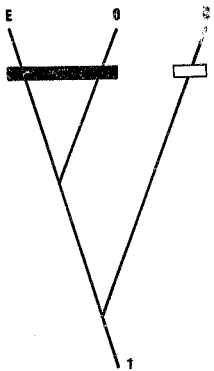
that they are more closely related to one another than to any other. Such taxa form monophyletic groups (as defined by Hennig 1965) which together form similar groups of higher rank and can thus be called sister groups. An important point made by Hennig is that in the phylogenetic system there can be no solely derivative nor solely primitive groups. At least one apomorphy must characterise a monophyletic group. In addition, a character state considered derived at one level becomes primitive when finer relationships within the group are sought (see Eldredge 1979).

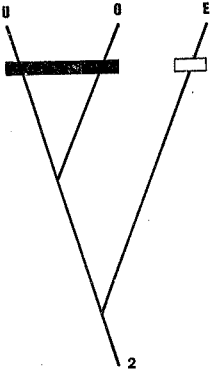
2.2 Criteria for proof of the Serial Endosymbiosis Theory

In order to prove the Xenogenous Theory directly, one has to show that mitochondria and plastids demonstrate synapomorphies with eubacteria (see fig. 1, p.10).

Alternatively, the Autogenous Theory could be corroborated by demonstrating shared derived features common to organelles and the nucleus, that are absent in the bacterial line (fig. 2, p.10). This method of proving either the autogenous or endosymbiotic origin is the main one, and many other approaches are, on closer examination, reducible to it.

Another way of demonstrating the xenogenous origin of mitochondria and chloroplasts is by means of analysis of the host (the potential eukaryote ancestor). This approach has been elucidated by Doolittle and Bonen (1981) and by Gray and Doolittle (1982). Taking into consideration the strong similarity between plastid cytochromes and cyanobacterial cytochromes one is led, from an autogenous standpoint, to accept that eukaryotes arose from cyanobacteria. By analogy, a similar position exists in the case of plastids of rhodophytes and chlorophytes (Doolittle and Bonen 1981; Gray and Doolittle 1982). A similar statement can be made with respect to the mitochondrion and purple non-sulphur bacteria and their aerobic derivatives. As Doolittle and Bonen (1981) have pointed out, these are mutually exclusive hypotheses. Thus, the endosymbiotic origin of at least one of the organelles would be 'nearly assured'. These arguments allow one to indicate an endosymbiotic origin





Figures 1 and 2

Three-taxon cladograms showing hypothesised relationships of eubacteria (= prokaryotes in this context)(E), organelles (mitochondria and plastids)(O), and urkaryotes (= eukaryotes in this context)(U).
In Figure 1, the Serial Endosymbiosis Theory is proved by showing synapomorphies in common between the bacterial and organelle lines. In Figure 2, the Autogenous Theory is proved (and the Serial Endosymbiosis Theory disproved) by demonstrating synapomorphies between the organelle and eukaryotic (nuclear) lines.

for at least one organelle, but not necessarily both. Which organelle one considers as the endosymbiont depends on various criteria, the most important of which are sequence similarities of proteins and nucleic acids. The present chapter concerns itself with the first method of proving the Serial Endosymbiosis Theory.

Uzzel and Spolsky (1974, 1981) have suggested that the features of organelles are primitive. In particular, they stated that 'if the features shared by plastids, mitochondria, and prokaryotes are all primitive states, then these may merely have been carried over from the primitive ancestor of eukaryotic organisms' (Uzzel and Spolsky 1974). Similarly, they wrote 'Regardless of how the eukaryotic cell got its organelles, their features are primitive'. Notwithstanding the fact that Uzzel and Spolsky's papers are important, the statements quoted above are unsatisfactory. As discussed by Hennig (1965, 1966), each group must be characterised by at least one apomorphy. The possession of plesiomorphies does not justify that those characterised by the *feature form a monophyletic group (Platnick 1979). The statement that* organelles are primitive is incompatible with cladistical theory. A good example of the perils associated with such reasoning is the case of the mycoplasmas. These were long considered to be primitive pre-bacterial prokaryotes, but Woese et al. (1980) showed that they are highly derived but degenerate derivatives of the clostridial bacteria (excluding Thermoplasma).

2.3 Origin of living cells

It is a common assumption that all cells today are derived from a common ancestral type (Ohno and Epplen 1983; Jukes 1983a; Kacser and Beeby 1984). The many important features in common between prokaryotes and eukaryotes, such as the genetic code, rRNAs, mRNA, as well as certain metabolic properties (Cavalier-Smith 1981), including energy transduction by ATP and NAD, suggest a common ancestry. From this point on, opinions start to differ. The traditional view is that eukaryotes evolved from prokaryotes (Carlile 1980; Van Valen and Maiorana 1980; Doolittle 1980). Cavalier-Smith (1981) also supports this idea on the basis that the

feasibility of a common ancestor either having a mixture of pro- and eukaryotic characters, or little resemblance to either, is very low. This is because 'the first evolutionary divergence after the formation of the first cell produced two separate lines, one with entirely eukaryotic and the other with entirely prokaryotic properties', a concept which Cavalier-Smith (1981) considered highly improbable.

The above view need not be the case, as it is clear that shortly after the first splitting event the two sister taxa were very similar. Subsequently, further splitting and possibly anagenesis (change within a lineage) transformed the lines into present-day pro- and eukaryotes. Apart from this point there are other reasons to believe that the traditional view is unsatisfactory. The differences between pro- and eukaryotes with regard to the translatory apparatus suggest that they had an ancestor in common which was much simpler. This does not need recourse to the explanation that a large amount of (rapid) modification of the ancestral prokaryote was necessary to yield eukaryotes. These considerations led Woese and Fox (1977) to postulate the existence of 'progenotes' in past primordial times. These simple organisms were characterised by the feature that the relationship between genotype and phenotype had not yet been finalised (Woese 1981). According to Woese (1983), the progenote was extremely primitive, containing RNA genes and characterised by inaccuracy of information transfer. The substantial differences between eubacteria, archaebacteria and eukaryotes demand that the progenote, as ancestor, was fairly 'undefined', with a 'plastic' translatory apparatus.

Analysis of the features in common between prokaryotes (= eu- and archaebacteria) and eukaryotes also shed light on the nature of the progenote and suggests that the progenote may not have been as primitive as generally thought. If a bipartite tree is assumed, comparison between eubacteria and eukaryotes suggests features of the progenote. Several proteins have been reported to be homologous between pro- and eukaryotes. Examples of enzymes are bacterial and vertebrate trypsin, dihydrofolate dehydrogenase and phosphorylase (Doolittle 1981) and possibly the RNA polymerases (see Huet et al., quoted in Mankin et al. 1984). The enzymes lipoamide dehydrogenase and glutathione reductase are very similar in the region of the oxidation-reduction cystine in E.coli and in eukaryotes

(Williams et al. 1982). Yeast glutathione reductase, for example, shares 13/14 residues with E.coli in this region. Homology between pro- and eukaryotic ribonucleases has also been reported (Hartley 1980; Hill et al. 1983). The last-named authors compared fungal and bacterial ribonucleases with positive results, but could find no significant similarity between the sequence of these enzymes and that of pancreatic ribonuclease. Pain (1982) gave detailed consideration to the relationship between a microbial ribonuclease and the pancreatic enzyme. The degree of similarity was poor and chain folding and active sites were completely different (Nakamura et al. 1982). Pain suggests that if the enzymes arose by divergent evolution, the common ancestral protein must have had a conformation that was relatively simple and unstable. The alternative (that of convergent evolution) cannot be excluded at the present time. A case in point is that of subtilisin and the serine proteases which seem to be unrelated (Wilson et al. 1973). However, James et al. (1978) compared the three-dimensional structures of bacterial serine proteases with those of pancreatic α chymotrypsin and elastase and concluded that the case for homology, that is, derivation from a common ancestor, is strong. Similarly, Weaver et al. (1985) conclude that goose-type, chicken-type and phage-type lysozymes arose from a common ancestor, despite the fact that their amino acid sequences appear to be unrelated. At least some relationship seems to exist between eukaryote and prokaryote heat shock proteins (Daniels et al. 1984). Pähler et al. (1984) have studied the three-dimensional structure of fungal proteinase K and bacterial subtilisin, and the two enzymes originate from a common ancestor. Very few proteins common to archae- and eubacteria have been sequenced. One, 3Fe:3S ferredoxin, demonstrates about 50% similarity (Hausinger et al. 1982). These authors believe that the original gene duplicated prior to the eubacteria-archaebacteria split, tending to indicate a common line of descent.

The existence of non-homologous but isofunctional proteins in pro- and eukaryotes argues against the suggestion that the latter were directly derived from the former. There is no reason to expect a proto-eukaryote emerging from a bacterial stock to develop entirely different enzymes to perform functionally equivalent tasks if the ancestor already had them. As Doolittle (1981) writes '... it is simpler to duplicate and

modify proteins genetically than it is to assemble amino acid combinations from 'random beginnings'. Mechanisms operative here include the fusion of parts of different genes to produce new ones (Nyunoya et al. 1985) and the shuffling of exons or introns (Doolittle 1985). Therefore, it would seem that the principles of information conservation have been adhered to throughout the evolution of proteins, including the origin of the first cells. Kacser and Beeby (1984) have proposed a mechanism whereby a '... precursor cell containing very few multifunctional enzymes with low catalytic activities leads inevitably to descendants with a large number of differentiated monofunctional enzymes ...' Perhaps a mechanism such as this would allow one to view the progenote at once as extremely primitive, yet potentially versatile.

Studies on tRNAs also point to an independent origin of pro- and eukaryotes. Certain tRNAs, such as those for phenylalanine and glycine, share a common ancestor whereas others, such as the tyrosine tRNAs, may have independent origins (Cedergren et al. 1980). Other data suggest that serine tRNAs diverged from each other only after the establishment of the eukaryotic line (Cedergren et al. 1981). Also, studies on the promoter region of the rRNA operon of Halobacterium (Archaebacteria) have revealed certain features in common between eubacteria, archaebacteria and eukaryotes (Mankin et al. 1984).

These data are contradictory with respect to the concept of a prokaryote, in the phylogenetic sense, as being the ancestor of the eukaryotes. The increasing number of homologous enzymes in bacteria and eukaryotes, however, is compatible with a prokaryote ancestor (in the organisational sense) of the eukaryote, and suggests that the progenote must have been a fairly 'respectable' cell with a basic metabolic foundation.

There are other difficulties associated with specifying prokaryote ancestors. In terms of systematics, specifying ancestral-descendant relationships is unsatisfactory because they rely on hypotheses concerning the evolutionary mechanism. They assume (Eldredge 1979) that an actual evolutionary event took place linking the taxa in question. This reliance on unjustifiable, false or inadequately tested hypotheses argues against their use (Gaffney 1979). Ancestral-descendant hypotheses also suggest that the putative ancestor must be more primitive than the

descendant. If the lines diverged from a common (hypothetical) ancestor, it is less clear which taxon is more primitive. The notion that eukaryotes are more advanced than prokaryotes needs to be avoided (Woese 1983).

2.4 Primitive features in eukaryotes and advanced characteristics of prokaryotes

While it is usually the case that one taxon is more plesiomorphic than the other in any sister group system (Eldredge 1979), this view has been exaggerated in the analysis of prokaryote-eukaryote relations. Prokaryotes are generally regarded as representing primitive forms of life (the ancestors of the advanced eukaryote). Bremer and Wanntorp (1981), for example, stated that all the defining characteristics of the prokaryotes are thought to be primitive, the other (eukaryotes), advanced. As Carlile (1982) states, it is inappropriate to regard one as primitive and the other as advanced since they represent alternative strategies: one line has become specialised metabolically and miniaturised, whereas the other is larger and more complex. Like eukaryotes, prokaryotes have evolved substantially (Reanney 1974). The genome of prokaryotes represents an extreme end point in the loss of superfluous DNA in the genome. Single gene copies for most products and the operon system could be viewed as advanced (Reanney 1974). The discovery of intervening sequences in eukaryotes led to a further development of this idea. As argued by Doolittle (1978) and Darnell (1978), it is very difficult to *imagine how non-coding stretches of DNA could be introduced into already* existing structural genes. Intervening sequences are probably primitive, and have recently been discovered in members of the archaebacteria (Kaine et al. 1983; Rogers 1983) and are known from mitochondria (see Mahler et al. 1982) and chloroplasts (Koller and Delius 1984; Koller et al. 1984). This idea is supported by the contention that RNA splicing (mainly associated with split genes) is an ancient metabolic feature. According to Reanney (1979), RNA-RNA interactions are the basis of the translatory process. Certain processing enzymes such as RNase P contain a large amount of RNA, and eukaryotic splicing enzymes seem to resemble the ribosome (Sharp 1981), and self-splicing RNA (representing great

antiquity) has been discovered (Abelson 1982; Cech et al. 1983; Van den Horst and Tabak 1985; Waring et al. 1985). In conclusion, split genes and RNA splicing are likely to be an ancestral feature.

Repetitive DNA is generally characteristic of eukaryotes and eubacteria generally lack such sequences, but it has been reported that repetitive DNA (comprising up to 1% of the genome) exists in the genome of E.coli and S.typhimurium (Watson 1985). Doolittle (1978) argues that the streamlining of the prokaryotic genome is an advanced feature. The problem in a restricted sense is most related to the manner in which increases in the size of the genome can be prevented, rather than encouraged (Reanney 1974).

The operon system of prokaryotes is most likely an advanced feature. Furthermore, it is difficult to envisage how, if operons are an aboriginal feature, the emergence of eukaryotes survived the breaking up of such a sophisticated system.

In conclusion, this section has discussed two main points. The first concerned the view that prokaryotes cannot be viewed as the direct ancestors of the eukaryotes. This conclusion was mainly based on the dissimilarities between the taxa which suggested that they may have a common ancestor rather than their being viewed as ancestors and descendants. This view was reinforced by the indication that many prokaryotic characteristics were in fact highly evolved and could not all be viewed as primitive. By the same token, eukaryotes seem to have some ancient features, such as intervening sequences.

The second main point dealt with indicated that the nature of the hypothesised progenote is somewhat contradictory. Some evidence (particularly the relationships between archaebacteria, eukaryotes and eubacteria) indicate that it was a very primitive cell, whereas other facts, such as the existence of homologous proteins in pro- and eukaryotes, indicated that the progenote may have been a more advanced cell.

2.5 Use of Cladistics in proving the Serial Endosymbiotic Theory

The demonstration of an organelle-bacterium relationship can be done in two ways. In one, the nature of the host can be assessed (see p.9). Alternatively, certain derived characters linking these organelles to a specific lineage of the bacteria, such as the purple non-sulphur bacteria, or cyanobacteria, can be identified. This approach could conceivably substantiate the Serial Endosymbiosis Theory without reference to the host cell. Concerning the chloroplast (which has a more conservative history), phenetic studies have been able to assign red algal chloroplasts to the cyanobacteria and chlorophyte and higher plant plastids to another lineage, perhaps represented by the chloroxybacteria (Prochloron) (Doolittle and Bonen 1981; Lewin 1981). By means of the process of resolution of lower level hypotheses of monophyly to higher level hypotheses (see Eldredge and Cracraft 1980), one ends up having to show that the mitochondrial/plastid line and the prokaryotes share apomorphies absent in the eukaryotes (fig. 1, p.10) and vice versa. This three-taxon tree is the basic phylogenetic statement of the problem. If one is confined to this particular state, one is dealing with a closed system (Wiley 1981). In such a phenetic system, parsimony has to be applied to select the hypothesis of relationship that, in the first place, minimises character conflict. Furthermore, parsimony within this three-taxon group may be unparsimonious in relation to higher level phylogeny (Wiley 1981). This state of affairs is unsatisfactory and as a result an (hypothesised) outgroup is needed which will allow one to determine the polarity of characters and sort synapomorphies from symplesiomorphies. The former are the relevant characters.

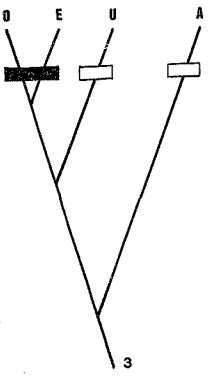
If ingroup comparison is used to determine polarities, uncertainties can arise. This comparison depends on the suggestion that the character state that occurs most frequently in the group is the primitive state (Stevens 1980). The proportion of lineages characterised by apomorphous traits depends on the numbers of cleavages after the origin of the apomorphous state. Consequently, because the number of cleavages is unknown, no conclusion as regards relationship can be drawn from the distribution (De Jong 1980). Watrous and Wheeler (1981) have also analysed this 'commonality principle' and conclude that it is impossible

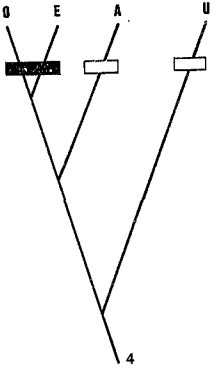
to differentiate the cases where common equals primitive from those where it does not, that is, without reference to the outgroup. Additional criticisms of this method are given by Underwood (1982).

Until recently there was little hope of solving this dilemma. The discovery of the archaebacteria, however, has presented a possible outgroup (fig. 3, p.19) or allowed the eukaryotes to become an outgroup (fig. 4, p.19). The outgroup criterion states that '... those character states occurring in other taxa within a larger hypothesised monophyletic group that includes the three taxon statement as a subset can be hypothesised to be primitive (plesiomorphous) and those character states restricted to the three taxon statement itself can be hypothesised to be derived (apomorphous)' (Eldredge and Cracraft 1980).

In order to do this, one accepts a higher level of synapomorphy as a working hypothesis which is based on other similarity criteria. This method is widely accepted (see, for example, Stevens 1980, De Jong 1980). If the archaebacteria are basal on the cladogram (fig. 3, p.19), they will serve as an outgroup allowing one to deduce apomorphies defining the eubacterio-organellar line, thus corroborating the Serial Endosymbiosis Theory. If the archaebacteria are more related to the eubacteria as in fig. 4 (p.19), one could show symapomorphies between mitochondria and eubacteria, using the eukaryotes as outgroup. If the archaebacteria are indicated as relatives of the eukaryotes as in fig. 5 (p.20) one would, cladistically speaking, be in trouble as there would be no outgroup and one would be operating in a closed system with its associated defects. It has been suggested that the demonstration of archaebacteria-eukaryote links would assist with corroboration of the Serial Endosymbiosis Theory (Gray and Doolittle 1982). Such evidence would certainly be suggestive of independent organelle-eukaryote lineages, and need not be incompatible with an archaebacterial outgroup (fig. 3, p.19).

A major question remaining is what, if any, taxon can be considered as an outgroup? In order to answer this question it is necessary to assess the nature of the archaebacteria.





Figures 3 and 4

Cladograms depicting hypothesised relationships among organelles (mitochondria and plastids)(O), eubacteria (E), urkaryotes (= eukaryotes in this context)(U), and archaebacteria (A).
In Figure 3 the Serial Endosymbiosis Theory is proved by using the archaebacteria as an outgroup and in Figure 4 by using the urkaryotes as an outgroup.

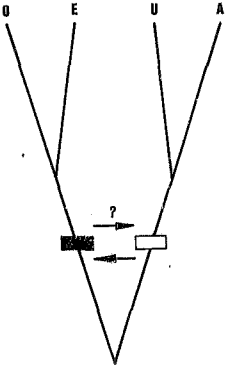


Figure 5

Cladogram depicting hypothesised relationships among organelles (mitochondria and plastids)(O), eubacteria (E), urkaryotes (U) and archaebacteria (A). In this cladogram, proof of the Serial Endosymbiosis Theory is not possible because synapomorphies cannot be established. This is due to the absence of an outgroup. The cladogram is hypothesised on the basis of phenetic indications.

2.5.1 The Archaebacteria - unique features

Having discussed the idea of a progenote as ancestor of pro- and eukaryotes, it is not difficult to introduce the idea of the archaebacteria as an additional lineage distinct from eukaryotes and true bacteria. It has become clear over the last few years that organisms comprise three distinct lineages which are very unlike one another (Doolittle 1980). The most probable explanation for this state of affairs is that they diverged at a very early stage from the progenote. From oligonucleotide catalogues of 16S and 18S rRNA, Woese and co-workers (Woese and Fox 1977; Fox et al. 1980; Woese 1981) concluded that living organisms could be divided into three groups: eubacteria, archaebacteria and urkaryotes (also known as the three primary kingdoms). The urkaryotes correspond to the cytoplasm of eukaryotic cells and represent the engulfing form (Woese and Fox 1977). The archaebacteria are ostensibly bacterial, but differ from 'other' bacteria in fundamental ways. The archaebacteria are a fairly heterogeneous group consisting of methanogens (Balch et al. 1977), halophiles (Magrum et al. 1978) and the thermoacidophiles (Fox et al. 1980). The heterogeneity extends to specific features in that some members of the kingdom may possess eubacterial versions of a character whereas others resemble eukaryotes. The thermoacidophiles have fairly deep phylogenetic divisions consisting of three branches represented by Sulfolobus, Thermoplasma and Thermoproteus (Thermoproteales) (Zillig et al. 1981; Cammarano et al. 1985).

The archaebacteria were first recognised on the basis of oligonucleotide catalogues which gave association coefficients significantly different from both eukaryotes and ordinary bacteria (Balch et al. 1977; Fox et al. 1980; Woese 1981). In addition, these groups are further defined by a distinct set of highly conservative oligonucleotides (see Doolittle 1980).

These initial results based on RNA catalogues have been backed up by a large volume of independent data pointing to the unique nature of the archaebacteria. The structure of the archaebacterial ribosome is different from that found in eubacteria and eukaryotes (Lake et al. 1982; Lake 1983; Cammarano et al. 1985). The cell walls are unique and lack

peptidoglycan and muramic acid (Kandler and Hippe 1977; Kandler and König 1978). The membrane lipids are peculiar in that they contain phytanyl side groups in ether linkage rather than acids in ester linkage (De Rosa et al. 1980; Kushwaha et al. 1981; Gray and Doolittle 1982). The RNA polymerases of the archaebacteria are different in being more complex subunits, having different stoichiometries and antibiotic resistances (Stetter et al. 1978; Zillig et al. 1982; Madon et al. 1983; Cammarano et al. 1985). The 5S rRNAs are different in sequence (Luehrsen and Fox 1981) and the 5S sequence of Sulfolobus is unique (Dams et al. 1983). The ribosomal proteins are distinctive (Matheson et al. 1978; Matheson and Yaguchi 1981; Schmid and Böck 1981, 1982). The tRNAs of archaebacteria are also peculiar. The initiator tRNAs show common unique sequence characteristics (Kuchino et al. 1982) and other tRNAs revealed unique modified nucleosides (Kilpatrick and Walker 1981; Pang et al. 1982). The unusual tRNA structure suggests that archaebacterial tRNAs have their own decoding properties and differing anticodon usage (Gu et al. 1983). The organisation of Methanobacterium DNA is unique (Mitchell et al. 1979). Finally, viruses discovered in the archaebacteria exhibit unexpected features (Janekovic et al. 1983; Martin et al. 1984).

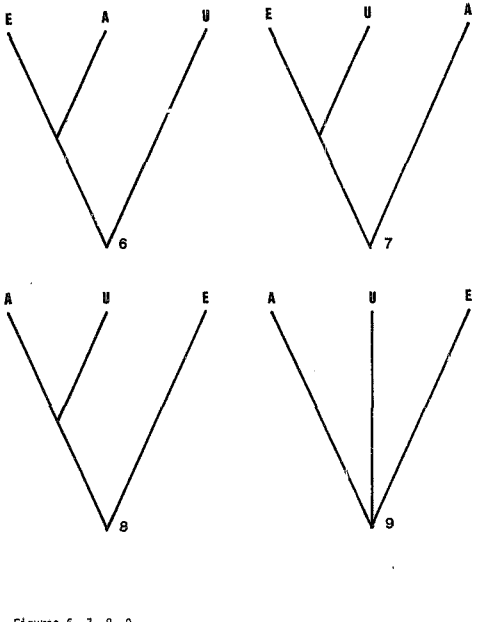
The archaebacteria also display unique metabolic capabilities, which set them apart from other forms of life. Some members of the thermophilic archaebacteria can live with carbon dioxide as their sole carbon source. This represents 'a new type of anaerobic purely chemolithoautotrophic metabolism, a possible primeval mode of life' (Fisher et al. 1983). Other types such as Sulfolobus brierleyi and a newly-discovered solfataric archaebacterium are unique because they are capable of growing strictly anaerobically by reduction or fully aerobically by oxidation (see Kelly 1985; Segerer et al. 1985; Zillig et al. 1985). The methanogenic bacteria have also been shown to use a novel pathway for the fixation of carbon dioxide (Hemming and Blotevogel 1985) and one member studied possessed a novel cyclic diphosphate, the function of which is not yet clear (Seely and Fahrney 1984).

On the basis of these substantial differences, Woese and colleagues have pursued the idea that living systems represent three aboriginal lines of

descent (Woese and Fox 1977). Such splitting is depicted as trichotomous (Fox et al. 1980; Woese 1981) (see fig. 9, p.24). In a cladogram a trichotomy is conceivably an unresolved dichotomy. While tri- or polychotomies are possible occurrences, analysis in terms of dichotomies has distinct methodological advantages (Hennig 1975). Given a trichotomous cladogram, the discovery of a single new character can dichotomise the trichotomy (Wiley 1981). Usually one of the three lines proves to be more related to another. This possibility as applied to the archaebacteria-eukaryote problem has been mentioned by Gray and Doolittle (1982), but is complicated by confusing character distributions Given the three taxa involved, four cladograms can be constructed (figs 6, 7, 8 and 9, p.24).

2.5.2 *A possible Archaebacteria-Eubacteria relationship*

In fig. 6 (p.24), the archaebacteria are considered more closely related to eubacteria. This view is favoured by Carlile (1982) and Van Valen and Maiorana (1980). 'Evidence' for this view comes from the fact that archaebacteria superficially resemble eubacteria in basic architecture (Woese et al. 1978). Archaebacteria lack most of the twenty-two crucial features of eukaryotes (Cavalier-Smith 1981) and have in common several with eubacteria. They are very small (see Zillig et al. 1982), possess cell walls (Woese et al. 1978) and have a small circular DNA chromosome (see Woese et al. 1978) the genome complexities of which resemble those of other prokaryotes (see Bollschweiler et al. 1985). They share with eubacteria other features such as small ribosomal subunits (30S and 50S ribosomes with 16S and 23S rRNA) (Woese et al. 1978), the presence of plasmids, phages (Yeats et al. 1982) and transposable elements (Xu and Doolittle 1983), as well as restriction enzymes (see Zillig et al. 1981; Daniels and Wais 1984). They also share certain metabolic pathways (see Gray and Doolittle 1982) and specific sequences such as are found in the initiator-interaction region of the 16S rRNA (Steitz 1978). The 16S rRNA sequence of the archaebacterium Halobacterium is closer in sequence to the eubacterial 16S rRNA than the eukaryotic counterpart (Gupta et al. 1983; Brimacombe 1984). In short, archaebacteria are characteristically members of the prokaryotic grade. Although evolutionary systematicists (e.g. Mayr 1974, 1981) attach great importance to grades, cladists do not



Figures 6, 7, 8, 9

Dendrograms showing hypothesised relationships between eubacteria (E), Archaebacteria (A) and urkaryotes (U). Figures 6, 7 and 8 are dichotomous, while Figure 9 represents a trichotomy or unresolved dichotomy.

rank them as unusually relevant to the determination of relationships (Hennig 1975; Gardiner et al. 1979). Evolutionary systematicists recognise grades by means of several criteria, notably morphological gaps and adaptive zones (Wiley 1981). Both these criteria produce paraphyletic groups (groups that include the common ancestor and some, but not all, of its descendants). Such groups are unacceptable as natural taxa.

