AN INQUIRY INTO THE NATURE OF SYMBIOSIS

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ABSTRACT: Evidence is reviewed which supports the derivation of several prokaryotic features in the order Kinetoplastida from bacterial endosymbionts. It is postulated that symbionts have become extinct in the majority of trypanosomatid species, but that the host has retained several probably adaptive features derived from the bacterial symbionts. A synthesis of general symbiology evolutionary principles is proposed which includes: the widespread and rapid evolutionary development of symbioses among free-living organisms; a frequent progression of types of symbiotic relationships through time (e.g., a symbiont population may progress from a pathogenic to a mutualistic relationship through time); the transfer to the host of adaptive symbiont traits; and the eventual extinction of the symbiont. A steady-state level of symbiotic relationships of various types is proposed, maintained by recruitment of symbionts from free-living species. Chloroplast and mitochondrial evolution are considered as special cases of this evolutionary model. Obligate symbioses are viewed as eventually adaptive to only one of the two species, the host. Evidence and consequences of this model are discussed.

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Questions in Symbiology

Symbiosis historically has been a very broad term, referring to any intimate interspecies interaction (De Bary, 1876). In practice, symbiosis usually refers to an interaction in which one species lives in or on the body of another (Schmidt and Roberts, 1981). There are many categories of symbiosis, generally defined by whether, in the short term, a given partner is harmed or benefited by the relationship. Parasitism, for example, is a widespread type of interaction in which the host is harmed, while the parasite benefits. Mutualism, on the other hand, is an interaction that benefits both partners. The distinctions between parasitism and mutualism are somewhat vague, however, and relationships can easily shift from one category to another, sometimes even in response to such factors as a change in diet (Trager, 1970).

Organisms from almost any group appear to be able to interact with, or to become, symbionts (Trager, 1970). Many of the mutualistic symbioses provide emergent capabilities not available to either partner alone, and are of fundamental importance to life on the planet. For example, algal-fungal interactions produce lichens, frequently the pioneers in terrains in which neither partner alone could survive. One of the greatest biomasses on earth is constructed from the interaction of coelenterates and dinoflagellates, which produces coral reefs (Barnes, 1974). The Rhizobium-legume association allows the agriculturally important fixation of nitrogen in the soil.

The study of the nature of symbiotic relationships may provide answers to several tantalizingly fundamental questions. How are such interactions established and controlled (e.g., how are the numbers and activities of the partners regulated)? Does the eukaryote always control in a prokaryote-eukaryote interaction, and if so, why? How often and how rapidly do such interactions evolve, and how fast does the relationship change? Given the premise that the type of interaction is subject to change, is there a 'typical' progression of types of interorganismal

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interactions through evolutionary time? What is the usual end point of such evolutionary progressions? We believe that preliminary answers to these types of questions are becoming available within one common type of symbiosis, i.e., bacterial-eukaryote endosymbiosis. Trends followed in these and other systems may suggest answers to the general questions posed above. In this essay, an attempt will be made to synthesize these answers into a general principle of the evolution of symbioses. Mitochondria and chloroplasts will be considered as special cases of such symbiont evolution.

Bacterial-eukaryote Systems

The resemblance between eukaryotic-bacterial symbiotic systems and the popular hypothesis of the symbiotic origin of mitochondria (Margulis, 1970) has been an underlying theme for much of the work done on these systems (Chang, 1975). The concept that this (presumably) more recent evolutionary event, i.e., the acquisition by a flagellate of a symbiotic prokaryote, might recapitulate the primordial evolution of the present-day eukaryote and its mitochondrion, is inherently intriguing. Our concept is that, whereas most of the evidence for the sequence and nature of the mitochondrial evolution(s) is lost, a series of more recent associations, especially a series showing gradations of types of interactions, may shed some light upon the usual sequence of events in bacterial-eukaryote evolution. By inference (with the assumption that current eukaryotes and prokaryotes resemble primitive ones) one or another of the hypotheses of mitochondrial origin may be made slightly more plausible. By extrapolation, some ideas about general principles of symbiology may be developed.