If cladograms depicted in figs 7 or 8 (p.24) are correct, the grouping eubacteria-archaebacteria is paraphyletic because the eukaryotes are excluded. Paraphyletic groups are often established on the basis of *convergent or plesiomorphic features. Thus*, the grouping of eu- and archaebacteria could, for example, be done on the basis of a small circular DNA genome (probably a primitive feature) and the presence of a cell wall (probably independently acquired). Wiley (1981) has criticised in detail the use of morphological gaps and adaptive zones in determination of rank for classifications. While an archae-eubacterial link is intuitively feasible, the evidence does not stand up to detailed analysis. Van Valen and Maiorana's view (1980) of archaebacteria as 'derived prokaryotes' does not escape these criticisms.

2.5.3 An Archaebacteria-Eukaryote relationship

As described above, archaebacteria have a great many unique features which would tend to indicate relationships as depicted in figs 7 or 9 (p.24). An alternative cladogram, fig. 8 (p.24) can however be constructed, indicating a common ancestor between archaebacteria and urkaryotes. If this is the case, derived features must define the archaebacteria-urkaryote group. In addition, both the methanogen-halophile group and thermoacidophile group must possess these features (unless loss or reversal has taken place). Several workers have, in fact, suggested a relationship between the archaebacteria and eukaryotes (see Gray and Doolittle 1982). Both the Thermoproteales and Halobacteria seem to have affinities with the eukaryotes. Thermoplasma resembles eukaryotes in that it has a histone-like chromosomal protein (Searcy 1975) as well as an actin-like protein (Searcy et al. 1981). The relationship of the actin-like proteins of Thermoplasma to that of the

eukaryotes is still obscure. The histone-like protein seems to be intermediate between pro- and eukaryote histone-like proteins and histones. The Thermoplasma protein also forms globular particles similar to eukaryote nucleosomes and differs from the nucleoprotein particles of E.coli (Searcy 1982). The work of Zillig et al. (1981) on the Thermoproteales also indicates a eukaryote affinity in that the component pattern of these archaebacterial RNA polymerases is strikingly similar to those of the eukaryotes. The Halobacteria, members of the methanogenic line, also show a variety of similarities to eukaryotes. For example, eukaryotic amino-acyl tRNA synthetases are able to aminoacylate halobacterial tRNAs but not those of other prokaryotes (Kwok and Wong 1979). Ribosomal A proteins from Halobacterium and Methanobacterium are distinct but resemble the eukaryote A protein in size and display a large amount of similarity at the N and C terminals. Furthermore, they contain tyrosine which is absent in eubacteria (Matheson and Yaguchi 1981). Other features include the eukaryote-like rhodopsin and glycoproteins, amino acid transport mechanisms and β-carotene synthetic pathways (see Gray and Doolittle 1982). A few 'eukaryote' characters seem to be present in most archaebacteria, such as sensitivity to diphtheria toxin (Kessel and Klink 1980), insensitivity to chloramphenicol and kanamycin, and sensitivity to anisomycin (see Woese 1983). The RNA polymerase subunit structure and RNA polymerase stimulation by silybin (Zillig et al. 1982) also connect eukaryotes with archaebacteria. The initiator tRNAs carry methionine in archaebacteria as in eukaryotes (see Woese 1983) and archaebacterial/eukaryote tRNA modification seems to be more modified than in eubacteria (Woese et al. 1978). The CCA termini are not encoded in Sulfolobus as in eukaryotes (Kaine et al. 1983). In some archaebacteria (but not Halobacterium, Methanobacterium, Methanococcus and Sulfolobus (Neumann et al. 1983; Wich et al. 1984)) the 5S rRNA cistron is unlinked to the rRNA gene clusters as in eukaryotes. This seems to be the position in Thermoproteus and Thermoplasma (Jarsch et al. 1983), while others, such as Desulfurococcus, have a variable degree of linkage (Neumann et al. 1983). The archaebacterial genome has also proved to be unusual in that repeat DNA sequences are found in the genome (Woese 1983). The Halobacterium genome, in particular, contains many repeat sequences although Thermoplasma and Methanococcus lack them (Sapienza and Doolittle 1982; Bollschweiler et al. 1985). The most recent discovery relevant here

is the indication that introns are present in archaebacteria (as demonstrated in Sulfolobus) (Kaine et al. 1983; Rogers 1983). On the basis of these features, relationships between archaebacteria and eukaryotes have been suggested, and Van Valen and Maiorana (1980) go as far as suggesting an archaebacterial ancestor to the eukaryotes.

The fact that eukaryote-like features are present in the different branches of the archaebacteria is a distinctly complicating factor. Both Halobacterium and Thermoplasma could represent a presumed ancestor. If this is the case, loss of Halobacterium or Thermoplasma features must have taken place in one of the lines. It is also possible that some of the characteristics of Thermoplasma are secondary and represent convergences. The histone-like protein may be associated with DNA stabilisation at high temperatures (Stein and Searcy 1978) and the 'actin' with absence of a cell wall. One must also be conscious of convergences due to similar selective pressures or constraints. This important point was made by Xu and Doolittle (1983) with respect to the archaebacterial transposable element ISH50. The archaebacteria are apparently very old (the methionine tRNA of Thermoplasma has 90% similarity to the ancestral quasi-species sequence predicted by Eigen (Kilpatrick and Walker 1981)) and seems to resemble that of the common ancestor (Gupta et al. 1983). In spite of this, one must not underestimate the amount of evolution that the archaebacteria themselves have undergone (Clarke 1982).

At the present time it would be inopportune to suggest a specific relationship between archaebacteria and eukaryotes (Woese and Gupta 1981; Woese 1983). Hori et al. (1985), using 5S rRNA sequences, argue strongly that the archaebacteria and eukaryotes separated after the split with eubacteria, but Gray et al. (1984) leave the order of divergence of the three lines open, despite the sensitivity of their methods. It does seem that two possibilities remain: either archaebacteria are the sister group to eubacteria-urkaryotes (fig. 7, p.24), or they are related to the urkaryotes (fig. 8, p.24). The former possibility is supported by the fact that the Halobacterium 16S rRNA resembles both the eubacterial and eukaryotic more than they resemble each other, suggesting that the archaebacterial sequence resembles their common ancestral sequence more than does either of the other two (Gupta et al. 1983). The common possession of characters between eukaryotes and archaebacteria does not

imply that they are sister groups: indeed, it remains possible that the urkaryote-archaebacteria pair are the sister group of the eubacteria (as in fig. 8, p.24). Shared urkaryote-archaebacteria characters may simply be plesiomorphies, which have been transformed to apomorphies in the eubacteria.

If the archaebacterial line represents the sister group of the eukaryotes only (fig. 5, p.20), it will make proof of the Serial Endosymbiosis Theory via cladistics a very difficult proposition. On the other hand, if the state illustrated in figs 3 or 4 (p.19) holds, cladistic proof of the Serial Endosymbiosis Theory should be possible.

2.5.4 The autapomorphy complication regarding organelle-prokaryote relationship

Even if the archaebacteria do prove to be an outgroup, further obstacles in the comparison remain. When an organism enters a new adaptive zone it is exposed to selective pressures which differ substantially from those that existed in the previous habitat. Features previously confined in the evolutionary sense are now freed to evolve in new directions. In such a case, particularly if a period of specialisation follows, derived features can be accumulated rapidly. Apomorphies accumulating in a line after a particular split (autapomorphies) can obscure the phylogenetic affinities of the organism. This results when the changes in a particular character are so substantial that resemblance to one or more lines becomes equivocal. While members of such a transformation series of characters remain homologous, the detection of this is difficult. The question is whether organelle features have remained sufficiently conserved to allow meaningful pointers as to their origins to be deduced.

Many nuclear-coded organelle features allow comparison with prokaryotes (Schwartz and Dayhoff 1978, 1981) but depend on the gene transfer assumption. This does not always hold for the organelle-encoded macromolecules because some of them seem to have undergone very substantial sequence changes. These differences sometimes leave doubt as to whether the sequence is truly homologous. On the inception of an endosymbiosis, selective forces on the cytobionts fall into two categories. Some (stabilising) selective pressures will be greatly increased (Smith

1979). In the case of the plastid, for example, genes involved with photosynthetic functions will be highly conserved. Other selective pressures may be entirely removed or even reversed (Harington and Thornley 1980). For example, genes coding for cell wall synthesis will degenerate and eventually disappear; the rudimentary wall present in some cyanelles (Trench et al. 1978) is a case in point.

A second and distinct factor also influences rate of change of mitochondrial and plastid base sequences. If gene transfer proceeds and only a small coding responsibility is placed on the organelle genome, constraints on the organelle translatory apparatus are reduced. A case in point is the mitochondrial genome which codes for a small number of proteins (13 in the human (Borst and Grivell 1981b)). Changes in such a system are easier to envisage as opposed to a larger, more complex system coding for thousands of proteins (see Jukes 1981; Cann et al. 1984). The mutation rate in mitochondrial DNA is substantially elevated compared with that in nuclear DNA. The rate of evolution of mitochondrial DNA is about ten-fold higher than that of nuclear DNA, and tRNA evolution is 100-fold elevated (Brown et al. 1982). The evolutionary rate of a gene is proportional to the mutation rate and is inversely related to the functional constraint. Studies by Miyata et al. (1982) indicate that silent changes in mitochondrial genes took place six times more often than in nuclear genes. This suggested that mutation rate is to a large extent responsible for this elevation in evolutionary rate. Cann et al. (quoted in Brown et al. 1982) have ascribed this increase to relaxation of functional constraint (in addition to higher mutation rate) as well as other factors such as poor repair and lack of recombination (Cann et al. 1984). The higher rate of evolution of mitochondria (and plastids) has resulted in substantial changes in sequence of macromolecules and seems to be responsible for the variant mitochondrial genetic codes.

2.5.5 Base sequence data

This large body of information is not analysed in detail since reviews such as those by Gray and Doolittle (1982) and Phillips and Carr (1977) deal with the results in depth.

In the plastid tRNA, sequences can be shown to be related to prokaryotes.

Phenylalanine tRNAs (LaRue et al. 1979; Cedergren et al. 1980; Hasegawa et al. 1981) and leucine tRNAs (LaRue et al. 1981) and tyrosine tRNAs (Green and Jones 1985) display such affinities. Other chloroplast tRNAs (valine and formylmethionine) also show typically prokaryotic features (McCoy and Jones 1980; Sprouse et al. 1981), although some chloroplast tRNAs display pro- and eukaryotic features (Ohme et al. 1984). Mitochondrial tRNAs show a different pattern and cannot be definitely related to pro- or eukaryote tRNAs (Cedergren 1981; Hasegawa et al. 1981). Mitochondrial tRNAs possess too many unique features to make their affinities obvious (Gray and Doolittle 1982), confirming the problem alluded to earlier. One of the most drastic unique features is the absence of the DHU loop and stem in mammalian serine tRNAs (De Bruijn et al. 1980). Other novel features include the absence of the sequence TCG (or A) in loop IV, which is replaced by UGCA, in the initiator methionine tRNA from Neurospora (Heckman et al. 1978). Interestingly, the Neurospora mitochondria tRNA tyr, although having other unique features, has the usual TψC sequence in loop IV. Plastid tRNAs, though much more conserved, also show some unexpected features (see Sprouse et al. 1981; Kashdan and Dudock 1982). The mitochondrial tRNAs have apparently evolved at fairly rapid and also different rates in different organisms, thus obscuring their phylogenetic affinities (see Cantatore et al. 1982).

Ribosomal RNA sequences, all in all, fit into the pattern described above. There is a substantial and significant similarity between plastid and prokaryote rRNAs (Phillips and Carr 1977, 1981). 16S rRNA comparisons between cyano- or other bacteria reveal a high degree of sequence similarity (Zablen et al. 1975; Bonen and Doolittle 1975, 1976; Bonen et al. 1979; Schwarz and Kössel 1980). The degree of change is small enough to allow specific plasto-prokaryote relations to be indicated (e.g. Doolittle and Bonen 1981). The 5S rRNA sequences studied also support a prokaryote-plastid link (Dyer and Bowman 1979; Phillips and Carr 1981; Takaiwa and Sugiura 1982). Küntzel et al. (1983) produced a phylogenetic tree which clearly indicates that plastid 5S rRNA clusters with the eubacterial group. Mitochondrial rRNAs generally show less prokaryotic affinity than their plastid counterparts. According to Doolittle (1982a) and Gray and Doolittle (1982), the only RNA sequence that strongly indicates an endosymbiotic origin for the mitochondrion is that of wheat

mitochondrial 18S rRNA. This molecule shows no detectable homology to *cytoplasmic 18S rRNA but is clearly homologous* to eubacterial 16S rRNA ($p<0.000\ 001$). Schnare and Gray (1982) have sequenced the 3'-terminal region of wheat mitochondria 18S rRNA. A 72% similarity to the E.coli molecule was found, contrasted to a 58% identity to the wheat cytosol molecule. In addition, residue 45 in both E.coli and wheat mitochondria 16S rRNA is 3-methyluridine or a related modification, whereas the cytoplasmic RNA had an unmodified U residue at the same position. Other mitochondrial rRNAs, such as those of the fungi and vertebrate animals, display a much greater sequence variability, indicating a rapid rate of evolution (Schnare and Gray 1982). In this regard, Bibb et al. (1981) indicated that the sequence and structural homologies could not delineate the evolutionary path of the mitochondrion. Sequences of human mitochondrial 16S and 12S rRNA genes do not indicate a specific affinity to the pro- or eukaryotes (Eperon et al. 1980). Van Etten et al. (1980) report mouse rRNA - E.coli homologies but these are much weaker than plastid - E.coli comparisons. Hybridisation of spinach chloroplast rRNA with mitochondrial E.coli and other chloroplast DNAs revealed little affinity between the mitochondrial sequence (20%) as opposed to E.coli (60-70%) (Bohnert et al. 1980).

A contentious sequence is that of 5S rRNA. Köchel and Küntzel (1982) place the mitochondria on the common organelle-bacterial line as opposed to that of the nuclear branch. This was more apparent for Paramecium, yeast and Aspergillus mitochondria than for mammalian mitochondria. Küntzel et al. (1983) maintain that the 5S rRNA sequence of wheat *mitochondria indicates a eubacterial origin for the organelle.* However, Gray and Doolittle (1982) state that ' is molecule cannot readily be classified as pro- or eukaryotic. Spencer et al. (1981) report that wheat mitochondrial 5S rRNA has a number of unique features and is as homologous to E.coli as is cytosol 5S rRNA. As before, the rapid evolution of wheat mitochondrial 5S rRNA genes seems to have obscured the phylogenetic ancestry of the organelle. Recent work by Gray et al. (1984) has provided more definite phylogenetic indications, and their results, based on small subunit RNA, confirm a eubacterial origin for plastids and the mitochondria of plants, animals and fungi.

2.5.6 The genetic code

The variant mitochondrial genetic codes are probably the most striking feature characterising these organelles. The animal and fungal mitochondria use UGA as a tryptophan codon instead of as a terminator. CUA is used to code for threonine in yeast, but specifies the standard leucine in mammalian and Neurospora mitochondria. AUA, which specifies isoleucine in the universal and Neurospora mitochondrial code, represents methionine in yeast and mammalian mitochondria. AGR specifies termination in mammalian mitochondria rather than the usual tryptophan. In maize mitochondria, CGG is used in addition to UGG to specify tryptophan but the rest of the coding seems to be standard. In Chlamydomonas mitochondria UGA functions as a stop codon and not as tryptophan (Pratje et al. 1984). The codes and their variants are discussed by various authors: Barrel et al. 1979; Bonitz et al. 1980; Mahler 1980; Borst 1981; Grivell 1981; Gray and Doolittle 1982; Hudspeth et al. 1982; Wallace 1982; Jukes 1981, 1982, 1983a,b). Further variants of the genetic code have been found outside the context of organelles. Analysis of the coding pattern of a mycoplasma showed that it also uses UGA as a tryptophan codon rather than for termination (Yamao et al. 1985). In several ciliates (Tetrahymena, Stylonychia and Paramecium), UAA and and UAG code for glutamine (Seilhamer and Cummings 1982; Caron and Meyer 1985; Helftenbein 1985; Preer et al. 1985). The mitochondrial codes therefore are not unique in deviating from the universal code (Fox 1985). In addition to the differences in decoding of specific codons, the mitochondrial tRNAs are peculiar in that they have an expanded codon recognition pattern so that a single tRNA species can recognise all the codons in the four families that each specify a single amino acid (Gray and Doolittle 1982). This allows the mitochondrial translatory system to operate with far fewer tRNAs than the 32 required by the wobble hypothesis. In particular, human and mouse mitochondrial DNAs code for 22 tRNAs (Anderson et al. 1981; Bibb et al. 1981; Wallace 1982; Jukes 1983a), and yeast mitochondrial DNA codes for 24 tRNAs (Bonitz et al. 1980; Wallace 1982).

The mitochondrial genetic code resembles a primitive genetic code, specifically, a '2 out of 3' system (Jukes 1981). This doublet code is

apparently what the early code consisted of. Jukes (1966) and Lagerkvist (1981) discuss the resemblance between this early code and the mitochondrial versions. Indications are (Jukes 1983a, 1983b) that the mitochondrial codes are simpler than the universal code, but not as simple as the proposed archetypal code (Jukes 1966). These observations led to suggestions (for example, by Harington and Thornley 1980; Mahler 1980) that the mitochondrial codes represent a primitive system originally possessed by the cytobiont. The now more feasible alternative, supported by the internal variability of the organelle codes (Borst 1981), suggests that present variant codes are secondary (Jukes 1981, 1983a, 1983b) or, specifically, accidental developments (Borst 1981). Construction of a phylogenetic tree based on the small subunit ribosomal RNA has strongly indicated that the divergences in the genetic codes are secondary. This is indicated by the fact that animal and fungal mitochondria branch after the plastid, and from the eubacterial lineages which use the universal code (Gray et al. 1984). Cedergren (1981) is of the opinion that the present data are contradictory as to whether the mitochondrial code preceded, or was derived from, the universal code.

In conclusion, it would appear (Borst 1981) that the small coding responsibility of the mitochondria has had at least two main and probably fortuitous results: accidental alterations in the code and relaxed evolutionary constraints on tRNA and rRNA sequences. At present it would seem that the complication in proving the endosymbiotic origin of mitochondria is not that they are too primitive (Uzzel and Spolsky 1974, 1981), but that they are too specialised (derived).

2.6 Summary

In this chapter several ideas pertaining to the proof of the Serial Endosymbiosis Theory were developed. It was argued that phenetic methods did not provide reliable evidence in favour of the Serial Endosymbiosis Theory. The main reason for this is that the organelles and prokaryotes could be considered to be similar on the basis of primitive features. Accordingly, the resemblance would not be indicative of a phylogenetic relationship.

It was pointed out that prokaryotes (in the phylogenetic sense) were unlikely to be predecessors of the eukaryotes. This is suggested by an analysis of the differences between pro- and eukaryotes. These differences are so substantial that it would appear that the pro- and eukaryotes diverged at an early stage from a common ancestor named the progenote. This organism was, in evolutionary terms, in a state of flux. A further consequence of this view is that prokaryotes have undergone substantial evolution since their presumed split from the eukaryotes and that they possess certain advanced features. The eukaryotes by the same token may have certain ancient features. Following this reasoning, a three-taxon tree relating the organelles, prokaryotes and eukaryotes was constructed.

In order to prove the Serial Endosymbiosis Theory more satisfactorily, cladistic techniques could be used. For such an approach to be meaningful, an outgroup is necessary to avoid working in a 'closed system'. The recent developments in prokaryote systematics due to the work of Woese and colleagues have defined a third line of organismic evolution distinct from eukaryotes and bacteria. This lineage represents the archaebacteria and is presumed to have split from the eubacteria and the urkaryotes (the eukaryote cytoplasm) at the progenote stage. This lineage could possibly provide an outgroup with which to compare prokaryotes, organelles and eukaryotes. If this is the case, one would be able to partition the features of the taxa into primitive and derived classes. The distribution of the last-named characters could enable one to prove a definite organelle-prokaryote or organelle-eukaryote relationship, thus allowing falsification of the Serial Endosymbiosis or Autogenous Theory.

If a cladistic approach proves to be feasible, another stumbling block remains. An analysis of organelle protein and nucleic acid sequences, as well as studies of the translatory apparatus of the organelles, suggests that the characters have undergone substantial change in evolution. As opposed to the 'primitive feature' problem, one is dealing with the reverse here - a case of extreme autapomorphy. The question is whether a particular character in an organelle has not changed so much that comparison with its presumed homologue is meaningless. It is possible that many organelle features, particularly those of mitochondria,

have diverged so much that their phylogenetic origin has been obscured. Therefore, in order to devise a more rigorous proof of the Serial Endosymbiosis Theory, at least two requirements are necessary - an outgroup and a series of homologous characters. While the questions raised in this section cannot be answered in much detail, it is hoped that the systematic problems have at least been more clearly stated.

CHAPTER THREE

ESTABLISHMENT OF SYMBIOSES

3.1 Metabolic and genic integration

There is little doubt that metabolic relationships are the cornerstone of symbiotic associations. Such associations can range from being casual to extremely intimate, and represent a spectrum of interactions from semi-parasitism to complete mutualism. These findings lend credence to the observation that the recruitment of an often taxonomically-unrelated organism to expand the metabolic complement of another is easier than de novo evolution of the pathways in question. This characteristic is well illustrated when an organism, by virtue of a symbiotic arrangement, is able to colonise a habitat that would be out of bounds otherwise. In addition, metabolic innovations absent in either of the members prior to the association can evolve due to interaction of their respective metabolisms.

Once a symbiotic relationship is established, the association commences with a period of metabolic integration (Margulis 1976, 1981). In many cases (particularly in the instance of intracellular symbionts), a period of genic integration follows (Margulis 1976, 1981). During this time, the genomes of at least one of the symbiotic partners becomes dependent on the other. This is partly the result of gene transfer, which is the essence of genic integration (Margulis 1976). Integrative factors are very important in symbiosis, and as Taylor (1979) says '...in terms of genetic novelty symbiosis represents a quantum leap of magnitude far greater than that arising from intrinsic sources such as mutation, hybridization or ploidy changes'.

Of all the symbiotic associations two are far more important than any others from a metabolic point of view. These are the association of the organelles of photosynthesis and of respiration with the host.

Most symbioses involve the passage of metabolites from one member to the other, and vice versa. Such compounds are usually of three different

kinds: polyhydric alcohols or glycerol, neutral amino acids and sugars (glucose and maltose) (Smith 1979). In time, the host usually specialises in providing the symbiont with simple metabolites in return for a synthesised substa.e. This is found in many of the symbioses involving cyanelles and chlorellae where there is substantial secretion of maltose and glucose by the endosymbionts (Brown and Nielson 1974; Jaynes and Vernon 1982). Other substances secreted here include malate and fructose. In other symbioses, the symbiont may provide the host with enzymes. It has been reported that certain haemo-flagellates possess bacteria-like endosymbionts which supply the host with the enzyme uroporphyrinogen I synthase (Chang et al. 1975). These enzymes allow the host to synthesise heme at greatly increased efficiency. The examples dealt with so far are reasonably simple. Many symbiotic relationships develop metabolites or pathways which are characteristic of neither partner alone (see Margulis 1981). This is probably a result of interactions between two different metabolisms and renders substantial diversification in synthetic pathways possible. The metabolic results of a symbiosis often allow an organism to occupy a niche that would be denied it in the absence of such an association. Certain bivalves possess symbiotic halophilic bacteria (Postgate 1983). Apart from being capable of microanaerobic nitrogen fixation, the bacteria are cellulytic. By virtue of the symbiosis, the shipworms are able to utilise a food source such as wood, which is nitrogen-poor and largely indigestible. Another example is the dense metazoan fauna associated with hydrothermal vents where sulphide-oxidising bacteria fix carbon non-photosynthetically and reduce nitrogen (Felbeck et al. 1981; Felbeck and Somero 1982). These bacteria are often symbiotic with vent worms and clams (Cavanaugh et al. 1981; Felbeck et al. 1981; Felbeck and Somero 1982). Symbiotic corals are generally the most successful and Wellington and Trench (1985) found specific reasons for the unusual success of a non-symbiotic coral. In insects symbiotic associations are widespread, and symbiotic gut flagellates in termites possess cellulase which permits the hosts to consume cellulose (Yamin 1981). When a symbiont allows the host to colonise a new habitat, the presence of the symbiont becomes obligatory. Other associations, such as the recycling of uric acid by nitrogen gut bacteria in termites, may be dispensable (Potrikus and Breznak 1981). In most cases, however (such as

cockroaches, bugs, lice and beetles), removal of the symbionts greatly decreases the fitness of the host (Steinhaus 1949).

An interesting form of integration that does not appear to be metabolic is the dependence of the host nucleus division on the presence of intracellular symbionts. This situation has been reported for Paramecium and its chlorellae (Margulis 1981) and in the case of the ciliate Euplotes and its endosymbiotic bacteria (omikron) (Heckmann 1975). Nuclear DNA replication in Chlamydomonas may also require a chloroplast gene product (Bennoun 1981).

In metabolic integration there is also duplication of metabolic pathways due to the fact that each member once had an independent existence. Such duplication is wasteful and a degeneration of genes in the symbionts as opposed to the dominant host usually takes place. In some cases the host could also lose certain genetic information. If, for example, a host had an inefficient and primitive respiratory system, the invasion of aerobic symbionts would lead to disuse and eventual loss of the genetic material carrying the information for the primitive system (see de Duve 1973 for details).

The process described above leads to obligate associations, but it is still not indicative of the next step in partner integration, namely, genic integration (Margulis 1976).

It is important to realise that a symbiotic association can increase the efficiency of an existing host metabolism and need not be a completely unique addition. Lack of attention to this point (coupled with the fact that some authors envisage an anaerobic host cell for the putative proto-mitochondrial endosymbiont) (Stanier 1970; Margulis 1976; Woese 1977), has led to criticism of the Serial Endosymbiosis Theory. Raff and Mahler (1972, 1975) argue that the eukaryotic cytoplasm is of an aerobic nature. In spite of the fact that Hall (1973), de Duve (1973) and Taylor (1974) pointed out that an aerobic host could very easily maintain aerobic endosymbionts, some authors, such as Keyhani (1981), persist in using the 'anaerobic host' as criticism of the Serial Endosymbiosis Theory. An aerobic host may enter into an association with aerobic cytobionts because the relationship increases the efficiency of overall

host respiration. In any case, it is of interest to note that anaerobic ciliates bearing endosymbiotic bacteria have been reported (Fenchel et al. 1977).

3.2 Genic integration

Genic integration implies that genes originally present in the genomes of one partner are transferred to the other (Margulis 1976). Unlike metabolic integration, this process is not a prerequisite for the establishment of the symbiotic relationship, nor is it of immediate advantage to either of the partners. In some cases genic integration is partial, with the host nucleus presumably being the recipient of endosymbiont genes. Examples of this (assuming a symbiotic origin) would be the mitochondrion, the plastid and cyanelles. It is still not known why genic integration has not gone to completion in these organelles, though it appears that the phenomenon of compartmentation may be implicated. The complexity of the symbiotic association could perhaps also be involved. In the case of the mitochondrion and plastid, hundreds of genes would be transferred to the nucleus and those remaining in the organelle could be involved in the biogenesis (Bogorad 1975; Bogorad et al. 1975; Thornley and Harington 1981). In simpler symbioses, that is, those involving few genes, genic integration could have gone to completion, in which case the endosymbiont would effectively cease to exist as an independent plasmon. It would then exist only as 'foreign' information in the host nucleus and be replicated by the host. Because the symbionts no longer exist, attempts to demonstrate their contribution are very difficult. Possible examples of 'extinct' symbionts could be the genes for tubulins in eukaryotes (providing the microtubules have a symbiotic origin, as speculated by Margulis)(see Margulis 1970), and possibly the presence of a ribosomal protein archaebacterial gene in the nucleus (Fox et al. 1980).

Although a gene transfer requirement is not exclusively a character of the Serial Endosymbiosis Theory (see, for example, Bogorad 1975), this question has been a major criticism of the theory (Allsop 1969; Raff and Mahler 1972, 1975; Uzzel and Spolsky 1974, 1981) and until recently

no satisfactory answers have become available. Unfortunately, Margulis (1981) did not consider the problem to any extent.

In the following section, the question of gene transfer will be discussed.

3.2.1 The problem

At least two observations suggest that a rearrangement in the location of genetic information between plasmons and the nucleus has taken place:

(i) Genome sizes

The genomes of mitochondria are variable in size, the largest mitochondrial DNAs being found in plants, the smallest in animal cells. All of these are substantially smaller than that of a 'minimal cell' such as a mycoplasma (Gillham and Boynton 1981). It has been established that mitochondrial DNA codes for about 5% of the organelle's protein (Borst and Grivell 1978), which would correspond to about 13 proteins in man (Borst and Grivell 1981b), about 20 in plants (Leaver and Gray 1982) and 8 in yeast (Borst and Grivell 1978). The number of proteins known to be coded for by the yeast mitochondrial DNA has increased due to the discovery of a limited spectrum of splicing proteins (maturases) encoded partly in the introns of the oxi3 and cob genes (Borst and Grivell 1981a; Jacq et al. 1982; Mahler et al. 1982). The mitochondrial DNA also encodes about 24 tRNAs as well as the small and large rRNAs of the mitoribosomes (Borst and Grivell 1978, 1981b). The mitochondria of plants code for some proteins, such as the α, β and ϵ subunits of F_1-ATPase that are not mitochondrially coded for in other organisms (Boutry et al. 1983). Plant mitochondrial DNA presumably also codes for the 5S rRNA characteristic of them (Leaver and Gray 1982).

The genomes of chloroplasts are also substantially reduced compared with those found in their presumed ancestors (cyano- and chloroxybacteria), and are roughly 15 times smaller than that of E.coli (Gillham and Boynton 1981). The cyanellar genome is similar in size to that of chloroplasts, being about 10% that of a free-living blue-green bacterium (Tanch et al. 1978).

Thus it is not surprising that the genome of the chloroplast is at least 'several-fold' too small to code for observed chloroplast polypeptides (Ellis 1981a). Chloroplast DNA codes for some proteins, such as elongation factors for chloroplast translation, which are not found in mitochondrial genomes.

In closing, it is of interest that the nucleus and organellar genome are able to contribute subunits of a single enzyme. In the yeast mitochondrion, joint contribution to an enzyme is found in the case of cytochrome C oxidase, cytochromes bc_1 and ATPase (Borst and Grivell 1978; Tzagoloff 1982). In the chloroplast, the small subunits of RuBP carboxylase are nuclear-encoded and the large subunits organelle-encoded (Ellis 1981a).

(ii) 'Prokaryotic' genes seem to occur in the nucleus

When an analysis of the sequences of mitochondrial and plastid proteins was done, it was found that they showed fairly strong resemblance to prokaryotic sequences, in spite of the fact that they were nuclear-coded (Schwartz and Dayhoff 1978). In particular, 'prokaryotic' proteins (in terms of sequence similarity), such as the mitochondrial c-type cytochromes (Dayhoff and Schwartz 1978) and the plastid ferredoxins (Schwartz and Dayhoff 1981), are encoded in the nucleus.

A similar situation holds for the superoxide dismutases (SOD). The mitochondrion has a Mn SOD that is encoded in the nucleus (see Raff and Mahler 1975). *The N-terminal sequence of this enzyme is very similar* (20/27 residues shared) to that of the bacterial protein (Steinman and Hill 1973), but bears no resemblance to the cytoplasmic Cu-Zn form (Steinman and Hill 1973). This is supported by Stallings et al. (1983) who have demonstrated further substantial structural differences between iron and copper/zinc SODs. Lee et al. (1985) have discovered that superoxide dismutase evolution rates vary: some values represent the fastest rates known for any protein, whereas others resemble slowly-evolving proteins with respect to rate. This complication implies that 'using the primary structure of a single gene or protein to ... reconstruct phylogenetic relationships is fraught with error' (Lee et al.

1985). This observation complicates the SOD interpretation. Piechulla and Küntzel (1983) have ascertained by protein analysis that the nuclear-located mitochondrial elongation factor gene (EF-Tu) is related to the corresponding protein from E.coli. The eukaryotic counterpart (EF-1) is also encoded in the nucleus and differs fundamentally from the mitochondria and E.coli proteins.

It is clear that the eukaryote nucleus bears genes that are very similar to those encountered in the bacteria, although the origin of these genes has long been a controversial issue.

3.2.2 Possible explanations for the presence of 'prokaryotic' genes in the nucleus

In terms of the Xenogenous Theory, the origin of genes coding for the majority of organelle proteins is a reflection of gene transfer (Raff and Mahler 1972; Uzzel and Spolsky 1974; Reijnders 1975; Margulis 1976; Dayhoff and Schwartz 1981; Ellis 1982), that is, genetic information once associated with the endosymbiont, has become integrated and maintained in the host nucleus.

Autogenous Theories account for the patterns described above in various ways which are discussed below.

(i) No transfer of genetic information required - the plasmid-based hypotheses

Some theories - for example, those proposed by Raff and Mahler (1972, 1975) and by Cavalier-Smith (1975, 1980) - suggest that a respiratory or photosynthetic plasmid was assembled bearing the appropriate RNA and enzyme-specifying genes. This is rather unlikely (Taylor 1974; Reijnders 1975). As Borst and Grivell (1978) note, no plasmids coding for essential functions are known and the mitochondrial DNA contains genes characteristic of prokaryotic chromosomes and not extrachromosomal elements (see also Davey and Reanney 1980).

Filer et al. (1981) found that gene transfer could not account for the similarities in sequence for the tuf gene (encoding peptide elongation factor Tu) among various prokaryotes. A similar conclusion for

cytochromes c was reached by Dickerson (1980). These observations suggest that the presence of essential translatory and respiratory genes on plasmids is unlikely.

(ii) Gene transfer required

Bogorad (1975) and Bogorad et al. (1975) suggested that this mechanism may be involved in intracellular gene dispersal. The gene transfer mentioned here is not associated with plasmids but is the same as that invoked by the Serial Endosymbiosis Theory.

(iii) Transfer of genetic information by a duplication-deletion process

Such theories as, for example, those of Reijnders (1975) and Keyhani (1981) require a duplication of the whole or part of the original genetic material. Theoretically, one of the two genomes diverges to become a proto-mitochondrial (or proto-plastid) genome, while the other becomes the nuclear genome. The degeneration of the proto-mitochondrial DNA presumably occurs in a similar fashion to that envisaged for the genome of a proto-mitochondrial endosymbiont. At the end of the process the organelle is left with a few, perhaps unique, genes. Keyhani (1981) suggests that a complete mitochondrial genome (a master copy) still exists in the nuclear DNA. This suggestion does not explain how natural selection maintains, over long periods of time, duplicated though unlinked genes. Duplicated genes generally diverge because the policing function of natural selection is absent (Ohno 1970). This does not apply to the multigene families, the homogeneity of which is maintained by specialised mechanisms (see Dover 1982; Amstutz et al. 1985). Neither Reijnders (1975) nor Keyhani (1981) considers the origin of the chloroplast and Bogorad (1975) does not discuss the origin of the mitochondrion. Neither of them considers the difficulties involved in organising the division of two or three unlinked identical genomes within a single cell in conjunction with a compartmentalisation process. The genome of E.coli seems to be composed of a duplicated (but linked) ancestral genome (Zipkas and Reilly 1975). In this case, duplicated genes have become changed to perform different, though related, functions. This example, however, differs from the situation described by Reijnders (1975) and

Keyhani (1981) in that the duplicated genomes remain linked. Therefore, in evolutionary terms, this is the same as standard gene duplication.

3.3 Physical mechanisms of gene transfer

If a host cell contains a large number of endosymbionts in a constant state of turnover it is likely that DNA will at times be released into the host cytoplasm. It is feasible that such genetic material could become integrated into the host genome by a transformation process. It is liable to have been e[illegible] earlier stages of evolution when the chromosomes and nuclear e[illegible] not evolved to their present specialised states, although [illegible]e degree of flux is characteristic of the eukaryote genome. Such transformation would have been random in terms of size of DNA translocated and gene type. The genotype of the host could have teen altered by the addition of new genetic information.

Data indicate that such transformation of eukaryotic DNA is at least feasible. Pellicer et al. (1980) report that the addition of pure DNA to recipient cells can lead to stable integration of this DNA. Deficient cells can be returned to the wild type condition by transforming with unmutated DNA. In addition, Mulligan and Berg (1980) have found that a bacterial gene (the xanthine-guanine phosphoribosyltransferase gene) can be expressed in the eukaryotic cell after transfection. More pertinent evidence involving the transfer of genes directly from bacteria to eukaryotic cells has also been published. It has been shown that E.coli carrying copies of SV40 genomes on a plasmid transferred the genes to mammalian cells (Schaffner 1980). Phagocytosis of bacterial cells or host-protoplast fusion allowed the entry of bacteria into the host. The latter process increased the frequency of transfer drastically. Rassoulzadegan et al. (1982) further analysed this type of system by showing that stable transformation was associated with the gene transfer. In particular, integration of SV40 DNA was at least as efficient as that observed after infection by virions. Schaffner (1980) suggested that this type of gene transfer from bacteria to higher organisms takes place continuously in nature. In plants, Fraley et al. (1983) and Horsch et al. (1984) have used the Ti plasmid Agrobacterium to transfer genes into the

plant nucleus, which are subsequently successfully expressed. In Ryegrass, which is not susceptible to Crown Gall, Potrykus et al. (1985) used protoplasts to transfer genes to the nucleus.

It is clear that the intracellular presence of many symbiotic bacteria (particularly those without walls) over long periods of time provides excellent possibilities for the physical transfer of DNA to the host genome.

3.4 General evidence for gene transfer

3.4.1 Evidence outside the context of organelle evolution

In at least three systems, gene transfer has been demonstrated. It is known that the bacterium Agrobacterium tumefaciens transfers part of a large plasmid to the eukaryotic cell (Drummond 1979). Such genetic colonisation (Schell et al. 1979) takes place by means of covalent integration of T-DNA into the host plant DNA (Yadav et al. 1980). It is interesting to note (Willmitzer et al. 1980) that T-DNA is absent from chloroplasts and mitochondria. Gene transfer between eukaryotes by means of retroviruses is well established (Benveniste and Todaro 1982). Finally, the movement of genes from one nucleus to another takes place in the fungus Schizophyllum commune when this organism is in the dikaryon state (Gaber and Leonard 1981). Such transfer seems to be associated with differentiation of the fungus for reproductive purposes (Gaber and Leonard 1981).

Although these examples clearly demonstrate that gene transfer is possible, they are not particularly instructive in the context of organelle origin. This is because they represent specialised mechanisms. The gene transfer phenomena in the Crown Gall bacterium and fungus are associated with genetic colonisation and developmental processes respectively. Such gene transfer is highly selective. Indeed, Raff and Mahler (1978) have suggested that selective gene transfer is required in terms of the Serial Endosymbiosis Theory, although there is no reason why this has to be so. Unlike the examples discussed here, gene movement from endosymbiont to host was most likely a random affair, with natural selection resulting in the fixation of certain transfers.

3.4.2 Evidence within the context of symbiotic evolution

Supporters of the Serial Endosymbiosis Theory have had in the past to be satisfied with unrelated or circumstantial evidence for proposed gene transfer events. Recent data have provided direct support for such a contention.

Prior to dealing with transfer from mitochondria per se, a recent example of gene movement from eukaryote to prokaryote will be considered. It is known that prokaryotes may contain both Fe and Mn superoxide dismutase (SOD) (Steinman and Hill 1973), while higher eukaryotes (as well as a few prokaryotes (Stallings et al. 1983)) have an unrelated Cu-Zn SOD (Asada and Kanematsu 1978). The luminescent bacterium Photobacter leiognathi engages in a specialised symbiotic association with its Ponyfish host. When an analysis was done on the amino acid composition of the bacterial SOD (Martin and Fridovich 1981), the enzyme differed dramatically from expected prokaryote SODs and in discriminant analysis clustered within the higher eukaryote SODs (Martin and Fridovich 1981).

These authors concluded that gene transfer from the eukaryote (fish) host to the bacterium is the most parsimonious explanation. In the context of the intimate symbiosis the possibility of gene transfer by transformation is not unlikely. Bannister and Parker (1985) investigated other features such as sequence similarity (taking mutation into account), active site residues and predicted secondary structure. The evidence confirmed the gene transfer explanation as opposed to a convergence or *divergence-based explanation. The interpretation of this discovery* has been complicated by the discovery of a Cu-Zn SOD in the free-living bacterium Caulobacter crescentus (Steinman 1982). It is also known from a few other prokaryotes (see Stallings et al. 1983). Unless this bacterium had a symbiosis in its past, the gene transfer explanation may not be applicable to it.

(1) Gene movement from mitochondrion to nucleus

Ellis (1982) has defined as promiscuous DNA those nucleotide sequences present in more than one of the three membrane-bounded organellar genetic systems of eukaryotes. Thus, any DNA shown to be transferred

to the main genome from the mitochondrion while retaining copies among them, conforms to the definition. It is important to realise that transfer where the organelle population has permanently lost the gene (since it is present in the host genome) would not be classified as promiscuous. Clearly only recent transfers, where such duplicates exist, would be promiscuous.

An early indication that mitochondrial genes had moved to the nucleus came from studies on the ATPase subunit 9(F_o) gene. This gene is mitochondrially located in yeast and maize, but resides in the nucleus in Neurospora and mammals (see Fox 1981; Dewey et al. 1985). An ATPase subunit 9 gene is present in the Neurospora mitochondrial genome (Van den Boogaart et al. 1982). These authors suggest that the nuclear gene could be the result of a gene transfer event. An analysis of the sequence similarities between the two Neurospora proteins and those from yeast and cattle suggest (a) that the gene transfer event must have taken place early in the evolution of metazoa, and (b) that the mitochondrial gene is still active. Dewey et al. (1985) have found substantial amino acid sequence similarity between all ATPase subunit 9 proteins, regardless of the coding site, indicating a common origin. Mahler (1981) has raised the point that one does not know whether the gene was transferred from nucleus to mitochondrion, or vice versa. Similarly, the α-subunit of the F_1 component is nuclearly coded in yeast, mammals and Neurospora, but mitochondrially coded in maize and bean (Boutry et al. 1983; Dewey et al. 1985). Sequences of the coupling factor complex subunit found in chloroplasts (βCF_1, chloroplast-coded), E.coli (βBF_1) and mitochondria (βF_1, nuclear-coded) have been compared. The amino acid similarity between these three proteins is 58% (Krebbers et al. 1982). Such a high degree of similarity argues against convergent evolution and supports the notion of gene transfer or dispersal (Krebbers et al. 1982). Not surprisingly, recent work has shown the presence of mitochondrial genes in the nucleus.

(ii) Fungi

It has been shown (Farrelly and Butow 1983) that the yeast nucleus harbours rearranged mitochondrial genes. The non-functional sequence found here has regions of strong similarity to parts of the var1,

cob/box and ori/rep regions of the yeast mitochondrial DNA. As a result, the authors suggest that the sequence originated from a petite mitochondrial DNA which subsequently became integrated into the nuclear DNA. This event is thought to have taken place relatively recently (about 25 million years ago), which effectively eliminates the idea that they may be 'conserved primitive sequences' dating back to the origin of the organelle from a postulated host genome duplication. Wright and Cummings (1983) have provided evidence for active mobilisation of genetic elements from the mitochondrion to the nucleus in Podospora. During the process of senescence, discrete mitochondrial sequences derived from an intron (see Kück et al. 1985) are excised and amplified. Such plasmids constitute virtually all the DNA in the mitochondria at the end of ageing, the normal genome being displaced. During senescence these plasmids transpose the genes oxi_2 and oxi_3 for subunits I and III of the mitochondrial cytochrome c oxidase to the nucleus where they are integrated (Wright and Cummings 1983).

(iii) Metaphyta

Little evidence of gene transfer from mitochondria or plastids to the plant nucleus is available. Siegel (quoted in Vacek and Bourque 1980) indicated that 1700 copies of the chloroplast genome were present in the tobacco leaf cell nucleus. Vacek and Bourque ruled out the possibility of active chloroplast rRNA genes in the nucleus, but it remains possible (as in the yeast example) that inactive genes exist in the nucleus. Recently, Watson and Surzycki (1983) found that both the chloroplast and nuclear genomes of Chlamydomonas share homology with E.coli genes for transcriptional and translational components, such as an elongation factor, ribosomal protein and the β subunit of RNA polymerase. As an explanation, gene transfer is suggested, but this remains circumstantial evidence. Direct evidence came from Kemble et al. (1983) and Timmins and Scott (1983), who discovered that sequences homologous to mitochondrial DNA existed in the maize and spinach nuclear genomes.

(iv) Animals

Evidence of movement of mitochondrial genes to the nucleus in animals has

also been forthcoming. Sequences homologous to the mitochondrial DNA (including rRNA genes) have been found in the locust nuclear DNA (Gellissen et al. 1983). These sequences were present in several locust tissues and were particularly abundant in the flight muscle which is very rich in mitochondria. The sequences show some divergence from those in the mitochondria (Gellissen et al. 1983). In the sea urchin, chromosomal sequences have been detected that are homologous to the cytochrome oxidase subunit I gene (apparently one complete gene plus a truncated version) and to a portion of the mitochondrial 16S rRNA (Fox 1983; Jacobs et al. 1983). As in the example of the yeast, the divergence time (mitochondrial-transferred gene) is recent, being about 25 million years. In mammals, rat liver nuclear DNA was shown to share a common sequence with the mitochondrial DNA (Hadler et al. 1983), and Nomiyama et al. (1985) revealed the presence of mitochondrial DNA-like sequences in the human nuclear genome. The degree of similarity was over 80% when compared to the corresponding bovine mitochondrial sequences, which indicated that the transfer of the segments to the nucleus took place after the human/bovine divergence, that is, fairly recently.

(v) Gene movement among organelles

If 'transforming organelle DNA' can mediate gene transfer between mitochondria and the nucleus, there is a possibility that DNA from either a mitochondrion or a chloroplast can invade the other. Such sequences, present in more than one compartment at a time, represent promiscuous DNA (Ellis 1982; Timmins and Scott 1984). It was the unexpected finding of Stern and Lonsdale (1982) that a 12-kilobase DNA sequence in the maize mitochondrial DNA was homologous (>90%) to part of the chloroplast genome. The sequence contains the coding sequences for isoleucine and valine tRNAs and the 16S rRNA gene. No major polypeptide is encoded in the sequence. It was also reported that the sequence may be essential for normal plant development. Lonsdale et al. (1983) reported that the maize chloroplast DNA sequence coding for the large subunit polypeptide of ribulose-1,5-biphosphate carboxylase was present in the mitochondrial DNA. Both coding and flanking sequences of this gene were demonstrated to be present, and in vitro transcription and

translation was possible. Lacoste-Royal and Gibbs (1985) discovered the same specific chloroplast protein (the small subunit of ribulose-1,5-biphosphate carboxylase) in the mitochondria of the protist Ochromonas. The authors suggest that the DNA sequence coding for this protein has migrated to the mitochondria from the nucleus or chloroplast and that it is expressed there. Translocation of such sequences could take place by a transformation/transposition process or by recombination after mitochondrio-chloroplast fusion (see Ellis 1982). In addition, Ellis suggests that plasmids or virus-like vector DNA could be responsible for movement of the DNA.

It is of interest to note that locust mitochondrion sequences in the nucleus are flanked by highly repeated sequences (Gellissen et al. 1983). In the yeast example (Farrelly and Butow 1983) some, but not all, nuclear mitochondrial DNA sequences lie next to a pair of transposable (Ty) elements. Linear transposon-like elements are known to be located in maize mitochondria (see Stern and Lonsdale 1982). In this regard, Cech et al. (1983) found structural homology between Tetrahymena rRNA and fungal mitochondrial intervening sequences. Cech et al. (1983) forward two explanations for this similarity: either the introns had their origin in transposable elements that were able to enter both nuclear and mitochondrial compartments of the cell, or, alternatively, that the introns had always been present in various mitochondrial genomes and are occasionally transferred to the nucleus. The general evidence points towards the fact that inter-organelle transpositions are common (Lonsdale et al. 1983; Osiewacz and Esser 1984; Nargang et al. 1984; Timmins and Scott 1984). This suggests that active, but not necessarily adaptive movement of DNA sequences from organelles to nucleus, or from organelle to organelle, may take place.

In conclusion, gene transfer as required by the endosymbiotic theories can no longer be regarded as implausible. On the contrary, evidence suggests that intracellular movement of genetic material, like movement of genetic elements elsewhere in the genome, may be a common occurrence.

(vi) The isozyme problem: gene transfer implicated

Evidence for gene transfer has also come from work on the distribution

and comparison of various enzymes. Plastocyanin and plant type 2Fe-2S ferredoxins are present in cyanobacteria and plastids but in no other eukaryotes (Weeden 1981). The presence of the genes for these proteins in the plant nucleus implies that gene transfer has occurred (Weeden 1981). There is strong evidence which suggests that gene transfer may lie behind the presence of plastid-specific isozymes. There are three possible ways of explaining the origin of such isozymes (Harington and Thornley 1982):

(1) One gene is originally part of the host genome and the other an addition by means of gene transfer from the endosymbiont;

(2) duplication of a host gene, followed by divergence and secondary incorporation of an isozyme into the organelle;

(3) transfer to the host and duplication of an endosymbiont gene, with secondary incorporation of an isozyme into the host cytoplasm.

Explanation (1)

This seems to be applicable to a substantial number of cases. Weeden (1981) has listed 18 isozyme pairs involved in carbohydrate metabolism in plants. In such cases, substantial differences between the two isozyme pairs are expected, with one showing a closer affinity to the proposed endosymbiont (cyanobacteria in this case). Such data have been reported for the isozymes of phosphoglucose isomerase (Weeden et al. 1982), glyceraldehyde-3-phosphate dehydrogenase, malate dehydrogenase and superoxide dismutase (Weeden 1981). In the case of the mitochondrion a parallel situation is presented by superoxide dismutase. An analysis by Steinman and Hill (1973) of the N terminals of these superoxide dismutases revealed a very great sequence similarity between the mitochondrial form and that of E.coli. Twenty out of 27 residues are identical. In contradistinction, the amino acid sequence of the cytoplasmic enzyme bears no resemblance at all to either the mitochondrial form or to that of E.coli. (In spite of the complications centred around SOD comparisons mentioned earlier, this basic argument still has some merit.) The only satisfactory explanation of the presence of a 'prokaryotic' gene in the nucleus, such as mitochondrial superoxide

dismutase, is that gene transfer has occurred from endosymbiont to host (Harinyton and Thornley 1980, 1982). In the context of endosymbiosis, Harris and Steinman (1977) and Gray and Doolittle (1982) have drawn attention to the gene transfer and 'primitive feature' (also see p.15 of the present thesis) objections to the above interpretation.

In conclusion, when the sequences, kinetic features and other biochemical details between the cytoplasmic and organelle isozyme are substantially different, one has good reason to suspect that gene transfer is involved.

Explanations (2) and (3) .

In order to distinguish between these and the previous explanation, one needs to compare the sequences involved with those found in free bacteria. This has not always been possible, although other evidence may suggest that the second explanation is more appropriate than the third (see below). Ohno (1970) has discussed in detail the fact that gene duplications can give rise to isozymes. One expects such proteins to be very similar with regard to sequence. In a detailed article on isozymes in plants, Gottlieb (1982) concludes: (a) that more than one enzyme for the same catalytic reaction in a single organelle or in the cytosol is the consequence of either gene duplication or addition of genomes in polyploids, and (b) that the products of duplicated genes will not be present in more than one compartment.

In my opinion, data on aspartate amino transferase (AAT) support these rules. In some plants, such as spinach, four isozymes are present and they are localised respectively in the cytoplasm, the plastids, the mitochondria and the microsomes (see Weeden and Gottlieb 1980). One could assume optimistically that the mitochondrial and plastid isozymes resulted from gene transfer. The microsomal isozyme is presumably the result of a gene duplication since the organelle has no known endosymbiotic origin.

In other eukaryotes, aspartate amino transferase (AAT) also exists as two isozymes: mAAT in the mitochondrion and cAAT in the cytosol, both being nuclear-coded (Sonderegger and Christen 1978). In spite of being analogous to those cases described in Explanation (1), the sequence

similarities are very different: mAAT and cAAT share a considerable sequence similarity, about 50% (Kagamiyama et al. 1977), and have very similar reaction mechanisms and kinetic features (see Sonderegger and Christen 1978). Evidence suggests that these two isozymes - which function in different compartments - are related through a duplicatory event and are thus homologous. Sonderegger and Christen (1978) estimate that this event took place about 600 million years ago, coincident with the origin of vertebrates. Similar duplications in fungi and plants are thought to have taken place independently (see Sonderegger and Christen 1978). Similarly, aldehyde dehydrogenase is also present as mitochondrial and cytoplasmic enzymes. In spite of a high extent of substitutions (about 50%), the two forms seem homologous (see Hempel et al. 1984). Creatine kinase is present as two isozymes, namely, cytoplasmic brain and cytoplasmic muscle. The two enzymes have an identity of 80% (Pickering et al. 1985).

It is possible in both the cases described that the third explanation could be involved and only comparison with prokaryotic will allow a definite decision to be made. It is suspected, however, that the ancestral gene was eukaryotic (Harington and Thornley 1982). This idea is supported by the fact that amino acid degradation (in which this enzyme (AAT) plays an important role) is poorly developed in bacteria (Lehninger 1975). The enzymes mAAT and cAAT are involved in the malate-aspartate shuttle, a specialised system involved in the co-ordination of mitochondrial and cytoplasmic metabolism. If the mitochondrion had a symbiotic origin, it would follow that the malate-aspartate shuttle is a subsequent (secondary) development.