Hypotheses of Mitochondrial Origin

Two fundamentally differing views are taken by workers on the origin of the mitochondrion. One alluded to above (Margulis, 1970) holds that the mitochondrion arose from a prokaryotic eubacterium after its capture by or invasion of a primitive eukaryote. The other (Raff and Mahler, 1975; Taylor, 1976) holds that the eukaryotic cell arose directly from prokaryotic-type progenitors, with the mitochondrion, for example, evolving from a pinched-off mesosome. An interesting recent variation on the capture and integration view holds that the mitochondrion arose from chloroplast(s) or captured green or blue-gree algae, which lost their photosynthetic capabilities (Woese, 1977).

Proponents of both views have used the same available information to support their conceptions of mitochondrial evolution. For example, the mitochondrion's generally histone-free DNA and chloramphenicol-sensitive translation system can be correlated with the bacterial nature of either the captured symbiont or a more conserved, specialized region of the progenitor prokaryote. A similar dilemma arises for almost any feature chosen for study; since both hypotheses predict some retained prokaryotic components, it is hard to decide what sort of evidence could exclude one of the hypotheses. Specific arguments or scenarios may be made less likely. For instance, the suggestion that a separate genome and translation system is necessary for making proteins which incorporate into the mitochondrion's inner membrane (Raff and Mahler, 1975) seems less likely, now that a mechanism for inner membrane insertion of cytoplasmically synthesized proteins has considerable support (Schatz, 1979; Anderson, 1981). Similarly, the finding of advanced types of information processing such as introns within mitochondria (Fox, 1981) make less likely the argument that new genes for mitochondrial functions were located in the nucleus because the mitochondrion had a primitive information processing system as suggested by Woese (1977).

Symbiosis in Nature

An "argument by analogy" approach seems less susceptible to the above objection that both major hypotheses of mitochondrial evolution predict both eukaryotic and prokaryotic components in the mitochondrion. Since the postulated ancestral bacterium was free-living before its capture by or invasion of a eukaryote, whereas the mitochondrion is highly dependent upon "host" factors, existence of a progression of intermediate forms could be interpreted as a recapitulation of such event(s), and would lend support to the hypothesis. Bacteria-eukaryote symbioses indeed occur frequently in nature, and show a diversity of types; one generality is that the host's nutritional requirements are often simplified by the bacterium (Margulis, 1970). Relationships can be found which show a plausible continuity from a quite facultative to a completely obligate nature (for simplicity, considered from the standpoint of the smaller symbiont), as would be expected during the evolution of a mitochondrion from a once free-living bacterium. There are, for example, associations which are reversible in naturally encountered conditions, such as many fungi-algae associations of lichens, and many dinoflagellate-coelenterate associations of corals (Muscatine, 1974). Rhizobium can exist either as a free-living bacterium or in the legume-Rhizobium nitrogen-fixing symbiosis. Bacteroids in cockroach mycetocytes, the kappa-type organisms in Paramecium (Trager, 1970), and the symbionts in the lower trypanosomatids, on the other hand, seem to be irreversibly committed to intracellular symbiosis.

Kinetoplastid Evolution

Similarities between the above progressively more obligate symbioses and a similar hypothesized progression during mitochondrial evolution seems to give the latter more plausibility. However, one problem is that such symbioses seem to appear fairly randomly across phyla, and display no traceable evolutionary development or progression. It is more meaningful to trace the apparent progression of a bacterial-eukaryote association within a group which has a defined set of evolutionary origins, a well-established "family tree". Members of the order Kinetoplastida may present such an evolutionary continuum (Hutner et al., 1979; Manwell, 1968). A preliminary tree for the evolution of the kinetoplastids is shown in Figure 1.