The other enzyme operating in this shuttle, malate dehydrogenase, is also present as three (sometimes four) isozymes in plants, located in the mitochondria, cytosol and microbodies (Gottlieb 1982). Studies by Birktoft et al. (1982) reveal that the nuclear-coded mitochondrial malate dehydrogenase and the cytoplasmic form are homologous. They are also homologous with lactate dehydrogenase. A definitive answer as to whether the original gene was of eukaryotic or symbiont origin is not possible, but the former is more likely. Comparison of the isozymes with the corresponding bacterial protein is necessary. Weeden (1981) mentions

that the enzyme, fructose 1,6-diphosphate aldolase in plastids, resembles the cytoplasmic form more than the prokaryotic type. Pichersky et al. (1984) studied the isozymes of triose phosphate isomerase (TPI). Their results indicate that the plastid and cytosol isozymes evolved from a common ancestral protein. They are also closely similar to sequenced animal TPIs (Pichersky et al. 1984). It is probable that Explanation (2) is relevant in this case. Furthermore, examples of cases where Explanation (3) would be applicable are not common. A possible candidate is cytochrome b_5. Here a mitochondrial and a microsomal form exist (Lederer et al. 1983) and are 58% similar. Cytochrome b is mitochondrially-coded in yeast (Borst and Grivell 1978). The microsomal protein may always have been there, but assuming an endosymbiotic origin, it is debatable that the two cytochromes maintained a 58% similarity from the point of splitting at the progenote stage. Alternatively, it could be possible that the eukaryotic gene evolved from a duplicated transferred prokaryotic gene.

In conclusion, one can state that not all organelle-specific isozymes need originate from transferred genes. There is no reason why a host gene cannot duplicate and have one of the enzymes specialise and become incorporated into an organelle.

(vii) Amino acid synthesis enzymes and gene transfer

Weeden (1981) has proposed that plants derived the genes for amino acid synthesis from cyanobacterial endosymbionts by gene transfer. In the present thesis, it is argued that this view is untenable for at least two reasons: (a) if the mitochondrion had an endosymbiotic origin, one would have expected genes for amino acid biosynthesis present in the ancestral bacterial endosymbionts to have been transferred to the animal (protozoan?) hosts. This does not seem to have taken place; (b) in addition, fungi (which have had no endosymbiotic association with cyanobacteria) are generally able to synthesise amino acids (Burnett 1976). The absence of genes for amino acid synthesis in animals is presumably a result of secondary loss. This is supported by the fact that some groups of bacteria (Lehninger 1975) and fungi (Burnett 1976) have lost the ability to make certain amino acids. In any event,

considering the central role proteins play in the metabolism of all living organisms, it is likely that amino acid biosynthesis is a primitive feature common to archaebacteria, eubacteria and the urkaryotes.

An analysis of sequence similarities of plant, fungal and prokaryote amino biosynthetic enzymes would indicate whether the gene transfer or the primitive feature hypothesis is correct. The same criticisms of Weeden's proposal apply to the genes coding for enzymes responsible for nitrogen and sulphur reduction. Most bacteria (possible endosymbiotic ancestors of mitochondria) and fungi are able to utilise nitrate and sulphate (see Lehninger 1975; Berry 1975; Burnett 1976).

3.5 Natural selection and gene transfer

In most opinions, gene transfer is generally viewed as the physical movement of a DNA segment from an endosymbiont to the nucleus. This, however, represents only half the process. The other, more interesting, part pertains to what selective forces not only make gene transfer a profitable arrangement, but also maintain such genes in the nucleus.

Two observations suggest that natural selection played an important role with respect to gene transfer. Firstly, the process seems to have taken place independently at least five or six times. The presumed endosymbioses of the mitochondria, three different types of plastids, the cyanelles and probably the hydrogenosome have probably been characterised by gene transfer. This is suggested by the small size and coding capacity of the organelle genomes. Secondly, Pelliçer et al. (1980) state that transformed cells express the biochemcial marker for hundreds of generations if the cells are maintained under selective pressure. *In neutral medium the selectable phenotype* is lost at frequencies which range from less than 0.1 to 50% per generation. Clearly, cells having transferred genes in the context of the Serial Endosymbiosis Theory would have to be at a distinct advantage in terms of fitness for them to have spread and to have become dominant in a population.

It is the purpose of this section to attempt an investigation into the advantages offered to host and endosymbionts through the process of gene transfer. This can be done by analysing the 'evolutionary potential' of gene transfer in the context of three types of host-endosymbiont relationship:

(a) A situation where there has been no gene transfer to the sexual host genome.

(b) The position where gene transfer to an asexual host genome has taken place.

(c) The position where gene transfer to a sexual host genome has also occurred.

3.5.1 No gene transfer

In this case, the interior of the cell is inhabited by a potentially large number of cytobionts; for example, the 4 000 endosymbiotic bacteria reported to be present in an amoeba (Chapman-Andresen 1971), and mitochondrial ploidy of 10^3 to 10^5 is reported from mammals (see Olivo et al. 1983). Two possibilities exist as far as the behaviour of the cytobionts is concerned, that is, either the presence, or the absence of cytobiont mating. Recent data (see Selander and Levin 1980) imply an extremely low rate of recombination being similar to mutation rates in bacteria. If this applies to an endosymbiont population, it follows that evolution will take place mainly in terms of clones. Here recurrent mutation (see Maynard Smith 1978) would be the main means of combining favourable mutations. In yeast mitochondria and some plastids, recombination is much more common and can be quite frequent (see Gillham 1978). In such cases recombination advantages would allow mutations from different cytobionts to be combined and deleterious mutations to be removed (Kondrashov 1982, 1984). Other mitochondria, such as in mammals, are not known to recombine (see Cann et al 1984). Gene conversion (see Birky and Skavaril 1976) would be an important phenomenon acting in such a system. Such conversion may result in the loss of a newly arisen mutant, and more rarely to the spread of a variant.

In both cases, segregation and intracellular selection are liable to play dominant roles in so far as gene frequency changes among the cytobionts are concerned. Segregation is a very inefficient process, and it is

estimated (Grun 1976) that about ten times the number of particles of consecutive divisions are necessary to produce homoplasy. Despite this (see Birky and Skavaril 1976), segregation in actual terms may be more efficient due to not entirely random partitioning at division or a lower effective number of segregating particles. In spite of the apparent inefficiency of the cytoplasmic genetic systems, the evolution of present-day cytobionts and organelles takes place under these conditions. The most significant disadvantage associated with this situation is the problem of producing a homoplasmon of a useful cytobiont mutant. The variant has to overcome the masking effect of other alleles before natural selection at the cellular level can act on it.

3.5.2 Gene transfer to an asexual genome

In this section it is initially assumed that a completed gene transfer has taken place. This implies that the gene is present only in the main genome, having been lost from the cytobiont population. Non-transferred genes behave in the manner described previously (section 3.5.1 above). An analysis of the possible evolutionary consequences may indicate whether such a process is feasible. As Mayr (1963) points out, an asexual organism lacks the capacity for recombination and depends on mutation for its variability. It also accumulates deleterious mutations by the Muller's Ratchet mechanism (see Maynard Smith 1978; Kondrashov 1982, 1984). The transferred gene has lost the recombination facilities that may have been provided by the intracellular mating system and is present in one copy per cell. Therefore the pool of genes has been reduced from a potentially large number to one. This has rather unfortunate consequences: (a) mutations cannot be combined; (b) deleterious mutations increase by means of the ratchet mechanism; (c) a deleterious mutation in the transferred gene destroys the host and associated endosymbionts. The advantages in this case are perhaps the fact that the gene is present in two copies in each cell at each generation and that the product is provided to the endosymbionts.

If one ignores the initial assumption, there is no reason why a gene could not be physically transferred to the host genome while copies of it remain in the endosymbionts. Assuming an endosymbiont loses the gene

in question and uses the host-supplied gene product, it may then spread due to the replicative advantage of a smaller genome, or become homoplasmic by segregation. This inevitably leads to the unacceptable state of affairs described above (see section 3.5.2). It is possible that the population would consist of a mixture of cells - those that have transferred some genes and those that have not. The former would perhaps originate continuously but be eliminated due to the inherent disadvantages in the system.

The advantage of the host nuclear genome being able to co-ordinate energy supply (and other factors) by possessing the gene cannot be realised in this environment since the consequent disadvantages are too great. Due to the asexual nature of the host, input of new genetic variation from other hosts is not possible. The variation generated has to be largely endogenous in origin and would depend on mutation, recombination and perhaps invasion of some free potential endosymbionts. It has been pointed out by Bodmer (1970) that recombination in small populations of prokaryotes is much more important than in large ones. Thus, the role of intracellular mating in small endosymbiont populations needs particular consideration in this case.

The conclusion drawn from this section is that gene transfer from cytobionts to an asexual host genome is, in terms of evolutionary potential, not advantageous. In order to maintain some degree of 'plasticity', it would seem better to have multiple copies of the genes located in the cytoplasm, as opposed to a single version in the nucleus. In this way the sources of variation are greater due to a larger population which increases the possibilities associated with mutation and recombination.

3.5.3 Gene transfer to a sexual genome

It has been noticed before (Grun 1976; Taylor 1979; Margulis 1981; Thornley and Harington 1981) that the movement of genes from endosymbionts to a sexual genome poses advantages for the partners in the symbiotic association. Most workers agree that there are distinct advantages associated with sex (see Kondrashov 1982, 1984, for example) and, as commented on by Mayr (1963), the exchange of genetic material between individuals vastly increases the production of variation. This is

coupled with the linearisation of chromosomes as envisaged by Cavalier-Smith (1975), which allows additional variability to be generated by chromosome assortment. The recombination of genes by means of the sexual system provides definite opportunities for a gene (and for the organism which harbours the gene). In association with such an arrangement, the importance of the cytoplasmic genetic system is reduced, and could even become a disadvantage. Intracellular mating and assortment of plasmons is unlikely to provide a better mechanism for inheritance and recombination than the sexual nuclear genome.

The advantages of the host sexual system can be divided into two, namely, that advantage derived from recombination, and those benefits gained from diploidy.

Recombination

Although certain aspects of the importance of recombination are controversial, many workers have come to the conclusion that the process is of advantage to an individual (see Crow and Kimura 1965; Maynard Smith 1968, 1971; Felsenstein 1974). In addition, it is important to note, as pointed out by Maynard Smith (1978), that 'selection must be acting today to maintain sex and evolution'. Clearly, if one cannot define advantages involved in the origin of recombination, there must be present benefits associated with the process. As mentioned in the section on the asexual genome (section 3.5.2), recombination increases variability, removes deleterious mutations and makes more efficient repair possible (Bernstein et al. 1984).

Diploidy

The main characteristic of diploidy is that it results in the presence of two copies of a particular gene in the cell. Accordingly, an asexual system bearing a single cell will evolve differently from a diploid one where two copies of the gene are present.

The role of gene duplication in the origin of proteins with novel functions is well known (Ohno 1970). Two views may be considered here: either duplication precedes the appearance of a protein with a new

function or, alternatively, the appearance of a new function in an existing protein precedes gene duplication (Orgel 1977).

In prokaryotes duplication usually has to occur first in order to create a spare copy, whereas in diploid or polyploid organisms two or more copies are always available (Orgel 1977). These observations do not hold for the endosymbionts, in spite of the fact that the cell is functionally polyploid. This is because the mutant and its 'spare copies' would not be co-ordinately transmitted as in the case with genes on chromosomes. Eventually, but much too quickly for significant evolution to have taken place, the mutant will segregate to form a homoplasmon and, as a result, all the spare copies will have been removed. Lewis and Wolpert (1979) have expanded upon Orgel's idea presented above. Their calculations show that diploids can enlarge their genomes with new genes for new purposes much more rapidly than in haploids. In diploids, due to the presence of a spare copy, duplication need not take place first. Such a spare copy, when mutated, could be maintained by heterosis in parallel with the old allele. After subsequent gene duplication, crossing over will lead to the conservation of the new gene in tandem with the old (Lewis and Wolpert 1979). Sexual reproduction always maintains the diploid state so that the two homologous parts of the genome are prevented from divergence (haploidisation). This ensures the continued existence of duplicate genes.

The expression of a mutant in a diploid is not as problematical as would be the case for a single mutant in a large endosymbiont population. In such a situation, homoplasy would have to be attained by segregation and other stochastic processes. A sexual system, however, can produce homozygotes by syngamy, assortment and recombination. The phenomenon of dominance would allow expression of a gene with the added benefits of concealment of deleterious recessive genes and heterosis.

It is important to point out that many unicellular organisms have a haploid dominant life style (Maynard Smith 1978) which detracts somewhat from the importance of the advantages of diploidy but not of sex. Some protozoa, however, do have a diploid dominant life style (Grell 1973). Margulis (1981) states that one would expect more gene transfer to have

taken place from endosymbionts to sexual than to asexual genomes. This is theoretically the case, but one must note that many asexual eukaryotes probably became so secondarily (Mayr 1963). Perhaps a previous period of sexuality may have been sufficient to allow substantial gene transfer from ancestral endosymbionts to the nucleus of euglenoids and other sexless protozoa. This would seemingly present the organism with most of the severe limitations described above (section 3.5.2). This problem, together with the reasons why sex was lost in the first place, remains unresolved.

3.6 Termination of gene transfer

As already mentioned, there seem to be substantial advantages associated with gene transfer. In spite of this, the process has failed to go to completion in several separate phylogenetic lines, that is, full genic integration has not occurred. This question is one of the main unresolved ones centring around the Serial Endosymbiosis Theory.

The importance attached to this question is borne out by the statement of Borst (1975) that '... we still do not know why these genes should be located on a separate mitochondrial DNA rather than on nuclear DNA. Before this question is answered, I consider further speculation on the evolutionary origin of mitochondria futile'.

The reasons for the termination of transfer are obscure (Borst 1981) and somewhat controversial. As Borst and Grivell (1978) have discussed, the maintenance of a mitochondrial genetic system is costly to the cell: ribosomal proteins, amino acid synthetases, DNA and RNA polymerases, ligases, RNA processing enzymes and those enzymes involved in recombination - at least 90 proteins in all - must be specified by nuclear genes. There is also use of the cell pool of nucleic acid precursors. Similar conclusions hold for the plastid. Also, the genes coding for components of the organelle protein-synthesising system are apportioned between the organelle and nuclear genomes in a highly specific manner (Gillham and Boynton 1981). Separated genomes represent a distinct load. Both mitochondrial and plastid genomes code for RNAs

involved in translation and also for a few proteins. The distribution of mitochondrial protein genes is highly conserved, the only exception being the DCCD binding protein which is mitochondrial in yeast (Gillham and Boynton 1981), but nuclear in Neurospora (Van den Boogaart et al. 1982). In the case of the chloroplast, the small and large subunits of RUBCase are consistently encoded in nuclear and plastid DNA respectively (Ellis 1981a). Finally, in mitochondria, plastids, cyanelles, and probably the hydrogenosome, gene transfer seems to have halted independently in all four cases. Extinction of the cytobionts, as discussed by McLaughlin and Cain (1983), has not taken place. The above considerations suggest that there must be some compelling reasons why the semi-autonomous organelles are maintained.

At least three solutions have been offered and these are dealt with below.

3.6.1 *The Biogenesis Explanation*

Based on the observation that most of the mitochondrially-synthesised proteins are located in the mitochondrial inner membrane (Van't Sant et al. 1981), coupled with the fact that they are very hydrophobic, it has been suggested that these components must be encoded at the site because they are untransportable (Bogorad 1975). A similar situation is present in plastids (Gillham 1978). The fact that no mitochondrial or plastid RNAs are nuclear-coded (with one exception, see Suyama 1982), can be viewed as circumstantial evidence for the contention that untransportability or perhaps difficulties in 'addressing' a product may play a role in organelle maintenance. This however is not applicable to organelle proteins (which, incidentally, force the RNA components to remain in the organelle). In a less specific form, which can exclude hydrophobicity as a reason, this hypothesis implies that certain inner membrane proteins need to be present in optional local concentrations in order to act as a scaffold on to which nuclear-coded proteins can be localised (Bogorad 1975). In conclusion, the basic idea involved here is that the specialised, unique, compartmentalised and double-membraned nature of these organelles requires the synthesis of some proteins at the site of assembly in order to ensure continuity. This explanation

has generally been a mainstay of the Autogenous Theories, whereas the Xenogenous Theories use it chiefly as an explanation as to why transfer is incomplete.

3.6.2 The 'Dead-End' Explanation

Borst and Grivell (1978) and Grivell (1982) have postulated that the mitochondrial genetic system represents an evolutionary dead-end. It would then appear as a frozen cross section of a point in evolutionary history in which the endosymbiont has transferred most, but not all, of its genes to the nucleus (see Mahler 1981). On examining the origin of various subunits of the ATPase complex it was noticed (Borst and Grivell 1978) (a) that at least one very hydrophobic protein is nuclear-coded and (b) that hydrophilic subunits of the F_1ATPase are mitochondrially-coded, but nuclear-coded in yeast. It thus appeared that the suggestion that untransportability lying behind the continued existence of a mitochondrial genetic system was implausible. These observations also cast some doubt on the supposition that there are specific evolutionary reasons explaining the existence of the organelle systems. The reason for the presence of the organelles may, in Borst and Grivell's opinion, be due to a 'dead-end' situation. Also, certain mitochondrial and plastid-located genes are known to have been transferred to the nucleus, indicating that, physically at least, the genes can be moved.

In conclusion, it should be noted that the 'dead-end' explanation is compatible with the Serial Endosymbiosis Theory but in direct opposition to the Autogenous Theories.

3.6.3 The Adaptive Explanation

Morpurgo (1975) suggested that organelles were maintained '... to conserve for some functions a system which permits rapid and efficient adaptation to a changing environment'. Similarly, Mahler and Perlman (1979) and Mahler (1981) maintain that mitochondrial genetic systems are maintained in order to ensure 'extreme conservation of functions while permitting the widest possible latitude and rapid changes in coding and amino acid sequences. This combination is hardly the one expected of

an evolutionary dead-end' (quoted from Mahler and Perlman 1979). Basically, the concept rests upon the idea that the organelle genetic system represents an adaptation, in other words, a selected evolutionary advantage. Mahler (1981) concludes '... only by this device and its inherent enormous flexibility and adaptability could they (mitochondria) have succeeded in maintaining functional invariance in a constantly evolving cellular background'. Sager (1965) has proposed a similar-in-principle reason. Since organelle replication is largely uncoupled from the cell cycle, the organelles can grow in response to environmental stresses. Separate organelle genomes are thus postulated to be maintained in order to provide flexibility in a changing environment.

In summary, these ideas are reconcilable with the Autogenous and Xenogenous Theories.

3.6.4 Appraisal

As has been described in the previous section, the biogenesis explanation in its extreme form can be regarded as untenable. Secondly, the 'dead-end' suggestion suffers from the defect that it begs the question as to why gene transfer ceased. Nor does it explain why the barrier that stopped gene transfer intervened in the origin of the mitochondria and plastids, the other presumed cytobionts which entered into endosymbiosis at different times.

The explanation presented in the present work incorporates elements of both these hypotheses. As has been argued earlier, apart from the empirical evidence, there is good reason to believe that there are distinct advantages in moving endosymbiont genes to the nucleus. Apparently a force of natural selection of some sort has countered the tendency of genes to migrate to the main genome. It is postulated here that the specialised compartmental and double-membraned nature of mitochondria and plastids requires the on-site synthesis of a few definite proteins in order to ensure continuity of the organelles. Thus the present bigenomic distribution of organelle genes can be regarded as an evolutionary stalemate and not a dead-end, where opposing forces of natural selection exist. The equilibrium point of such a

stalemate can differ depending on (a) the prevailing balance of the two selective forces, and (b) stochastic differences. The first consideration could perhaps account for plastids having undergone less gene transfer than animal cells. For instance, animal cells may require rapidly replicating mitochondrial genomes, whereas rate of replication in plants may not be as important. Secondly, certain transfers may differ in the distribution on a random basis, and thus be non-adaptive (but not deleterious). The equilibrium point would become frozen once the divergence of the organelle genetic code from that of the host took place. At this stage it could be considered a 'dead-end' situation because it is to all intents and purposes irreversible. The mitochondria can therefore be considered to be 'dead-end' in the sense that gene transfer is effectively stopped. On this basis, the plastid with a standard code would not be a 'dead-end' situation.

While such an explanation is fairly feasible, [illegible]ive view requires further analysis. With regard to this, at le[illegible]spects may be considered. If the mitochondrial genetic system [illegible] maintained in order to allow 'enormous flexibility and adaptability', why does it apply only to a few subunits of certain enzymes? In addition, it can be argued that the main genome with its sophisticated sexual system has perfectly adequate recombining mechanisms to allow the degree of flexibility required as in immunoglobulins, and advanced repair systems to ensure extreme conservation as in histones.

The rate of mitochondrial DNA evolution is about ten times higher than that of nuclear DNA (Brown et al. 1982) and the silent substitution rate is at least six times greater than that in the nuclear genes for protein coding genes (Miyata et al. 1982). The rate of tRNA evolution is elevated 100-fold. The rate of substitution at replacement sites, however, is as low as for nuclear genes (Miyata et al. 1982).

This increased rate of sequence change is attributable to two main factors. It is probably related to an increased rate of mutation in mitochondria (Brown et al. 1982; Miyata et al. 1982; Cann et al. 1984). The increased mutation rate is probably also related to the transfer of (proto) mitochondrial DNA polymerase to the nucleus (Hasegawa et al. 1984). As these authors point out, once the polymerase coding responsibility is

removed from the (proto) mitochondrion, tRNA genes are freer to mutate because these errors cannot positively feed back on the replication machinery. This is mainly applicable to proteins.

A removal of functional constraints is another reason and applies particularly to RNAs (Brown et al. 1982; Cann et al. 1984). Hasegawa et al. (1984) estimate that the constraints operational on animal *mitochondrial tRNA are about 1/30 of those that apply to cytoplasmic* tRNA. Hasegawa et al. (1984) also mention that the small size of the mitochondrial DNA increases the speed of replication which consequently increases the mutation rate.

Williams (1966) argues strongly against the idea of selection for increased mutation rates and, on the contrary, suggests that natural selection tends to reduce mutation rates towards zero. Rose and Doolittle (1983) have also attacked the idea that mutations are advantageous (that is, selected). Population geneticists have analysed the possibilities of optimised mutation rates. In asexual organisms mutation rates can be optimal rather than minimal (Leigh 1970). In the absence of recombination, mutation rates can increase, possibly due to a hitch-hiking effect. This mechanism acts in such a way that a mutator gene may be spread by being linked to an advantageous mutant gene. In this view, mutation rates may have been optimised in an asexual population of cytobionts.

Once recombination takes place, mutation rates are reduced towards a minimum (Leigh 1970). In yeast mitochondria (perhaps representative of primitive forms) recombination is very substantial and one could conclude on the basis of population genetics theory that the high mutation rate manifested is not a consequence of selection; that is, an adaptation. It is instructive to consider mutation (in organelles at least) as an effect of unrelated trends. In this regard Rogers et al. (1985) believe that there is an inverse relationship between genome size and mutation rate as exemplified in the mycoplasmas. Thus gene transfer (an adaptation) may be responsible for the high rate of change in mitochondrial DNA sequences by means of several secondary effects such as: small genome size, small coding responsibility, freedom from the great error loop (Hasegawa

et al. 1984), inefficient repair, high turnover rate (see Brown et al. 1982), and drift in the genetic code (Gray et al. 1984; Rogers et al. 1985). As argued cogently by Reanney et al. (1983), these phenomena, particularly elevated mutation rates, are probably the result of noise generated in the replicative and translational mechanisms. The intrinsic noise-generated pressure is a problem which most systems have eliminated as far as possible and not a factor that is encouraged by selection.

If the transfer equilibrium point occurred where it is today, that is, with a mitochondrial coding task of about 13 proteins (Brown et al. 1982), a lack of functional constraint would occur fortuitously. As pointed out by Jukes (1981) and Cann et al. (1982) (quoted in Brown et al. 1982), such a tiny genome coding for a few proteins only, is much less restrained than a system coding for thousands of proteins, particularly if fewer regulatory elements are present. This reasoning also applies to the mycoplasmal use of UGA as a tryptophan codon (Rogers et al. 1985). The final result is rapid change in tRNA sequences and drift in the genetic code (Cann et al. 1984).

Gray et al. (1984) have noticed that the loss of genetic information by the cytobiont may be related to the origin of the variant genetic codes. If the genes coding for modification enzymes are lost, uridine in the wobble position on certain tRNAs would remain unchanged and would subsequently provide an important mechanism in the reversion of the code. Clearly the divergence of the code may have been caused by different but related conditions such as:

(a) loss of a large part of the genome, that is, reduced coding responsibility (quantitative effect)

(b) loss of specific genes, such as error repair, replication or tRNA modifying genes (qualitative effect).

The non-adaptive phenomenon of code divergence would thus freeze the transfer process at that point. The present-day pattern of codon usage in mitochondria (that is, with UGA being a tryptophan instead of a termination codon universally in the organelles) suggests that a great deal of gene transfer took place very early in eukaryote evolution. The reduced genome drifted to fix this codon as a tryptophan specifier prior to phylogenetic divergence. Other changes (see Jukes 1981) took place

later. With respect to Sager's suggestion that extrachromosomal systems are adaptive in order to respond to environmental stress, there is little justification for such a view. Even single cells such as E.coli can respond notoriously quickly to a new environment such as an elevated sugar concentration. Reaction in terms of gene control systems should suffice.

The final conclusion drawn here is that the features of rapid change are fortuitous and that constraint is enforced by natural selection on the remaining genes. Lack of such leads to extinction. The properties of the mitochondrion (and the plastid for that matter) already discussed are possibly the result of the organelle being forced to maintain a few protein genes for reasons of biogenesis.

3.7 Functions and effects associated with extrachromosomal plasmons and the process of gene transfer

Given the fact that the eukaryotic cell contains semi-autonomous plasmons, certain consequences follow. These are related to the basic phenomenon of endosymbiosis and gene transfer. The former process confines individual symbionts within a cell which subsequently have to be selected as a group. Gene transfer conversely 'individualises' a great many endosymbiont genes.

3.7.1 Indirect selection acts on the cytobionts

Due to the confinement of a population of cytobionts within a cell, it is virtually impossible to select against an individual endosymbiont at a particular instant. This allows a degree of freedom with respect to mutation and selection. Selection pressure is not as severe because the system is functionally polyploid. To quote Ohno (1970) 'Policing by natural selection becomes very ineffective when multiple copies of the gene are present'. Basically, selection acts on the average properties of the group unless a homoplasm is formed. The free drifting nature of much of the mitochondrial genome in yeast, for example, is well documented (see Harington and Thornley 1980), whereas the human mitochondrial DNA is more 'streamlined' (Borst and Grivell 1981b).

3.7.2 Evolutionary rates in the nucleus and cytoplasm must be co-ordinated

The plastid and mitochondrion represent a unique situation in that the two genomes (the organelle and the nucleus) code for different components of the same enzyme. Because the subunits of the enzyme interact with each other, changes in one must be reflected by accommodations in the other. The nucleus, which codes for most of the proteins involved, will dictate the lower evolutionary rate. The high rate of sequence change in mitochondria could conceivably cause difficulties in maintaining coevolution between organelle and host encoded enzyme subunits. The clearest data known with regard to this point is the work of Cann et al. (1984) on the human mitochondrial genome. These authors report that the cytochrome oxidase subunit 2 is evolving unusually fast in primates as opposed to rodents or ungulates. Cytochrome c, which interacts directly with subunit 2, is nuclearly-encoded and has also evolved faster in primates than in rodents or ungulates. Secondarily, the nuclear-coded mitochondrial ribosomal proteins have changed more rapidly than is known for conventional ribosomal proteins, reflecting the high rate of change of mitochondrial rRNA (Cann et al. 1984). A study of the restriction endonuclease patterns of mitochondrial and chloroplast DNA in Zea led Timothy et al. (1979) to conclude that organelle DNAs evolve in concert with the nucleus, but not necessarily with each other.

3.7.3 Parasexuality

Presumably as a consequence of recombination in free prokaryotes, both yeast mitochondria and chloroplasts fuse and recombine genetic material (Gillham 1978). While mutation can generate the raw material for evolution, recombination brings together independent favourable mutations and helps to eliminate deleterious variants. This process was probably more important in the earlier stages of endosymbiosis. The recombination of yeast mitochondrial DNA does not require mitochondrial protein synthesis (Strausberg and Birky 1979) and the genes for this process are presumably found in the nucleus.

The significance of parasexuality seems to be greater in unicells such

as yeast and Chlamydomonas than in higher organisms where it may be absent as in mammals (see Cann et al. 1984). Many eukaryotes are uniparental with regard to mitochondrial or plastid inheritance, as indicated by many authors (Bogorad 1975; Tilney-Bassett and Abdel-Wahab 1979; De Francesco et al. 1980; Giles et al. 1980; Reilly and Thomas 1980; Grant et al. 1980; Sears 1980b; Ferris et al. 1982; Kuroiwa et al. 1982). It is possible that the proteins enclosed in the organelle are sufficiently conserved and have reached a stage of stability or are already changing that makes the input of more variation redundant. While any nuclear-coded gene products that interact with the organelle-coded versions will have to evolve in concert, other nuclear-coded proteins are free to evolve as rapidly or as slowly as required. In opposition to the above, it is important to point out that uniparental inheritance may be an effect and not adaptive in the first place. Birky (1983) has found that uniparental inheritance may be due to drift but in other taxa, such as Chlamydomonas, specific mechanisms to ensure that the process takes place, seem to be at work.

3.7.4 Gene constancy and size variation

There seem to be distinctly different forces of natural selection that govern the nature of genes maintained in the organelle DNA, as opposed to those affecting the size. The presence of particular genes in organelles (as argued earlier) is perhaps related to reasons associated with biogenesis. As expected, the presence of genes in the mitochondrion is conserved in many eukaryotes (see Mahler 1981; Borst and Grivell 1981b), and Stern and Newton (1985) found a 'core' of DNA present in all cucurbit mitochondrial DNAs examined, in spite of other variations in size. Similarly, the size of other mitochondrial (and plastid) DNAs is very variable (Gillham 1978; Mahler 1981). In particular, fungi demonstrate a wide range of size (see Clark-Walker et al. 1983), ranging from 19 kbp in Torulopsis, through 68-78 kbp in yeast (Saccharomyces) to 108 kbp in Brettanomyces. In the Cucurbitaceae seven-fold mitochondrial size variation is known (Stern and Newton 1985).

In yeast the large size is largely due to substantial non-coding sectors which are often AT-rich (Borst and Grivell 1978; Fox 1981), and due to

introns (Borst and Grivell 1978). The mitochondrial genome of Aspergillus is smaller than those of other fungi studied and the smaller size is due to the fact that the genome has shorter intergenic spacers and fewer introns (Brown et al. 1985). Even more extreme is the human mitochondrial genome which is very compact (Borst and Grivell 1981b; Fox 1981). It was suggested (Thornley and Harington 1981) that the repetitive nature of many mitochondrial DNAs may be due to the absence of natural selection, with consequent duplications being caused by unequal crossover along the lines described by Smith (1976). Lonsdale et al. (1983) stated that intramolecular recombination could be responsible for the fact that the maize mitochondrial genome can exist as one or three circular molecules. Sites of homologous intragenomic recombination at repeated sequences in several plant mitochondrial genomes have been postulated (see Stern and Palmer 1984; Falconet et al. 1984).

Apart from intragenomic recombination, other mechanisms have been postulated to contribute to the variation in size of organelle genomes. Tzagoloff et al. (1979) thought that AT-rich regions are formed by degeneration of vestigial genes, and Lonsdale et al. (1983) suggest that the uptake of foreign DNA may be involved. Whatever the origin of the spacer DNA (which is responsible for size variations), it is postulated here that they are either caused by fortuitous molecular events (ignorant DNA) or that they are parasitic sequences and that a certain laxity of selection allows them to develop. In keeping with this view is Clark-Walker et al.'s (1983) suggestion that a small ancestral mitochondrial DNA gave rise to the larger genomes known.

Several factors are involved in the primary reduction in the size of the cytobiont DNA. Gene transfer and degeneration of genes due to host takeover are perhaps the most important. Further than this, the size appears to be dependent on the precise selective regime present which may be stringent (as in human mitochondrial DNA) or less so (as in yeast).

CHAPTER FOUR

PROTEIN TRANSFER FROM TRANSFERRED GENES INTO ORGANELLES

Gene transfer pertains to the physical integration of endosymbiont DNA into the host nucleus as well as to the maintenance of such sequences in the genome. Maintenance itself also depends on two conditions: a selective advantage ought to be associated with the transfer, and proteins encoded by the transferred gene must be able to enter the proto-organelles. Thus, the development of a system which allows Transferred Gene Encoded Proteins (TGP) to enter the endosymbiont is a prerequisite in the transformation of prokaryotic cytobionts into DNA-containing organelles.

In spite of the fact that this is a crucial part of the Serial Endosymbiosis Theory, it has been given very little attention. Reijnders (1975) noted it as a complication of the Serial Endosymbiosis Theory and Bogorad (1975) discussed it mainly in the context of biogenesis. Cavalier-Smith (1980, 1982) and Harington and Thornley (1982) deal with the problem in more detail.

4.1 Nature and origin of delimiting membranes

Both mitochondria and plastids consist of a double membrane surrounding a matrix. In the case of the mitochondria the outer membrane resembles the endoplasmic reticulum (Tzagoloff 1982) while the inner membrane is more prokaryotic in nature. In particular, the inner membrane is rich in cardiolipin (Gillham 1978), a phospholipid characteristic of bacterial cell membranes (Lehninger 1975). Cholesterol and phosphatydyl inositol show the opposite distribution to that described above (see Lehninger 1975; Tzagoloff 1982 for details). The outer membrane of mitochondria is impermeable to compounds with a molecular weight of 2 000 or less (Tzagoloff 1982) and the inner membrane is even less permeable to molecules (Chua and Schmidt 1979). A similar situation is found in the

chloroplast (Chua and Schmidt 1979). According to the Autogenous Theory these differences were in the past ascribed to two factors: that the mitochondrial DNA codes for the inner membrane, and that such genes have undergone divergent evolution (Uzzel and Spolsky 1974). As has been discussed in the previous section (Ch. 3), the mitochondrial DNA codes for so few proteins that this cannot be a viable explanation. In terms of Xenogenous Theories the inner membrane of the organelle is derived from the cytobiont whereas the outer, as a consequence of phagocytosis, is host-derived (John and Whatley 1975). Taylor (1974) is more specific in that he considers the inner organelle membrane to correspond to the outer endosymbiont membrane, whereas Whatley et al. (1979, fig. 1) suggest the opposite. The latter is probably more accurate considering that the respiratory chain is located in the inner membrane (John and Whatley 1975). This contention is supported by the fact that the majority of present-day endosymbionts are enclosed within a host vacuole and very few examples are known where encircling host membranes are absent (Smith 1979). Cyanelles (which are so organelle-like that some consider them as chloroplasts, such as Trench 1981) are housed within a host vesicle (Trench et al 1978; Jaynes and Vernon 1982). The chlorellae *in Paramecium are also found within vacuoles (Karakashian and Rudzinska* 1981).

With respect to mitochondrial-like symbioses, the bacterial cytobionts of the amoeba Pelomyxa are surrounded by a host vesicle (Whatley et al. 1979) and in the Homoptera a host membrane is known to envelop the cytobiont (Houk and Griffiths 1980). The reasons for this widespread envaculation are unclear, but it is possible that it protects the cytobionts and allows the host a measure of control over the endosymbionts (Smith 1979), such as regulation of metabolite movement (Taylor 1974). After observing that lysosomes fail to fuse with symbiont-containing vacuoles in Paramecium, Karakashian and Rudzinska (1981) postulated that the cytobionts altered the vacuole membranes around them to prevent fusion. The presence of three, four or five membranes around the thylakoids of chloroplasts (Whatley 1981b) has also been interpreted in terms of phagocytosis of symbionts.

In chromophyte algae, four bounding membranes to the plastid are present. It has been proposed that from the inside, the membranes represent those

of the prokaryote symbiont, the vacuole of the first host, the plasmalemma of the first host and the vacuolar membrane of the second engulfing host (see Whatley 1981b for a detailed analysis of this point).

Recently, the assumption that the inner and outer membranes of the mitochondrion and plastid are derived respectively from the prokaryote and the host, has been questioned. Gibbs (1981) considers the double membrane of the chloroplast as representing the two membranes bounding bacteria. Trench (1981) stated that '... the concept that the outermost chloroplast envelope is homologous with the host vacuolar membrane is little more than speculation, even though the outer and inner chloroplast envelope membranes have distinct properties'. Cavalier-Smith (1982) agrees with this and discusses the problem in detail. In such cases the phagocytically-derived vacuolar membrane has been lost or never existed in the first place.

Concerning the eukaryotic nature of the outer membrane, it is feasible that such a character may be a secondary development. It is notable that while the mitochondrion synthesises cardiolipin (for the inner membrane), the other major phospholipids are synthesised in the endoplasmic reticulum. With the outer membrane continually in contact with the cytosol it may acquire some of the characteristics of the endoplasmic reticulum. As a possible example, the enzyme NADH-cytochrome b_5 is immunologically identical with that found in the Endoplasmic Reticulum (ER) (see Chua and Schmidt 1979). The two membranes in mitochondria and chloroplasts are closely and regularly spaced (about 60Å), which is distinctly different from vacuolar membranes around endosymbionts (Taylor 1974) which are less narrow. From a bioenergetic point, two closely spaced membranes such as those found in bacteria would be more efficient than a widely-separated arrangement. Perhaps the loss of the vacuolar membrane is relatrd to the specialised chemiosmotic functioning characteristic of mitochondria and chloroplasts.

In conclusion, the matrix of the mitochondrion is surrounded by two membranes while the chloroplast may be bounded by two, three, four or five (Whatley and Whatley 1981).

Whatever the origin of the membranes, Transferred Gene Encoded Proteins (TGP) had to pass through these barriers for genic integration to proceed and to allow the degeneration of the endosymbiont genome.

The presence of a prokaryotic cell wall initially would not have been too problematical since it is largely permeable to large molecules and metabolic compounds. The cell wall would cease to be of much use once the prokaryote was established in an osmotically buffered environment and would become an impediment to transfer processes and be metabolically wasteful. In present-day cyanelles a highly reduced cell wall remains (Cyanophora), or is absent (Glaucocystis) (Trench 1981). In the metazoa a highly reduced cell wall is found around aphid symbionts thought to be derived from gram negative bacteria (Houk and Griffiths 1980).

4.2 Protein movement across membranes - general features

It is interesting that the import of proteins into mitochondria and chloroplasts differs largely from that elucidated for secreted proteins. In the latter case, the signal hypothesis (Blobel and Dobberstein 1975) in general has proved to be a viable explanation. The mechanism proposed is a cotranslational one, and transport across or into the membrane is dependent on, and takes place simultaneously with, the elongation of the protein (Blobel and Dobberstein 1975). Secreted proteins bear a signal sequence on the amino terminal, which is composed of a substantial number of hydrophobic amino acids (Ellis 1981b). The signal sequence consists of two parts (Von Heijne 1981), a basic N terminus and a highly non-polar central part. The length of the signal sequence is usually between 20-40 residues (Watson 1984). All signal sequences can be divided into three basic regions: a positively charged N-terminal region, a central hydrophobic region and a more polar C-terminal region that seems to define the cleavage site (Von Heijne 1985). Because signal sequences lack homology, it is thought that the secondary or tertiary structure is important in recognition interactions (Emr and Silhavy 1983; Walter et al. 1984). To complicate the issue, Brown et al. (1984) have shown that alterations, which would have caused significant changes in the secondary structure of the leader, did not affect secretion. As the signal

sequence emerges from the ribosome it binds to the membrane via an interaction with specific receptors located in the membrane (Blobel and Dobberstein 1975). This receptor binding is mediated by a signal recognition particle (SRP) which contains 7S RNA and six proteins (Walter and Blobel 1982). The SPR binds to the SRP receptor, also known as the docking protein (Walter et al. 1984). Once the nascent polypeptide penetrates through the membrane, a signal peptidase removes the signal sequence and the protein assumes its proper conformation (Ellis 1981b; Walter et al. 1984). This description accounts for the basics of the process. Less common complications such as internal signal sequences (see Von Heijne 1981; Talmadge et al. 1981) or the fact that this sequence may be necessary, but not sufficient to allow export (Moreno et al. 1980), are not considered here.

An important aspect of the signal hypothesis as outlined above is the fact that it involves the presence of specific receptors in the appropriate membrane. These receptors (Blobel and Dobberstein 1975) may form a tunnel in the membrane.

An alternative to the usual signal hypothesis is discussed by Von Heijne (1981). Although signal sequences have an overall hydrophobic character in common, it is not clear how so little information allows a specific protein-protein interaction to take place. It was further postulated that the non-polar part of the signal sequence partitions into the lipid bilayer as it emerges from the ribosome. On translation the rest of the protein is pushed through and the signal cleaved off. In this model, the signal sequence assumes a passive role as opposed to that expressed in the earlier hypothesis. Ellis (1981b) does not like this idea on the grounds that receptors are required to allow sufficient specificity for correct compartmentation. Randall and Hardy (1984) also believe that a strict cotranslational requirement is unnecessary in the signal hypothesis. These authors have proposed a model that owes elements to both the standard signal hypothesis and to the membrane trigger hypothesis of Wickner (1976, 1980). The trigger hypothesis involves post-translational or partly post-translational insertion into the membrane. The main function of the signal sequence is to confer solubility on a protein and not to mediate binding (see Randall and Hardy 1984). Some data oppose this view, for example the observation that post-translational export of

some periplasmic proteins in E.coli is not possible after cotranslational export was prevented (Pages et al. 1984).

It is of interest that cotranslational secretion in pro- and eukaryotes is very similar, implying that the system is conserved and very ancient (Talmadge et al. 1980; Pages et al. 1984; Walter et al. 1984). Watts et al. (1983) provided additional pointers to the conserved character of the signal system. They found that bacteriophage M13 coat protein and mouse IgG kappa chain fragment precursor are both processed by E.coli leader peptidase. This implied evolutionary conservation of signal peptidases and the properties of the pre-proteins which make them cleavable. In bacteria most secretion takes place across the cytoplasmic membrane into the periplasmic space and in eukaryotes into the endoplasmic reticulum (Davis and Tai 1980).

4.3 Incorporation of proteins into organelles

The fact that synthesis of organelle proteins and import are not linked (Borst and Grivell 1978) suggested that a signal type translocatory mechanism may not be involved. Unlike the signal hypothesis, a completed polypeptide chain is released into the cytoplasm (Neupert and Schatz 1981). Import of proteins into the DNA-containing organelles is significantly different from secretion processes characteristic of pro- and eukaryotes.

4.3.1 Precursors of proteins

Cytoplasmically-made mitochondrial proteins can be divided into two groups: those that are made as larger precursors and others that lack extensions. Basically, these groups correspond to proteins which are translocated into the matrix, the inner membrane and the intermembrane space, and those integrated into the outer membrane (Gasser and Schatz 1983). *Outer membrane proteins (OMM) do not require an energised inner* membrane nor a trypsin-sensitive component and are not processed (Schatz and Butow 1983). The other group of proteins requires N-terminal cleavage and an energised inner membrane for translocation (Schatz and Butow 1983; Gasser and Schatz 1983). A substantial number of differences

distinguish these two groups of proteins.

(i) Absence of extensions

This group is composed mainly of outer membrane proteins. The yeast OMM porin, studied by Mihara et al. (1982), does not appear to have a larger precursor. The authors believe that an uncleaved signal sequence is present to allow for its positioning as a protein spanning the OMM. A similar position exists for other proteins such as the yeast 29-kilodalton OMM (Gasser and Schatz 1983). Hase et al. (1984) found that all the information required for targeting and anchoring a specific OMM (the 70 kd protein) was contained within the amino terminal 41 amino acids. A foreign protein such as β-galactosidase fused to this stretch was correctly targeted. Some proteins in this group are not OMM. Cytochrome c (Schatz and Butow 1983) has no larger precursor but is imported into the intermembrane space as the apoprotein upon which the prosthetic group is added. The Neurospora mitochondrial ADP/ATP carrier has no larger precursor but, unlike the cytochrome c case, requires a mitochondrial membrane potential for import into the inner membrane (Zwizinski et al. 1983). 3-Ketoacyl-CoA thiolase, 2-isopropylmalate synthase and carbamoyl-phosphate synthase are matrix enzymes and are not made as larger precursors (see Hampsey et al. 1983; Mori et al. 1985).

(ii) Larger precursors

Studies using cell free systems demonstrated that most mitochondrial proteins are synthesised as larger precursors (Tzagoloff 1982). These protein molecules usually have an N terminal extension and are more hydrophilic than the mature protein (Van't Sant et al. 1981). Such precursors are known for matrix inner membrane and intermembrane space proteins (Neupert and Schatz 1981). As one example, mitochondrial RNA polymerase (a matrix enzyme) is synthesised as a precursor 2 000 daltons larger than the mature product (Lustig et al. 1982) and the citrate synthase precursor is between 2 and 4 kd larger (Suissa et al. 1984), and Neupert and Schatz (1981) list several other proteins made as larger precursors. Yet another example of such a protein is rat liver ornithine carbamoyltransferase, the precursor of which has a 3 400 - 4 000 dalton

N terminal extension (Miura et al. 1983). Sulphite-oxidase an intermembrane space, is synthesised as a precursor, 3 000 daltons heavier than the final product (Ono and Ito 1984a).

The sequence of the N terminal extension of a matrix protein ATPase proteolipid is known (see Schatz and Butow 1983) and consists of 66 amino acids which render the protein more basic and hydrophilic. A similar situation is noticed in the case of the chloroplast. The RUBCase small subunit of the chloroplast is synthesised as a precursor which has a 44 amino acid extension on the N terminal (Chua and Schmidt 1979). This extra piece renders the protein more basic. Plastocyanin is made with a transit peptide 66 residues long (Smeekens et al. 1985). Precursors are also known for other chloroplast proteins, namely ferredoxin and the apoprotein of the chlorophyll-protein complex II (Schmidt et al. 1979). The extension of plastocyanin is thought to have a weight of 15 000, making it the longest extension known for either the chloroplast or for mitochondria (Bennet 1982). The 22-kd heat shock protein of Chlamydomonas that is associated with the chloroplast is made as a 26-kd precursor (Kloppstech et al. 1985).

(iii) Nature of the extension

The signal sequences studied in secreted proteins have a few common features and a marked degree of hydrophobicity (Waksman et al. 1980) is the most noticeable. Within the central part of the sequence a highly non-polar part of ten to 15 residues is generally present (Von Heijne 1981) but no similarity in primary structure is discernible. With regard to secondary structure, Emr and Silhavy (1983) have demonstrated the importance of a stable α-helical conformation in the central hydrophobic region of the signal sequence. The above facts apply both to pro- and eukaryotes.

The pre-sequences or transit peptides (Schmidt et al. 1979) that are characteristic of organelle proteins synthesised in the cytoplasm are also variable in sequence. In chloroplasts the sequence is not conserved (Ellis 1981a) and the transit peptides of Pisum and Chlamydomonas reveal no homology (similarity), although the protein proper is very similar. The transit peptide is highly charged and hydrophilic as opposed to the

signal sequence (Anderson 1981). The pre-sequence plays a crucial role in targeting and can be located at either the N or C termini, or at both (Ellis 1981b) but is usually present as an N terminal extension. Fusion experiments done by Van den Broek et al. (1985) (see also Ellis 1985) have shown that a foreign protein connected to a transit peptide is correctly targeted into the chloroplast. Similarly, Hurt et al. (1984) fused the amino terminal 53 amino acids of subunit four of yeast cytochrome c oxidase to the mouse cytosolic enzyme dihydrofolate reductase. The non-mitochondrial enzyme was successfully transported into the mitochondrion. More recently, Hurt et al. (1985) found that fusion of less than half of the pre-sequence was effective as described above.

4.4 Importation of organelle proteins

After the precursor has been synthesised it is released and binds to a receptor on the organelle envelope (Schmidt et al. 1979; Hennig and Neupert 1981; Schatz and Butow 1983). Recognition is perhaps mediated by the extra sequence (Chua and Schmidt 1979) or some other feature of the tertiary structure (Ellis 1981a). Cytochrome c, for example, is recognised by a receptor via structural features characteristic of the apoprotein (Matsuura et al. 1981) rather than as a larger precursor. Extra-mitochondrial precursors have to interact with receptors on the mitochondrial surface as a first step of their transfer into the organelle (Neupert and Schatz 1981; Hennig et al. 1983).

Particularly interesting with respect to the role of overall structure in transport is the observation that the mitochondrial isozyme of aspartate amino transferase has evolved more slowly than the cytosolic isozyme (Sonderegger and Christen 1978). Similarly, the plastid isozyme of phosphoglucose isomerase is far less variable than the cytosolic version (Gottlieb 1982). It is thought that this difference (and probably that in the AAT example) may be related to requirements for transport across the organelle envelope (see Gottlieb 1982).

4.4.1 Receptors

Together with the transit peptide, the receptors are thought to ensure that the protein is transported to specific destinations within the organelle. Neupert and Schatz (1981) have suggested that different receptors may be involved, depending on whether a protein is transported across one or both membranes. Competition experiments suggest that there are different receptors corresponding to different compartments (Schatz and Butow 1983). Lately, more specific data on mitochondrial receptors have become available. Work on the intermembrane space (apo)cytochrome c (Hennig et al. 1983) has indicated that a limited number of highly-specific binding sites for the protein are present. The density of the receptors was found to be 600 per μm^2 of mitochondrial surface. Transfer into the mitochondrion took place directly from these binding sites.

Other receptors have also been reported, such as the Neurospora inner mitochondrial membrane ADP/ATP carrier (Zwizinski et al. 1983) and the rat liver mitochondrial matrix protein, ornithine carbamoyltransferase receptor (Argan et al. 1983). The last-mentioned protein also seems to require a cytosol factor for recognition and/or uptake. Similarly, the import of the β subunit of mitochondrial ATPase is dependent on the presence of a cytosolic factor (Ohta and Schatz 1984). Gasser and Schatz (1983) have presented evidence to suggest that receptors for the yeast 29-kd outer mitochondrial membrane protein exist, and Mihara et al. (1982) have noticed a membrane specificity for the yeast mitochondrial protein, porin. Ono and Ito (1984b) have detected specific binding on the outer *mitochondrial membrane for pre-sulphite oxidase. Mori* et al. (1985) present evidence which suggests that more than one protein may share a common receptor. By means of competition experiments they demonstrated that 3-ketoacyl-CoA thiolase, ornithine carbamoyltransferase, medium chain acyl-CoA dehydrogenase and acetoacetyl-CoA thiolase are transported into mitochondria by a common pathway. Interaction between the precursor and the receptor is thought to open a pore which allows the protein to pass through the membranes. This translocation requires energy, either ATP (Borst 1981; Neupert and Schatz 1981) or, more likely, an electro-chemical gradient (Schatz and Butow 1983), as in the ADP/ATP carrier protein (Zwizinski et al. 1983). Finally, the precursor (if larger) is

converted to the mature protein by proteolytic cleavage or by the addition of a heme or similar group. These effects result in conformational changes which trap the final product (Neupert and Schatz 1981). The processing is done by a highly specific protease (Neupert and Schatz 1981). This protease itself is imported from the cytoplasm (see Yaffe et al. 1985) and although essential for the growth and assembly of mitochondria, is not so for import. In chloroplasts the cleavage is performed in two steps by a soluble protease (see Robinson and Ellis 1984; Van den Broek et al. 1985). Similarly, in mitochondria the processing of the ATPase subunit 9 occurred in two sequential stages via an intermediate-sized polypeptide (Schmidt et al. 1984). Ono and Ito (1984a) could not detect an intermediate in the processing of sulphite oxidase but this could have been because the size difference was too small to have been detected (Ono and Ito 1984a).

Intermembrane space proteins such as cytochrome b_2 and c_1 are first transported across the inner membrane where they are processed prior to release into the intermembrane space (Gasser et al. 1982). Outer membrane proteins are different from others in that import seems to be energy- and receptor-independent and requires no proteolytic processing (Schatz and Butow 1983) as in the 29-kd outer mitochondrial membrane protein (Gasser and Schatz 1983).

The type of protein import described here is called vectorial processing (see Waksman et al. 1980) and results from the (scalar) modification of precursors on only one side of a membrane. This drives the transport in one direction. The processing mechanism can be proteolytic removal, disulphide bridge modification or covalent modification of a prosthetic group.

In conclusion, one can consider some important features of vectorial processing:

(1) The mechanism operating in mitochondria and chloroplasts is very similar;

(2) Precursors are usually larger than the mature protein due to the presence of a transit peptide;

(3) Receptors are thought to exist on the organelle envelopes;

(4) The mechanism bears little resemblance to vectorial translation (the signal hypothesis).

4.4.2 Transmembrane movement of Transferred Gene Encoded Proteins

Although the mechanisms described in the previous section allow translocation of organellar proteins, the central question that arises is how host-translated TGP penetrated the endosymbionts. Because evolution cannot anticipate, it is unlikely that TGP-receptors were present on the inner and outer membranes of the endosymbiont, which previously existed in isolation of the host. The conversion of endosymbiont into proto-organelle can conveniently be considered under four headings:

(i) The initial mechanism of import of TGP into the organelles
(ii) The origin of organelle protein receptors
(iii) Possible contamination of proto-organelles by host proteins
(iv) The relationship between mitochondria and plastids.

(i) The initial mechanism of import of TGP into the organelles

Since receptors were not present on the proto-organelle membranes, by elimination is left the alternative, namely, that TGP spontaneously translocated across one, or both, the membranes encapsulating the endosymbiont. While most workers on organelle and secretory proteins stress the importance of receptors, others such as Wickner (1976, 1980), Engleman and Steitz (1981) and Von Heijne (1981), emphasise the role of spontaneous insertion into membranes. If part of a protein is partitioned from an aqueous environment into the non-polar membrane interior, a release of free energy can take place when a hydrophobic helix is buried (Von Heijne 1981).