The superscript (S) in Figure 1 indicates the presence of an intracellular bacterial endosymbiont in at least some members of the taxonomic group. The bodonids are biflagellated, largely free-living members of the order Kinetoplastida (Vickerman and Preston, 1976), to which the family Trypanosomatidae also belongs. The Euglenida is a diverse order in the class Phytomastigophora, of the same subphylum as the kinetoplastids (Mastigophora). Many taxonomists place the euglenoids evolutionarily near the kinetoplastids (Taylor, 1976; Levine et al., 1980). Support for the euglenoid-bodonid common ancestor (step 1 in the figure) includes the following euglenoid-bodonid cytological similarities, which are also shared with the trypanosomatids: 1-2 flagella, deformable pellicle with underlying rows of microtubules, and a similar type of paraxial rod (Vickerman and Preston, 1976). Biochemical similarities between euglenoids and trypanosomatids include the occurrence of trimethyllysine in both euglenoids and trypanosomatids, retention of α-linolenic acid (an algal, fungal, and plant lipid) in lower trypanosomes (Meyer and Holz, 1966; Beach et al., 1979), polyphosphate storage both in some trypanosomatids and in euglenoids, a similar cytochrome C sequence, and an anomalous pyridine haemochrome of cytochrome C_{555} (Bowman and Flynn, 1976).

Although bodonids have not yet been characterized biochemically, support for the relationship between the bodonids and trypanosomatids (step 2 in the figure) includes the above mentioned cytological similarities, and most convincingly the presence of a unique organelle, the kinetoplast. Finally, support for a

Naturally, to maintain such a unidirectional process, new symbionts must be recruited. Of course, these would come from among free-living species. Given the concept that obligate parasites and pathogens cannot reevolve to free-living status, the continual falling into such obligate relationships would have long ago made virtually all species into parasites. Since this is not so, we see that obligate symbionts eventually become extinct. There are naturally energetic and other considerations which help to explain why the host would not long maintain a parasite, or even a mutual as an entire organism, if it could otherwise accomplish the needed tasks (for instance, by stealing the capacities from its symbiont). The symbiont is at a disadvantage in the host-symbiont relationship because lines of free-living organisms must continue, to provide a home for the symbiont; the host lacks this important restraint (recall a symbiont lives on or in a host, in our definition of symbiosis). We would propose that there is an approximately constant steady state flow in the evolution of organisms, from free-living organisms, to facultative symbionts, to obligate symbionts, and eventually to extinction, through evolutionary time. We propose this as a fundamental unifying concept underlying the evolution of symbioses.

Within this schema, we do not postulate that very well-adapted organelles such as mitochondria are usually bumped out by newcomers, but that in specialized environments, intracellular niches are rapidly filled to fit host needs. Other organelles such as hydrogenosomes in Trichomonas vaginalis (Honigberg, 1978) arose from incorporation of originally free-living, appropriately competent microbes into the cytoplasm, for instance, as the host invaded the environment in which such microbes live. The diversity of symbiont types is thus seen as part of the same process which produced other cell organelles. A susceptibility to invasion and a hospitality even to pathogens may be adaptive for a population of free-living organisms, as a mechanism to rapidly acquire necessary (perhaps unusual) capacities. It is a source of radical recombination events. The captured organisms may have evolved a given capacity only after millenia of selection, whereas the initially hospitable host may steal them within a brief (recall Jeon's ameoba-bacterial mutualism established within 100 generations) evolutionary time period. Some insight into the underlying reason for gaps in immunity may thus be gained from this perspective.

It is important to reemphasize the dynamic nature of this postulated set of evolutionary steps. Intracellular symbioses progress not only to a stage wherein it becomes difficult to distinguish an intracellular symbiont from a de facto organelle (Trench, 1980), but eventually to oblivion, also, generally, even as organelles. Establishment of an obligate mutualism is not an end point, but simply another step of a more general process. It is also important to reemphasize the universality of the principle (see Figure 2). Although this discussion has so far centered upon bacterial-eukaryote systems, the evolutionary principle (which one might summarize, from the standpoint of the smaller partner, as the black hole of symbiology) is believed to apply not only to other microbes, such as algae, but to metazoan symbiosis as well. We would also postulate that the evolutionary forces underlying this general principle act also on older organelles such as the mitochondrion and the chloroplast. Indeed, mitochondria of higher eukaryotes possess less DNA than do those of protists (Anderson et al., 1981). Mitochondria and chloroplasts may thus be seen as rocks in the stream of symbiont evolution; other symbionts flow around their well-filled niches, as functions are only slowly removed from the control of these primordial symbionts.