A similar, but less restrictive, idea is the membrane trigger hypothesis of Wickner (1976, 1980), which suggests that the signal sequence might alter the folding of a protein so that it refolds on coming into contact with a membrane. This change in conformation induces the protein to pass through the membrane, with polar groups perhaps being shielded. As Von Heijne (1981) has remarked, this hypothesis requires no translation machinery since the ability to become wholly or partly translocated would be an inherent property of the protein. No requirement for

ribosome-membrane interaction is required. The best example of this mechanism is that of the phage, M13 coat protein (Wickner 1976, 1980). The most detailed exposition of a spontaneous insertion model has been given by Engleman and Steitz (1981). They have divided membrane proteins into three classes: secreted proteins, anchored membrane proteins, and globular membrane proteins. Organelle proteins fall into all three classes but all matrix, inner membrane and intermembrane space proteins can be regarded as being secreted. Engleman and Steitz (1981) have proposed the Helical Hairpin Hypothesis to explain how proteins could spontaneously enter and cross a membrane. According to this idea, the polypeptide chain folds in an aqueous environment to form an anti-parallel pair of helices. Such helices can be formed anywhere within a polypeptide but in proteins with a leader peptide, one of the helices will be formed by it. The helical hairpin inserts spontaneously into the lipid interior due to hydrophobic interactions. When this occurs the free energy arising from burying hydrophobic helical surfaces must be greater than the free energy cost of burying charged and hydrogen-bonded groups. The two arms of the hairpin must be 3_{10} or α-helices to allow insertion. If the second helix is polar, secretion will take place until a hydrophobic segment is encountered. Integral membrane proteins without a leader may insert single hydrophobic helical segments as a spontaneous process.

The folding of a polypeptide after synthesis may prevent insertion. Thus, cotranslation (or perhaps a transit peptide) could stop the new polypeptide from folding in a stable fashion. It is important to stress that the helical hairpin can arise within a protein and not involve the C or the N terminal. Therefore 'because of the distribution of polar and non-polar sequences in the polypeptide sequence, secretion and the insertion of membrane proteins are spontaneous processes that do not require the participation of additional specific membrane receptors or transport proteins'(Engleman and Steitz 1981).

The mechanism described here may have allowed TGP to enter the endosymbionts. The TGP that form part of the outer membrane, the *intermembrane space and the inner membrane were perhaps best suited for* transfer as they must have previously possessed characteristics (such

as proto-transit or signal sequences) making this possible, although insertion here would take place from the opposite direction (symbiont cytoplasm to membrane). Matrix proteins would have presented a difficulty because they are not secreted or perhaps even membrane associated. If a spontaneous system either as described here, or a variant of the membrane trigger mechanism existed, evolutionary time would have been created for natural selection to improve the import of the organelle proteins.

In spite of the crucial role spontaneous transport is suggested to have played, it is unlikely that the mechanism would have proved adequate in the long run. A transport system based on spontaneous processes suffers from the serious drawback that it is not specific. A large degree of specificity is needed to steer a protein to the correct compartment. This applies not only in terms of localisation of non-organelle proteins, but also with respect to location within the organelles. Considerable difficulties might be expected in consigning a protein specifically to the inner membrane or intermembrane space. Proteins capable of passing through the membrane would not necessarily stop in the intermembrane space but could continue to the matrix. Similarly, embedded proteins would probably stop in the outer membrane.

The second serious problem with the system is that it is not selective. Any protein present in the cytoplasm which had the appropriate distribution of polar/non-polar sections would cross the membrane of the proto-mitochondrion and proto-plastid. Consequently the intracellular pool of TGP, possibly severely taxed due to the many cytobiunts, would be diluted by spontaneous loss across cellular membranes. Finally, certain TGP, lacking the features for spontaneous translocation, would be excluded.

These considerations, together with evidence for present-day receptors, indicate that such receptors would have improved the endosymbiosis a great deal.

(ii) The origin of organelle receptors

Due to the close similarities between pro- and eukaryote secretory

processes (see Von Heijne 1985 for structural similarities in the signal sequence), it is thought that the signal system is a very ancient mechanism (Talmadge et al. 1980; Watts et al. 1983). As a result it is probable that the host and cytobiont both had functional secretory systems. Therefore receptors for cytobiont membrane proteins probably existed. Two problems arise here, namely (1) the presence of signal sequences on TGP would probably have resulted in TGP being secreted into the endoplasmic reticulum. It has been thought that proinsulin can be secreted and processed in bacteria when a bacterial signal sequence is connected to it (Talmadge et al. 1980). This is presumably due to the similarities existing between pro- and eukaryotic signal systems; (2) the polarity of the receptors would be incorrect in that the precursors would be entering from the reverse side of the two membranes. Schatz (1979) has alluded to this point. Because a signal type translocation is not found in two presumably independent organellar import systems, once can conclude that the system was incapable of providing a direct mechanism for TGP import.

The existence of these impediments to transport required that sooner or later receptors would had to have appeared. The evolution of a receptor corresponding to a particular protein is a tricky evolutionary innovation. The system, when completed, is a co-adapted one and effectively connects two genes in a functional sense. Once a rudimentary receptor-protein relationship is established, the recognition between the two can be improved in small steps. The protein and the receptor must maintain a close stereochemical relationship and neither can alter too rapidly. How the initial association arises is more difficult to envisage. It is highly probable that in this (and in most, if not all, co-adapted systems), pre-adaptation plays a central role. In such a view either the protein or the 'receptor' would by chance be predisposed to bind the other. Once a rudimentary binding took place, natural selection would further the process. This mechanism allows existing receptors to be transformed into new ones with different specificities or receptors to evolve from scratch. The latter would be a less common occurrence due to the conservative nature of evolution. Duplication of receptor genes could also have taken place. Once receptors appeared, 'excluded' TGP, that is, those incapable of spontaneous import would start entering the endosymbionts.

As a digression, it is of interest that Watts et al. (1983) have reported an interesting case where two different proteins have a processing specificity in common but use different membrane receptors. Receptors are probably more easy to vary than the signal system and offer a flexible mechanism of specifying the address of a protein.

(iii) Origin of the transit peptide

Most organelle proteins possess a transit peptide and this peptide is similar to the signal sequence in that it is usually an N-terminal extension that is removed, and differs from it in that it is more hydrophilic. Two hypotheses can be proposed to explain the presence of such transit peptides. Schatz (1979) has suggested that such pre-pieces (transit sequences) may have evolved from signal sequences operative in vectorial translation. Alternatively, such extensions may have been added to organelle proteins during their evolution (Ellis 1981b), perhaps by means of exon shuffling (see Harington and Thornley 1982) which has also been implicated in the origin of a variety of vertebrate proteins (Doolittle 1985). Stochastic effects influencing 'pre-piece shuffling' may render this mechanism of origin less feasible than a signal sequence transforming mechanism. A major difficulty exists, however, for matrix proteins such as, for example, RNA polymerase. Such proteins (some of which have larger precursors) would not have had signal sequences that could have been transformed into transit sequences.

As Tzagoloff (1982) has stated, two problems face a prospective membrane or organelle matrix protein with reference to importation: recognition of target membrane, and translocation. Both, but more so the first, are dependent on receptors. The recognition of the target membrane has been termed the 'product specificity corollary' by Weeden (1981). For gene transfer to be successful, TGP must return to the specific organelle (and to the precise part) that used to house the TGP gene. For example, the isozymes of aspartate aminotransferase in plants have to be assigned to the cytosol, plastid, mitochondrion or microbodies (see Weeden and Gottlieb 1980).

In closing, it is important to stress that in the case of mitochondria, and in the standard chloroplast situation, the TGP may have to cross

two membranes and up to six (as in cryptomonad algae). In a situation where a protein has to cross six membranes there may not be six distinct protein specific receptors present at each barrier. Perhaps a combination of receptors and spontaneous import makes it possible for a protein to cross so many membranes.

(iv) Possible host contamination of proto-organelles

In 1978, Borst and Grivell reported that 'there is no reason to think that the mitochondrial compartment in the cell has any protein in common with the rest of the cell'. This statement has proved to be almost exclusively correct. In general, the organelle is furnished with its own characteristic spectrum of proteins - apparently mostly derived from *gene transfer* (see Weeden 1981, for example) - which remain specific to the organelle. In some cases, proteins characteristic of one type of organelle may be found within another (see Lacoste-Royal and Gibbs 1985) but this is usually due to the invasion of the gene.

Three proteins are suspected to be shared between mitochondria and the cytoplasm (see Fox 1982), being nuclear-coded tRNA dimethyltransferase, tRNA methyltransferase and leucyl tRNA synthetase. Mutations in these genes abolish the activity of both cytoplasmic and mitochondrial tRNAs (Hopper et al. 1982). Surguchov et al. (1983) suggest the existence of a common mitochondrio-cytoplasmic ribosomal protein, and mutation studies by Polakowska et al. (1983) have defined a nuclear locus (cdc 8) in yeast which affects replication repair in both the nucleus and mitochondria. One of the complications restricting the existence of shared proteins is presumably the fact that the organelle version requires specialised features to ensure its transport across the organelle envelope (see Gottlieb 1982).

There are two ways of solving this problem. One (see Fox 1982) is to place the same gene under two different modes of translation and transcription which would leave shared common sequences, but change the termini, as happens in the case of secreted and cytoplasmic invertase in yeast (Perlman and Halvorson 1983). Another way is to duplicate the gene in question and allow one to specialise for the introduction into

the mitochondrion or plastid. This mechanism would be preferable if the functions within and without the organelle are significantly different, as seems often to be the case.

The adding of transit peptides to organelle-bound proteins probably places restrictions on bona fide shared proteins and renders difficult the production of two different enzymes from the same gene by differential transcription or translation.

The paucity of present-day shared proteins is presumably due to two factors, namely, difficulty in transport of a typical cytosol enzyme across the organelle envelope, and the specialised organelle functions required. If a spontaneous import system, or alternatively, relatively non-specific receptors existed at one time, it is quite feasible that host proteins entered the organelles. Relevant to this question are the presence of the enzymes existing in the mitochondrion and cytoplasm as isozymes, such as aspartate amino transferase, malate dehydrogenase, *isocitrate dehydrogenase, malic enzyme, phosphoenol-pyruvate* carboxykinase and aconitate hydratase (see Hopper et al. 1982).

If it is assumed that the original gene belonged to the host - which seems to be the case for AAT - it is clear that the organelle isozyme was derived from that gene. A subsequent duplication would allow an organelle-specific form to evolve. For this to take place, it is necessary that the product of the duplicated gene (sooner or later) entered the endosymbiont.

(v) The relationship between mitochondria and plastids

It is striking that the mechanisms of protein import into mitochondria and plastids are remarkably similar: transit peptides are usually involved, and the transport is decoupled from translation. Cavalier-Smith (1980), assuming a non-symbiotic origin for the mitochondrion, and the reverse for the plastid, has suggested that the transport system that evolved for the former was adapted for the latter. This could be possible - and would apply equally if the mitochondrion evolved symbiotically - if *receptors between the plastid and the mitochondrion are or were shared*

(see Cavalier-Smith 1982, for a discussion of this point). This solution, however, does not alleviate the problem of the evolution of transit segments either de novo or from existing signal sequences for plastid proteins. Such peptides are probably of independent origin in the two systems.

If the mitochondrion originated autogenously, it is peculiar that the vectorial translation and signal sequence mechanism does not apply there. It is significant that such similar transport processes and transit peptides have evolved independently in the mitochondrion and one or probably more plastid origins. Clearly, the problem has been solved in an identical way indicating that it is the most feasible solution to the import problem. It is likely that inherent constraints limit the evolutionary solutions to the problem, making the present import system the most feasible.

4.5 Degeneration of the endosymbiont genome

When a free-living prokaryote enters into a symbiosis with a host cell the selective emphasis will be substantially altered. One view is that the selection pressure is greatly reduced (for example, Harington and Thornley 1982), the other that it is greatly increased (Smith 1979). Both are probably accurate, depending on what aspect of the symbiont genome one concentrates. Those genes involved directly in the symbiosis will be under strong stabilising selection, initially within the endosymbiont and subsequently, after transfer, in the host nucleus. Such genes would also be under pressure for improvements or specialisations. Conversely, a large proportion of the genome will be freed from natural selection if the functions they were involved with become redundant, either by host substitution, gene transfer, or by becoming irrelevant (such as cell wall synthetic enzymes). Gene transfer is an important factor in the degenerative process. As described in earlier sections, the presence of a functional endosymbiont genome plays a crucial role in the early stages of the symbiosis. It provides a backing system which creates evolutionary time for the development of receptors and transit peptides. Only once these systems have originated

can degeneration of the cytobiont genome proper commence. It is important to stress that transport per se probably did not reduce the size of the genome. The process results in redundancy between a nuclear gene (derived from a lysed symbiont, for example) and other genes within normal intact symbionts. The creation of the redundancy is contingent upon the import of TGP. Host substitution is a major cause of redundancy but appears to be restricted mainly to the supply of metabolites and lipids and not enzymes. Once such metabolites are freely available, repressed operons and other genes are no longer policed by natural selection and as a result, degenerate. It is likely, considering that bacteria can take up metabolites (via specialised transport systems), as well as the diffusible nature of most small molecules, that degeneration via host substitution and irrelevancy preceded loss due to gene transfer. The latter process affects the most important genes and would be delayed till TGPs entered the symbionts in force.

Another aspect of degeneration (which is caused by removal of natural selection in one form) is the presence of strong selective forces encouraging a decrease in proto-organelle genome size. Once maintaining selection is removed, processes of deletion erode the size of the endosymbiont genome. Such endosymbionts could well be at an advantage compared to others lacking deletions by being able to replicate more rapidly. They would also be most acceptable to the host because they would represent less of a load.

It must be pointed out that the nature of selection favouring a decrease in organelle DNA size *is poorly understood (see p.70)*. Selection clearly acts in most circumstances since organelle genomes are usually substantially reduced in size. That other factors are involved here is clear on comparison of mitochondrial DNA sizes in humans and yeast. Hickey (1982) has put forward the suggestion that a biparental pattern of host genome reproduction allows selfish DNA to spread. This, it is further stated, is a possible reason why biparentally inherited yeast mitochondria contain more non-functional DNA than uniparentally inherited human mitochondria.

4.6 Conclusion

In this section the consequences of having membrane-bound organelles within the cytoplasm were considered. Both plastids and mitochondria are bounded by two selectively permeable membranes. If a gene is transferred, the protein product of this gene has to enter the endosymbiont. If this is impossible, two crucial features of the endosymbiosis cannot take place, namely, gene transfer and degeneration of the cytobiont genome.

An analysis of present-day systems of protein import into plastids and mitochondria revealed two interesting facts. Firstly, the import mechanisms differ fundamentally from the cotranslational type known for prokaryote and eukaryote secreted proteins (the signal system). In the organelles, import is generally mediated by a process of vectorial processing. The basis of this system is that transported proteins usually have an extension known as the transit peptide. The proteolytic removal of this small peptide 'locks' a protein into the organelle. In virtually all carefully studied cases, a protein specific receptor co-responsible for import was identified. Organelle outer membrane proteins generally lacked the extensions and seem to insert directly into the membrane.

In the second place, the vectorial processing mechanism is present in both mitochondria and plastids which had independent endosymbiotic origins. Unless one system was derived partially from the other (which is unlikely), it would seem that common evolutionary constraints characteristic of the endosymbiosis resulted in the origin of a very similar system in both cases.

Having dealt with the systems characteristic of organelle protein import, the origin of receptors was considered. A receptor is an essential component in a system that mediates protein specific import. A major problem centres around the evolution of receptors. It was argued that three main factors played a role in the development of the protein specific receptor system. There was a prerequisite for a functional endosymbiont genome to provide proteins while receptors evolved to

mediate the entrance of the same components but encoded in transferred genes. The spontaneous import systems described in this section were suggested to have made initial import possible. This import, it should be stressed, probably was not significant in terms of the supply of proteins in functional terms, this responsibility resting with the cytobiont genome. However, the spontaneous import did create favourable circumstances allowing natural selection to cause the origin of receptors. Once this took place, the importance of transferred genes would increase dramatically and would relieve the cytobiont genome of most of its coding responsibility. Following this, degeneration of the cytobiont genome would take place, representing a further integrative stage.

Finally, it should be noted in closing, that the selectively permeable nature of membranes represented the biggest passive complication in the origin of mitochondria and plastids.

CHAPTER FIVE

SYNCHRONISATION OF HOST ENDOSYMBIONT DIVISION

Mutualistic relationships evolve when two organisms are brought into close proximity. With respect to endosymbiosis, ingestion is a starting point (Boucher et al. 1982). The prospective cytobionts require to resist the digestive or other assault of the host. Once the endosymbiont is established, parasitism can conceivably become a problem (Boucher et al. 1982). Certain accepted mutualisms are possibly parasitisms. Ahmadjian and Jacobs (1981), for instance, showed that a lichen consisted of controlled fungal parasitism of the algal partner. In terms of endosymbiosis, the regulation of cytobiont growth rates is essential for a stable association. In addition, the number of cytobionts has to be kept within limits at an optimal number. If the proliferation of the endosymbionts is greater than that of the host, lysis of the cell will take place, causing the extinction of the host and thus ending the endosymbiosis. Alternatively, if division of the host is faster than that of the cytobionts, the production of symbiontless cells by segregation will begin and eventually go to completion. Because free-living prokaryotes generally divide rapidly it is likely that the former case is the more common one.

5.1 Control of cytobionts

A variety of mechanisms can be employed in order to maintain endosymbiont numbers at a steady state. Expulsion, digestion and inhibition are possible candidates (Muscatine and Pool 1979). Ejection does not seem to be a very important mechanism and is thought not to be involved in regulation of intracellular symbionts in Hydra. Similarly, digestion is not a prominent mechanism. Inhibition differs substantially from the above described regulatory systems in that it operates at the chemical level. Endosymbiont growth can be inhibited by nutrient limitation or by secreted inhibitors (Muscatine and Pool 1979). The former does not seem to play a role in Hydra (McAuley 1981a) and inhibition is largely

unknown there (Muscatine and Pool 1979). In the Homoptera (Insecta) control of symbionts can take place by confining them to a specific organ (the mycetome), lysosomal breakdown, engulfment by hemocytes, action of endogenous lysozyme and hormonal influence on symbiont reproduction (Houk and Griffiths 1980). In the cockroach the division of the mycetome is host-controlled (Grigolo et al. 1984).

In the context of Endosymbiosis Theory, gene transfer presents a mechanism by which the population size of cytobionts can be regulated. Once a gene transfer is complete the cytobiont population lacks the gene and must obtain the product from the host. In this context certain transferred genes such as those coding for cytobiont replicatory enzymes would be better candidates than others for control. However, such gene transfers take place only after, or perhaps as a consequence of, steady state endosymbiosis. Inhibition is a more likely control mechanism operating at the initial stages, and would also be most effective if it acted on some part of the genome involved in replication.

In Hydra (McAuley 1981b) and Paramecium (Margulis 1981), a close relationship is noticed between host mitosis and the division of the endosymbiotic Chlorellae. When the host cell is not dividing the cytobionts also do not propagate. The reverse situation, that is, where the cytobionts divide on stimulation of host division, also takes place.

5.2 Host and endosymbiont replication rates

Within a cell bearing plasmons, potential for conflict between the host DNA and the cytoplasmic replicons arises (Lewontin 1970; Grun 1976; Eberhard 1980, 1981; Cosmides and Tooby 1981). If an endosymbiosis is to be stable and efficient, such conflict has to be kept to a minimum. A convenient parameter to consider here is the relative rate of replication of host DNA and endosymbiont DNA.

It is important that the rate of replication of endosymbionts takes place in accordance with the rate of increase of the environment, that is, the cell. In some ciliates and a chlorophyte, division of the mitochondria takes place synchronously with that of the host nucleus

(Margulis 1981). Similarly, the division of the two cyanelles of the amoeba, Paulinella, is closely linked to that of the nucleus (Smith 1978). The cyanelles of Cyanophora display what may be a more primitive situation. The cytobionts do not divide in synchrony with the host at all times (Trench et al. 1978) and decrease in number when the host is dividing rapidly. When stationary phase is entered by the host the cyanelles continue to divide and increase in numbers, but during log phase of the host the numbers of cyanelles are constant. A similar state of affairs pertains to Glaucocystis and its cyanelles (see Trench et al. 1978).

Present-day endosymbioses have generally achieved the goal of co-ordinated division. In the mitochondria, plastids and cyanelles, genic integration has proceeded to a substantial degree. What is now discussed further are the details of the plasmon-nucleus relationship within the context of early establishment of the symbiosis.

6.3 The problem of 'selfish' DNA

Because natural selection does not operate on DNA through the organismal phenotype only (Doolittle and Sapienza 1980; Doolittle 1982b), DNA sequences within the cell can conceivably compete via mutation and replication. This competitive process results in more efficient replicators increasing their numbers at the expense of the less efficient competitors, eventually causing them to become extinct (Orgel and Crick 1980). This conclusion poses problems concerning the establishment of 'co-operative' systems of replicators. The Qβ phage system illustrates the results of such replication competition. Among the wild type and its variants, the former predominates because it replicates more efficiently than the mutants (Mills et al. 1967; also see Ninio 1982). In an in vitro system mutants can be tolerated and in selection experiments (Mills et al. 1967) certain very small variants outcompete the wild type due to an exceedingly efficient and rapid replicatory ability. Whatever the selection regime may consist of, such competition is important in that it allows a self-replicating unit to compete with error copies and maintain its information.

In certain circumstances competition can become an inhibitory factor. In the origin of life as conceived by Eigen (Eigen and Schuster 1979), small RNA polymers had the ability to self-replicate. Due to an error threshold the useful coding length of such molecules is unable to grow and information content is fixed at a maximum of about 100 nucleotides. This length is too small to code for replication machinery of a higher fidelity. Compartmentation of such entities aggravates competition (Eigen et al. 1980) and together with the error threshold resulted in 'evolution almost coming to a standstill' (Eigen and Schuster 1979). To overcome this hurdle, a system of hypercyclic co-operation had to develop. Escape from 'selfish' sequences was necessary to allow further evolution.

The main point made here is that competition without co-operation can lead to a dead end situation. Furthermore, as stressed by Bresh et al. (1980), and conceded by Eigen et al. (1980), the hypercycle is under constant threat from parasitic sequences. Such sequences can either maintain their replicase specificity and lose coding/tRNA function or, worse still, possess a total functional defect while maintaining a higher replicase affinity. Such 'selfish' sequences would be expected to proliferate at the expense of other slower, but more functional, replicators, and thus undermine the whole system. When multiple genomes are present within a system, the spread of parasitic DNA sequences has to be counteracted to allow for further evolution.

In analogy with the above discussion, a host cell bearing endosymbionts could feasibly suffer from 'selfish' cytobionts. Such plasmons would be parasitic (sequence dependent) in the sense that they spread intracellularly due to a higher efficiency of replication (Orgel and Crick 1980; Doolittle and Sapienza 1980; Doolittle 1982b). Selfish DNA is generally thought of as making no specific contribution to the phenotype (Orgel and Crick 1980) and without major effect on the organismal phenotype (Doolittle and Sapienza 1980). There is reason, however, to suspect that selfish DNA can have deleterious effects on the host (Hickey 1982; Doolittle et al. 1984): an example of this may be the origin of phages from plasmids or transposons (Doolittle and Sapienza 1980; Flavell 1981).

5.3.1 Examples of inter-endosymbiont/organelle competition

The replication of RNA viruses within a host cell provides a suitable system for the manifestation of competitive effects between replicons. Within a cell containing a certain RNA virus strain an equilibrium can be reached during which the particular strain predominates (see Holland et al. 1982). During such an infection, defective interfering (DI) particles can arise. These are subgenomic particles formed by deletion recombination which are able to replicate with the aid of helper virus enzymes. Furthermore, the DI particles interfere with the replication of the helper virus. Soon these particles completely outreplicate the original virus and displace it. Escape from the DI particles can only take place when a resistant mutant arises. This strain then predominates until another DI strain arises (Holland et al. 1982). The example described above illustrates that 'parasitic' replicators as discussed in theory can arise and displace larger, more functional replicators.

In organelles two levels of competition can be distinguished. One exists between organelles or cytobionts. In addition, intergenomic competition can take place within polyploid organelles. Competition between organelles can be classified into two artificial groups, namely, naturally-occurring competitive phenomena, and intracellular selection. The latter type is discussed on p.106.

Competition has been demonstrated to take place amongst chloroplasts. In crosses between Pelargonium species with wild type or white chloroplasts, interplastid competition has been indicated (Abdel-Wahab and Tilney-Bassett 1981; Tilney-Bassett and Birky 1981). Such competition apparently occurs both between wild type and mutant plastids and amongst the mutants themselves. Differences in replicative abilities and stabilities seem to be involved in altering the competitiveness of plastids. Within the zygote each type has a specific probability of replicating first, determined by some feature of the plastid or an interaction between such a feature and the nucleus (Abdel-Wahab and Tilney-Bassett 1981).

In mitochondria, documented examples of replicative competition exist. As alluded to previously, two levels of competition can be discerned: among sequences within a mitochondrion, and then between the mitochondria

themselves. The phenomenon of senescence in fungi is apparently related to 'parasitic' sequences. Senescence is not adaptive and has no function; it is more accurately the subversion of function (Williams 1966). The vegetative multiplication of cells in fungi is limited by the process of senescence. In Podospora anserina, senescence is characterised by the appearance of circular DNA molecules of mitochondrial origin called SEN-DNA (Jamet-Vierny et al. 1980). Recent work by Osiewacz and Esser (1984) indicates that the plasmid is derived from an intron of the cytochrome c oxidase gene and, importantly, that it contains an autonomously replicating sequence. This amplified mitochondrial DNA is the result of two processes (Bernardi 1982a): excision of part of the genome, and amplification of the sequence. The arrest of growth is eventually caused by the progressive elimination of the true mitochondrial genome due to preferential replication of the SEN-DNA (Jamet-Vierny et al. 1980). The property is contagious (Belcour 1981). Detailed analysis of the sequence of the rapidly-replicating SEN-DNA reveals that it plays a role in the initiation of replication of the mitochondrial chromosome (Vierny et al. 1982). A similar phenomenon is noticed in the ragged mutants of Aspergillus (Kück et al. 1981) as well as in the poky and stopper mutants of Neurospora (see Bernardi 1982a). A different cause of senescence is known in Neurospora intermedia. Here, the insertion of a 'foreign' DNA transposable element into the mitochondrial DNA (often within the open reading frame in the intron DNA of the mitochondrial 25S rRNA gene) is associated with senescence (Bertrand et al. 1985). Although the mechanism responsible for senescence is not clear, the authors suggest that the insertion results in the affected mitochondrial genomes increasing in number relative to normal mitochondrial genomes.

Plant mitochondria display a condition known as male sterility. This 'cytoplasmic' sterility can be divided into different groups T, C and S, all of which are associated with the mitochondrial DNA (Leaver 1980). In S Male-Sterile maize and sorghum, linear plasmid-like molecules are found in the mitochondria (Kim et al. 1982; Pring et al. 1982). These DNAs appear to originate from normal mitochondrial DNA (Pring et al. 1982), are able to amplify (see Kück et al. 1981) and have short inverted terminal repeats as in transposons (Kim et al. 1982). In maize, the two

plasmid-like DNAs S-1 and S-2 are about five-fold more abundant than mitochondrial DNA (Levings and Sederoff 1983).

Analysis of the nature of petite mutants in yeast serves as a very good example of parasitic DNA. Petite mutants are formed when a segment of the wild type mitochondrial DNA is excised and amplified in tandem or in inverted repeats (Tzagoloff 1982). Excision is associated with direct repeats within areas of replication origin or intergenic sequences (see Bernardi 1982a) and is the result of unequal site-specific crossing over events (Bernardi 1982b). The success of a particular petite depends on its ability to replicate and the efficiency with which it does so (Rank 1970b). Petites lacking proper replication origins can be found but are very rare (Bernardi 1982a). Nevertheless these petites have surrogate origins. Thus, the presence of sequences allowing for replication are essential if a petite is to survive competition with other petites or wild type mitochondrial molecules. The phenomenon of suppressivity is a manifestation of replication competition. In this case crosses between yeast cells with suppressive petites and those with wild types can result in a variable proportion of wild type to petites in the progeny (Gillham 1978). Some petites are hypersuppressive and crosses with wild types result in only petites in the progeny (Blanc and Dujon 1980). The work of Bernardi and associates (see De Zamaroczy 1981) has indicated clearly that the nature of the ori sequences in petites determines the level of transmission of petites to the progeny. This relates both to the efficiency of a particular ori sequence as well as to the density of this sequence on a petite genome. The suppressive petites spread because they have a replicative advantage over wild-type mitochondrial DNA. This suggestion, now confirmed (De Zamaroczy 1981), was best developed by Rank (1969, 1970a, 1970b, 1972). Reid (1980) has since then related the phenomenon to that of selfish DNA. The production of petites is not adaptive because the yeast cell, being without functional mitochondria, can survive only by its anaerobic capacities.

In the 'selfish' examples described above, a particular sequence eventually spreads throughout the population of organelles and therefore host cells as well, providing that no counter-selective pressures come into existance. This spread is a sequence-dependent process and suggests (see Dover and Doolittle 1980) that such sequences are 'parasitic'.

In conclusion, it is important that a critical approach must be adopted when referring to DNA sequences as 'selfish' without careful consideration. For example, Biel and Hartl (1983) found evidence for natural selection of transposons in E.coli and Schwarz-Sommer et al. (1985) have proposed that plant transposable elements generate sequence diversity important in evolution. Similarly Levin and Lenski (1983) argue that plasmids are not 'parasitic' but are selected for. A functional role for plasmids of Paramecium endosymbionts has been indicated by a high degree of conservation amongst various Paramecium strains (Quakenbush 1983).

Prior to dealing with the mechanisms involved in the elimination of 'selfish' (organelle) DNA, the population genetics of plasmons is briefly considered.

5.4 The population genetics of organelles

5.4.1 Stochastically influenced interactions amongst organelles and their genomes

Intracellular mating, segregation and replication are the main events strongly influenced by chance that can take place in a cell bearing organelles.

(i) Bacterial mating

The mating of bacteria (and presumably that of early prokaryotic endosymbionts) depends on various factors, in particular, the probability of collision which is related to density, the probability of plasmid transfer, and the probability of integration of the transferred DNA (see Hayes 1974). While rates of recombination in artificial circumstances are sufficiently high to be useful analytically, indications are that natural rates of recombination are extremely low (Selander and Levin 1980; Levin 1981; Levin and Lenski 1983), being similar to the mutation rate. This suggests that intracellular bacterial mating is/was a very rare event and unlikely to have had much influence on allele frequencies within the cell in the face of other processes such as segregation (host division) and random replication (cytobiont division). Because

recombination is so rare in bacteria, interactions centred on clones of individuals play an important role in population genetics. Whittam et al. (1983) demonstrated the importance of chance extinction of lines (drift), and selective differences between particular genetic combinations in natural populations of E.coli.

(ii) Recombination of organelle genomes

As opposed to the low rates reported for bacterial populations, some organelles recombine at high frequencies. Yeast mitochondria are extremely promiscuous (Margulis 1981) and recombine so actively that all the products of multiple recombination are produced in a short time (Dujon, see Margulis 1981). This property is extensively used in genetical studies (Dujon et al. 1974, for example). Similarly, recombination is known for Chlamydomonas chloroplasts (Adams et al. 1976). In contrast, mitochondrial recombination in Paramecium is apparently very rare (see Gillham 1978) and is not known to take place in mammalian mitochondria (Aquadro and Greenberg 1983; Cann et al. 1984). Intra-genomic recombination is reported from plant chloroplast and mitochondrial DNAs (Stern and Palmer 1984).

Recombination between chromosomes of higher organisms is generally considered reciprocal, that is, classical crossing over. In yeast mitochondria and Chlamydomonas chloroplasts asymmetric (non-reciprocal) recombination is more characteristic (Van Winkle-Swift and Birky 1978). During such an event, DNA molecules pair and form a heteroduplex molecule. Mismatches resulting from heterozygosity are corrected and this can lead to gene conversion (see Williamson et al. 1977).

If pairing and conversion take place repeatedly and randomly, they can lead to random drift of gene frequencies within the organelle, providing more than one genome is present within the organelle, or within the cell. Simulations by Birky and Skavaril (1976) suggested a homogenising effect as a result of conversion which maintains or pushes a cell towards homoplasy. As a result, new mutants are usually rapidly lost and pure mutant populations are rarely produced (Birky and Skavaril 1976; Williamson et al. 1977). In particular, with numbers of interacting genomes around 50 or 100, mutants went to fixation very slowly and

rarely. In the case of smaller numbers such as four, mutants went to fixation at significant frequencies (Birky and Skavaril 1976). The probability of fixation of a mutant via conversion is equal to the initial frequency (Ohta 1977). Conversion can thus be effective 'in maintaining the genetic homogeneity of nucleoids or organelles in the face of immigration of DNA molecules from nucleoids or organelles of other genotypes' (Birky 1978). Gene conversion is best studied in yeast mitochondria (Dujon et al. 1975; Birky and Skavaril 1976; Williamson et al. 1977; Butow et al. 1982; Birky 1983) but is also thought to take place in Chlamydomonas chloroplasts (Van Winkle-Swift and Birky 1978 Sears 1980a; Birky et al. 1981).

Recently, the role of gene conversion in random drift in mitochondria has been questioned (Birky et al. 1982). This follows from the fact that the conversion process changes gene frequencies very slowly when large numbers of molecules are present. In addition, conversion effects are not entirely consistent with data pertaining to co-conversion and recombination (Birky 1978; Thrailkill et al. 1980; Birky et al. 1982).

Despite the questionable importance of conversion in mitochondria the role of this process is now becoming apparent in the homogenisation of multigene families and duplicated genes (Baltimore 1981; Jackson and Fink 1981; Klein and Petes 1981; Hayashida and Miyata 1983; Amstutz et al. 1985). Several population genetics studies (Gutz and Leslie 1976; Ohta 1980; Nagylaki and Petes 1982; Nagylaki 1984; Ohta 1984; Walsh 1985) have suggested a role for conversion. Conversion may also play a role in the process of molecular drive (Dover 1982; Dover and Flavell 1982). A particularly interesting study is that of Lamb and Helmi (1982) which indicates that conversion could have important effects in evolution and very large effects on selectively-neutral alleles or slightly-selected alleles. Where selection coefficients are not low, conversion will still have a large influence on the frequency of recessive alleles, but less influence on a dominant allele (Lamb and Helmi 1982). A study by Walsh (1985) on conversion in multigene families indicated that selection was more important than conversion, provided that selection was not very weak. In the last-mentioned case, a slight conversion bias could greatly alter

fixation probabilities. Some authors, however, have presented models which indicate that conversion can, in some cases, successfully oppose selection (Lamb and Helmi 1982; Hickey 1982).

An important concept is that of disparity of conversion which provides a directional drive to conversion. If no disparity is present, conversion is purely stochastic.

(iii) Random replication

In eukaryotes, [illegible]ation of chromosomal DNA molecules is non-random in that repli[illegible]ns only once in the vegetative cell cycle (Birky 1978). In co[illegible]e replication of extra-chromosomal DNA is uncoupled from the co[illegible]bling of nuclear DNA (Eberhard 1980). In addition, extra-chromosomal DNA molecules can be selected randomly for replication. Because certain molecules may by chance be selected for replication more often than others (Birky et al. 1982; Birky 1983), stochastic fluctuations in gene frequencies result in zygote clones that show a large variance of gene frequencies. Stochastically-based uniparental inheritance may be partly caused by this mechanism.

Birky et al. (1982) regard random replication, or perhaps random turnover or degradation, as the major cause of random intracellular drift. Random replication has been postulated for yeast mitochondria (Birky 1978; Birky et al. 1982; Birky 1983) and Chlamydomonas chloroplasts (Birky et al. 1981). As with conversion, random replication will not have large effects on allele frequencies in cells if the total number of genomes is large (Birky 1978; Birky 1983). As before, it may operate most actively within nucleoids or within organelles where it might be important in maintaining genetic homogeneity.

(iv) Vegetative segregation

Segregation is a consequence of having plasmons in the cytoplasm that are partitioned at division. Given a heteroplasmic cell, segregation produces homoplasmic daughter cells, eventually resulting in complete sorting out. Given large numbers of segregating units, simple segregation is a rather inefficient process. According to Michaelis

(see Grun 1976), about ten times the number of segregating particles of consecutive cell divisions are necessary to give a homoplasmic population. This holds regardless of the ratio of the two types of particles in the original cell. However, three important assumptions are made, namely, that the two types of particles must divide at an equal rate, that they double uniformly between mitoses so that their numbers remain constant and that they occupy zero cell volume (see Birky and Skavaril 1984). In segregation models, including those of Dujon et al. (1974) and of Dujon and Slonimski (1976), the probability that a particular molecule in the sample (bud or daughter cell) will be of a particular genotype is determined by the frequency of that genotype in the original pool of molecules. In practice, sorting out takes place much more rapidly than expected on the basis of random segregation (see Dujon et al. 1974; Dujon 1975; Dujon and Slonimski 1976; Williamson et al. 1977; Birky et al. 1978b; Birky and Skavaril 1984). It is therefore postulated that the segregating unit is the nucleoid or whole mitochondrion (Treat and Birky 1980). With regard to evidence for segregation rates, Solignac et al. (1984) estimated that 500 generations were required for sorting out in Drosophila mitochondria. This value corresponds to 400 segregating units which in turn correlated to the number of mitochondria reported for animal cells (Solignac et al. 1984). A similar position to that described above is found in the chloroplast (Van Winkle-Swift 1980). Chloroplast DNA molecules in Chlamydomonas, numbering between 70 and 110, are organised into a smaller number (1-16) of densely packed nucleoids. The partitioning of these units may be random (Birky et al. 1978b; Treat and Birky 1980; Van Winkle-Swift 1980; Birky 1983). Recently (Birky et al. 1982; Waxman and Birky 1982) a new model of vegetative segregation in yeast mitochondria has been proposed. In contrast to the examples discussed above, some heteroplasmic genes in Chlamydomonas chloroplasts do not segregate and appear to be stable (Spreitzer et al. 1984). Substantial importance is attached to intracellular random drift, that is, stochastic mating and conversion and particularly random replication, acting in concert with segregation. Random drift is suggested to be more important than segregation in the production of homoplasmons.

5.4.2 Intracellular selection

This process is one of the most important phenomena of intracellular population genetics and is particularly relevant in the context of competitive interactions of cytoplasmic genes.

The selection of certain plasmon variants within a cell is an important mechanism operating in the spread of mutant genomes. As remarked by Williamson et al. (1977), *the altering of the replicative capacity of a* mitochondrial DNA molecule is very important in its effect on the spreading of a certain type. Directed intracellular selection is the process whereby the replicative superiority of a mitochondrial genome, for example, allows this genome to increase its frequency to unity. Birky (1973) found that spontaneous erythromycin-resistant mitochondrial mutants reproduce more rapidly in the presence of the drug than do sensitive wild types. In the absence of the drug the reverse situation occurs. Given a selective pressure, certain spontaneous mutants can go to fixation. Intracellular selection can be random with respect to genotype as opposed to the directional type described above (Van Winkle-Swift and Birky 1978) but does not result in net gene frequency changes. Intracellular selection has been recorded for mikamycin-resistant mitochondria (Beale 1973) as well as for chloramphenicol and erythromycin-resistant mitochondria (see Gillham 1978) in Paramecium. Putrament and Ejchart (1981) found that intracellular selection was responsible for the increase of mit-mutants in yeast mitochondria. Intracellular selection is a direct consequence of inter-plasmon competition as described earlier.

5.4.3 Uniparental inheritance

Uniparental inheritance is a very common phenomenon and, together with vegetative segregation, represents one of the laws of organelle population genetics (Birky 1978). Uniparental inheritance does not seem to have a general mechanism behind it at the cellular level (Birky 1978). Monogametic transmission and selective silencing of organelle DNA from one parent are important mechanisms, particularly in plants and animals (Sears 1980b; Birky et al. 1982; Lansman et al. 1983; Birky et al. 1984).

An interesting form of uniparental inheritance is known in yeast (Birky

et al. 1978a; Thrailkill et al. 1980; Birky et al. 1982). Here the phenomenon is associated with random changes of gene frequencies. Birky et al. (1981) also showed that random drift in allele frequencies in Chlamydomonas had a major influence on uniparental inheritance. Therefore, given a certain input frequency of alleles from the two parents (see Gillham 1978 for details), deviations from these initial gene frequencies can take place, leading to an increase or decrease in frequencies in the zygote and its progeny (Birky et al. 1982). To a large extent it seems that uniparental inheritance is an incidental effect of molecular events taking place in the zygote, but this is by no means always the case.

5.5 A possible mechanism for the elimination of parasitic endosymbionts

Those that have concerned themselves with the spread of parasitic DNA within a cell have noticed that two levels of natural selection affect the outcome in terms of cytoplasmic genes (Lewontin 1970; Doolittle and Sapienza 1980; Dover and Doolittle 1980; Orgel and Crick 1980; Orgel et al. 1980; Eberhard 1980, 1981; Cosmides and Tooby 1981). These two opposing forces are intragenomic or, in the present context, intracellular selection and phenotypic (cellular) selection. The former kind is responsible for the spread of 'parasitic' sequences which are in competition with each other. This competition eventually takes place to the detriment of the host. When such sequences start to affect the *fitness of the host, phenotypic selection comes into play.* Here the success of a disadvantageous sequence is linked to the reproductive success of the host. Yeast cells, lacking intramitochondrial ATP, for example, lose the ability to grow and multiply (Gbelska et al. 1983), and yeast nuclear gene expression during sporulation requires mitochondrial protein synthesis (Marmiroli and Lodi 1984).

Another interesting example of the feedback of mitochondrial on host fitness is that of malate dehydrogenase. Plants cannot develop if the mitochondrial MDH isozyme is incapacitated, but survive in the absence of the cytosol isozyme (Goodman et al. 1981). Therefore, once the

organismal phenotype is involved the frequency of the parasitic sequences can be affected. If the host fitness is reduced, parasitic sequences are liable to decrease. For this reason, self-restraint in selfish DNA is a logical expectation (Doolittle et al. 1984). It is important to note, as pointed out by Evans (1984), that 'selfish' DNA will be eliminated if it imposes any cost, those with self-restraint included. However, it is possible that 'selfish' DNA could spread in spite of deleterious effects on the host (Hickey 1982).

In the context of endosymbiosis, groups of cytobionts are organised in such a way that individual interests are compromised by a 'subordination to group interest'. The crucial question is whether such an organisation is adaptive, that is, a biotic adaptation (sensu Williams 1966), a mechanism designed to promote the success of a biota. In the long term an endosymbiont would have the highest fitness if it replicated in phase with the host. Therefore, the syste... (host and endosymbionts) maximises the number of descendants of the individual cytobiont. The success of the group could be an incidental consequence of selection of alternate alleles rather than of group selection, the latter referring to the differential mortality and replication of groups (Leigh 1983). Williams (1966) states that one can 'expect cooperative mutualistic mechanisms to arise between any two species in which each constitutes for the other an important aid to survival'.

These objections to group selection in an endosymbiosis need to be seriously considered.

A significant complication centres around the fact that 'evolution is not anticipatory' and that 'structures do not evolve because they might later prove useful' (Doolittle and Sapienza 1980). The question is how selection of alternate alleles could have succeeded in establishing slow-replicating endosymbionts in opposition to those capable of quicker replication. Even a replication rate that is slightly higher than another should eventually allow the spread of that particular type of endosymbiont. In the long term, slow replication is of advantage to an individual but this does not explain how escape from immediate ...acellular (interreplicon) selection took place. That this is

potentially a problem is indicated by the fact that examples of 'parasitism' are known and that control mechanisms exist (discussed on p.94).

From a mechanical point of view, group selection is clearly involved. Due to the polyploid nature of an endosymbiont-containing cell, individual cytobionts cannot be rigorously selected as individuals on selecting a cell. The whole group is involved in the selection process. Any qualities required in the endosymbiont population have to be obtained by elimination of groups of endosymbionts, that is, individual host cells; or, alternatively, by making a population of cytobionts an individual (a clone).

Three forms of natural selection can be distinguished in these circumstances (definitions modified from *Wilson 1980*):

(1) Intracellular selection: natural selection that operates on the differential fitness of individual endosymbionts within a local host cell

(2) Group selection: natural selection that operates on the differential fitness of local populations of endosymbionts within a global population

(3) Phenotypic selection: natural selection that operates on the differential fitness of individual host cells within local host cell populations.

The phenotypic selection is the vehicle by which group selection is effected. The fitness of the host is dependent on the summed effect of individual endosymbionts. Group selection, however, does not require the existence of homoplasmons to operate. The probability of extinction of the group is equivalent to the probability of extinction of the host cell. It is expected that group selection usually requires several cell generations to produce homoplasmons because individual cytobionts cannot be eliminated or selected at once.

5.6 The relationship between group selection, spread of faster replicators and stochastic processes

Segregation is a process central to the group selection mechanism described.

Stochastic events at the time of host division can lead to unequal segregation of different types of symbionts to different daughter cells. This mechanism alters at a specific time the fitness of a group (the probability of survival from generation to generation). Segregation in isolation is largely useless because it is not selective. Although segregation is random, the population of endosymbionts is not randomly inherited, but is a function of previous selection events. If segregation were acting alone, attainment of homoplasy would be greatly retarded. This is not only because segregation is related to the numbers of endosymbionts involved, but also because elimination of rapid dividers (with numbers always on the increase) is practically an impossibility. Group selection is essential if cells homoplasic for in-phase replicating symbionts are to be produced at a significant rate. As is indicated by the description above, group selection as opposed to individual host or cytobiont selection cannot produce an effect at a particular instance in time but requires a succession of selectional events.

5.7 Does group selection of endosymbionts meet the requirements?

The idea of group selection in the context of endosymbiosis has not been dealt with extensively in the literature. Lewontin (1970) mentions it and Eberhard (1980) and Cosmides and Tooby (1981) deal with the concept in less specific terms. Harington and Centner (1982) and Leigh (1983) deal with the problem explicitly as applied to cytobionts. Levin and Lenski (1983) and Stewart and Levin (1984) have done an in-depth study on the origins of lysogenic phages. These authors also discuss the possibility that interdemic (= group) selection may be involved in the maintenance of temperance in a phage (an individual disadvantage) as opposed to the lytic life cycle. Lastly, Doolittle et al. (1984) have proposed that group selection could account for the characteristic of self-restraint in 'selfish' DNA. Consequently, it is useful to investigate whether the process as envisaged here meets the requirements Williams (1966) and Maynard Smith (1976) have discussed. Williams (1966) wrote that 'natural selection can produce significant cumulative change only if selection coefficients are high relative to the rate of change of the selected entity'. Genic selection coefficients acting on the

genome are high relative to mutations and as a result organic adaptations evolve. A high extinction rate of groups is a requisite if group selection is to take place. Because group extinction here is directly linked to cell extinction, a high turnover is assured.

An important parameter to be analysed is the amount of migration between groups. The more migration takes place, the lower are the chances of group selection (Maynard Smith 1976). Migration is a central concept in the paradox of group selection (Alexander and Borgia 1978). The requirements of the paradox are as follows and likely to be met in an asexual population of cytobiont-bearing host cells. Neighbouring groups (cells) must be independent enough to develop differences what will account for differential extinction but near enough so that extinguished groups can be replaced by emigrants (daughter cells) from surviving groups (cells). Leigh (1983) has shown that group selection can take place under conditions of no migration and on assumption that a given host is infected by parasites from only one other host individually. In an asexual host cell line, group selection is likely, since migration is zero. Thus, in the early stages of endosymbiosis, perhaps involving asexual hosts, group selection may well have been a potent influence. If one considers a state where the host is sexual, the question of group selection becomes more complex. In this case migration is no longer zero. Group selection has a reasonable chance of overriding individual selection 'if a population's chance of producing another population is higher than its chance of producing an emigrant that mates successfully in an already established population' (Leigh 1983).

This is a difficult condition to assess but is presumably associated with the relationship between fission and fusion in the organism. In purely mechanical terms, group selection will continue to take place but the efficiency of the process may be reduced. Hickey (1982) has described how selfish DNA with major deleterious effects can spread through a population. In an asexual population, colonisation of new genomes is not possible and their fate depends on the host. For this reason group selection acts particularly well. If the host is sexual, spread of parasitic sequences is greatly enhanced (Hickey 1982). It is clear that if the time between divisions of cytobionts is much shorter than the time

between sexual fusions of the host, group selection will act before faster replicators had the chance to spread to hosts with a higher proportion of slower replicators. This expectation also follows from Leigh's (1983) work. It is interesting to note that group selection becomes once again efficient when maternal inheritance takes place due to the exclusion of migrants (Leigh 1983). Perhaps the complication of sex can account for the fact that many symbiont control systems are located in the nucleus which is unaffected by fusions. A population of synchronous cytobionts is more likely to be 'infected' by a rapid divider (via sex) if the regulation of division is cytoplasmically centred.

5.8 The simulation of group selection in an asexual population of hosts

These data are presented in Appendix 1.

5.9 Selfish DNA, gene transfer and the concept of fitness

Reproductive success of the symbionts can best be increased by furthering the success of the host. This is the 'classical' phenomenon of parasitic self-restraint (see Doolittle et al. 1984). This means that two levels of selection (individual selection on the host, and individual selection of cytobionts) have become linked. Gould (1982) has mentioned the importance of the interaction between levels in a hierarchical system. Due to the cost of maintaining separate genomes (Ninio 1975; McLaughlin and Cain 1983), selection acts against the independent existence of the cytobionts. The host is most favoured when it can (a) reap the benefits that the presence of cytobionts can result in, and (b) minimise the load the last-named places on it. Gene transfer is a possible compromise. Since evolution is not anticipatory, the cytobiont fitness is increased by stepwise loss of its individuality as an organism. This process is encouraged by group selection of cytobionts (death of the host) and by individual selection of cytobionts (because cytobionts with smaller genomes and with less coding responsibility will outcompete larger-genomed cytobionts). When this process goes to its logical end, the

cytobionts become extinct (McLaughlin and Cain 1983). The extinction of symbionts by gene transfer (McLaughlin and Cain 1983) is in one sense a prime example of 'selfish' propagation of genes at the expense of the previous genetic community they existed in, and contributed to. Clearly at least some genes can be selected as units and are not necessarily bound within an epigenetic system or organism as suggested by Ho and Saunders (1981). The main driving force between individual host and individual cytobiont selection levels is group selection.

Fitness can be defined in various ways. Williams (1966) states that it is 'effective design for reproductive survival', and furthermore that it is the basis of natural selection. The entry of proto-cytobionts into the host cell presumably reduces their fitness because it depresses their reproductive rate. This holds until the moment the cytobiont becomes obligatorily dependent on the host. When this takes place the cytobionts are no longer comparable to free-living forms, and their fitness is dependent on, and must be assessed with respect to, the host. Using this argument, it can be shown that cytobionts can be considered extinct the moment they become obligatorily dependent on the host 'Extinction' as used earlier in this section and by McLaughlin and Cain (1983) is thus reduced to the mere mechanical dismantling of the cytobiont genome. The event of obligatory dependency is of crucial importance in the functional sense because it is at this point that the individuality of the cytobionts is irreversibly lost. Whether the cytobiont genes are located within their somas or scattered on the host genome does not really affect the argument, except in the mechanical sense. They are part of a new system, the host. Similarly, the moment the host becomes obligatorily dependent on the cytobionts it has lost its individuality and can be considered as extinct. At this time a third organism is created which cannot be compared to a host which is not dependent on its cytobionts. The appearance of a new functional system represents the destruction of the two genetic communities that contributed to its formation.

The above reasoning can lead to extreme viewpoints. For example, the suggestion that a host line becomes extinct when it becomes obligatorily dependent on the cytobionts. Similarly, by McLaughlin and Cain's (1983) criterion, a cytobiont which had functionally transferred 99% of its genes physically to the host would still be viewed as extant.

CHAPTER SIX

CONCLUSION

6.1 The interplay between natural selection and non-adaptive change which together have shaped the mitochondrion and plastid

Over the last 40 years, evolutionary thought has been dominated by the idea that natural selection is an all-important optimising agent which *fits an organism to its environment by means of a series of adaptations* (Gould and Lewontin 1979). According to this view, natural selection assumes the role of a creator (Gould 1982). This approach (the 'adaptationist programme' of Gould and Lewontin 1979) generally views adaptation as a response to environmental *changes mediated by natural* selection. Proponents of this view often make use of ad hoc explanations to account for the presence and adaptive nature of a particular organic feature (Lewontin 1978). Such ideas, although not necessarily incorrect, are unsatisfactory because they are difficult to test. There is little doubt that adaptive evolutionary change is important in organic evolution though over-emphasis of this point has led to the eclipse of evidence for ideas that non-adaptations, chance effects, and effects of other adaptations have been a powerful force in the shaping of the evolutionary history of organisms (Williams 1966; Lewontin 1978; Gould and Lewontin 1979; Gould 1982; Gould and Vrba 1982).

As in other sectors of evolutionary studies, theorising over the origin of DNA-containing organelles has been influenced by adaptation-orientated explanations. In the following section two main ideas will be developed: (a) that the Autogenous Theories are unadulterated products of the 'adaptationist programme' in that they place overdue emphasis on natural selection as a creator; (b) that the Xenogenous Theory serves as a good example of the far-reaching effects non-adaptive factors can have in *evolution*.

6.1.1 The Autogenous Theories

These theories can be divided into three main classes: the plasmid invagination type (Allsop 1969; Cavalier-Smith 1975; Raff and Mahler 1972, 1975; Taylor 1976); the genome duplication type (Uzzel and Spolsky 1974, 1981; Reijnders 1975; Keyhani 1981), and the cluster-clone variants (Bogorad 1975; Bogorad et al. 1975; see also Taylor 1976).