Elaboration of the Model

At this point it should be cautioned that even valid general trends usually have specific exceptions. Our evolutionary model does not predict the exact nature of a symbiont's evolutionary progression in the short term. Symbionts

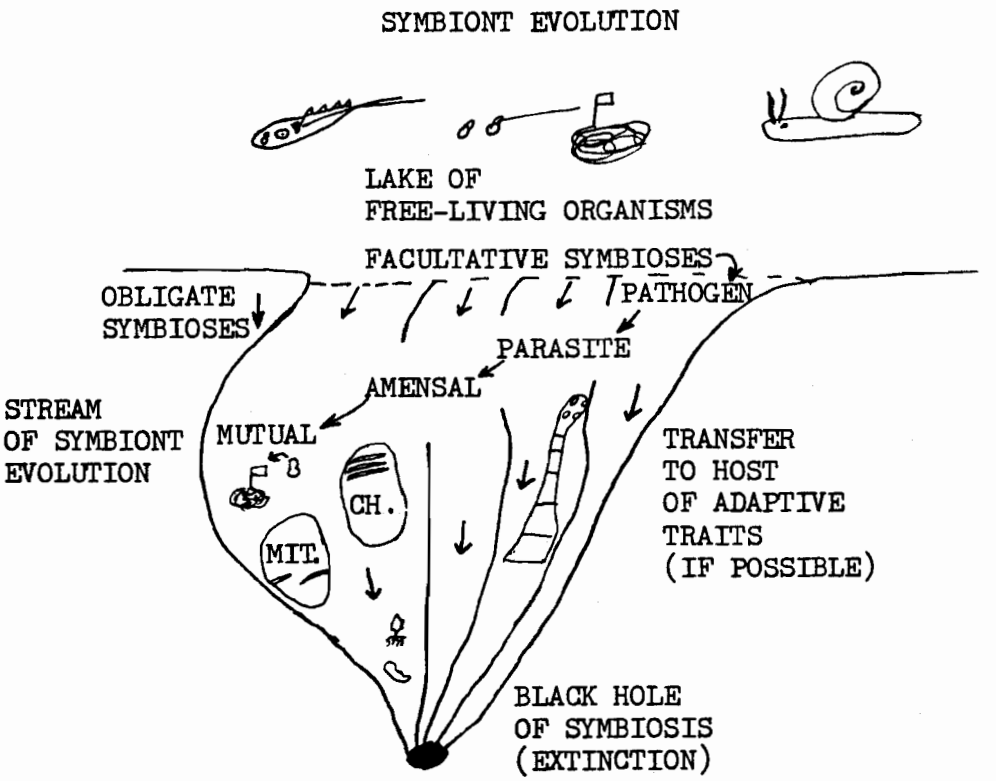

Figure 2. Shown is a sketch of the postulated model of symbiont evolution. At the top are represented free-living organisms, including a kinetoplastid protozoan recently invaded by a bipolar bacterium, similar bipolar free-living bacteria, and a snail (the latter to emphasize that any phylum is susceptible to invade or to be invaded). Within the bipolar bacteria is postulated to exist an enzyme (scrawl with a raised flag) able to catalyze a reaction needed by the protozoan in a given habitat. As it progressively loses autonomy over more functions, the symbiont typically becomes a pathogen, parasite, amensal, mutual, and extinct. The necessary information regarding the adaptive trait may be transferred to the host at any time; in the flagellate-bacterium example, while the bacterium is a mutualist, the DNA specifying the crucial flaglike part of the enzyme has been recombined to make a hybrid enzyme in the host. The mitochondrion (MIT) and chloroplast (CH) fill major niches in the crowded cellular habitat. Other habitats include the gut (as in the parasitic tapeworm sketched) and almost every other organ. All obligate symbionts progress toward the black hole of symbiosis, extinction, often becoming unrecognizable as organisms (viruses, plasmids, jumping genes, organelles) during the process.