These theories all have in common three principles: (a) natural selection is postulated to lie behind the development of the organelles; (b) the creation of separate specialised linkage groups and membrane compartments for the organelles, and (c) the original main genome is viewed as the source of all genetic information. It is necessary to emphasise the fact that, in accordance with the first principle, Autogenous Theories rely almost solely on natural selection as a creator and driving force responsible for the origin of compartmentalised linkage groups. Examples of the reasoning behind this type of hypothesis are numerous; a single quotation from Keyhani (1981) will suffice: 'The collapse on each side of the respiratory membrane system and the site of attachment of chromosomes to plasma membrane produced, respectively, the mitochondria and the nucleus'. In this typical example, natural selection can be seen as playing a creative role in the production of membrane compartments and the forming of separate linkage groups.

In the case of plasmid-based Autogenous Theories, the evolution of respiratory or photosynthetic plasmids bearing appropriate genes for translation (RNAs) and metabolic functions is envisaged, although two important criticisms have been levelled against this concept. The first centres around the fact that mitochondria (and chloroplasts also) carry genes characteristic of bacterial genomes and not of plasmids (Borst and Grivell 1978). In addition, plasmids do not generally carry essential genes (Borst and Grivell 1978), an observation supported by the fact that lateral gene transfer has not affected phylogenetic relationships based on analysis of genes such as 16S rRNA and cytochromes (Dickerson 1980). Secondly, the large number of excisions and insertions required to place many unlinked main genome-located genes on to a single linkage group is without precedent (Reijnders 1975) and is unacceptably teleological.

The plasmid-based theories also make implausible assumptions regarding organelle membrane permeability. This faulty logic has escaped attention, yet it is probably one of the most serious flaws of the Autogenous Theories. Let us assume that a small plasmid coding for a limited number of unique organelle proteins arises and, further, that it is successfully compartmentalised. In order to replace non-organelle encoded enzymes or proteins lost in turnover (and perhaps to allow replication of the proto-organelle DNA), nuclear-coded proteins would have to cross one or, more likely, two semi-permeable membranes. Because receptors are not present (evolution is not anticipatory), these proteins en masse could only have penetrated the proto-organelle with a low or negligible success rate. An analogous situation was described in Chapter Three (p.36) of this thesis, with reference to the Serial Endosymbiosis Theory. A crucial difference is noticeable, however: the fact that a complete, fully-operational bacterial chromosome provides a back-up, that is, a supply of essential proteins while receptors evolve. This is not the position in the case of the plasmid as described above. The postulation of partial compartmentalisation does not avoid these problems because a selective force for receptors arises only on complete compartmentation (apart from the fact that it makes chemiosmosis impossible).

If duplicated whole genomes are implicated in the autogenous origin of the organelles as described by Keyhani (1981) and Reijnders (1975), the problems of plasmid assembly and protein importation (as described above) are avoided. Other illogical assumptions, however, cannot be circumvented. Let us assume that two complete genomes arise in the cell which are subsequently compartmentalised. (This implies that the problems involved in the co-ordination of the division of the two genomes and continued compartmentation are solved.) At this stage, natural selection is postulated to work in two opposing ways (see Reijnders 1975): 'one pressing for elimination of redundant DNA-information' and 'the other pressing for the maintenance of the situation'. Analysis of the postulated selective forces reveals that unacceptable requirements are made with respect to the specificity of protein import. One can divide the genes of each genome into two subsets: O for genes able to become organelle genes and subset H for prospective host genes. One compartment must

thus lose subset H (this compartment eventually would become the organelle), the other must lose subset O. For this to occur, the prospective organelle compartment must exclude proteins encoded by subset O of the prospective host genome but permit import of host subset H proteins. The reverse argument holds for the envisaged host compartment. If import of subset O proteins from the presumed host genome into the envisaged organelle compartment does take place, incapacitation of the presumed organelle subset O genes would take place and terminate the possibility of differentiation. The reverse also holds. No directed change is possible. In short, the duplicated genome origin for organelles depends on the prior existence not only of receptors, but a protein specific set of such receptors, that is, the presumptive organelle membrane must allow the presumed host subset H products in, but exclude presumed host subset O proteins.

The prior existence of, or de novo evolution of, such a selectively permeable protein specific barrier between the two compartments with initially identical genomes is an unacceptable requirement and unlikely to have been realised in nature. It is important to realise that the above argument applies to any genes that are in common between two compartments and not only to the extreme situation of duplication (where all genes are in common). Because autogenous theories assume a common original pool of information for proto-organelle and nuclear DNA, the chances of 'common' genes are high.

It is clear that both the plasmid origin and duplicated genome autogenous origins for organelles are fundamentally flawed in that they make unacceptable requirements so far as membrane permeability is concerned. These logical defects (aside from other evidence) render the Autogenous Theories unlikely.

The examples described above have also emphasised the fact that natural selection acting as creator is a force postulated to account for origin of the organelles. [illegible] ore not surprising that the four objections raised against the [illegible] ionist programme' by Gould and Lewontin (1979) are characteristic of the autogenous explanations. The main weaknesses are failure to distinguish current utility from reasons

of origin; unwillingness to consider alternatives to adaptive expositions; and reliance on plausibility alone as a criterion for acceptance.

6.1.2 The Serial Endosymbiotic Theory (and Xenogenous variants)

In contrast to the Autogenous Theories, the Serial Endosymbiosis Theory depends much more on alternatives to direct adaptation. This can be seen by tracing the origin of the organelles through various hypothesised stages. In a symbiotic origin the entrance of the prokaryotes into the host is accidental, being an effect of ingestion. These prospective cytobionts were co-opted for a new function and *their current utility does* not imply the reasons for their origin. Once the cytobionts became established a period of secondary adaptation was entered. This is the most important period in the history of the endosymbiosis and, as a result, the present thesis has concerned itself primarily with this period in the evolutionary progress of the organelles.

6.1.3 The period of secondary adaptations

The first modification that free-living prokaryotes had to undergo was to regulate their rate of division in accordance with the division of the host. In the last part of the thesis it was indicated that group selection may have been a significant force involved in the origin of a stable host-cytobiont relationship. *This form of* selection acted in opposition to individual selection between cytobionts. In this way parasitic replicators were eliminated. Group selection of endosymbionts is partly dependent on crucial non-adaptive random processes such as segregation, replication and gene conversion for its efficiency. These processes play very important but undirected roles in interactions with natural selection. A simulation of group selection acting on an asexual *population has indicated* that it could be significantly efficient in opposition to individual selection.

Once a stable endosymbiosis had been established, the most drastic and important feature of the period of secondary adaptation took place: the *movement of genetic material*, in a physical and a functional way, from

the endosymbiont population to the host genome. The physical transfer and integration of the cytobiont DNA into the main genome was almost certainly an effect of molecular processes such as liberation of endosymbiont DNA into the cell interior followed by subsequent transformation. Following these accidental events, natural selection became involved by taking advantage of the transfer accident and using it to increase the fitness of the host and endosymbionts.

In spite of the fact that the origin of sex is a controversial issue (Rose 1983), it would appear that advantages associated with the process lie behind the maintenance of it. Sex consists of two basic phenomena: recombination and diploidy, and both seem to have advantages associated with them (Williams 1975; Maynard Smith 1977). Such advantages may be individual and immediate (Williams 1975) or useful to a group or species (Stanley 1979). Sex is advantageous in terms of genome expansion (Lewis and Wolpert 1979) but this is dependent on diploidy which is not characteristic of protist life cycles (Maynard Smith 1978). In conclusion, the transfer of endosymbiont genes to the sexual host genome was postulated to increase the fitness of the host and endosymbionts. In addition, the centralising of formerly cytoplasmically located genes in the nucleus frees these genes from those processes affecting extranuclear genes. Such effects would include group selection and stochastic effects. The transferred genes would become subject to the rules of Mendelian inheritance.

Because of the compartmented nature of the organelles, transferred gene encoded proteins had to enter the proto-organelles to permit degeneration of the cytobiont genome. Due to the selectively-permeable nature of the proto-organelle envelope, Transferred Gene Products could not easily penetrate into the proto-organelle interior, thus negating possible advantages of gene transfer. As before, an accidental effect, namely, spontaneous transport, is postulated to have allowed initial import of Transferred Gene Products until bona fide receptors had evolved. During this period, the original endosymbiont genome provided a back-up system while proper receptors evolved. Receptors were necessary to help Transferred Gene Products to cross membranes as well as to guide them to specific locations within the proto-organelles. As was argued, the

origin of receptors themselves was the result of adaptation, although initially even this process of receptor-protein recognition must have been based on pre-adaptations and chance effects, for example, the already-present signal system coupled with chance affinities.

Information flow from the mitochondrion and plastid to the nucleus appears to have ceased. While physical transfer may continue the functional integration of such DNA genes in the host nucleus seems to be less probable. The distribution of mitochondrial and chloroplast genes is fairly constant in different taxa indicating that there may be a reason for the present distribution of organelle-host encoded genes. It has been argued in this thesis that reasons of biogenesis may be responsible for the continued existence of organelle DNA. The organelle genome in this view would represent an evolutionary stalemate, not a 'dead end' but an equilibrium point between two opposing selective forces. The mitochondrial genome with an altered genetic code is definitely frozen while successful gene transfer in the case of the plastid could be possible.

It is important to note that gene transfer from the cytobionts to the nucleus is in all probability an irreversible process. Once a gene is lost from the cytobiont population and fixed in the nucleus, reintroduction of the gene into the original population is difficult. This is so because the single cytobiont bearing the host gene would have to become homoplasmic. Furthermore, coupling a cytobiont to a specific nuclear background is difficult since the cytobiont may not be co-ordinately transmitted with the nucleus. The end result of the partial gene transfer was a physically small genome coding for a few components. The evolution of this small genome was relatively unconstrained due to a greatly reduced coding responsibility. As a result of this, the organelles, and mitochondria in particular, are characterised by high mutation rates resulting in rapid sequence change, altered tRNAs and altered genetic codes (Jukes 1982). The intrinsic noise described by Reanney et al. (1983) manifested itself strongly. These characteristics were seen largely to be the properties of a small genome rather than adaptations, as some supporters of the Autogenous Theories (see Mahler 1981) have claimed for some of the phenomena.

An important concept developed in the final chapter lies in the fact that the fitness of the host and cytobionts is linked. Arguments were presented that suggest that genes can be selected as units, that is, they can be removed from their original bearer and allowed to evolve further elsewhere. Once the relationship between cytobionts and host is obligatory, the cytobionts can only increase their fitness meaningfully (in the long term) by favouring the spread of the host. Apparently, gene transfer increased the fitness of host and cytobionts. This transfer tendency could have resulted in a cytobiont becoming extinct as an organism (McLaughlin and Cain 1983). The immediate benefits of gene transfer eventually result in the dissolution of the endosymbionts as discrete organisms. In a converse argument it was suggested that extinction, viewed in terms of organisation and fitness, took place when the cytobionts became obligatorily dependent on the host (or vice versa). This extinction was accompanied by the creation of a new individual. The creation of this new individual perhaps represents the establishment of a new hierarchical level in the sense discussed by Vrba and Eldredge (1984). Whether or not this individual could be considered a new hierarchical level, it is instructive to list the principles of hierarchical evolution (Vrba and Eldredge 1984) with their applications to endosymbiosis.

(1) Each new hierarchical step is initiated by the origin of recognition and interaction among entities that are already present.
[Establishment of symbiosis, gene transfer, protein translocation, stochastic population genetical effects]

(2) Lower level phenomena are preadapted for higher organisation.
[Metabolic potential of cytobionts]

(3) Organisation at one level facilitates, constrains and directs possibilities of emergent novelties.
[Host dominance, action of group selection, import of organelle proteins]

(4) Non-random sorting of heritable variation at all levels.
[Individual, group, and 'selfish' selection at the levels of host, cytobiont group and individual cytobionts]

(5) Loss of autonomy at lower levels as autonomy at higher levels increases. Change from 'selfish' to mutually interacting entities.
[Gene transfer, host-located control, evolution of epigenetic system, and change from group to individual selection as far as possible]

In conclusion, it can be pointed out that the endosymbiotic origin of organelles is a very good example of how incidental effects in conjunction with natural selection can interact to have far-reaching consequences.

6.2 The remaining problem

As Gould and Lewontin (1979) have remarked, the division of an organism into parts, and then explaining each as a direct adaptation, is one approach to understanding evolutionary origins and inter-relationships. If the Serial Endosymbiosis Theory is correct, one is presented with a case in which two different genetic units are placed into a position where fusion is not only a possibility but a probability. Such a process of co-adaptation and co-evolution involves loss of functional freedom of the two partners (Riedl 1977, 1978). Initially, loss of functional independence takes place while independent genetic units persist linked through fitness. This would correspond to the position before genic integration or control-related interactions evolved between host and cytobionts. In this state the 'alteration of the genetic information of each of the two structures will only be accepted by selection if they change in a simultaneous and corresponding manner' (Riedl 1977). Such genetically independent but functionally dependent systems (linked) are difficult to change by adaptation because each of the genetic units alters on its own, whereas a common function has in fact developed. This drastically reduces the chances of successful adaptation. If these independent genetic units can become epigenetically dependent, the

adaptive advantage increases very rapidly. Such systematisation involves the evolution of an epigenetic system. A genetic unit superimposed on the others has to develop and this system would mediate between various genes in the two systems. Presumably, in an endosymbiosis, such a system would have to evolve in order to co-ordinate, at the controlling level, the relationship between host genes, transferred cytobiont genes and those remaining in the cytoplasm.

No doubt the meshing of a eukaryote with one or more prokaryotes required unique and sometimes bizarre solutions to unusual gene regulation problems. In the case of the mitochondrion, it would appear that gene regulation by means of interaction of splicing enzymes has been one possible solution. On the other hand, the plastid ancestor seems to have been more advanced, and in the chloroplast and organelle a fusion of eukaryote gene control systems with the prokaryotic operon system may have taken place.

Once the epigenetic controlling system co-ordinating the three separate systems (that is, the host genes, the nuclear-located cytobiont genes, and those remaining in the organelle) has been elucidated, we will be able to view the eukaryotic cell in a holistic fashion instead of having to analyse it in terms of the component units it once was.

APPENDIX 1

THE BASIS OF THE COMPUTER SIMULATION OF GROUP SELECTION IN AN ASEXUAL POPULATION OF HOSTS

As was explained in earlier sections, group selection of cytobionts depends on two critical factors: Random distribution of endosymbionts at division of the host (segregation) and elimination of groups of cytobionts due to a reduction in host fitness (host death). This decrease in host fitness depends on the cytobiont composition of the host at the time. The action of group selection takes place over successive generations and, in conjunction with segregation, produces its effects over a period of time.

A model was designed to demonstrate the elimination of cytobionts with properties which adversely affected host fitness. The characteristic chosen to monitor 'selfish behaviour' on the part of the cytobionts was an elevated replication rate. This was a convenient feature which represented the individual fitness of a cytobiont.

An asexual host population was assumed. By following the fate of lineages arising from a mother cell, an extrapolation could be made to what would be taking place in a population at large.

The mathematical details of the model were largely developed by M R Centner.

Elements of the model

1. Definition of an initial host cell containing a certain total number of cytobionts, together with the proportion of fast and slow replicating cytobionts.

2. Replication of the two types of cytobionts according to their growth rates by means of growth formulae of the type $N_1 = N_0.P_0.(1 + R)$.

3. *Division of the host into two daughter cells.* This division was random in terms of number and proportion of cytobionts. This random variation was produced by using the standard deviation of a binomial distribution ($\bar{p} \pm \sqrt{pq/N}$) in conjunction with random numbers. In this way, normally distributed values of $\bar{p}$ were obtained.

4. The two daughters were thereafter assessed for fitness. A linear fitness curve was constructed as in fig. 1. This allowed a fitness to be determined for a particular cell based on the total number of cytobionts. The fitness curve started with a 'basal' host fitness, representing a symbiont-free individual. After this, an increase in numbers of cytobionts led successively to an increase in fitness, constant fitness and finally a decrease in fitness to zero.

5. After determination of fitness, each of the two daughter cells was subjected to a mortality test. Random numbers between 0 and 1 were used to represent mortality as

   If the random number was smaller [illegible] fitness value, the cell was regarded as a survivor. If both daughters were regarded as extinct, the line had ceased. In the case of one surviving, it formed the next mother cell. Thus, the fitter of the two cells based on numbers, formed the base of a new line.

6. Finally, if both daughters survived one was selected according to different optional criteria. Here one of the two surviving lineages was followed. The other, in theory, also persisted. These choices were very important in the demonstration of group selection.

   (a) Choice of cell with higher fitness, irrespective of composition.

   (b) Choice of cell with highest proportion of fast cytobionts i.e. with higher q value.

   (c) Choice of cell with highest proportion of slow cytobionts i.e. with higher p value.

7. The data were stored in arrays and plotted when a certain number (100) of cycles had been completed or both daughters became extinct. Fig. 2 represents the main elements of the program as described above.

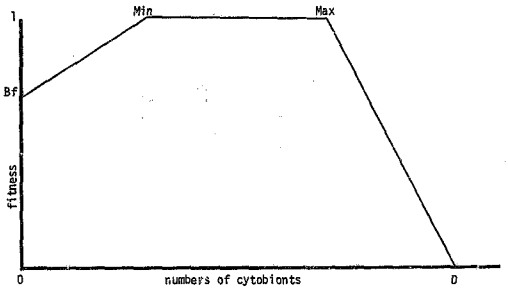


Figure 1

Diagram of the *fitness curve* used to determine the fitness of a cell based on total number of cytobionts. The ordinate *represents fitness* from 0 to a maximum of 1. The abscissa represents total number of cytobionts. Bf represents the basal fitness, that is, the fitness of a cell without cytobionts. Min and Max are the minimum and maximum optimal numbers respectively, correlating to a fitness of 1. D is the death number, which corresponds to a fitness of 0.

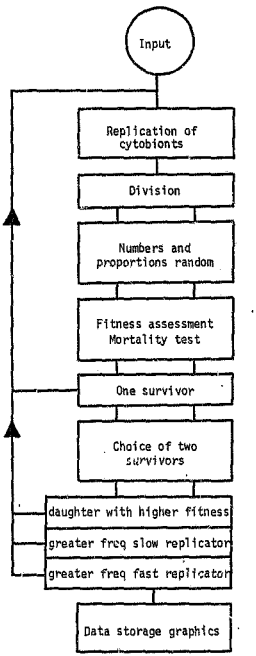


Figure 2

Flow diagram of the program demonstrating the action of group selection of cytobionts in an asexual population of Host cells.

(a) Choice of cell with higher fitness, irrespective of composition of cytobiont population

As can be seen in fig. 3, this selection criterion generally led to extinction. This is so because selection on the rising part of the fitness curve tends to favour the daughter with a higher proportion of fast cytobionts. In some cases, especially when the growth rates of the cytobionts was made similar, p fluctuated more or less randomly.

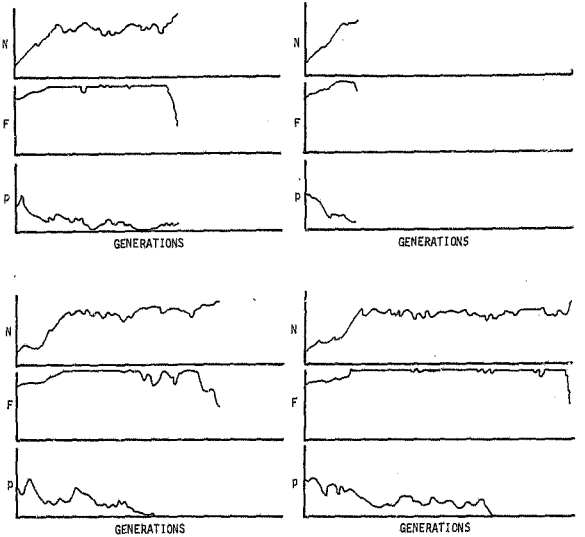


Figure 3

Graphs showing number of symbionts (N), fitness of host (F) and frequency of slower replicating cytobiont (p) against generations. To produce these graphs, the daughter cell with the highest fitness (irrespective of cytobiont composition) determined by the total number present was selected to start the next cycle if both survived. Note that p fluctuates with a tendency to be lost, leading to an increase in numbers and drop in fitness. Selection on the rising part of the fitness curve, favouring cells with more symbionts, in turn favours cells with a higher proportion of 'fast' symbionts eventually leading to the loss of the p gene. These lineages are fairly short-lived. F,p on scale 0-1, N on scale 0-120.

(b) Choice of cell with highest proportion of fast cytobionts

This selection criterion represents, in effect, the 'control'. As is apparent from fig. 4, these lines are short-lived and characterised by a rapid fixation of the q symbiont (fast). After this has occurred, N rises rapidly and fitness drops precipitously. This represents the group selection against hosts bearing the 'selfish' cytobiont.

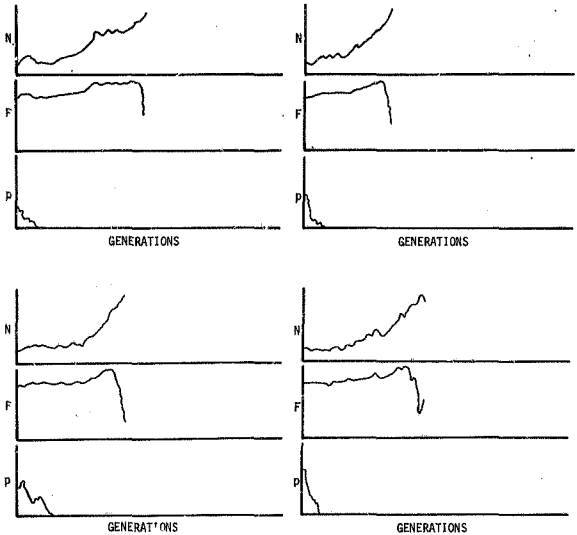


Figure 4

Graphs showing number of symbionts (N), fitness of host (F) and frequency of slower replicating cytobiont (p) against generations. To produce these graphs, the daughter cell with the higher frequency of the fast cytobiont was selected to start the next cycle if both survived. Note that p soon reaches a frequency of zero, whereafter N rapidly multiplies, leading to a steep drop in fitness causing extinction of the line. These graphs represent the 'control'. F,p on scale 0-1, N on scale 0-120.

(c) Choice of cell with highest proportion of slow cytobionts

These curves, depicted in fig. 5, represent the 'experiment' and are characterised by an increase in p, the frequency of the slow cytobiont, to fixation. Fitness and numbers remain relatively constant, being affected mainly by drift. This drift component sometimes causes numbers to fluctuate randomly in unfavourable directions leading to extinction. While all the lines did not persist indefinitely, a much larger proportion continued for more rounds than was the position observed with selection of fast cytobionts. These curves were never (with the exception of that mentioned below) characterised by precipitous decreases in fitness. In some cases, if the fast cytobiont had a much faster replication rate than that of the slow counterpart, selection of the daughter with the larger proportion of the slow cytobionts could not counteract the excessive replication of the fast and the line gave an appearance normally associated with selection of the fast cytobiont.

The nett longer persistence of lineages as shown here represents the survival of hosts bearing the higher frequency of 'in-phase' cytobionts.

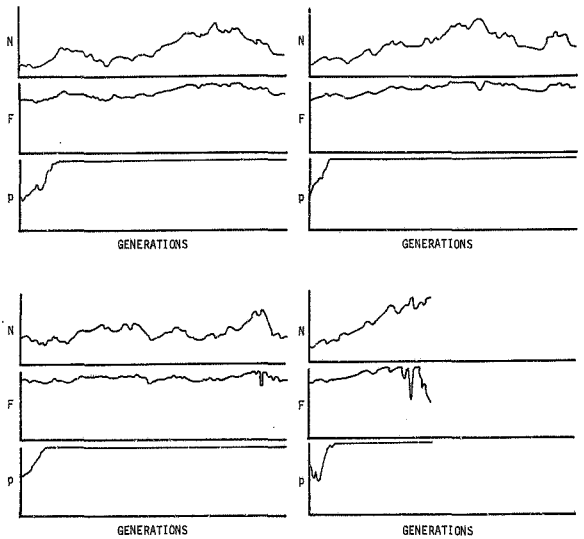


Figure 5

Graphs showing number of symbionts (N), fitness of host (F) and frequency of slower replicating cytobiont (p) against generations. To produce these graphs, the daughter cell with the higher frequency of slow cytobiont was selected to start the next cycle, if both survived. Note that p goes to fixation while N and F tend to be fairly stable. Random fluctuation of N is apparent, and in one example (bottom right) it leads to extinction of the line. These graphs represent the 'experiment'. F,p on scale 0-1, N on scale 0-120.

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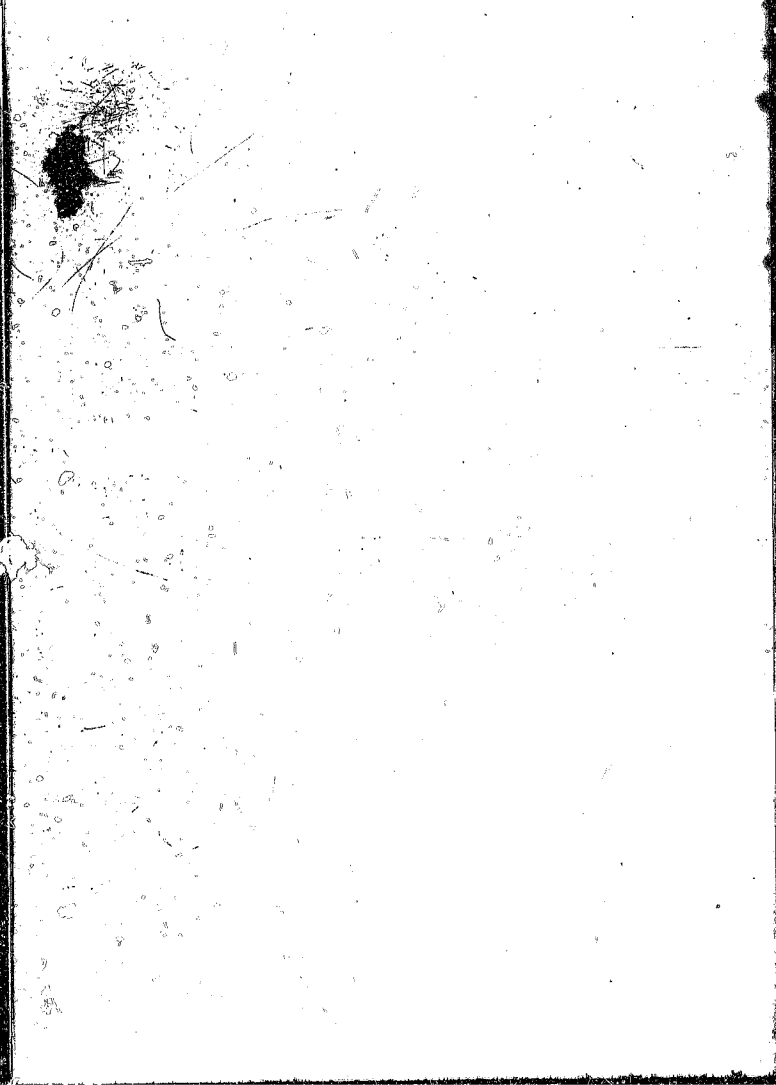
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Author Harington Alexis
Name of thesis The Role Of Natural Selection In The Endosymbiotic Origin Of Organelles. 1985

PUBLISHER:
University of the Witwatersrand, Johannesburg
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