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respond to the individual selection pressures of the environment in which they are found. If it is adaptive to become larger, this can occur (how else to explain the existence of Ascaris?). Similarly, numbers of individuals can increase, or the life cycle can become more complex; in fact these appear to be common trends among parasites (Schmidt and Roberts, 1981). These are, as it were, eddies in the stream of symbiont evolution.

The more unique feature of symbiont evolution is the eventual progression (though the rates of such progressions may differ) toward extinction as organisms. Extinction is not necessarily the end point for free-living organisms; though many free-living species do go extinct (probably even without becoming symbionts, unless one counts ingestion of their remains as a symbiosis), free-living lines are maintained. According to the black hole model just outlined, virtually all lines of symbiont organisms will become extinct, and previous lines have repeatedly done

so. We would like to discuss briefly some of the possible consequences of this symbiont extinction principle.

First, if obligate symbionts progress toward extinction, then becoming an obligate symbiont is highly nonadaptive. Why, then, does symbiosis occur so frequently in nature? A trivial explanation is that evolution is a blind process. One could imagine that a positive feedback of many progeny produced, coupled with the genetic isolation inherent in life within a host, accidently leads to an obligate life style in a given population and eventually a new symbiotic species.

Although the above scenario of positive feedback and genetic isolation superficially explains the evolution of symbioses, we reiterate that obligate symbioses are eventually strongly nonadaptive to the symbionts. The tendency to become symbionts must have been repeatedly selected against through evolutionary time; mechanisms resisting a symbiotic life style should have evolved. So again, why do obligate symbioses occur so frequently in nature? We suggest that organisms might have evolved mechanisms which help to encourage and control the evolutionary development of other species into symbionts. Why this may be so will be discussed later. We would also suggest that early symbiotic relationships between similar-sized species may be seen as a struggle; the winner (eventual host) has either the best cross-species control mechanism(s), and/or the best mechanism(s) for resisting its own incorporation. Recall that several of the crucial features of the relationship which will eventually be controlled are the number and the activities of the symbiont. DNA regions controlling autonomy-related functions such as the cell cycle would be both the target of takeover mechanisms by a potential host, and the regions over which a potential symbiont would attempt to retain control.

In this context, existence both of mechanisms allowing rapid recombination, and of DNA regions in which recombination is restricted (Thompson and Woodruff, 1978; Watson, 1976) in the genome of almost all organisms is interesting. A list of all possible recombination mechanisms is beyond the scope of this discussion, but several features may give advantages of eukaryotes over prokaryotes (besides the obvious size differential and phagocytic capacity which usually favor the eukaryote). The nucleus is partially protected by the nuclear membrane (perhaps the DNA is further protected by histones); and more DNA, scattered in multiple chromosomes, allows more rapid and varied recombination events. Specialized mechanisms such as mutator genes appear to exist which can increase the rate of recombination, for example in response to stress (such as temperature; but we suggest, including the stress of a parasitic invader), even in a single generation (Thompson and Woodruff, 1978).

Capacity for recombination may be important not only in the struggle for control of the relationship, but also as a mechanism for extracting a symbiont's adaptive trait, for instance a gene for a useful enzyme or part of such an enzyme. One would not necessarily require that the host be able specifically to remove the adaptive gene, although gene removal mechanisms may have some specificity. Additionally, symbionts containing adaptive traits would survive longer in the host (there is less selective pressure for their removal), and probably amplify genes which sustain the host; both of these processes would increase the host's overall chance of successfully stealing the trait. Even assuming a high degree of randomness in the removal mechanisms, those removed characteristics which are useless to the host would be at least slightly nonadaptive and would thus eventually be removed from among the eukaryote's progeny. If this seems at all a slow or energetically expensive process, it should be compared with the alternative of evolving the trait de novo. The negative effects of parasites or even pathogens on a host population may be evolutionarily cheap, considering the potential for novel recombination events made available from such symbioses.

Vectors of the Metazoa

Because of the ubiquity of disease organisms and parasites, one concerned with eliminating them might be tempted to assign the shedding of debilitating parasites as one function of the return by metazoa of each generation to a single cell. However, it should also be realized that the cleansing return to unicellularity also provides an opportunity for a novel assortment of symbionts in the host's offspring. Such new symbionts can provide potentially novel recombination opportunities, even as the offspring are provided with novel arrangements of previously assimilated genes of both parents. There is evidence that DNA from viruses and isolated DNA injected into embryos can enter the germ lines of the resulting animals and be transmitted to their progeny (Marx, 1982; Mintz, 1978).

It is possible that only parasites infecting germ-line cells can contribute directly to the gene pools of metazoa. If this is true, only viruses and perhaps rickettsia would be likely vectors of permanent alterations in the hosts' chromosome-located DNA. However, the apparent ease of recombination between various groups of viruses and bacteria might still allow gene flow from nongerm-line infecting organisms.

It appears less likely, but it would be more interesting if communication (i.e. gene transfer) between somatic cells and germ-line cells is possible. In this case, even information used to modify tissues or organs in an individual, acquired not from its gametes but from its symbionts, could be retained in subsequent generations.

Chloroplasts and Mitochondria

As mentioned earlier, the authors consider chloroplast and mitochondrial evolution to be special cases of symbiont evolution. It is possible that these organelles have neared the end point of any more or less stepwise simplification which allows their continued function. This is not to say that a future multiple alteration could not make these organelles obsolete. Indeed, we would argue that there are inherent energetic and adaptive disadvantages to having two genomes and translation systems are energetically expensive to maintain. Furthermore, the host endures the complications of coordinately evolving at least partially separated genomes. There is also the potential for at least brief spurts of aberrant, independent evolution, e.g. favoring organelles rather than the host. Eventually, given time and fortuitous events, these considerations lead to the prediction that even the well-adapted mitochondrion (or certainly, all potential for its autonomy) will eventually be lost.

It has recently been reported that the coding specificity of mitochondria involves a decreased number of different tRNA codons and tRNA molecules. Furthermore, both eukaryotic (e.g. introns) and prokaryotic (e.g. initiation codon) features have been found in mitochondria (Borst and Grivell, 1981). Explanations for these data have included a polyphyletic origin of mitochondria (with perhaps some deriving from the Archaeobacterium group of Woese (1980)) and genetic drift. We would suggest that besides genetic drift and direct energetic considerations, there is further selective pressure toward simplification related to the essentially symbiotic nature of the mitochondrion. Specifically, as discussed above, it is adaptive to have a minimal number of parameters for the host to control.

It is, of course, possible that mitochondria directly arose from quite diverse groups (Woese, 1980). However, this does not explain the great number of mitochondrial homologies (e.g. the same 8-10 peptides translated in the mitochondria of organisms from several phyla). We point out that the concept of a continual incorporation and dissolution (the steady state flow) of symbionts through evolutionary time within the bodies of hosts makes unsurprising the acquisition of unusual characteristics in diverse groups or in their mitochondria. A mixture

of prokaryotic and eukaryotic characteristics is probably one of the more common results of such associations.

Epilogue

During and after the great plagues of Europe, observers were amazed to see processions of ill people dancing in a delirium of apparent joy (Deaux, 1969). Many people are familiar with the unique feeling of well-being after recovery from a serious illness. It may be an instinctive wisdom of life, that an illness constitutes not only a danger to the individual, but an opportunity for the species.

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